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Multiple factors contribute to the spatially variable and dramatic decline of an invasive snail in an estuary where it was long-established and phenomenally abundant

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Abstract Boom-bust dynamics of invasive species have long intrigued scientists and managers alike, but quantification of such dynamics, let alone their causes, is rare. We documented the decline of a previously prolific invasive mudsnail, *Batillaria attramentaria*, at Elkhorn Slough estuary in central California, USA. The mudsnail was the most abundant epibenthic invertebrate in the estuary, maintaining very high densities for many decades before declining heterogeneously throughout the estuary over the past decade, decreasing in density by three orders of magnitude at some sites. We used field and laboratory experiments to test several possible mechanisms for its demise. We show that the crab *Pachygrapsus crassipes* can prey heavily on *Batillaria*. We detected high dissolution rates of *Batillaria* shells, and we measured greater predation rates on tethered snails with dissolved versus intact shells. Warm water temperatures and high water levels coincided with the period of most dramatic *Batillaria* declines (2013–2015). Localized water impoundments appear to buffer environmental drivers of the decline because *Batillaria* remained abundant at sites with artificial tidal restriction, while the population crashed at one site after full tidal exchange was

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restored. We also investigated trematode parasite prevalence and molluscicide applications to the surrounding watershed as possible causes of mudsnail declines, but they had little explanatory power. Our findings illustrate the potential for population crashes even for long-established introduced species at pest levels of abundance, and demonstrate that such declines can exhibit spatial heterogeneity. Both of these results highlight the value of investigating population dynamics of invaders across multiple temporal and spatial scales.

Introduction

Only a small fraction of species that are introduced to new ranges by human activities become highly abundant in the new ranges (Williamson 1996). Yet species that reach outbreak levels of abundance are likely to have significant ecological impacts on the communities they invade because abundance scales positively with impacts (Parker et al. 1999). Species that persist at extremely high abundances may continue to exert strong ecological influences indefinitely (Sakai et al. 2001). However, dramatic population declines can occur after invasive populations have achieved high abundance. These so-called boom-bust dynamics have long intrigued invasion scientists (Elton 1958; Simberloff and Gibbons 2004; Cooling and Hoffman 2015; Aagaard and Lockwood 2016). From a conservation perspective, boom-bust dynamics are important because they suggest that outbreak or boom phases may be transient, and negative ecological impacts may be reversible if the bust is persistent (Strayer et al. 2017).

Many factors can lead to rapid, precipitous population declines of invasive species (Strayer et al. 2017). By definition, an invader has an initial abundance of zero, and must achieve positive population growth to establish and become common in a new range. Sometimes, the same factors that facilitate a rapid transition from zero to high abundance may later cause the reverse trend. For instance, a non-native species that is not initially recognized and attacked by predators and parasites in the introduced range may initially undergo rapid population growth because it faces fewer enemies (Keane and Crawley 2002). However, an established non-native population may later crash when new non-native enemies are introduced, or native ones adapt to the invader (Strayer et al. 2006; Diez et al. 2010; Strickler et al. 2016). Rapidly growing populations may be prone to density-dependent crashes following high abundance, as modeling with time-lags suggests (May et al. 1974). Busts may also occur because the reduced genetic diversity common in populations of many invaders attenuates the capacity for rapid adaptation to sudden environmental shifts (Aghighi et al. 2014).

Quantitative documentation of declines in abundant, well-established introduced species is rare, and

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rigorous examinations of potential causes are even rarer (Simberloff and Gibbons 2004; Strayer et al. 2017). Understanding the conditions under which abundant invaders may crash is important for shaping our conceptual understanding of invasion dynamics, and for informing management. Multiple approaches have been used to unravel causes of population declines in endangered species, such as comparing spatial distributions of populations under different environmental conditions, correlating temporal patterns of decline with environmental changes, and experimentation (Peery et al. 2004). These approaches are relevant to investigating changes in invader populations, although the conservation goals for endangered and non-native species tend to be opposite. The challenge to obtaining a robust understanding of spatial and temporal patterns is having sufficient data collected broadly over space and time.

At the Elkhorn Slough estuary in central California, the invasive Japanese mudsnail *Batillaria attramentaria* was once estimated to number a billion individuals (Wasson et al. 2001). The first field record of *Batillaria* in Elkhorn Slough dates to 1951 (Carlton 1992), though the snail may have arrived as early as 1930 (Bonnot 1935; Byers 1999). By the end of the 1950s, *Batillaria* had established densities of thousands of individuals per m^2 (McClean 1960). After several intervening decades, *Batillaria* continued to be documented at such high densities at multiple sites within the estuary (Byers 1999; Fabian 2016). The striking abundance of this invader was a familiar feature to visitors at the Elkhorn Slough Reserve, and was highlighted in the Reserve's visitor center and educational tours. Then, around 2013, anecdotal observations of decreased abundance of the snail were recorded. In 2015, we were stunned to discover that live *Batillaria* (and most of their shells) had entirely disappeared from a site that Byers (1999, 2000a) had studied and Wasson et al. (2001) had dubbed "*Batillaria* Heaven" because of the remarkable snail densities there (Fig. 1). For many familiar with Elkhorn Slough, this decline was the biggest "ecological surprise" (*sensu* Doak et al. 2008) of our careers: an extreme and unexpected biotic change likely to have far reaching ecosystem effects. We thus launched a multi-faceted investigation of the *Batillaria* decline.

The first goal of this investigation was to quantify the population trajectories of the invasive mudsnail across multiple sites in the estuary, documenting patterns of density over time by compiling historic data and conducting new surveys. Our aim was to determine whether the decline was of sufficient magnitude and duration to qualify as a collapse (Aagaard and Lockwood 2016), and if so, where and at what scale. The second goal was to explore multiple causal mechanisms that might have driven the decline, singly or acting in concert. We employed the "multiple competing hypotheses" approach to examining population declines, drawing on various datasets to evaluate support for different causal factors (Peery et al. 2004). We used correlative studies to determine whether the temporal or spatial patterns of decline matched patterns of change in environmental variables, and we conducted manipulative experiments to test hypotheses. At the outset, we considered many equally plausible causal factors that could have driven or contributed to the decline. Predation by crabs was one focus because (1) crabs seemed particularly abundant at sites where *Batillaria* had crashed (Fig. 1); (2) crabs consumed large numbers of *Batillaria* when they infiltrated field enclosures in Elkhorn Slough during a separate experiment (Fabian 2016); and (3) crabs were recently documented preying on *Batillaria* further north in Washington (Grason et al. 2018). Acidification was also of interest because we noted dissolution of *Batillaria* shells, and ocean acidification has been identified as an emerging driver of changing coastal conditions (Doney et al. 2009). Parasitism was also identified as a possible mechanism, because a recent study (Fabian 2016) found higher infection prevalence of larval trematode parasites that sterilize the snails than had been documented in earlier observations of prevalence in Elkhorn Slough (Torchin et al. 2005). Also, since Elkhorn Slough is surrounded by extensive agriculture, we considered a potential role of molluscicides, as well as other water quality conditions, in *Batillaria*'s decline. By quantifying patterns across multiple decades and sites, and exploring multiple causative factors with correlative and experimental studies, we provide a thorough investigation of a remarkable boom-bust episode of a long-term, well-established, widespread invader.

Methods

Study site

Elkhorn Slough is a 1200-ha estuary, extending about 10 km inland from the town of Moss Landing in the Monterey Bay region of central California. The history

Fig. 1 Dramatic change in abundance of *Batillaria* at two sites. Left top: Jeb Byers conducting experiments in 1998 at the site called "*Batillaria* Heaven" (4-BH) due to the striking densities there—photo shows the ground carpeted by mudsnails; Left bottom: the same site in 2018, with zero snails present, and numerous *Pachygrapsus* crabs (in black circles); Right top: high densities of snails at Whistlestop Lagoon (5-WL) documented in 2011 by Rachel Fabian; Right bottom: the same area in 2018, after years of strong declines

and ecology of the estuary are well described (Caffrey et al. 2002). The region has a Mediterranean climate, with all appreciable rainfall occurring between October and May. The estuary receives limited freshwater input, and salinity in undiked regions is usually close to marine concentrations. Tides are semi-diurnal, with a maximum tidal range of ~ 2.5 m. Extensive portions of the estuary have been diked (Van Dyke and Wasson 2005), and areas with restricted tidal exchange have impaired water quality and symptoms of extreme eutrophication, including night-time hypoxia and extensive algal growth (Hughes et al. 2011). About 60 non-native invertebrate species have been documented in Elkhorn Slough, mostly introduced during attempts at non-native oyster culture and by boats traveling from major ports to Moss Landing Harbor near the estuary mouth (Bonnot 1935; Wasson et al. 2001, 2005).

Study species

The Japanese mudsnail *Batillaria attramentaria* is a highly successful invader of intertidal habitats in North American estuaries from Boundary Bay, BC, to San Diego, CA (Byers 1999; Crooks et al. 2016). Its native range extends from Hong Kong in the south to Russia's Kuril Islands in the north (Prozorova et al. 2012); the snail is often the dominant invertebrate in salt marshes and on mud flats along the northeastern coast of Asia (Hasegawa 2000). On the North American West Coast, *Batillaria* displaces its native ecological analog, *Cerithideopsis californica*, where the species overlap, due to its superior resource conversion efficiency and lower mortality (Byers 2000a; Byers and Goldwasser 2001). *Batillaria* is a direct developer, and its lack of a planktonic stage limits its dispersal and likely the extent of its introduced range in the eastern Pacific, but it remains a risk, with recent invasions in San Francisco Bay (2005), Bodega Bay (2007) (Weiskel et al. 2007), and the Tijuana River Estuary ($\sim$ 2016) (Crooks et al. 2016).

Batillaria is a deposit feeder, primarily consuming epibenthic diatoms in the upper and middle intertidal zones (Whitlatch and Obreski 1980, Byers 2000a). It is long-lived ($\sim$ 6–10 years), growing to 2–3 mm in the

first year, and maturing at 2–3 years at sizes of ~ 5–17 mm (Whitlatch 1974; Yamada 1982). In its native range, *Batillaria* is the first intermediate host to the larval stage of at least nine trematode parasite species (Hechinger 2007) with complex life cycles that subsequently parasitize a variety of second intermediate hosts (e.g., crabs, fishes), with birds as definitive hosts. Only one of these trematode species infects *Batillaria* in its North American invasive range (Torchin et al. 2005; Miura et al. 2006). Infected snails are typically not killed, but are sterilized by the infection, thereafter only producing parasites (Hechinger 2010).

General approach of study

In July 2015 we noticed the complete absence of *Batillaria* from a site (dubbed "*Batillaria* Heaven") where it had been extremely abundant for decades, where we had regularly taken visitors to show them its remarkable abundance between 2000 and 2013 (Fig. 1). This striking change motivated a series of subsequent surveys and experiments, conducted over a period of 3 years. In late summer 2015 we began consistent surveys of *Batillaria* abundance at eight focal sites (Fig. 2) so we could track further changes; these surveys continued through 2018. We compiled earlier data on mudsnail abundance from other sources to enhance our analysis of longer-term changes. Between 2015 and 2018, we conducted various small-scale experiments to explore potential causes of the decline, which we describe in detail in the sections below. In summer 2018, we conducted more extensive experiments and surveys building on the earlier work, and these form the centerpiece of our spatial comparisons of sites. We also explored possible correlations of decline with factors such as disease or molluscicide use. For the sake of brevity and to enhance clarity, the methodology and results are summarized here and reported upon in greater detail in the Supplementary Information (SI). All statistical analyses were conducted using R version 3.5.2 (R Core Team 2018).

Documenting *Batillaria* distribution and abundance trends

To quantify *Batillaria* abundance for spatial and temporal comparisons, a permanent transect was designated in 2015 at each of eight sites with varying population densities and trends (Fig. 2). At five sites the transects were established within a few meters of transects established for a different study in 2011, so that we could examine changes in density at the same locations. Transect locations were initially chosen haphazardly at each site (either in 2011 or in 2015) and were sampled at intervals spanning *Batillaria*'s intertidal distribution, beginning just below the seaward edge of the marsh and extending to low mudflats. Data were also obtained from 1997 density sampling at two of the eight sites. Temporal patterns were assessed with linear regressions of log-transformed density over time and Pearson Correlation Coefficients.

We also synthesized changes in *Batillaria* distribution and abundance, drawing on data from a long-term monitoring program developed to track bank erosion at 26 sites around Elkhorn Slough. A 10-m long stretch of mudflat was rapidly searched and relative abundance of conspicuous epifauna was scored at each site, using five index categories. Sites were surveyed in alternate years from 2005 to 2017.

Tethering and transplant experiments

We conducted experiments with tethered *Batillaria* in three different years. In summer 2015, soon after having discovered *Batillaria*'s absence from a site where it was formerly abundant (4-BH), we collected snails at a nearby site where they persisted in high abundance (5-WL) and tethered them at both sites (Fig. S2). Our hypothesis was that mortality would be much higher at the site where they had disappeared than the site where they remained abundant, due to a cause such as disease or predation. To compare vulnerability of different size classes, we tethered large (average size 26 mm) and small (17 mm) snails at each site for about 4 weeks (Table S2).

From winter 2016 through spring 2017, we attempted a different technique, marking and transplanting snails to again characterize survival at the same two sites, and to examine predation risk. We again collected snails from site 5-WL, where they were still abundant, and marked and transplanted them to site 4-BH as well as back to site 5-WL. We marked small (10–15 mm) versus large (23–30 mm) size classes with different colors, placing some in cages while leaving others uncaged, to test whether protection from predation provided by cages would increase
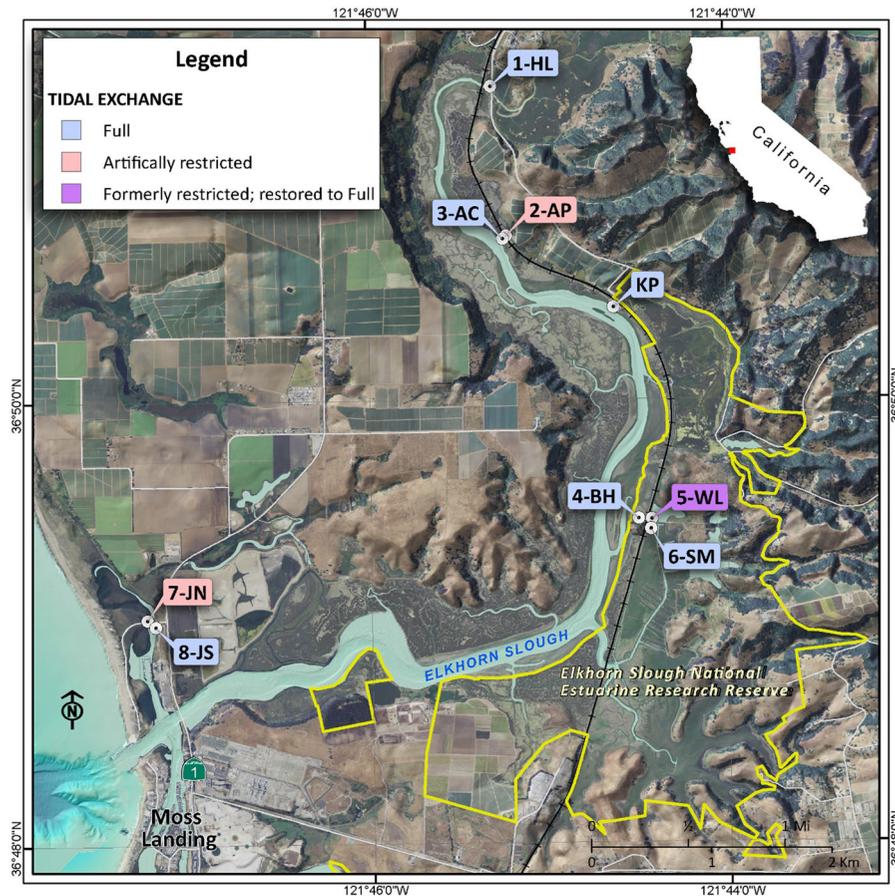
Fig. 2 Focal study sites. Hudson Landing (1-HL), Azevedo Pond North (2-AP), Azevedo Creek (3-AC), *Batillaria* Heaven (4-BH), Whistlestop Lagoon (5-WL), South Marsh at Hummingbird Island (6-SM), Jetty Road North at Bennett Slough (7-JN), Jetty Road South in North Harbor (8-JS). Kirby Park (KP) was used to collect crabs for laboratory experiments. Site 2-AP, just east of a railroad line, has restricted tidal exchange; site 3-AC is along the outflow channel for this restricted lagoon. Site 5-WL is just north of a berm and formerly had restricted tidal exchange. Site 7-JN is just north of a road and has restricted tidal exchange. Right inset shows location of study area in the state of California, USA

survivorship, and to test whether large snails had better survivorship than small snails (Fig. S2).

In spring 2017, we failed to relocate many marked *Batillaria* at site 5-WL in the just-described transplant experiment. To investigate this pattern more closely, we conducted another tethering experiment at this site in summer 2017 for about 5 weeks (Table S2). We noticed that the shells of some snails at site 5-WL looked weak or dissolved, and hypothesized that this might be related to the population decline. We thus tethered snails at 5-WL collected from this site and from a site with a more stable snail population (2-AP) with less dissolved shells. Our hypothesis was that mortality of the snails with more dissolved shells would be higher than those with less dissolved shells

because of increased susceptibility of dissolved shells to shell-cracking predators.

In summer 2018, we conducted our most extensive tethering experiment (Table S2). We tethered snails at six different sites for about 8 weeks. We collected similarly sized snails from two sites, one site with snails with generally intact shells (8-JS) and one with fairly dissolved shells (7-JN) and tethered snails collected from both sites at each experimental site. We hypothesized that predation rates would be higher on the snails with more dissolved shells.

We used Pearson's Chi squared tests with Yates' continuity correction to compare proportions of tethered snails that were intact versus those that remained alive or dead but whose shells showed signs of

predation as a function of snail size category and site in the 2015, 2017, and 2018 tethering experiments (see Table S2 for all counts and categories). We ran the same test on the snails from the 2016–2017 transplant experiment, analyzing marked snails that were relocated versus not, as a function of site, caging treatment, and size category. We also used a generalized linear model with a binomial distribution to examine whether snail survival varied as a function of shell condition in the 2018 tethering experiment.

Crab abundance: spatial and temporal patterns

Crab abundance was indexed in summer 2018 by quantifying their burrows at each of the eight focal study sites where *Batillaria* density transects were conducted. Within a 10-m stretch of shoreline encompassing the location of the *Batillaria* transect, we haphazardly placed three replicate quadrats (0.25 m^2) immediately below the seaward edge of the marsh and counted all burrows larger than 1 cm. We used a linear regression to examine whether predation rate in the 2018 tethering experiment varied as a function of crab burrow density. In Elkhorn Slough, only grapsid crabs make burrows > 1 cm in high intertidal mudflats. The grapsid crab *Pachygrapsus crassipes* is the only crab found regularly in Elkhorn Slough salt marshes (Wasson et al. 2019) and is by far the most abundant crab found in the mudflats just below the salt marsh, where *Batillaria* densities are highest. The smaller grapsid crab *Hemigrapsus oregonensis* also overlaps with the lower elevational end of the *Batillaria* distribution and could have created some of the smallest burrows observed. We assume that burrow density correlates with crab abundance, as has been found for other species (e.g. Angelini et al. 2015). Other potential crab predators on *Batillaria* in this system include cancrid crabs and European green crabs (*Carcinus maenas*), however, these are rarely found in the Slough in crab monitoring surveys with baited traps or pitfall traps (Elkhorn Slough Reserve, unpublished data).

To assess longer term trends in crab abundance, we examined crab burrow densities with data from 2005 to 2017 that are part of the long-term bank erosion monitoring study at 26 sites in Elkhorn Slough where *Batillaria* abundance was scored categorically (described above: *Documenting* Batillaria *distribution and abundance trends*). We used analysis of variance to compare average site-level crab burrow density across different years, and linear regression to examine the relationship between burrow density and *Batillaria* abundance (categorically scored) at these sites.

Crab feeding trials

To more closely examine potential predation by *Pachygrapsus* on *Batillaria*, we conducted a series of three laboratory feeding trials with the crabs and mudsnails. We examined effects of crab size, snail size, snail shell condition and presence of alternate foods in different trials.

In May 2018, we investigated how two size classes of *Pachygrapsus* affected predation on *Batillaria*. For treatments in this experiment we offered a crab a small and large *Batillaria* together, or a small *Batillaria* with algae (the green alga *Ulva lactuca*, which is an alternative food for *Pachygrapsus*). We reported predation rates (proportion of shells damaged, proportion of snails killed) in order to compare the outcomes of the treatments.

In July 2018, to complement the tethering experiments, we explored the hypothesis that crabs from site 4-BH, where the snails had been absent for 3 + years, would attack fewer snails because they were unfamiliar with *Batillaria* as a potential prey item. We conducted a brief feeding trial to compare snail predation rates by crabs from site 4-BH (naïve predators encountering novel prey) versus 5-WL (previously exposed predators encountering common prey), and used a Pearson's Chi squared test with Yates' continuity correction to compare the numbers of snails consumed from the different predator source populations.

In October 2018, we used a prey choice experiment to examine whether *Batillaria* shell dissolution affected crab predation rates on snails. We gave individually-contained crabs one snail with a dissolved shell and one with an intact shell of the same size class, and again used a Pearson Chi squared test to compare outcomes for the two shell conditions.

Batillaria shell condition assessment

To evaluate shell condition, live *Batillaria* were collected in 2018 from our eight focal sites with the exception of 4-BH, where no mudsnails were present.

We scored shell dissolution according to a standardized preservational or taphonomic index used in paleontological research (Fig. 8, Fig. S8; Cai et al. 2006; Powell et al. 2011). We also examined each snail for signs of predation (Fig. 8, Fig. S9; i.e., shell repair, peeling and punctures; Walker 2001; Walker et al. 2002) and for presence of algae on the shell. To determine whether the high shell dissolution observed at some sites at Elkhorn Slough was unusual in this region, we also collected snails in northern California (Bodega Bay and San Francisco Bay) and examined them similarly.

We extracted pore water samples in 2018 at seven of the eight focal sights to determine whether its chemical properties correlated with shell dissolution (difficulty extracting pore water prevented our sampling from site 6-SM). The pH and total alkalinity values were measured for the pore water samples. Additional carbonate chemistry parameters were calculated in the software application CO2-calc (Robbins et al. 2010) using the pH and total alkalinity values, as well as in situ temperature and salinity data that were collected with the samples.

Earlier, we had also evaluated the shell condition of dead mudsnails collected at the transect locations at five sites in summer 2017, with emphasis on determining frequency of shell repair (a sign of unsuccessful crab predation) or lethal damage where crab predation was the likely cause of death.

Parasites and diseases affecting *Batillaria*

We examined *Batillaria* for presence of trematode parasites in July 2018 at all of our focal sites, with the exception of 4-BH, which had no mudsnails present. Snails were collected and immediately dissected in the laboratory to look for patent trematode infection. Patent infections are identified by the presence of either of two larval stages, rediae and cercariae, that colonize the tissue of snails serving as the first intermediate host species in the trematodes' complex life cycle. We additionally compiled a summary of all past published trematode survey data for *Batillaria* in Elkhorn Slough to detect potential changes in infection rates over time.

We also considered whether a novel parasite or pathogen not previously documented could contribute to the dramatic decline in mudsnail abundance at Elkhorn Slough. To investigate this possibility, we collected snails at Elkhorn Slough sites with stable versus declining populations, and at two sites in other estuaries with stable/high populations: Bodega Bay and Tomales Bay. We fixed the soft tissue in Davidson's solution and prepared 5 μm, hematoxylin- and eosin-stained tissue sections for microscopic examination. Histological sections were examined for evidence of infection with protozoan and metazoan parasites, bacterial and viral pathogens, and non-infectious disease that can result from causes such as toxicant exposure.

Trends in environmental factors potentially affecting snails or crabs

We examined monthly trends over the past decade in water quality data from a long-term monitoring station near survey site 6-SM to determine whether any distinct changes had occurred over time, such as decreases in pH, which might explain *Batillaria* declines. This is the in situ monitoring station closest to most undiked sites with *Batillaria*, and due to strong currents, is representative of a large portion of the estuary. We examined water level data from the nearby NOAA tide station in Monterey; water levels and tidal dynamics are very similar within the undiked portions of the estuary as along the open coast.

Because Elkhorn Slough is in a watershed surrounded by intense agricultural activity, and its wetlands are known to have high levels of nutrients and pesticides (Caffrey et al. 2002), we considered whether contaminants in run-off could affect the mudsnails. We focused on metaldehyde, a molluscicide of emerging global concern that is used on agricultural fields to reduce slug and snail abundance. It is water-soluble and highly mobile in soils and is known to affect marine molluscs (Moreau et al. 2015). Metaldehyde is typically applied in fall and winter, and it may be transported via run-off into adjacent wetlands (Castle et al. 2017). It has a half-life of 3–223 days depending on environmental conditions (Castle et al. 2017), and has been shown to be transported quickly during rain events (Asfaw et al. 2018). We obtained data on the quantities of metaldehyde applied to fields in the subwatersheds around our sampling sites from the California Department of Pesticide Regulation Pesticide Use Reporting program. We determined whether metaldehyde usage patterns correlated with patterns of *Batillaria* decline.

Results

Documenting *Batillaria* distribution and abundance trends

Batillaria density decreased from thousands per square meter to zero or near zero at four of the eight study sites (1-HL, 4-BH, 5-WL, 6-SM; Table 1, Fig. 3). At one of these sites (4-BH), the snail appears to be entirely absent; not a single individual has been seen in 300 m of shoreline around the focal transect in repeated searches from 2015-present. At other sites where we detected zero snails in the permanent transects (i.e., the data reported in our tables and figures), occasional individuals were still detected in the general area. Most of these declines occurred prior to the more intensive sampling in 2015–2018, but during this latter period there was a strong decline at one site (5-WL, Fig. S1). This site was converted from artificially restricted to full tidal exchange in summer 2014 as part of a restoration project. Of the three sites that maintained moderate or high numbers over time, two had tidal exchange restricted by water control structures (2-AP, 7-JN), and one was the outflow channel associated with one of these restricted sites (3-AC).

Surveys of 26 mudflat sites in the estuary from 2005 to 2017 revealed a marked decrease in *Batillaria* distribution, declining from the highest number of sites with *Batillaria* (15) recorded in 2007, to the lowest number of sites (3) in 2017, with a concurrent decrease in abundance across sites where it remained (Fig. 4; Table S1). *Batillaria* was consistently absent from the lower estuary, so most of this change occurred in the upper estuary.

Tethering and transplant experiments

Taken together, the four experiments indicated that predation rates varied markedly at different sites over time, and with different mudsnail sizes or shell conditions.

In summer 2015, survivorship was high at both sites (Table S2, Fig. S3) and did not differ for small versus large snails ($\chi^2 = 0.05$, df = 1, $p = 0.82$). Mortality of tethered snails did not differ between the site where *Batillaria* had recently disappeared (4-BH) and the site with abundant *Batillaria* (5-WL), counter to our hypothesis ($\chi^2 = 2.5$, df = 1, $p = 0.11$). At both sites, a single small snail died of apparent predation (broken shell remnants remaining on tether). One snail died with its shell intact (no sign of predation) at the site with abundant snails (5-WL). The low mortality

Table 1 Summary of *Batillaria* densities and potentially related factors at eight sites

Site	Tidal Exchange (F=full, R=restricted)	Densities over time (live snails per m²)						Crab burrows (per m²)	Predation of tethered snails (percent)	Shell taphon-omic index	Shell repair (percent of snails)	Trematode parasites (percent of snails)	pH	Aragonite saturation state (Ω)
		1997	2011	2015	2016	2017	2018							
1-HL	F	-	3255	0	-	-	0	4	-	2.8	60	53	7.1	0.72
2-AP	R	-	3239	705	-	731	509	13	14	2.4	30	26	7.1	2.38
3-AC	F	-	2708	147	-	164	723	47	55	2.9	10	8	6.8	0.40
4-BH	F	1064	-	0	0	0	0	28	30	-	-	-	6.7	0.21
5-WL	F (was R)	1078	-	2418	1529	55	1	87	100	3.1	20	0	7.0	0.51
6-SM	F	-	2272	0	0	0	0	8	-	2.6	27	20	-	-
7-JN	R	-	4575	1849	-	1637	5234	9	10	3.5	20	75	7.2	0.48
8-JS	F	-	-	0	-	5	42	21	5	2.5	20	8	7.3	0.79

Details on how each parameter was measured and summarized is in the Methods and SI. For Tidal Exchange, blue formatting indicates full exchange; pink artificially restricted exchange, and purple a site where restricted exchange was restored to full exchange in 2014. For densities, conditional formatting was used to highlight higher (green) versus lower (red) densities. (Density data from 1997 were collected less thoroughly than in all subsequent years and may represent an underestimate.) For the remaining variables, data are from summer 2018. Conditional formatting showing high values in darker shades of green was applied separately to each parameter. In all cases, average values for the site are shown. Blank cells with dashes indicate that no data were available
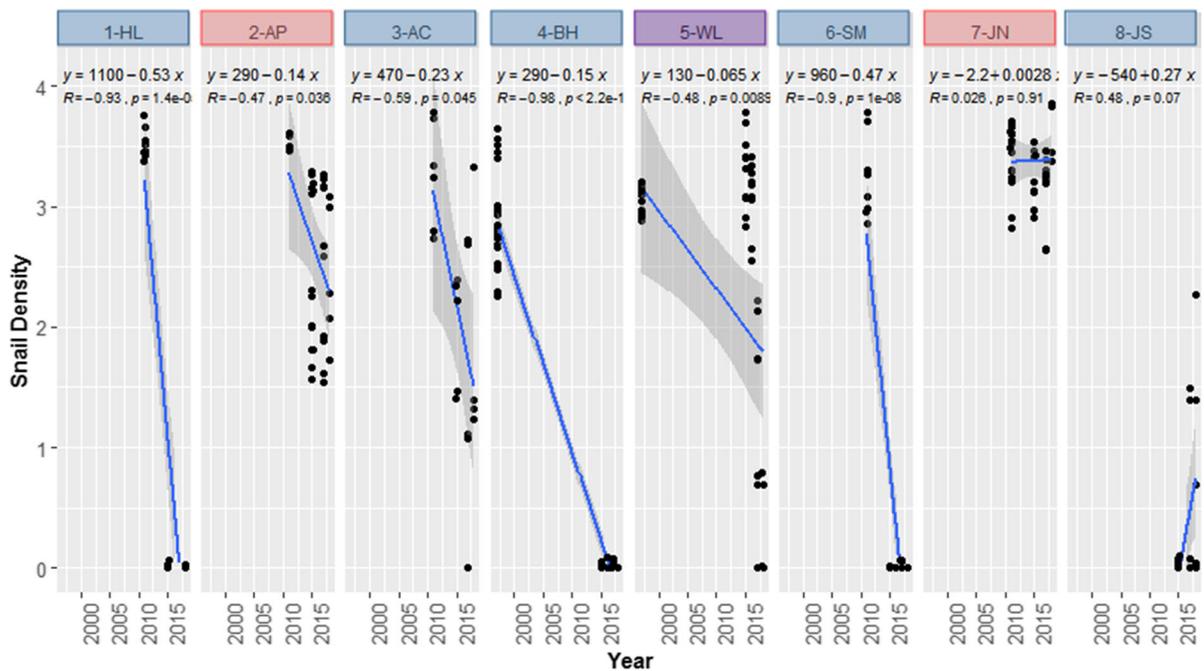
Fig. 3 *Batillaria* density over time at eight sites, 1997–2018. Each point represents a single location along a transect sampled for density. Due to the high range in magnitude of densities per m^2 (0–15,000), we used log(density + 1) for the Y axis. Sampling periods differ by site as explained in the SI. Changes over the more recent intensive study period, such as the dramatic decline at 5-WL following restoration of full tidal exchange, are shown in Fig. S1. Smoothed regression lines are shown in blue, with gray shading for 90% confidence intervals. The equation of the line, Pearson Correlation Coefficient and significance level are shown for each site. Color-coding of site labels is as in Fig. 2

occurred despite the experimental period including some extremely hot temperatures during low tide exposure, and despite high numbers of *Pachygrapsus* crabs observed at one site during the experiment (4-BH; Fig. 1 lower left).

In winter 2017, survival at site 4-BH showed similar patterns: high numbers of marked, transplanted snails were recovered, with no effect of caging ($\chi^2 = 1.7$, df = 1, $p = 0.19$) or snail size ($\chi^2 = 2.4$, df = 1, $p = 0.12$). However, survival at site 5-WL showed a completely different pattern (Fig. S4): the experiment was ended after 3 months because fewer than 15% of uncaged marked snails could be found at site 5-WL (while around 80% were relocated at 4-BH). The difference in proportions of recovered snails between sites was highly significant ($\chi^2 = 121$, df = 1, $p < 0.0001$). At 5-WL, caging had a very strong effect on the proportion of snails that were recovered ($\chi^2 = 42$ df = 1, $p < 0.0001$), while snail size was not significant ($\chi^2 = 0.13$, df = 1, $p = 0.73$).

In summer 2017, mortality of tethered snails at 5-WL was very high for the first 2 weeks of the experiment (Table S2, Fig. S3). Thereafter, mortality slowed considerably and most remaining snails survived. Counter to our hypothesis that mortality rates might be higher for snails collected from the site undergoing the decline, survival patterns of snails tethered at 5-WL were similar for snails from both origin populations (5-WL and 2-AP, $\chi^2 = 0.67$, df = 1, $p = 0.41$).

In summer 2018, we documented remarkably varied trajectories of *Batillaria* survival across the six sites where snails were transplanted and tethered (Fig. 5, Table S2); survival was significantly different among sites ($\chi^2 = 27$, df = 5, $p < 0.0001$). Predation rates were low at three sites, moderate at two sites, and extremely high at one site. For example, at two sites (7-JN, 8-JS), no snails with shells in good condition at the start of the experiment had evidence of predation after 7 weeks, while 100% were dead by predation within 3 weeks at the site with recent declines (5-WL). At four sites, the shells in better condition had higher survivorship than fairly dissolved shells. A general linearized model revealed that survival rate varied
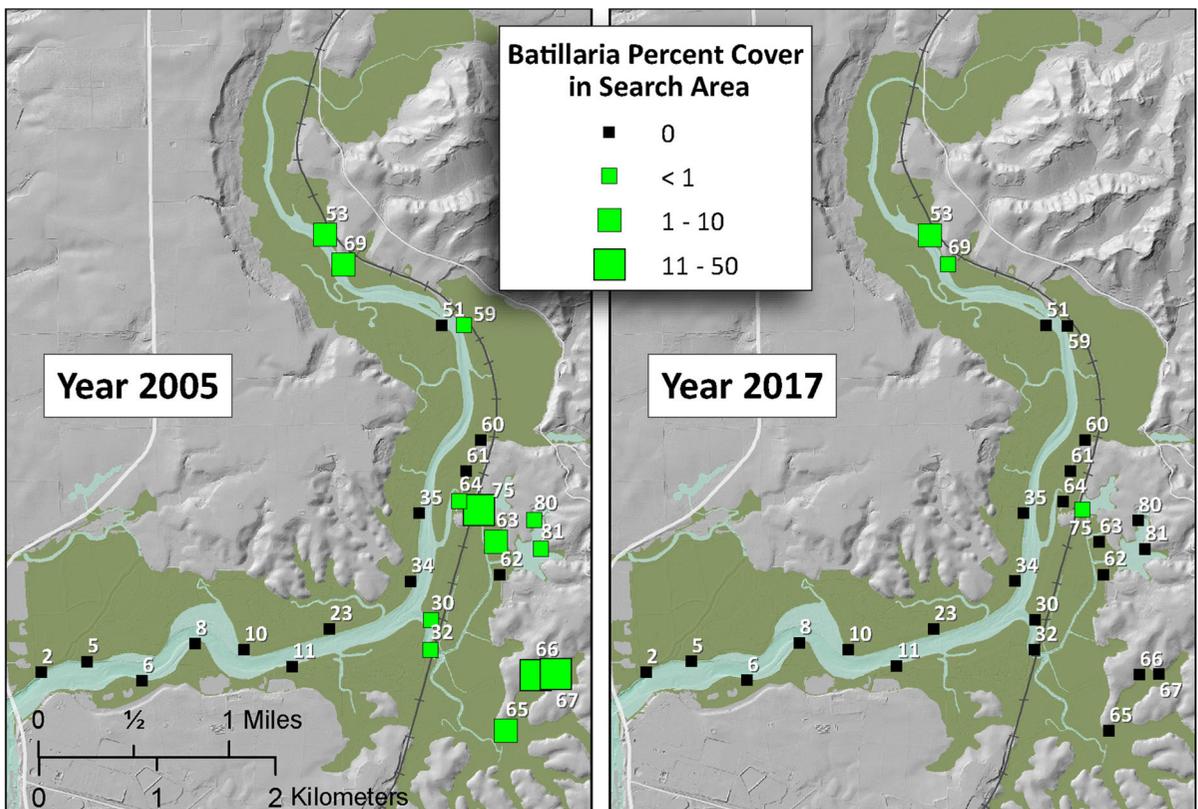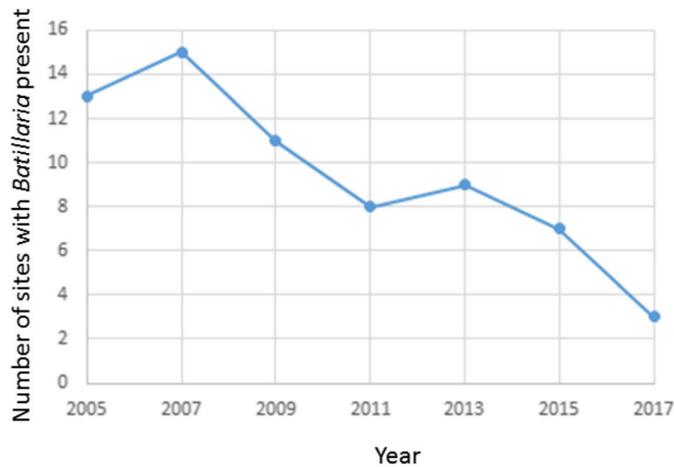
Fig. 4 Change in *Batillaria* relative abundance at sites across the estuary. Top: Number of 26 survey sites in Elkhorn Slough with *Batillaria* present over time. Bottom: Relative abundance of *Batillaria* at these sites in 2005 (left) and 2017 (right). These sites are monitored in alternate years as a part of a long-term study of bank erosion (site numbers are non-consecutive as some monitoring stations were dropped over time for logistic reasons). *Batillaria* abundance is scored into 5 categories in a rapid assessment of a 10 m stretch of shoreline; in the 2 years shown, no site had the highest abundance (> 50% cover). Note that these are representative sites throughout the estuary and different than the focal *Batillaria* study sites shown in Fig. 2, with the exception of site 75, which is focal site 5-WL

significantly as a function of snail shell taphonomic index ($p = 0.03$, $Z = 2.1$).

We did not directly observe predation on live *Batillaria* in the tethering studies. However, the peeled
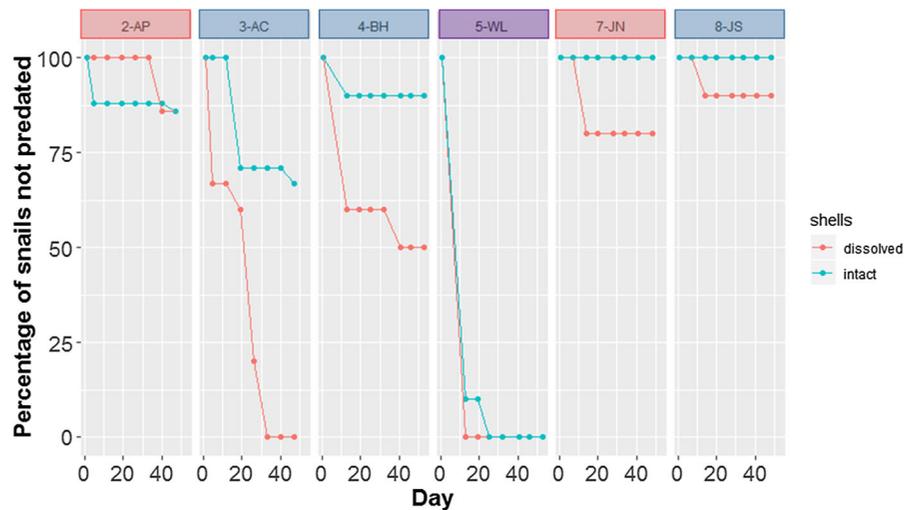
Fig. 5 Outcomes of Summer 2018 tethering experiment at six sites. Sites are labeled at the top and correspond to Fig. 2 and Table 1. The y-axis shows the percentage of non-missing shells that showed no signs of predation; the x-axis is days since the experiment started. Blue dots and lines correspond to snails collected from 8-JS, with relatively intact shells (low taphonomic index); red dots and lines correspond to snails collected from 7-JN, with fairly dissolved shells (high taphonomic index); see examples in Fig. 8. Sites 2-AP, 3-AC, and 7-JN had moderate to high ambient *Batillaria* densities; sites 5-WL and 8-JS had very low densities, and site 4-BH had zero ambient *Batillaria* present (see Table 1 for 2018 densities at these sites)

apertures and shell punctures observed on the shells were consistent with those seen in laboratory feeding trials with *Pachygrapsus* (see below). *Pachygrapsus* was abundant at sites with high predation on snails, and no other predator large enough to break snail shells was observed to be common on high intertidal mudflats at these sites. Shorebirds may occasionally eat *Batillaria,* but we have never observed this at Elkhorn Slough, despite the high abundance of both shorebirds and mudsnail at many sites.

Crab abundance: spatial and temporal patterns

At the eight focal sites where *Batillaria* density was monitored, crab burrow densities varied widely (Table 1). Crab burrow density was strongly positively related to predation rate in the summer 2018 tethering experiment (Fig. 6).

At the 26 bank erosion monitoring sites, crab burrow density was lower in the early monitoring period (2005–2011) than in the later period (2013–2017). Burrow counts exceeding 100 per m^2 were only observed in the later period (Table S1). At sites where *Batillaria* was present during at least one survey, there was a clear peak in crab burrow density in 2013 (Fig. S5). There was a significant negative relationship between crab burrow density and *Batillaria* abundance across these 26 sites (Fig. S6).

Crab feeding trials

The crab feeding trials demonstrated the potential of *Pachygrapsus* as an effective predator on *Batillaria*. Some crabs, in some trials, readily attacked and killed *Batillaria* (Fig. S7, S. Video 1). The crab trials, taken together, also showed high variation in predation rates (Table S3).

In May 2018 trials, 11.1% of snails showed evidence of predation, with about 6.5% surviving attack and 4.6% dead. The proportion of small snails with evidence of predation was greater when paired with large snails (16.7%) versus when they were paired with algae (13.9%; Fig. 7a). Nevertheless, when offered both snails and algae, crabs consumed algae more often (36.1% of trials) than attacking snails (13.9%). Predation by large crabs (25%) was significantly higher than by small crabs (4%) across treatments. Only large crabs killed and consumed snails, though both large (n = 4) and small crabs (n = 1) caused shell damage.

In July 2018 trials, 37.5% of snails showed evidence of predation, with 18.75% of snails surviving
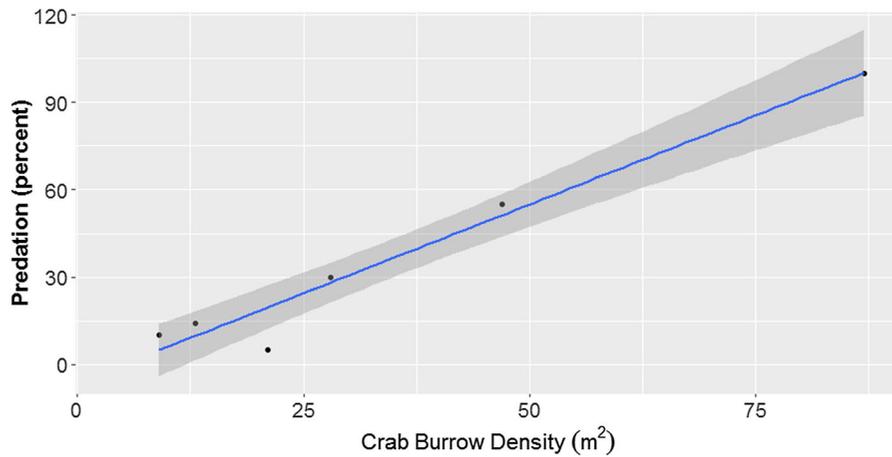
Fig. 6 Percentage of snails preyed on as a function of crab burrow density. There was a highly significant positive relationship between the percent of tethered *Batillaria* with evidence of predation and the crab hole burrow density in adjacent areas, $R^2 = 0.96$, $p = 0.0006$. Each point represents one of the six focal sites where snails were tethered in summer 2018. Smoothed regression line is shown in blue, with gray shading for 90% confidence intervals
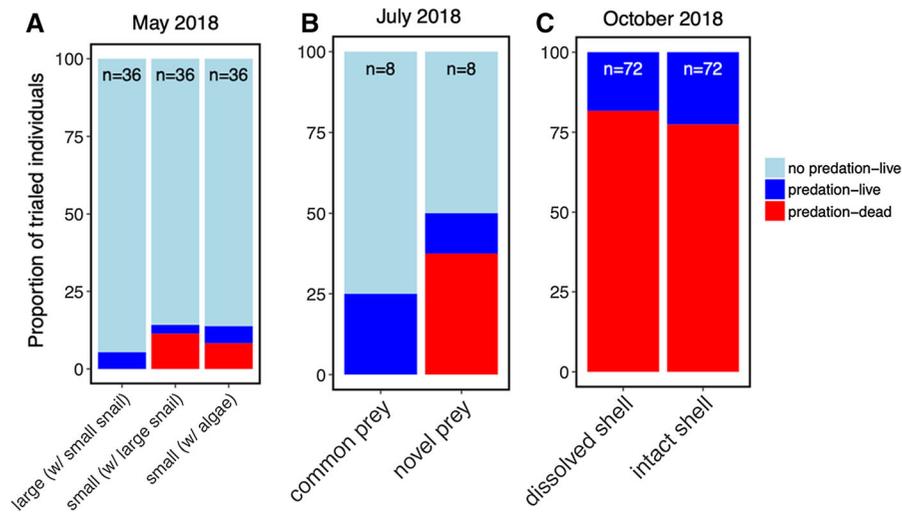
Fig. 7 Crab predation across laboratory feeding experiments. *Batillaria* predation by crabs varied across feeding experiments. **A** Experiment in May 2018 had two treatments, small and large *Batillaria* offered together, or small *Batillaria* offered with algae; to facilitate comparison with other experiments, which only used small snails, outcomes are shown separately for small and large snails. **B** Experiment in July 2018 had two treatments, crabs from a site where *Batillaria* was a common prey item versus a site where the snail is absent, so *Batillaria* was a novel prey item. **C** Experiment in October 2018 had two treatments, snails with very dissolved versus intact shells. Blue shading represents snails that were still alive at the end of experiment; light blue had no evidence of predation, while dark blue showed signs of predation (shell breakage). Red shading represents snails that died due to predation

attack and 18.75% dead. We rejected the hypothesis that lower predation observed in the tethering experiment at site 4-BH vs. site 5-WL was due to difference in crab familiarity with *Batillaria*. "Naïve" crabs from the site that had no snails for the past 3 + years attacked them readily, at rates higher than crabs from the site where snails are a commonly available prey item (Fig. 7b), though differences among source populations were not significant ($\chi^2 = 1.1$, df = 1, $p = 0.30$).

In October 2018 trials, 100% of snails showed evidence of predation, with 20.1% surviving attack

and 79.7% dead (Fig. 7c). Crab predation did not vary as a function of *Batillaria* shell condition ($\chi^2 = 0.17$, df = 1, $p = 0.68$).

Batillaria shell condition assessment

Our 2018 assessment of mudsnails collected at the focal sites revealed that shell condition (taphonomic index) varied across sites (Table 1, Fig. 8, Table S4) and within sites (Fig. S10), but overall, shells generally showed substantial dissolution (a high taphonomic index), with the older part of the shell (spire) in considerably worse condition than the most recent growth (last whorl). The site with highest snail density (7-JN) had the highest taphonomic index (Table 1, Fig. S10). Pore water parameters varied markedly across sites (Table S5), but showed no clear relationship to snail density or taphonomic index. However,
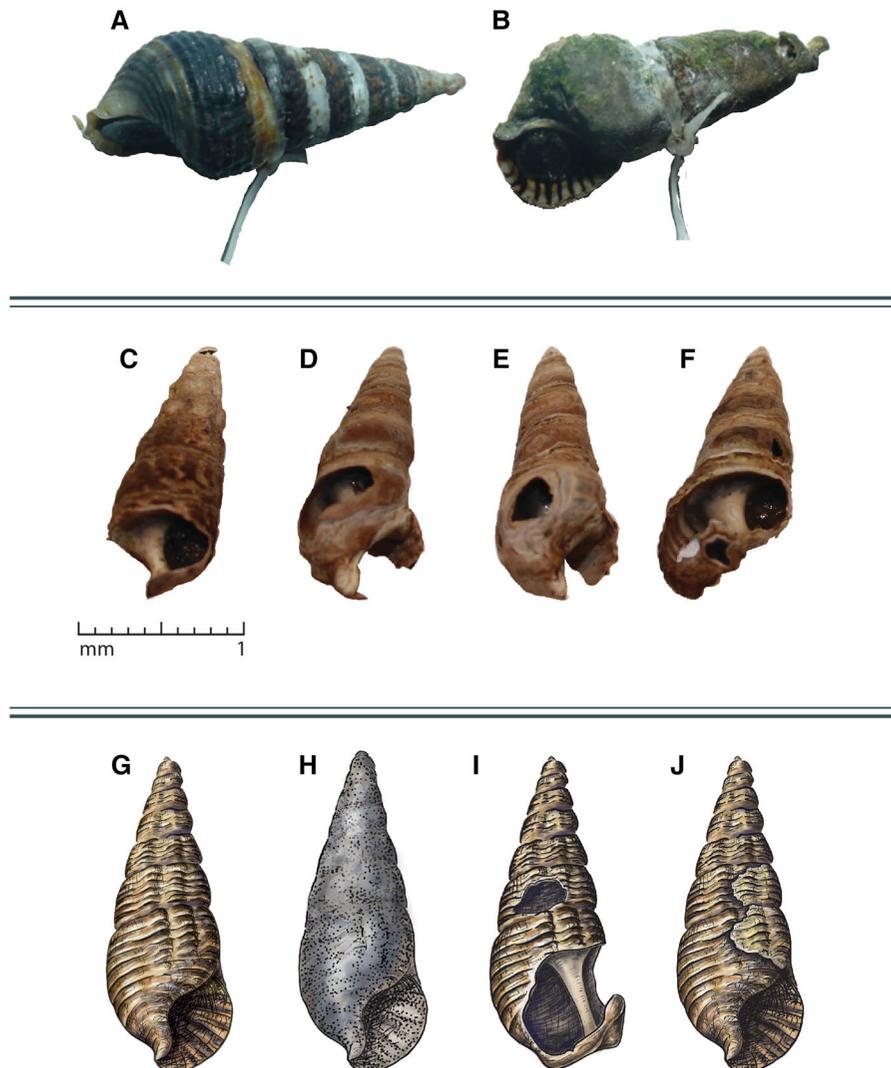
Fig. 8 Shell dissolution and crab damage to shells. A: Live mudsnail with intact shell with low taphonomic index; B: live mudsnail with very dissolved shell with high taphonomic index and algal growth; both A and B were used for tethering experiment and show attachment with dental floss. C–F: crab damage on empty shells with typical peeled apertures and puncture wounds. G–J: illustrations by D. Jolette (see complete series in Figs. S8-9). G: idealized intact shell with taphonomic index of 0; H: highly dissolved shell with taphonomic index of 4; I: peeled aperture and puncture wound typical of crab predation; J: shell repair typical following crab predation

aragonite saturation state was very low at various sites with recent *Batillaria* declines, and lowest ($\Omega = 0.21$) at one of the sites with the most striking crash (4-BH), while highest ($\Omega = 2.38$) at the site with best shell condition and quite high snail density (2-AP, Table 1).

The 2018 assessment of live snails revealed a high frequency of shell repair at many sites (Table 1). Dead snail shells collected in the field in 2017 also showed a high frequency of previous predation attempts that had been survived (shell repair), as well as a high frequency of predation as the apparent cause of death (peeled apertures, punctures). The damage was consistent with crab predation, similar to the damage seen in the laboratory trials with *Pachygrapsus* (Fig. S11).

Parasites and diseases affecting *Batillaria*

Infection rates by larval trematode parasites varied widely among sites in 2018, from 0 to 75% of mudsnails at a site being infected (Table 1). The site with the highest parasitism had the highest *Batillaria* density (7-JN), and the site with the lowest parasitism had the strongest recent *Batillaria* declines (5-WL). It appears that trematode parasitism rates may have increased over time at Elkhorn Slough. The earliest documented prevalence rates from the late 1990s are lower than all subsequent periods (Table S6). Trematode prevalence was also high (68%) in snails we examined from Tomales Bay where population densities are high and stable. Besides the trematode parasites, we found no other parasites, disease agents, or pathological conditions in the Elkhorn Slough *Batillaria* (Table S7, Figs. S12-13).

Trends in environmental factors potentially affecting snails or crabs

Characterization of water quality data for all measured parameters revealed high interannual variability but no clear directional trends for any except water temperature, which increased significantly by about 0.5 °C at approximately the same time as crab burrow densities increased and strong *Batillaria* declines occurred (Fig. S14). There was a concurrent increase in water levels and thus inundation time associated with an El Niño event (Fig. S15). There was no trend of acidification over the past 17 years (Fig. S14).

Application of the pesticide metaldehyde, designed to kill terrestrial snails and slugs, was variable spatially and temporally (Fig. S16, 17). There was no indication that the application rates were higher near the areas with declining snail populations, or increased in those areas with declines. Overall for the region, metaldehyde application peaked in 2 years prior to the observed decline (2009, 2011), and decreased during the period of *Batillaria* declines.

Discussion

Lack of predictability and importance of scale in invasion dynamics

Quantitative data on boom-bust dynamics are rare (Simberloff and Gibbons 2004; Strayer et al. 2017). We provide a clear quantitative example of a species that was established for many decades at high densities, and moreover was the most abundant epifaunal species in Elkhorn Slough. *Batillaria attramentaria* has broader environmental tolerance and faster growth than a confamilial native mudsnail (*Cerithideopsis californica*) that resides in many estuaries on the northeastern Pacific coast (Byers 1999, 2000a, b). Given the high abundance of *Batillaria* over decades and its broad distribution in Elkhorn Slough, we would not have predicted its dramatic decline, including its complete disappearance from some sites. Our results illustrate the challenge of predicting invasion success even for a well-established, seemingly stable introduced species at pest levels of abundance.

Boom-bust dynamics can occur at various temporal and spatial scales (Strayer et al. 2017), but scale is not often explicitly considered or defined in boom-bust investigations. In this case, we documented population crashes at the spatial scale of individual wetland sites within an estuary. All seven of the sites for which we had older data had densities of 1000s of *Batillaria* per m², qualifying as a boom phase of the invasion. At one of these sites, density remains similarly high; at two sites, density has decreased but is still high (100s per m²); at the other four sites, populations have collapsed and densities are zero or near zero (Table 1). The trends at these latter sites certainly meet definitions of invasive collapse, such as > 90% decline in abundance in < 10 years that persist for > 3 years (Aagaard and Lockwood 2016). At the scale of the whole estuary, there has also been a decline in

distribution and abundance of this non-native snail (Fig. 4), but across the entire invaded range of the species on the northeastern Pacific coast, *Batillaria* abundance remains high at many estuaries, and new invasions continue (Weiskel et al. 2007; Crooks et al. 2016; Grason et al. 2018). Our study documenting variable temporal patterns of decline across multiple scales highlights the importance of considering scale in boom-bust dynamics. It will be fascinating to track *Batillaria* populations at different sites in Elkhorn Slough in the future to determine whether declines persist, or whether the snails recover, heralding cycles of boom-bust dynamics.

Restoration of tidal exchange can drive switch from boom to bust phase

In comparing population trajectories, the most conspicuous pattern was the contrast between sites with full versus artificially restricted tidal exchange. Although *Batillaria* densities over the past decade crashed at many sites with full tidal exchange, densities remained high (100s to 1000s per m^2) at three sites that had long had artificial tidal restriction resulting from water control structures. At one of these (7-JN), high densities were reported nearly 60 years ago (McClean 1960), so the boom phase has been very sustained. Both this and the other tidally restricted site in our sampling (2-AP) presently have the highest *Batillaria* densities in the estuary (and the sites immediately adjacent to them, 3-AC and 8-JS, which have had fairly stable populations at lower densities, may be affected by spillover). At another of our focal sites (5-WL), the natural tidal range was restored in 2014, precipitating a bust phase, and current *Batillaria* densities there are near zero. To be clear, full tidal exchange can be compatible with high *Batillaria* densities, as was observed for many decades at sites around Elkhorn Slough and still is the case in many other estuaries. But apparently, at least at Elkhorn Slough, populations in natural tidal conditions are more vulnerable to crashes than populations in tidally restricted sites.

Tidally restricted sites in the estuary have reduced biodiversity (Ritter et al. 2008) and degraded water quality (Hughes et al. 2011) compared to adjacent fully tidal sites. It appears that a change unfavorable to *Batillaria* occurred, affecting fully tidal sites in the estuary, but sites with artificially restricted tidal

exchange were buffered from this change. At site 5-WL, restoration of natural hydrological processes unexpectedly reduced a super-abundant invasive species to near extinction. Such hydrological restoration can be desirable to enhance many ecological functions (Williams and Orr 2002; Raposa and Roman 2003), including reduction in invasive species (Buchsbaum et al. 2006), as illustrated by our study. We have also noticed that abundance of another invasive species, the European green crab (*Carcinus maenas*) has generally declined in the estuary, but remains higher at a tidally restricted site in the estuary (Elkhorn Slough Reserve, unpublished data), and postulate that this is also due to reduced predation risk (in this case, from southern sea otters). Since diking and water control structures are some of the most pervasive human alterations to estuaries (Kennish 2002), the possibility of reducing the abundance of non-native species as demonstrated by our study exemplifies another dimension to the importance of hydrological restoration.

Evidence for important role of crab predation in *Batillaria* decline

Multiple lines of evidence suggest that crab predation may exert strong top-down effects on *Batillaria* populations, particularly at sites with full tidal exchange (Table 2). Tethering experiments showed the potential for high and rapid mortality in the field. There was a strong correlation of predation rates of tethered mudsnails with the density of crab burrows across sites (Fig. 6), and the site with the strongest decline in recent years had the highest predation rates. Over the past decade, a peak in crab burrow density coincided with the onset of *Batillaria* decline at many sites in the estuary (Table S1B). Examination of live snails and dead shells in the field revealed high frequency of damage characteristic of crab attacks, and of shell repair, although patterns varied by site in ways that were not obviously related to burrow density. Laboratory trials revealed that *Pachygrapsus* can readily attack and consume *Batillaria*. Grapsids have relatively small claws with limited biomechanical strength for cracking large snail shells (Yamada and Boulding 1998), however, multiple studies have demonstrated grapsid crab predation on *Batillaria*'s native analogue, the California horn snail (*Cerithideopsis californica*) (Sousa 1993; Armitage

and Fong [2006](#); Lorda et al. [2016](#)). Furthermore, other studies have documented crab predation on congeneric *Batillaria sp.* in the native range (Örstan [2006](#); Miura et al. [2012](#)), and even *Batillaria attramentaria* itself in Washington (Grason et al. [2018](#)).

Predation effects can vary dramatically over space and time (Vermeij [1982](#)), and this was certainly the case for crab predation on *Batillaria* in our study. We found strong differences in mortality of tethered snails across six sites monitored in the same summer, and at the same site (5-WL) in different years, and high variation in shell damage and repair across sites. Even within our controlled lab trials we measured high variation in predation rates (< 5% of snails killed in some trials, > 75% in others). We hypothesize that high variability in the field is driven by *Pachygrapsus* switching from preferred algal food sources to *Batillaria* when the preferred food becomes scarce or metabolic demands are particularly high. Similarly, Sato and Chiba ([2016](#)) attributed a decline in *Batillaria* in the native range to prey-switching by a predator whose preferred food became rare. While an increase in crab abundance or prey-switching seems to have affected snails in the majority of the estuary with full tidal exchange, these crab effects appear not to have manifested themselves at the two sites with artificial tidal exchange where *Batillaria* populations remain high. At the site where full tidal exchange was restored, crab burrows dramatically increased following restoration, coincident with the *Batillaria* declines (Table S1).

Intriguingly, earlier studies had demonstrated that *Batillaria* invests much less in its shell mass per unit size than *Cerithideopsis* (Byers [2000a](#)). Low investment in the shell allows the invader to convert food resources into growth more efficiently than the native mudsnail, and gives it a competitive advantage (Byers [2000a](#)), but this competitive advantage depends on low predation pressure since a thin shell makes the snail more vulnerable. Byers ([2002](#)) hypothesized that as humans dramatically alter habitats and food webs, selection regimes are modified and systems transform faster than the evolutionary responses of native species. A thinner shelled non-native snail like *Batillaria* might thrive over its thicker shelled native competitor in estuaries modified by human impacts where predator abundance has been diminished and a thicker shell is maladaptive. If selection regime modification (SRM) explains *Batillaria*'s prior success over its native competitor, our study may represent an example where SRM has been reversed. Specifically, previously low crab predation in Elkhorn Slough may have allowed *Batillaria* to establish and thrive (and possibly outcompete *Cerithideopsis*). But if the predatory environment has now increased, the formerly successful thinner-shell strategy may now be a vulnerability.

Other potential factors contributing to *Batillaria* decline

Detecting causes of population declines is challenging for native as well as non-native species, and can involve multiple causal agents (Peery et al. [2004](#)). Predation, disease/parasites, competition, and anthropogenic impacts are the most frequently invoked drivers of population crashes of invasive species in the literature, though often without strong evidence (Strayer et al. [2017](#)). Although there is compelling evidence that crab predation can exert strong pressure on *Batillaria* populations, it is likely that multiple interacting factors contributed to *Batillaria* declines at Elkhorn Slough. Biotic impacts are often density-dependent, and thus would not explain the complete disappearance of snails from site 4-BH. We employed experimental and correlative approaches that ruled some mechanisms out, while others were deemed likely contributing factors (Table [2](#)).

The substantive shell dissolution we observed led us to consider the possibility that local acidification was behind the decline. However, this factor was ruled out as the main driver, since the sites with highest snail density had the highest shell dissolution. Nevertheless, dissolution may interact with predation, as we found higher predation rates on snails with dissolved versus intact shells. Low aragonite saturation state can negatively impact shelled organisms (e.g., Busch et al. [2014](#)), so the extremely low aragonite saturation state of some of the pore water samples collected, including at the two sites with most dramatic declines (4-BH, 5-WL) suggest this factor may contribute to *Batillaria* declines.

Long-term water quality monitoring data revealed that the water temperatures and levels increased approximately at the time as the *Batillaria* population crash at fully tidal sites, as a part of a larger regional oceanographic phenomenon dubbed "the Blob," where unusually warm water was pushed against the

Table 2 Summary of evidence for different potential factors contributing to *Batillaria* decline

Factor	Data type	Evidence for	Evidence against
Full tidal exchange	Density over time	The two most abundant and stable populations have artificially restricted tidal exchange (2-AP, 7-JN); site (5-WL) that formerly had abundant and stable population during restricted tidal exchange saw dramatic crash following restoration of full tidal exchange (Fig. 3, Fig S1)	None
Crab predation	Tethering experiment	High mortality due to predation in 2017 at site 5-WL (Fig. S3) and at three sites in 2018 (Fig. 5)	Low mortality at 4-BH and 5-WL in 2015 (Fig. S3)
	Crab burrow density	At 6 focal sites, burrow density correlated with tether mortality (Fig. 6); at 26 sites across estuary, higher numbers of crab burrows 2013-2017 coinciding with snail decline (Fig. S5); negative relationship between burrow density and snail abundance (Fig. S6)	None
	Lab feeding trials	*Pachygrapsus* attacks and consumes *Batillaria,* at variable but sometimes high rates (Fig. 7)	None
	Shell assessment	High frequency of evidence of crab attacks (shell repair in live snails, peeled or cracked shells in dead; Table S4, Fig. S11)	None
Shell dissolution	Tethering experiment	More predation on dissolved than intact shells in 2018 (Fig. 5)	Sites with most dissolved shells (highest taphonomic index) had most abundant populations (Table S4); stable northern California populations also had dissolved shells (Fig. S10)
Water quality and levels	Tethering experiment	None	Virtually no mortality of snails in any tethering experiments (2015, 2017, 2018) due to causes other than obvious predation
	Water quality data	Increase in water temperature and water level coincident with *Batillaria* decline (Fig. S14-15)	None
Molluscicide	Pesticide data	None	Spatial and temporal patterns of metaldehyde application do not match patterns of *Batillaria* decline (Fig. S16-17)
Trematode parasites	Microscopic examination	None	Weakly positive relationship between parasite infection prevalence and abundance of *Batillaria* across sites; high infection rates at stable sites at Elkhorn and elsewhere (Table S6)
Disease	Histological examination	None	Besides trematode parasites, there was no evidence of any other parasites, disease agents, or pathological conditions in *Batillaria* from Elkhorn Slough, and no differences in condition among stable versus declining populations
	Tethering experiment	None	Virtually no mortality of snails in any tethering experiments (2015, 2017, 2018) due to causes other than obvious predation

Each potential factor is listed in the first column, the type of data used in the second, and evidence from the data that provides support for or against this factor contributing to *Batillaria* declines is shown in the third and fourth columns. Factors are ordered from strongest to weakest evidence for contribution to decline

northeastern Pacific coast (Peterson et al. 2015). It is possible that this warming led to a regime shift in algal communities that provide food to both *Batillaria* and *Pachygrapsus*, and thus could have decreased food availability for the snail while increasing crab predation rates on snails. We also observed dieback of the vegetation in low marsh elevations during the Blob due to waterlogging, which may have somehow enhanced burrowing opportunities and thus crab densities. Relative water level increases due to earthquake-driven subsidence have been identified as the cause of a predator-driven *Batillaria* decline in Japan (Miura et al. 2012; Sato and Chiba 2016). The two sites with water control structures where *Batillaria* density remains high would have been buffered from regional changes in water level, since water levels there are dictated by water control structures. These two sites are always lagoonal, with narrower bands of intertidal habitat resulting from a maximum tidal range of about 1.0-1.5 m, in contrast to the 2.5 m of sites with natural tidal exchange.

We found no clear role for other potential drivers of the *Batillaria* crash. Trematode parasites, pathogens, and pesticides seem to have little relationship with the snail's decline. Trematode parasites sterilize their snail hosts, and thus potentially decrease population growth rates. Although parasitism rates appear to have increased over time, recent spatial patterns of parasite infection did not coincide with patterns of decline. However, population recovery following an initial decline may be hampered in a population with high infection prevalence, which removes a high proportion of adult snails from the reproductive pool. The sudden and complete dieback observed at sites such as "*Batillaria* Heaven" (4-BH) is consistent with a density-independent factor such as a toxic chemical. However, the spatial and temporal patterns of applications of the most common molluscicide used in regional agriculture, metaldehyde, did not coincide with patterns of *Batillaria* decline. The dieback also appears consistent with spread of a new emerging disease or pathogen, but histological studies failed to find any indicators of such issues, and no differences between the boom and bust populations.

Ecological consequences of *Batillaria* decline

There could be strong ecological ramifications of the decline of *Batillaria* in Elkhorn Slough. *Batillaria* strongly affects mudflat communities (Wonham et al. 2005), particularly benthic diatom communities (Byers 2000a; Fabian 2016). Given the formerly high densities of the mudsnail, we may expect a rebound of benthic diatoms on high intertidal mudflats, which could in turn support population growth by other epibenthic foragers as well as benthic infauna. One of the most dramatic results of *Batillaria*'s decline at Elkhorn Slough is likely to be a sharp reduction in the parasitism rate in fish and birds that have been serving as the second and definitive host species for the snail's trematode parasite for decades (Torchin et al. 2005). The one trematode species that infects *Batillaria* within North America (*Cercaria batillariae*) is itself a non-native species, likely introduced with the snail from Japan (Torchin et al. 2005; Miura et al. 2006). Because *Batillaria* is the only snail species that can host the larval stage of *Cercaria batillariae*'s complex life cycle, the trematode will go locally extinct if its host snail does. The resulting relaxation of infection, especially on second intermediate fish hosts, is thought to be especially strong given that the metacercarial cysts attach to the pericardium (Torchin et al. 2005). Future studies should quantify ecological consequences of the *Batillaria* decline.

Conclusions

The decline of *Batillaria* at Elkhorn Slough provides a clear, quantitative example of a boom-bust sequence. The crash was especially surprising after the phenomenal success of the invasion and its apparent stability for decades. The majority of observations of boom-bust dynamics do not provide strong evidence demonstrating the cause (Strayer et al. 2017). As we show here, demonstrating the cause(s) can be difficult, even with substantial investment in field and laboratory experiments and correlative observations carried out over many years. Spatial and temporal variability in patterns made detection of causal factors more challenging, yet simultaneously, boosted insight into the causal mechanisms. That is, contrasts among sites over time allowed us to discover that only sites with full tidal exchange experienced population crashes, while sites with artificial tidal restriction had persistent boom phase populations. Contrasts among sites also enabled us to establish a relationship between grapsid crab burrow density and mortality of tethered snails,

while long-term data allowed us to detect increases in temperature, water levels, and grapsid crabs coinciding with the *Batillaria* decline. Understanding boom-bust dynamics thus can be enriched by investigations occurring across multiple temporal and spatial scales.

Many important questions remain to be answered in future studies. Most puzzling perhaps is what exact changes in environmental conditions precipitated the crashes in fully tidal sites after decades of stability. It is possible that climate-driven changes might be responsible, in which case other *Batillaria* populations along the coast may be similarly affected in the future; understanding interactions between climate change and invasions can be fruitful (Hellmann et al. 2008). Or perhaps the changes at Elkhorn Slough were related to the unusually high agricultural nutrient-loading there (Caffrey et al. 2002), which could facilitate invasion success (though see Weiskel 2012). Another key question is whether *Batillaria* populations will recover in places where they have crashed. If so, what is behind the recovery? If not, what are the ecological consequences for the system of losing such high numbers of a grazer? At the very least it seems the microphytobenthos should be strongly affected. Clearly the enigmatic invasion dynamics of this mudflat snail merit further attention.

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Each section below provides additional detail on the Methods and Results for the section of the main paper with the same title.

Documenting Batillaria *distribution and abundance trends*

Batillaria density

      For the density sampling from 2015-2018, a single transect was sampled at each site. Each transect began just below the seaward edge of salt marsh (approximate elevation of 1.3 m NAVD88) and continued to about +0.3 m above Mean Lower Low Water (approximately 0.3 m NAVD88). This is the elevational zone where *Batillaria* is most abundant at Elkhorn Slough, though some mudsnails are found above and below this range at low densities. A quadrat (0.25 m^2) was placed every meter along the transect, which, depending on slope, resulted in 3-5 quadrats sampled per transect. Live and dead mudsnails in each quadrat were counted and the sizes of the first haphazardly collected 30 individuals of each category were measured. When densities were very high, counts were made in smaller (0.0225 m^2) quadrats. Data from this intensive, consistent monitoring period is shown in Figure S1.

      Density sampling methods in 2011 were similar, except that multiple transects per site were conducted, and cores (diameter 8.4 cm, 0.0055 m^2) were used to sample mudsnails. The sizes of all mudsnails in the core were measured and recorded in the field, or in the laboratory for samples with many mudsnails. Density sampling methods in 1997 were less rigorous. Density was estimated from 10-15 0.05m2 quadrats haphazardly placed along the shoreline.

      Sampling in all years occurred in summer or fall (July-August 1997, June-August 2011, October 2015, December 2016, August-September 2017, and June 2018). Anecdotally, we have noticed no marked seasonal trends in abundance of this long-lived mudsnail. Figure 3 in the main paper contains all data from every sampling period for these sites.

Figure S1. *Batillaria* **density over time at eight sites, 2015-2018.** Data partially overlap with Fig. 3, but focus on higher-resolution temporal trends over the most recently sampled four years. Each point represents a single quadrat sampled for density. Due to the high range in magnitude of densities per m^2 (0-15,000), we used log(density+1) for the Y axis. Smoothed regression lines are shown in blue, with gray shading for 90% confidence intervals for significant relationships.
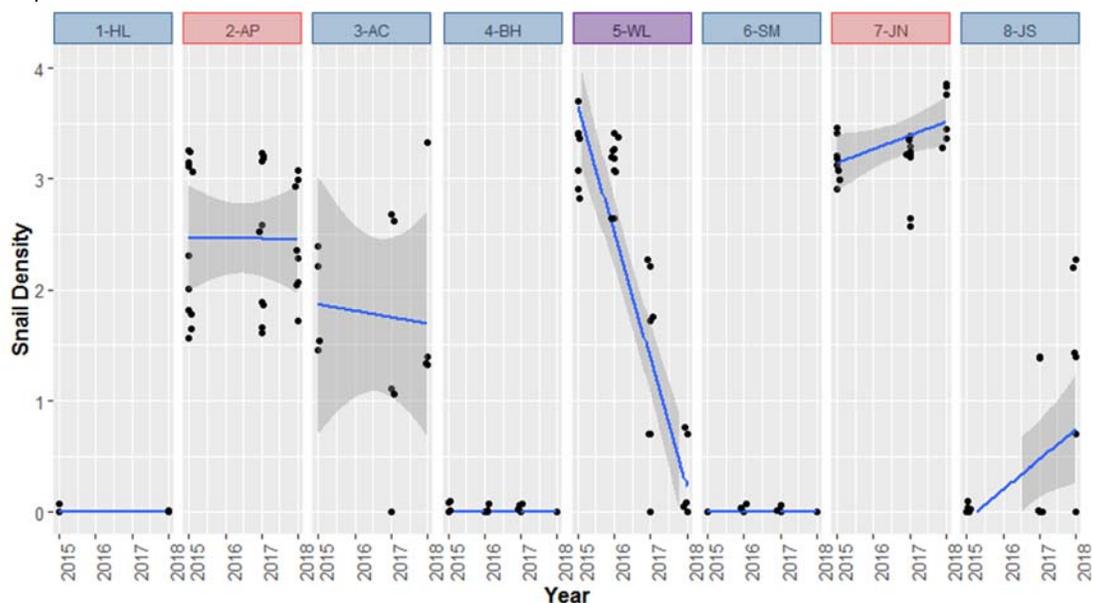
Batillaria distribution and abundance index

In alternate years, a rapid search was conducted in a 10-m stretch of mudflat at 26 sites in the estuary. The search area was marked on both ends with PVC stakes that are used to monitor bank erosion. The mudflat search area extends from the seaward edge of the marsh (at approximately +1.3 m NAVD88) down to approximately +0.15 m above MLLW (+0.15 m NAVD88), where two more PVC stakes mark the lower search boundaries. At many sites, the width of the search area is approximately 10 m, such that a 100 m² area is searched; at sites with very gradual slope the width is as much as 30 m (300 m² searched), and at sites with very steep slopes it is just a few meters (20-30 m² searched). The entire area was searched for approximately 10 minutes, for all animals and algae > 1 cm, which were scored into an abundance index with categories based on percent cover in the entire search area (0=absent, 1=present in <1% of search area, 2=present in 1-10% of search area, 3=present in 11-50% of search area, 4=present in >50% of search area). As a part of the same survey, a quadrat (0.25 m²) was placed on the bank immediately below the seaward marsh boundary and burrow holes >1 cm in diameter are counted; in Elkhorn Slough, the only species that creates such large holes in the high intertidal is the crab *Pachygrapsus crassipes*. The *Batillaria* and crab data are shown in Table S1.

Table S1. Changes in *Batillaria* abundance and crab burrow density at 26 sites in Elkhorn Slough over time. Top (A): Scores for *Batillaria* are percent cover recorded as index categories. Data for 2005 and 2017 are presented spatially in Fig 4b. **Bottom** (B): Crab burrow density in quadrat just seaward of marsh boundary. Sites are numbered non-consecutively, 2-81, because some sites were reinstated from an earlier master's research project investigating bank erosion. Site 75 is one of the focal *Batillaria* study sites, called 5-WL in Fig. 2 and elsewhere, with restored tidal exchange; all other sites are different than ones used by other study components, and have full tidal exchange (hence their blue coloration). Conditional formatting was used in Excel to highlight changes; darker shades of green represent higher numbers.

A - *Batillaria* abundance across sites. 0: no *Batillaria* present; 1: <1% of search area had *Batillaria*, 2:1-10%; 3 = 11-50%, 4: >50%

Year	Sites with snail	Lower Main Channel									Upper Main channel								Parsons complex								
		2	5	6	8	10	11	23	30	32	34	35	51	53	59	60	64	69	61	62	63	65	66	67	75	80	81
2005	13	0	0	0	0	0	0	0	1	1	0	0	0	2	1	0	1	2	0	0	2	2	3	3	3	1	1
2007	15	0	0	0	0	0	0	1	0	0	1	0	0	3	1	0	3	2	2	1	2	3	3	4	2	1	1
2009	11	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	2	1	0	1	2	1	1	0	2	1	0
2011	8	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	1	1	1	1	3	0	0
2013	9	0	0	0	0	0	0	0	0	0	0	0	0	1	0	0	1	1	0	0	1	0	1	1	3	1	1
2015	7	0	0	0	0	0	0	0	0	0	0	0	0	1	1	0	0	1	0	0	0	0	1	1	1	1	0
2017	3	0	0	0	0	0	0	0	0	0	0	0	0	2	0	0	0	1	0	0	0	0	0	0	1	0	0

B - Crab burrow density across sites. Density per m² of crab burrow holes in marsh banks are shown.

Year	avg. crab density	Lower Main Channel									Upper Main channel								Parsons complex								
		2	5	6	8	10	11	23	30	32	34	35	51	53	59	60	64	69	61	62	63	65	66	67	75	80	81
2005	27	20	20	72	78	72	54	4	6	64	28	46	20	6	56	12	0	44	2	30	22	50	0	0	0	0	0
2007	13	0	10	10	18	34	0	10	8	30	4	16	4	26	14	54	20	32	8	30	20	0	0	0	0	0	0
2009	26	4	26	32	8	70	66	24	70	18	64	88	48	14	6	46	22	14	10	28	14	10	0	2	0	0	0
2011	30	18	32	36	76	32	44	48	48	90	18	60	22	50	56	24	10	8	0	46	22	12	20	18	0	0	0
2013	44	6	74	78	54	46	66	14	72	84	32	86	28	8	54	62	20	72	20	126	28	54	38	34	0	0	0
2015	45	60	64	102	148	84	62	18	24	62	4	60	32	134	66	16	22	68	10	8	32	4	46	26	10	0	0
2017	34	44	0	0	158	128	0	20	44	38	10	48	18	112	32	30	4	60	0	4	30	16	12	30	34	0	0

Tethering experiments

For all three tethering experiments to assess mudsnail survival, we used dental floss attached to 15 cm metal ground stakes for the tether (Fig. S2). The floss was tied around the middle of the snail shell and a dab of superglue added on top of the knot. Tethered snails were distributed at approximately 1 m intervals along the shoreline about 30 cm seaward of the seaward edge of the marsh in all experiments (at an elevation of about 1.2 m above MLLW). This zone just below the marsh is typically where maximum densities of *Batillaria* and large crab burrows occur. The tethering experiments were conducted in

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summer, for three to seven weeks (8/21/2015-9/23/2015; 7/12/2017-8/7/2017; 6/7/2018-7/30/2018). Tethered mudsnails were checked approximately weekly. Snails were categorized as follows:

- AI: Alive, intact (live snails have visible operculum)
- AP: Alive with signs of recent predation (aperture peeled, puncture holes in shell)
- DI: Dead, intact (no operculum)
- DP: Dead with signs of recent predation (broken shell remaining on tether)
- M: missing (fate unknown; could have been predated or tether could have slipped off)

The numbers of snails remaining in each category at the end of the experiment are summarized in Table S2. For survival over time (Fig. 5; Fig. S3), we calculated the percent that was not affected by predation as follows (see above abbreviations):

Percent not predated = (AI + DI) / (total deployed – M) x 100

For the summer 2015 tethering experiment, we used mudsnails from a single source (5-WL) and tethered them at two sites, one with zero *Batillaria* abundance (4-BH) and one with high *Batillaria* abundance (5-WL). We used two size classes, large (range 25-30 mm, average 26 mm shell height) and small (range 12-18 mm, average 17 mm), deploying 10 large and 10 small at each site. Each time we checked the tethered snails, we estimated the number of *Pachygrapsus* visible on the mudflat around the tethered mudsnails (approximately 20 m by 2 m swath of shoreline). As these crabs are typically wary in daytime, there were usually none present, but on one foggy early morning we encountered 17 crabs in the study area at site 4-BH (see Fig. 1, lower left); we never saw any crabs at site 5-WL. Because we thought high temperature associated with the past years of drought might play a role in the *Batillaria* decline, we measured sediment temperatures adjacent to the tethered snails during low tide on one of the hottest afternoons of the year (8/15/2015), which coincided with this experiment. We measured a maximum temperature of 33°C at 4-BH (maximum of 28°C at 5-WL); sediment temperatures measured on other days of the experiment were typically 20-22 °C. Despite experiencing such high temperatures that could have resulted in thermal stress, no snails died intact (with no signs of damage) at the site that had recently experienced loss of the *Batillaria* population (4-BH). At each site, a single small snail died from apparent predation. The snails began losing their tethers at high rates after about three weeks so the experiment was ended.

For the summer 2017 tethering experiment, we focused on one site, 5-WL, which was undergoing a strong population decline. We hypothesized that mudsnails at this site might be especially vulnerable to predation because we noted that many had partly dissolved shells. We thus collected 20 mudsnails from both site 5-WL and from a site with a more stable population, 2-AP, to tether at site 5-WL. We attempted to use similarly sized snails from each site, but snails were smaller at 5-WL, and so average size of the 20 tethered snails from 5-WL was smaller (22±1.5 mm) than the 20 from 2-AP (26±2.4 mm; Table S2). This tethering experiment yielded very different results than the 2015 experiment: there was high predation in the first weeks (Fig. S3). At the end of the experiment, 2 live snails remained from the former source, and 4 from the latter. The average size of these survivors was around 21 mm for both sites, which was smaller than the average deployed size from either location. This pattern was unexpected, since we expected that the largest snails would have a greater rate of survival.

For the summer 2018 tethering experiment, we deployed mudsnails to six different sites. We collected mudsnails from two sites, one with generally intact shells (8-JS) and one with fairly dissolved shells (7-JN). We chose the better shells from the former and the worse shells from the latter to maximize contrast in shell condition, while maintaining a similar size (average 21-23 mm in shell height, see Table S2). We deployed 10 mudsnails with intact shells and 10 with dissolved shells to each site. We assessed the taphonomic index of each snail prior to deployment; the average index for the intact shell treatment was 1.01 (± 0.4), while the average index for the dissolved snail treatment was 3.55 (± 0.4).

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Figure S2. Field methods for tethering and transplant experiments. Left: *Batillaria* tethered with dental floss and superglue to a metal ground stake labelled with a numbered tag and inserted partially into the mudflat. Right: hardware-cloth cage covered with screen used for transplant experiment; snails spray-painted red and green are just visible inside.

Table S2. Summary of the three tethering experiments. For each category of snails, both number and percent of total snails at the end of the experiment is shown. When snail numbers were zero, table cells were left blank to allow for easier review of patterns in the table. Conditional formatting was applied to percentages; darker shades represent higher percentages. For the 2018 experiments, snails from source site 7-JN had fairly dissolved shells while snails from source site 8-JS had relatively intact shells. Site labels are color-coded: full tidal exchange = blue, artificially restricted tidal exchange = pink; restoration of tidal exchange from restricted to full = purple.

year	site	source	size (average mm)	total # deployed	intact snails				snails with signs of predation				missing snails	
					live		dead		live		dead			
					#	%	#	%	#	%	#	%	#	%
2015	4-BH	5-WL	26	10	8	80		0		0		0	2	20
2015	4-BH	5-WL	17	10	5	50		0		0	1	10	4	40
2015	5-WL	5-WL	26	10	10	100		0		0		0		0
2015	5-WL	5-WL	17	10	7	70	1	10		0	1	10	1	10
2017	5-WL	5-WL	22	20	2	10		0		0	14	70	4	20
2017	5-WL	2-AP	26	20	4	20		0		0	13	65	3	15
2018	2-AP	7-JN	22	10	6	60		0	1	10		0	3	30
2018	2-AP	8-JS	21	10	6	60		0	1	10		0	3	30
2018	3-AC	7-JN	23	10	4	40	1	10		0	1	10	4	40
2018	3-AC	8-JS	21	10		0		0	1	10	4	40	5	50
2018	4-BH	7-JN	21	10	9	90		0		0	1	10		0
2018	4-BH	8-JS	22	10	5	50		0	3	30	2	20		0
2018	5-WL	7-JN	22	10		0		0		0	10	100		0
2018	5-WL	8-JS	22	10		0		0		0	10	100		0
2018	7-JN	7-JN	22	10	10	100		0		0		0		0
2018	7-JN	8-JS	21	10	8	80		0	1	10	1	10		0
2018	8-JS	7-JN	21	10	10	100		0		0		0		0
2018	8-JS	8-JS	22	10	9	90		0		0	1	10		0

Figure S3. Summer 2015 and 2017 tethering experiments. The graphs show the percentage of *Batillaria* that had no signs of predation out of the total number of non-missing mudsnails at each date. **Top**: summer 2015 experiment conducted at two sites, 4-BH (left) and 5-WL (right). Red lines are large snails (26 mm average, n=10 at each site) while blue lines are small snails (17 mm average, n=10 at each site). **Bottom**: summer 2017 experiment conducted at one site, 5-WL, with snails collected at that site and at a site with a stable population, 2-AP (n=20 from each source).
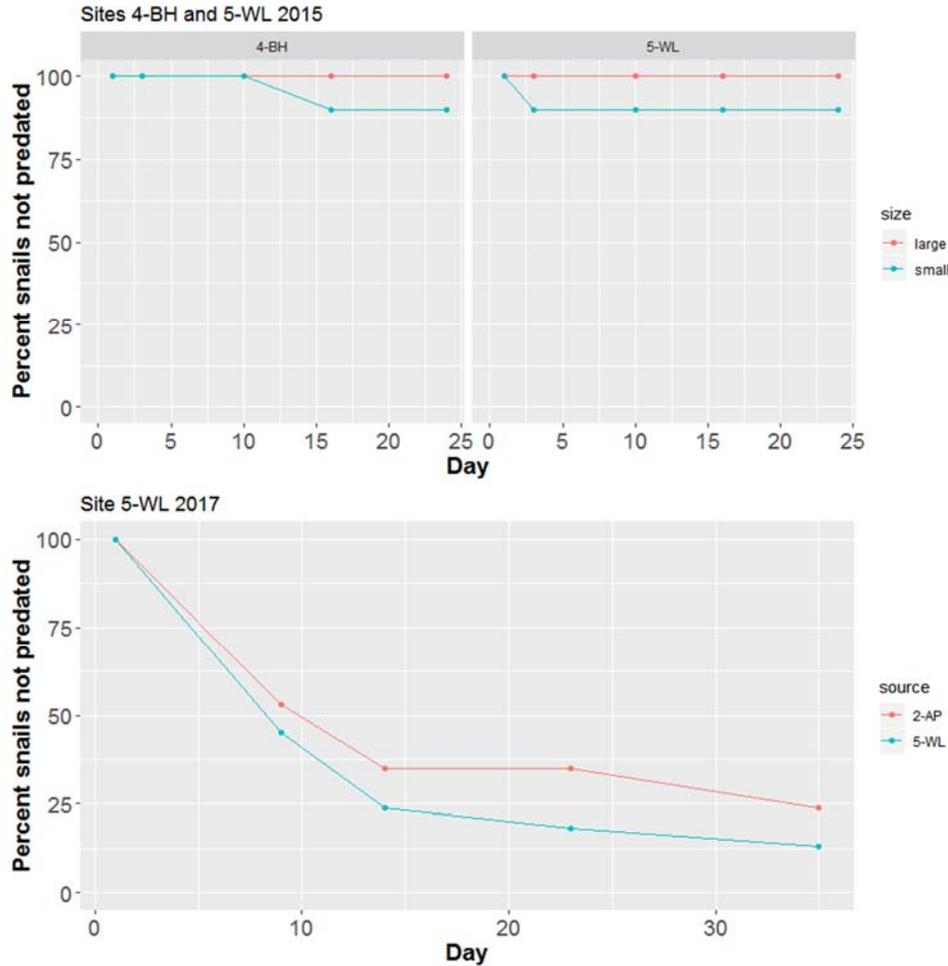
Transplant experiment

In winter 2017, we conducted a transplant experiment to characterize survival and predation at the same two sites as the first tethering experiment, 4-BH and 5-WL. On 12/14/2016, we collected mudsnails from site 4-BH, where they were still abundant. We selected two size classes to examine, small snails ranging from 10-15 mm, and large ones ranging from 23-30 mm. We found fewer large snails, so used lower sample sizes for them than for small snails. We poked the snails through holes in a box lid so just the spire emerged and then spray painted all the spires (different colors for large vs. small). At each site, we deployed two cages (built of hardware cloth covered with screen), measuring approximately 30 x 30 x 30 cm. The cages were open at the bottom and placed into soft mud with ground staples so that the bottoms were in the mud (Fig. S2). The cages were intended to exclude crabs but when we checked them later small grapsid crabs were sometimes in the cages, presumably having burrowed underneath the edges. The cages were placed about 2 m apart, around 1 m seaward of the seaward edge of the marsh. In each, we initially deployed 10 large and 25 small snails. In the uncaged area between the cages at each

site, we placed 50 large and 100 small snails. When we checked on the experiment, we attempted to find all mudsnails in the cages and between them. We very rarely found dead mudsnails or found signs of predation, therefore we report results only for marked live mudsnails (Fig. S4). Some of the decrease over time was likely due to burial in mud or dispersal away from the search area. We ended the experiment on 3/22/2017, when we had difficulty locating 15% of the uncaged mudsnails from site 5-WL. At that point we collected all the mudsnails we could find on the mud surface at site 4-BH (which had no other mudsnails present except the marked experimental ones) and returned them to site 5-WL.

We did not quantify crab abundance during this experiment, but crabs were noted to be abundant at both transplant sites during the experimental period. When recapturing the marked mudsnails, we noticed that mudsnails at site 4-BH were generally within 1 m of the area they'd been placed (between the two cages), while mudsnails at site 5-WL had travelled farther on average, up to 4 m.

Figure S4. Winter 2017 caged and uncaged transplant experiment. The graphs show the percentage of marked *Batillaria* that were located on each date. All snails were originally collected from site 5-WL, from two different size classes, large (red) and small (blue). **Left**: snails that were placed in wire cages (25 small and 10 large in each cage, 2 cages per site, results for both cages combined); **Right**: uncaged snails (100 small and 50 large deployed at each site in the area between the two cages). **Top**: snails transplanted to site 4-BH; **Bottom**: snails transplanted to site 5-WL.

6
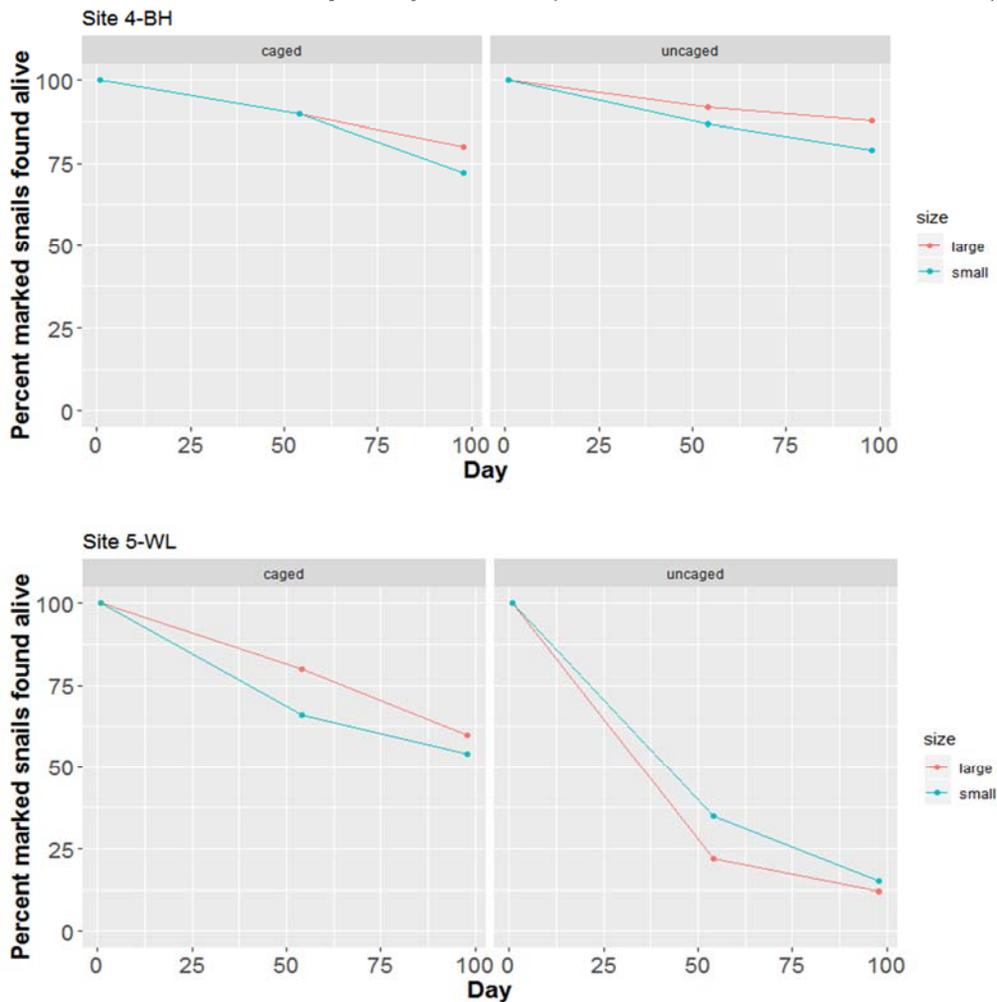
Crab abundance: spatial and temporal patterns

Crab burrow densities were monitored at the 26 bank erosion sites as described above and summarized in Table S1. To more closely examine these patterns, we limited the analysis to the 13 sites that ever had *Batillaria* present (omitted sites 2-11, 35, 51, 60). The resulting densities over time are shown in Figure S5. ANOVA revealed that year was a significant factor affecting burrow density (P = 0.0015, df=6, F = 3.87), but the only pairwise comparisons that were significant in a Tukey HSD test were 2013 vs. 2005, 2007, and 2009. Thus, the high burrow densities in 2013 represent a significant peak.

We also examined the relationship between crab burrow density and *Batillaria* abundance at these sites. We used averaged data from 2005 and 2007, the years where *Batillaria* occurred at the most sites (Table S1). There was a weak but significant, negative relationship between crab burrow density and *Batillaria* abundance index across these sites (Fig, S6).

Figure S5. Crab burrow densities over time. Crab burrow densities adjacent to mudflats at erosion monitoring sites that had *Batillaria* present in at least one monitoring year.
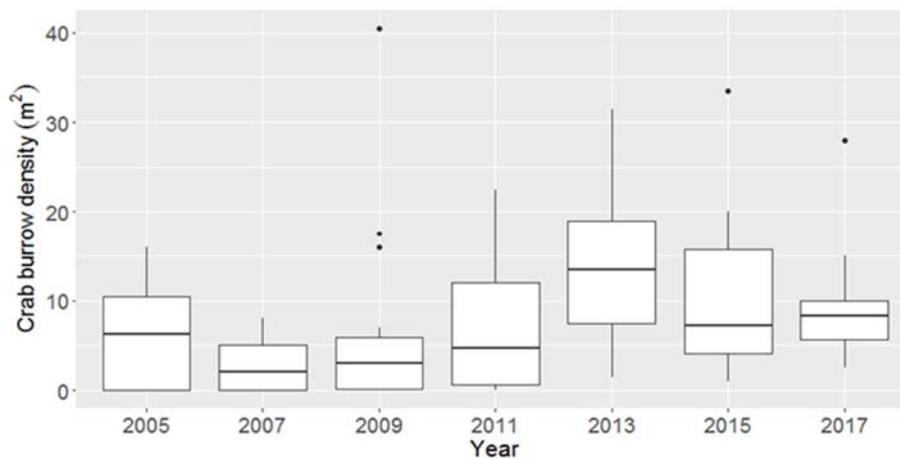
Figure S6. Crab burrows vs. *Batillaria* abundance index. Crab burrow densities adjacent to mudflats at erosion monitoring sites that had *Batillaria* present in at least one monitoring year. Data were averaged from 2005 and 2007 monitoring of crabs and mudsnails (data shown in Table S1). Smoothed regression line is shown in blue; R^2 = 0.19, P = 0.02.
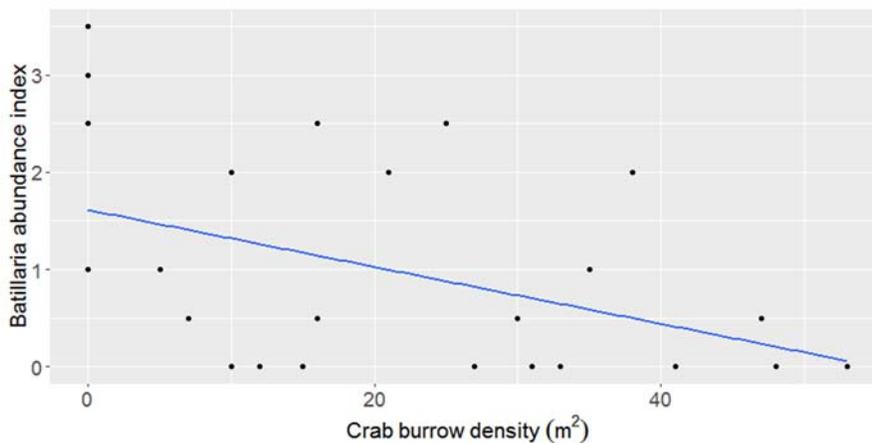
Crab feeding trials

Laboratory trials were conducted with the primary aim of a) determining whether *Pachygrapsus crassipes* consumes *Batillaria*, and b) examining distinctive shell damage caused by crab predation on the mudsnails. The various crab feeding trials each had additional hypotheses that were examined, as described below.

The crab feeding trials were conducted in May, July and October 2018 and varied somewhat in design (Table S3) but all involved captive crabs offered mudsnails and/or algae in enclosed containers over a 2-3 day duration. Because this species of crab is difficult to trap (e.g., drowns in pitfall traps, does not typically enter baited traps), we collected individuals from under rocks at a site in the estuary with extensive rip rap (site KP, Fig. 2), for all except the July 2018 trials (see below). Crabs were held in running seawater drawn from Monterey Bay, at a marine laboratory, without additional food for a period before the experiments were conducted (three days in May 2018 at Long Marine Laboratory; seven days for July 2018 at Moss Landing Marine Laboratory, and seven days at Long Marine Laboratory in October 2018). For all except the July 2018 feeding trials (where crabs and mudsnails were simply placed at the bottom of moist buckets in a laboratory at the Elkhorn Slough Reserve), we placed corrugated plastic sheets in free-flowing seawater tables at Long Marine Laboratory under plastic containers that housed individual crabs. Wooden blocks were attached to the plastic containers and corrugated plastic to secure the containers and provide area for the crabs to perch outside of the water. Plexiglass was placed over the tops of the containers to prevent crab escape while still allowing technicians to visually monitor the trials. Mudsnails were tethered using dental floss around the middle of the shell with a dab of superglue on top of the knot and wrapped around the screw in the wooden blocks. This was to prevent the snails from moving out of the crabs' range. The experiments were conducted over 72-hr periods where the crabs were checked twice a day, once in the morning and once in the evening, to see if there were any interactions between the crabs and either the snails or algae (*Ulva lactuca*).

Evidence of predation attempts included the presence of shell fragments, peeling of the shell, damage to the aperture, damage to the spire, or soft tissue damage. Sometimes predation attempts resulted in the death of the snail; the snail's soft tissue was typically removed and eaten by the crab (Fig. S7, Video S1). We separately report proportion of snails that were never attacked (no evidence of predation), those still alive with evidence of predation, and those that were dead resulting from predation (Table S3).

For the May 2018 feeding trials we used an orthogonal experimental design where we offered crabs of two size classes (small, 29-32.9 mm and large, 33-44 mm) either mudsnails of two size classes (small, 10-19mm and large, ≥20mm) or a small mudsnail and algae (*Ulva lactuca* cut into 1.5 g portions). We used mudsnails, crabs and algae from a single source (KP). The treatments were categorized as follows:

- small crab with both large and small snail
- large crab with both large and small snail
- small crab with small snail and alga
- large crab with small snail and alga

This design allowed us to determine whether 1) consumption of mudsnails varies as a function of crab size, 2) consumption of mudsnails varies as a function of mudsnail size, 3) crabs prefer algae over mudsnails, and if so, whether this preference is stronger for small versus large crabs. We hypothesized that a) predation attempts will be higher for large crabs versus small, b) crabs of all sizes will prefer small mudsnails over large and c) small crabs will prefer algae over small mudsnails more frequently than large crabs will.

In these trials (n=108), few *Batillaria* were attacked by crabs; 4.6% *Batillaria* were damaged and alive while 6.5% were dead. Only large crabs ate mudsnails and those that interacted with mudsnails showed a preference for small mudsnails over large. Preference was inferred because small mudsnails

were the only individuals that were killed by crabs (n=7), though two large mudsnails did show evidence of predation (i.e., shell damage). When offered both snails and algae, crabs consumed algae more often (36.1% of all encounters) than attacking snails (13.9% of all encounters), irrespective of crab size. Predation by large crabs (25%) was higher than that of small crabs (4%) across treatments.

In July 2018, we conducted a very brief feeding trial to follow up on results of the summer 2018 tethering experiment. We were puzzled why so many snails were eaten at site 5-WL, while fewer were eaten at nearby site 4-BH, despite high crab abundance at both sites. We speculated that naïve crabs at 4-BH might not recognize *Batillaria* as food, since mudsnails had been absent from this site for 3+ years. We thus collected eight crabs each at site 4-BH and site 5-WL and, after holding the crabs in running seawater for one week, offered them mudsnails with fairly dissolved shells (taphonomic index 3-4 from site 7-JN) for a two-day feeding experiment conducted in buckets with wet paper towels at the bottom. Counter to our hypothesis, more crabs from 4-BH attacked mudsnails (50% of crabs) than from 5-WL (25% of crabs), and the only mudsnails killed were attacked by crabs from 4-BH. Thus the lower predation rate at 4-BH in the tethering experiment was apparently not because crabs at 4-BH were not recognizing *Batillaria* as prey.

For the October 2018 trial we explored how shell condition may influence crab predation. We hypothesized that more mudsnails with dissolved shells would be eaten or damaged by crabs than mudsnails with intact shells. We collected mudsnails from two sites, 7-JN and 8-JS, and crabs from a single site (KP). Previous trials showed that evidence of predation on *Batillaria* was more likely when mudsnails were paired with large vs. small crabs, therefore larger crabs were selected for this trial. We visually assessed and categorized the snail shells using the taphonomic index (TI) in Figure S8. Each crab was given a snail with a dissolved shell (TI of 3-4) and a snail with an intact shell (TI of 0-2). To distinguish the two types of snails we marked their tethers accordingly. All of the snails (n=144) in the 3 day October trials were either killed (79.7%, n=115) or damaged (20.1%, n=29) by crabs. There was no significant difference between rates of consumption among dissolved vs. intact snails (χ^2=0.17, P=0.68) nor did crab predation (attempts and successes) vary as a function of shell condition.

Table S3. Summary of laboratory crab feeding trials. Conditional formatting was applied to the percent of snails showing no predation vs. evidence of predation on shell but snail still alive vs. evidence of predation on shell and snail is dead. Algal pieces were offered as alternative food in some May 2018 trials (p=present), but not other trials (a=absent). Snail shell condition was only considered and assessed in the final October 2018 experiment. Snail source sites are color-coded as elsewhere in this paper (full tidal exchange = blue; restricted tidal exchange = pink).

| | | | | | | | | | no predation | | snails with signs of predation | | | |
| | | | | | | | | | live | | live | | dead | |
trial	crab source	crab size (average)	snail source	ulva p/a	snail condition (d=dissolved; i=intact)	snail size (average)	# replicates	trial duration (days)	#	%	#	%	#	%
2018-May	KP	32	KP	p	?	23	15	3	15	100	0	0	0	0
	KP	37	KP	p	?	22	21	3	16	76	2	10	3	14
	KP	31	KP	a	?	20	13	3	12	92	1	8	0	0
	KP	31	KP	a	?	28	13	3	13	100	0	0	0	0
	KP	37	KP	a	?	20	23	3	18	78	1	4	4	17
	KP	37	KP	a	?	32	23	3	22	96	1	4	0	0
2018-July	4-BH	26	7-JN	a	?	19	8	2	4	50	1	13	3	38
	5-WL	26	7-JN	a	?	20	8	2	6	75	2	25	0	60
2018-Oct	KP	37	7-JN	a	d	21	69	3	0	0	13	19	56	81
	KP	33	7-JN	a	d	21	3	3	0	0	0	0	3	100
	KP	37	8-JS	a	i	22	69	3	0	0	15	22	54	78
	KP	33	8-JS	a	i	21	3	3	0	0	1	33	2	67

Video S1. Crab feeding trials footage shows further evidence of predation on *Batillaria*. Footage in the video was gathered from May and October trials. The link is to a 73 second video that shows the varied response by crabs to *Batillaria*. https://www.youtube.com/watch?v=-wGUlJoXd-g&t=2s

Figure S7. Shell damage from crabs. Compilation of photos of *Batillaria* damaged by *Pachygrapsus* in feeding trials.
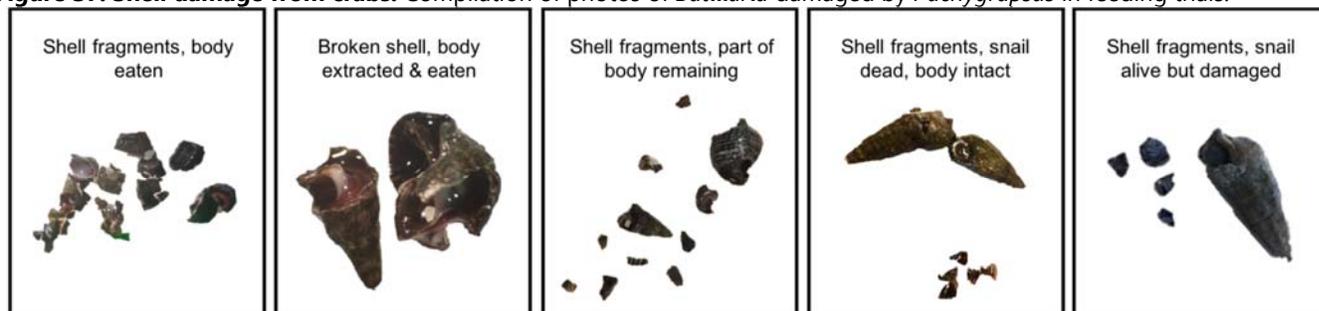
Batillaria *shell condition assessment*

To examine shell condition, we collected 20 live *Batillaria* from each of our focal sites in July 2018, with the exception of 4-BH, where no mudsnails were present, and 6-SM, where we only found 11 mudsnails (which we collected). At the sites with abundant mudsnails (2-AP, 3-AC, 7-JN), we collected mudsnails from the area spanned by our permanent transect monitoring locations. At sites with very low abundance (1-HL, 5-WL, 6-SM, and 8-JS), we searched an area of about 100 m around the transect location to obtain the mudsnails. To compare the condition of *Batillaria* shells from populations outside of Elkhorn Slough that were not experiencing similar rapid decline, we also collected 55 mudsnails from Doran Spit, Bodega Harbor in August 2018 and 30 mudsnails at Loch Lomond Marina (city of San Rafael) in San Francisco Bay in September 2018. Since we collected snails haphazardly, they represented the typical size distribution for each site, and sizes ranged from 12-37 mm for individual snails.

We examined shells microscopically in the laboratory. We separately evaluated the spire and the last whorl (most recently produced shell where repairs are possible) and scored the shell's dissolution on a scale of 0-4 (Taphonomic Index, Fig. S8). We determined if there was any evidence of a peeled aperture (indicative of a recent predation attempt) and if there was evidence of shell repair (older predation attempt, but repaired by the snail) (Fig. S9). Because many shells had algae on them, which can cause bioerosion that in turn facilitates dissolution, we also noted whether each shell had algae present or not.

Since shell condition generally worsens with size/age, we were concerned that size differences among sites might bias taphonomic index results. We thus conducted all summary analyses in two ways, once with all snails collected and once with just the subset that ranged from 18-28 mm, which resulted in lower variance in site averages, but smaller sample sizes (Table S4). However, the patterns we found (in terms of site differences) were generally similar whether the full data set or size-controlled dataset were used. Therefore, for the main summary table (Table 1) and statistical analyses linking shell condition to *Batillaria* density or pore water, we used the full data set with the larger sample size representative of snails at the site.

Overall, we found that shell condition was fairly poor at many sites (Table S4), with the average taphonomic index ranging between 2.4 and 3.5 for the different Elkhorn Slough sites; the mudsnails from Bodega Bay scored in the middle of this range with an average index of 2.8 while the mudsnails from San Francisco Bay scored an average of 2.4. There was high variation within sites as well (Fig. S10). The spire, which is the older part of the shell, consistently had a higher taphonomic index (poorer condition) than the last whorl, which is the youngest part of the shell. A high percentage of snails had algae on shells that could facilitate dissolution, because algae can erode the shell surface and/or cause pitting (Walker and Carlton 1995). A high percentage of shells showed evidence of past repairs, but this varied strongly by

site. Only a small percentage had peeled whorls indicative of a recent predation attempt (Table S4). These indicators of predation were consistent with damage caused by *Pachygrapsus* in feeding trials.

We used linear regression to explore the relationship between taphonomic index and *Batillaria* density (square-root transformed) using site (n=7, since one focal site, 4-BH, had no mudsnails to assess) as replicate. Unexpectedly, there was a weakly positive relationship between taphonomic index and *Batillaria* density (R^2=0.41, P=0.12), but this relationship was driven by one site, 7-JN, which had by far the highest taphonomic index and density.

Prior to the intensive studies in 2018, we evaluated the death assemblage (shells of dead mudsnails) from three focal sites in summer 2017 to determine the incidence of peeled shells and shell repair. For each site, we haphazardly selected 25 shells ranging in size from 19-23 mm and evaluated frequency of peeled apertures and shell repair. The results of these assessments are summarized in Figure S11. Peeled apertures were common at all sites, and were seen in 100% of dead mudsnails at 7-JN, suggesting crab predation was likely the primary cause of death at this site. Shell repair varied across sites, with no evidence of repair at the site with the highest peeling, perhaps because mudsnails were eaten before they could undergo repair.

We also examined pore water as a potential correlate of shell condition. We attempted to sample all eight focal sites but could not extract pore water from one of them (6-SM). The other sites were sampled twice (7/2 and 7/19/2018 on morning low tides), except for 5-WL and 8-JS, which were only sampled on the first date, and 1-HL, which was only sampled on the second date. To extract pore water, we used an MHE PushPoint sampler and plastic syringe. Extracted water was placed in a glass borosilicate bottle and poisoned with 4 x 10-4% $HgCl_2$ fixative. The samples were transported in a cooler and refrigerated until they were processed at the Coastal Sustainability Lab at the University of California, Santa Cruz. The samples were analyzed for pH and total alkalinity following best practices (Dickson et al. 2007). Pore water pH was measured (total scale; Zeebe and Wolf-Gladrow 2001) via spectrophotometric analysis (Shizmadzu) using a pH-sensitive dye (m-cresol purple), and total alkalinity was measured using potentiometric acid titration (Metrohm, 905 Titrando), *In situ* carbonate chemistry parameters (including DIC) were calculated using the computer software CO2SYS v2.1 (Robbins et al. 2010), with K1 and K2 dissociation constants based on Mehrbach et al. (1973) as refit by Dickson and Millero (1987), and $KHSO_4$ based on Dickson et al. (2007). This also allowed us to calculate the sulfate concentrations and aragonite saturation state. Pore water results are summarized in Table S5. Strong contrasts were found among sites. There were very high values for total alkalinity, aragonite saturation, carbon dioxide and dissolved inorganic carbon at site 2-AP. Aragonite saturation state was unusually low at site 4-BH.

We used a general linear model (GLM) to examine whether *Batillaria* density varied as a function of all the pore water variables combined, using Gaussian distribution and additive effects only; no pore water variables emerged as significant. Likewise, a GLM relating *Batillaria* shell condition (taphonomic index) to all pore water parameters (using Gaussian distribution and additive effects) yielded no significant relationships. For these analyses we averaged values by site and used site as replicate, which resulted in limited power (only six of the focal sites were included in the analysis, because one site had no *Batillaria* and at another we could not extract pore water). We square-root transformed the following variables with outliers: density, dissolved inorganic carbon, carbon dioxide, aragonite saturation state and total alkalinity. For shell condition, we used the combined taphonomic index (averaging spire and last whorl) as well as the spire-only taphonomic index, in case the older part of the shell was a more sensitive indicator. To more closely examine the pairwise individual relationships, we conducted linear regressions for each pore water variable vs. average *Batillaria* density at each site; none were significant. Likewise, we calculated Spearman rank correlations for each pore water variable vs. average *Batillaria* taphonomic index at each site. Only the relationship between aragonite saturation state and taphonomic index was marginally significant (ρ=-0.083, P=0.058), but this relationship was driven by one site, 2-AP, with the lowest taphonomic index in the estuary and by far the highest aragonite saturation state.

We were surprised that pore water parameters did not correlate more strongly with shell condition and speculated that perhaps bioerosion by algae on shells is a stronger driver. We conducted a t-test and found that average taphonomic index of snails with algae is higher than without algae (3.0 vs. 2.7), but this difference was not significant (Welch's t-test: t=-1.63, df=123, P=0.11).

Table S4. Summary of shell condition analysis. Average values are shown for each parameter. Conditional formatting was applied, with darker shades indicating higher values (which represent poorer shell condition). Color coding sites as in Table S2.

A. Representative sample of all snails at site

site	number assessed	length (mm)	taphonomic index			algae (percent of shells)	repair (percent of shells)	peeled whorl (percent
			whorl	spire	combined			
1-HL	20	26.8	2.4	3.1	2.8	20	60	10
2-AP	20	24.4	2.0	2.6	2.4	40	30	10
3-AC	20	20.2	2.7	3.1	2.9	40	10	10
5-WL	20	20.6	2.7	3.3	3.1	55	20	0
6-SM	11	19.4	1.9	2.9	2.6	0	27	0
7-JN	20	25.1	2.8	3.9	3.5	55	20	0
8-JS	20	21.9	2.2	2.7	2.5	50	20	0
9-BB	55	20.3	2.1	3.2	2.8	96	15	11
10-SF	30	21.4	1.7	2.8	2.5	n.d.	43	0

B. Only snails 18-28 mm

site	number assessed	length (mm)	whorl	spire	combined	algae	repair	peeled whorl
1-HL	10	21.5	2.2	2.5	2.4	10	50	10
2-AP	18	23.3	1.9	2.5	2.3	44	28	6
3-AC	16	21.3	2.9	3.3	3.1	50	13	6
5-WL	17	21.3	2.8	3.4	3.2	53	24	0
6-SM	7	19.6	2.0	3.0	2.7	0	14	0
7-JN	15	23.6	2.7	3.8	3.5	53	7	0
8-JS	19	21.5	2.2	2.7	2.5	47	16	0
9-BB	51	20.6	2.1	3.2	2.8	98	14	12
10-SF	26	21.1	1.7	2.8	2.4	n.d.	35	0

Figure S8. Taphonomic index. *Batillaria* shells were scored according to the following guide illustrated by Danielle Jolette.

Taphonomic Index

0 : Pristine
color intact; sculpture intact; no pitting; no dissolution

1 : 1-25% altered
up to 25% of the shell surface determined for the three different shell parts (protochonch, spire, last whorl) separately, have color loss, sculpture loss, pitting or dissolution

2 : 25-50% altered
between 25-50% color loss, sculpture loss, pitting or dissolution determined for each of the shell parts separately

3 : 50-75% altered
between 50-750% color loss, sculpture loss, pitting or dissolution determined for each of the shell parts separately

4 : 75-100% altered
between 75-100% color loss, sculpture loss, pitting or dissolution determined for each of the shell parts separately

Figure S9. Signs of crab predation on shells. Crab predatory damage examined on *Batillaria*, including peeled and punctured holes indicative of unrepaired crab predation and shell repair, indicative of past crab predation.

Crab Predatory Damage
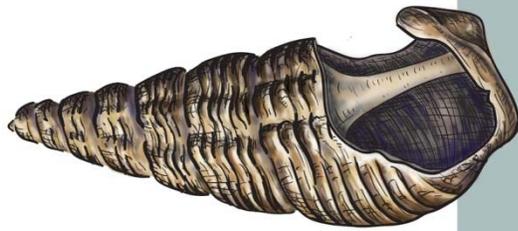
Aperture peeled

Aperture peeled and puncture wound on penultimate whorl
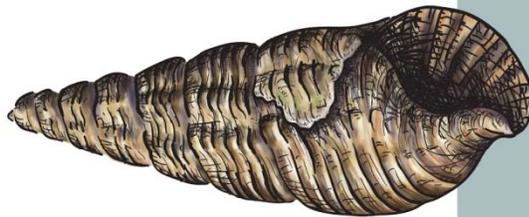
Shell repair visible on last whorl
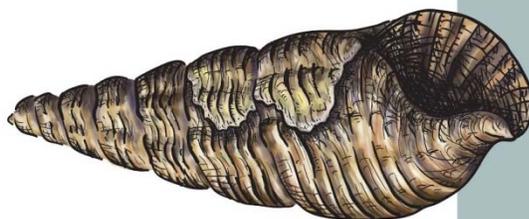
Shell repair on last and penultimate whorls

Figure S10. Variation in shell taphonomic index across sites. The first sites are the eight focal ones at Elkhorn Slough (except for 4-BH which has no *Batillaria* currently present to assess). Site 9-BB is Bodega Bay and 10-SF is Loch Lomond in San Francisco Bay; these are comparison sites outside of Elkhorn Slough. Color-coding is as in Table S2.
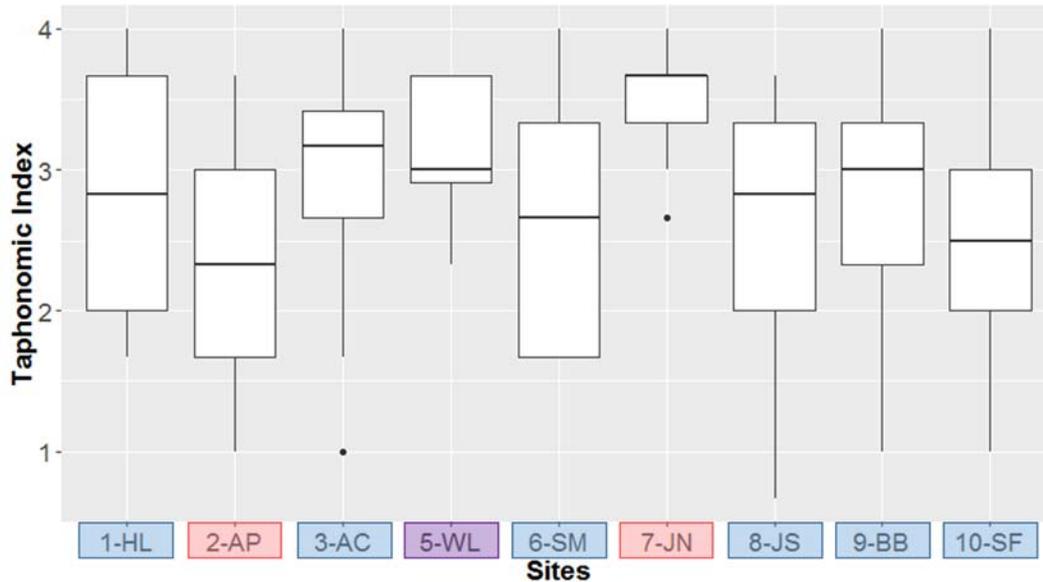
Table S5. Summary of pore water chemical parameters. Physiochemical parameters of pore water samples. Temperature and salinity were measured *in situ*, total alkalinity (TA) and pH were measured in the lab, and the partial pressure of carbon dioxide (pCO2), the dissolved inorganic carbon (DIC) and the aragonite saturation state were calculated using CO2-calc. The pH values below have been adjusted to the *in situ* temperature and salinity. Color-coding of sites is as in Table S2. Color-coding of pore water parameters goes from low (red) to high (green).

Site	Temp (°C)	Salinity (ppt)	TA (umol/kg SW)	pH (adj)	pCO2 (uatm)	DIC (uM/kg SW)	Sulfate (adjusted)	Aragonite Saturation (Ω)
1-HL	21.1	33.7	4150	7.09	8117	12274	27179	0.72
2-AP	20.3	37.5	20853	7.05	34448	49750	30224	2.38
3-AC	19.6	37.4	4410	6.85	13440	17490	30204	0.4
4-BH	19.1	38.0	3364	6.65	17841	21212	30619	0.21
5-WL	21	35.9	3185	7.05	6769	9961	28953	0.51
7-JN	17.3	33.6	2692	7.17	4141	6840	27070	0.48
8-JS	17.1	33.2	3170	7.33	3382	6559	26751	0.79

Figure S11. Frequency of crab predation on shells. We assessed two indicators of crab predation, peeling of the aperture and shell repair, with shells of 25 dead mudsnails collected at each of three sites.
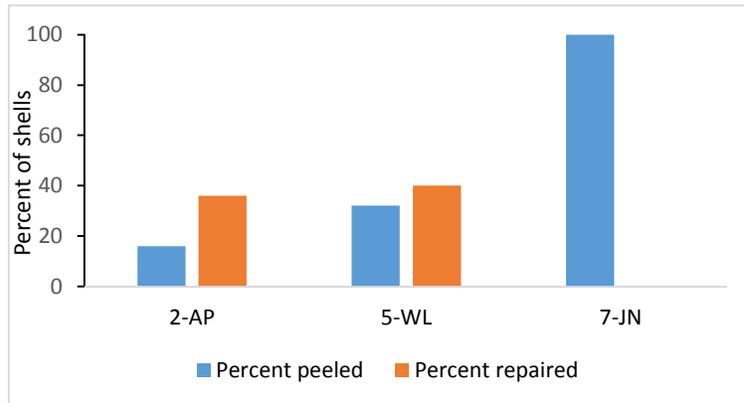
Parasites and diseases affecting Batillaria

Trematode prevalence in mudsnails

In July 2018, when we collected mudsnails at our focal sites to examine shell condition as described above, we collected an additional 20 mudsnails per site for an assessment of trematode parasitism. We examined both the 20 collected for shell taphonomy and the 20 additional ones, thus 40 mudsnails per site at our eight focal sites, with the exception of site 4-BH, where no mudsnails were present, and 6-SM, where only 10 were obtained. Snails were dissected and examined microscopically for patent trematode infection, indicated by the presence of rediae (mobile, larval stage of trematode in snail host tissue) and/or cercariae (free-swimming, infective trematode stage released from infected snail hosts into the water column). Parasitism rates varied considerably by site. A linear regression analysis revealed a weak, non-significant positive relationship between prevalence of trematode parasitism and *Batillaria* density across the focal sites in 2018 (R^2 = 0.41, P=0.12), but this was driven largely by one site, 7-JN, with the highest parasitism and density.

We compared our 2018 data with all previous Elkhorn Slough data on trematode parasitism rates (Table S6). Because trematodes rarely kill their infected mudsnail hosts, parasitism rates increase with snail size; thus, it would be ideal to compare size-standardized prevalence across the same sites over time, but such data were not available. The most striking trend is the markedly lower prevalence rate in the late 1990s relative to later dates.

Table S6. *Batillaria* infection prevalence, from all data available 1998 to 2018. Trematode infection prevalence in snails varied greatly among sites and overtime within sites, but infection was observed at all sites surveyed when snails were present. Site locations and color-coding are shown in the main paper in Fig. 2.

Year		Site 1-HL	2-AP	3-AC	4-BH	5-WL	6-SM	7-JN	8-JS	KP	Source
1998-2001	prevalence	–	–	–	3.9	–	–	2.7	–	4.0	Torchin et al. 2005
	n	–	–	–	102	–	–	75	–	50	
	size (avg)	–	–	–	21.4	–	–	19.9	–	19.8	
	size (sd)	–	–	–	2.2	–	–	2.0	–	4.8	
2006	prevalence	43.1	–	–	55.8	–	–	–	43.5	58.0	Lin 2006
	n	51	–	–	52	–	–	–	69	50	
	size (avg)	NA	–	–	NA	–	–	–	NA	NA	
	size (sd)	NA	–	–	NA	–	–	–	NA	NA	
2014	prevalence	–	–	41.7	–	–	–	–	–	72.9	Fabian 2016
	n	–	–	228	–	–	–	–	–	340	
	size (avg)	–	–	25.7	–	–	–	–	–	23.9	
	size (sd)	–	–	17.2	–	–	–	–	–	4.9	
2018	prevalence	52.5	25.6	7.5	NA	0.0	20.0	75.0	7.5	–	This study
	n	40	40	40	0	40	10	40	40	–	
	size (avg)	26.1	23.9	20.4	NA	20.8	19.4	24.9	21.6	–	
	size (sd)	5.9	4.0	3.3	NA	2.3	4.8	4.4	2.3	–	

Histological examinations

To determine whether the dramatic declines at Elkhorn Slough might be from a novel disease or pathogen not present in the other estuaries, we also collected additional *Batillaria* at Elkhorn Slough, Bodega Bay and Tomales Bay for thorough histological examination at Bodega Marine Laboratory. The sampling dates and other collection data are shown in Table S7. The Bodega Bay and Tomales Bay samples were stored overnight in seawater-saturated towels at 4°C and processed the following day. The Elkhorn Slough samples were shipped overnight to Bodega Marine Laboratory with gel ice and processed the following day (June 2017 samples) or after storing an additional night in seawater at 4°C (February 2017 samples). After measuring shell height, each body was removed from its shell by cracking the shell open with a hammer. The bodies were then placed into histological cassettes (4-6 per cassette) and fixed in Davidson's fixative (Shaw and Battle 1957) for 48 hours, followed by routine production of hematoxylin- and eosin-stained 5 µm paraffin tissue sections. The prevalence of snails infected with trematodes at each site was determined by dividing the number of snails with observed trematodes by the number of snails for which gonad tissue was present in the tissue section. Since this method of tallying from histological slides differs from the examinations on a per-snail basis with a dissecting microscope, the results are presented in Table S7 and not included in Table S6. Similarly, the prevalence of individuals with gill prokaryote inclusions was determined as the number with the inclusions divided by the number of snail samples for which gill tissue was present.

Our histological examination of *Batillaria* provided close examination of the trematode infections, which were present as rediae containing cercariae (Fig. S12). Levels of infection were higher in individuals examined from Tomales Bay than Elkhorn Slough (Table S7). We also observed a *Rickettsiales*-like prokaryote (bacterium) in the gill of four individuals from Tomales Bay (Fig. S13). It formed intracellular inclusions but only one to a few were present in each tissue section and were unlikely to be causing significant harm to the host mudsnails. No host response to the bacteria was present.

Figure S12. *Batillaria* **gonad illustrating trematode infections.** A: Normal male gonad. Arrows point to developing male gonad; lighter-stained tissue is digestive gland. B: Normal female gonad. Arrows point to bright pink oocytes; lighter-stained tissue is digestive gland. C: Trematode-infected gonad. Arrow points to gonad completely filled with trematode rediae; lighter-stained tissue is digestive gland. D: Higher magnification of trematode-infected gonad, showing cross section of one redia (arrow points to its circumference) containing multiple developing cercariae.
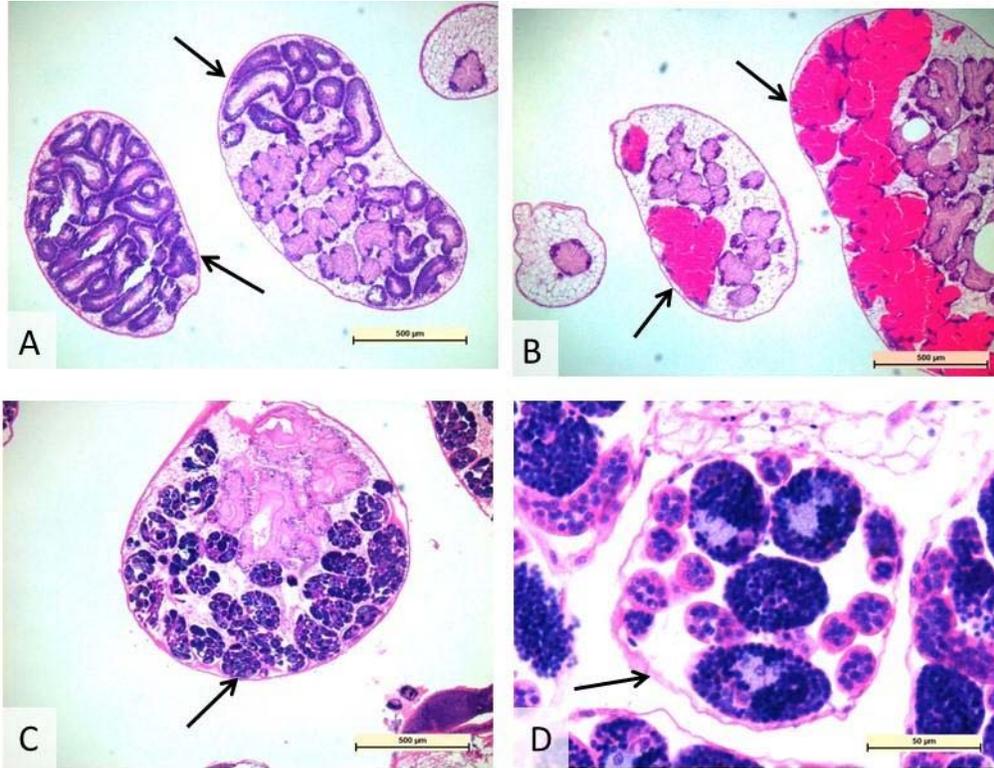
Figure S13. Intracellular prokaryotic inclusion in gill tissue, *Batillaria* from Tomales Bay. A: Large basophilic inclusions (arrow) in tissue forming the base of the gill. B: Higher magnification of inclusion (arrow), showing uniform basophilic staining typical of prokaryote intracellular inclusions in molluscs.

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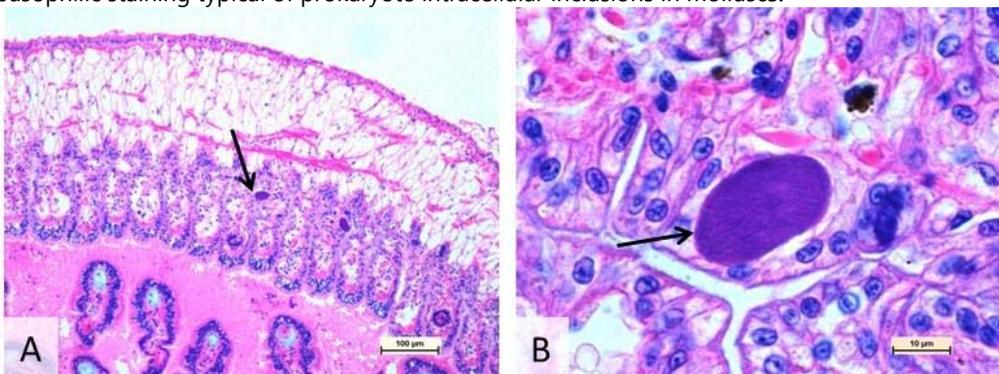
Table S7. Prevalence of trematode infections in gonadal tissue and intracellular prokaryote infections in the gill in *Batillaria* samples from histological examination. Number of animals infected/number with appropriate tissue (gonad for trematodes, gill for prokaryotes) in parentheses. Bodega and Tomales Bays are two sites external to Elkhorn Slough for comparison.

Estuary	Bodega Bay	Tomales Bay	Elkhorn Slough				
Site	Doran Beach	Walker Creek	6-SM	KP	5-WL	5-WL	2-AP
Month/year collected	Jan-17	Mar & Jun-2017	Feb-17	Feb-17	Feb-17	Jun-17	Jun-17
% with trematode infections	7 (3/40)	68.4 (52/76)	16.7 (1/6)	50 (9/18)	0 (0/19)	10 (2/20)	66.7 (4/6)
% with gill prokaryote inclusions	0 (0/52)	7.1 (4/52)	0 (0/7)	0 (0/17)	0 (0/18)	0 (0/12)	0 (0/10)
Mean shell length (mm±s.d.)	15.8±1.9	25.3±4.7	19.8±4.1	26.2±6.7	21.3±1.9	20.6±3.1	23.7±7.4

Trends in environmental factors potentially affecting snails or crabs

We examined water quality trends using data collected consistently over time as a part of the National Estuarine Research Reserve System-wide Monitoring Program (see https://cdmo.baruch.sc.edu/ for more information on data collection or to download data). A YSI sonde has been continuously deployed at Elkhorn Slough Reserve's South Marsh about 600 m south of *Batillaria* sampling site 6-SM since 1995, collecting standard water quality parameters *in situ* (dissolved oxygen, pH, temperature, salinity, turbidity). As of 2005, monthly water quality samples have also been collected hourly for 24 hrs using an ISCO sampler at this station (near 6-SM), with subsequent laboratory analysis for nutrient and chlorophyll-*a* concentrations. We examined temporal trends in all parameters using on-line tools generated by Marcus Beck (https://beckmw.shinyapps.io/swmp_summary/), which include visualization of annual and monthly means and anomalies.

Most of the measured parameters varied considerably interannually but showed no clear temporal trends over the long term and no relationship to the decline in *Batillaria*, which was first quantified in 2015 but anecdotally began a few years before this. Due to the potential for estuarine acidification (especially as it might affect shell condition), we were particularly interested in pH trends, but observed none (Fig. S14-top). The only parameter that showed a pattern consistent with the *Batillaria* decline was water temperature, which showed a clear increase in the more recent years (Fig. S14-bottom).

Water level data from the NOAA tide station revealed that water levels were unusually high during this same period of elevated temperature (Fig. S15). Previous analyses revealed that water levels in Elkhorn Slough and Monterey are extremely tightly correlated (Elkhorn Slough Reserve, unpublished data). An analysis of water level data from Elkhorn Slough suggests that the average 7 cm increase in water depth would increase inundation time for mudsnails living just below the marsh edge (elevation 1.3 NAVD88) from 36.6% to 41.3% of the time (Elkhorn Slough Reserve, unpublished data).

Analysis of metaldehyde application on neighboring farmland was conducted by obtaining data from the California Department of Pesticide Regulation Pesticide Use Reporting Program. These data are made available to the public as annual reports based on township and section. Using ArcGIS Desktop v10.4, we performed a spatial join of the data for each 1 square mile section and plotted metaldehyde application (lbs/yr) over a total of 125 sections for years 2005 through 2015. Additionally, total metaldehyde application was summed for all years in the time range and plotted on a map to identify any patterns or trends in the Elkhorn Slough and Moro Cojo watersheds.

Spatial patterns were variable over time (Fig. S16, 17), but did not show especially high application near the mid-estuary sites that had the strongest *Batillaria* declines. Temporal patterns show a peak in usage of this pesticide in the region during 2009-2012, and a decrease in usage during the subsequent period when the greatest *Batillaria* declines occurred. It is possible that there could be a delay between application of the molluscicide on farm fields and its arrival and mobilization in the adjacent wetlands, but it is not clear why sites such as 7-JN and 2-AP that continue to have abundant mudsnails would be less affected than sites with declines, as these two sites both receive substantial agricultural run-off, while a site like 5-WL, which is on a nature reserve, does not. However, considering the relatively high application

rate of metaldehyde on farms just west of Castroville (i.e., three times the rate of all other farms combined in the local area), and given the hydrologic connectivity between this area and Elkhorn Slough (via the Old Salinas River Channel), it is possible that high concentrations of metaldehyde are carried into Elkhorn Slough during incoming tides. In this scenario, low to mid-estuary sites would likely be more impacted than upper estuary sites.

Figure S14. Water quality data. Data for two parameters sampled in South Marsh (near *Batillaria* site 6-SM). Annual anomalies are shown. Trend lines are shown in blue dashes. pH (top) shows no long term trend 2000-2017, while water temperature shows a marked increase corresponding to the period of *Batillaria* declines.
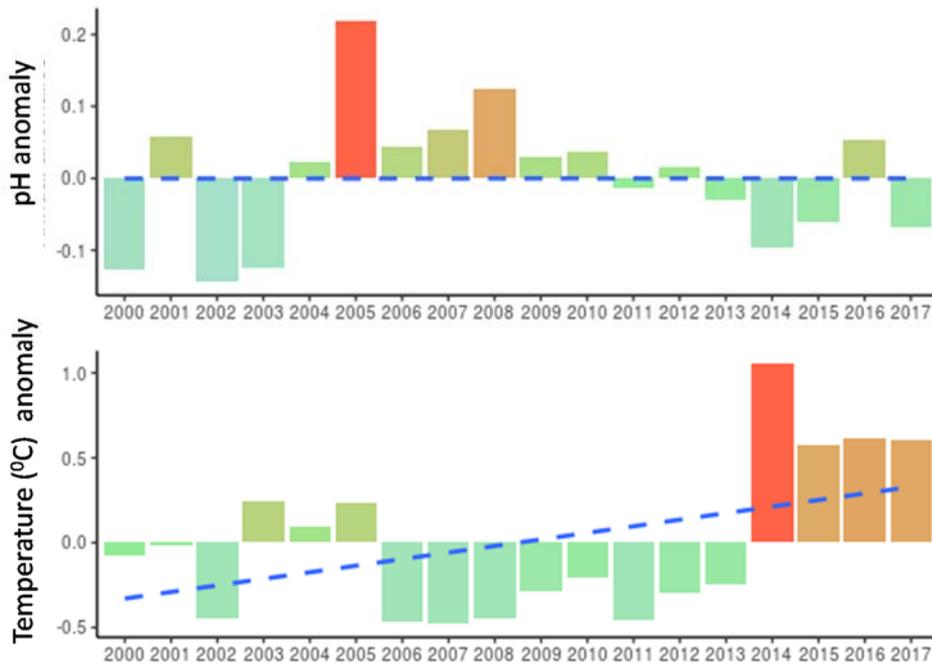
Figure S15. Water level data. Water level data measured at the NOAA-COOPS tide station in Monterey, California.
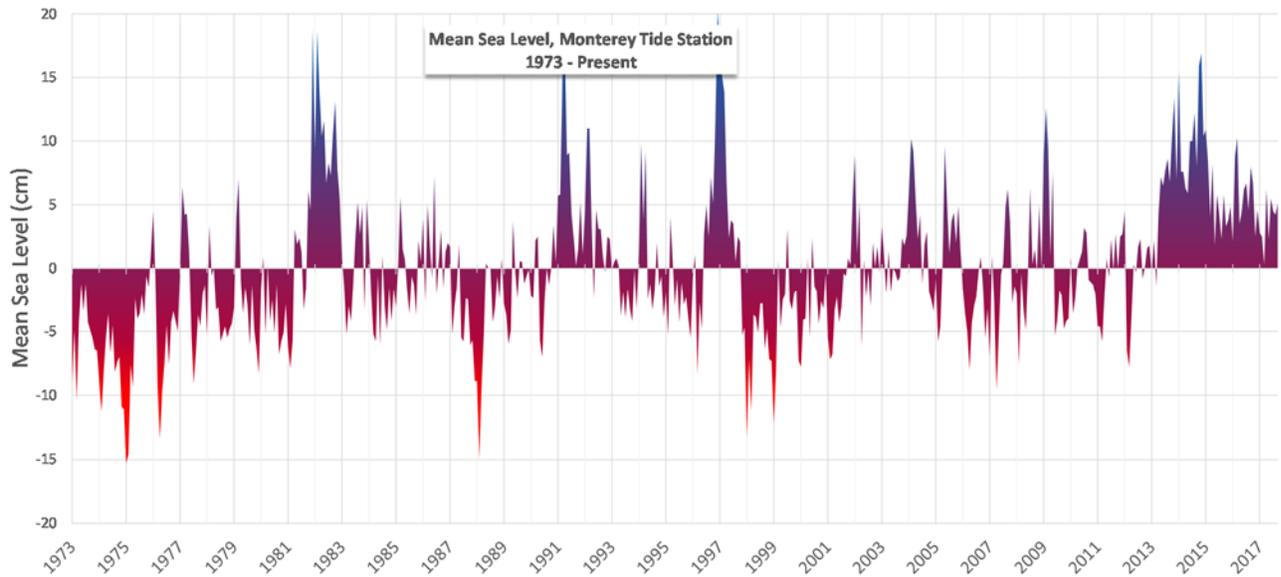
Figure S16. Metaldehyde application summed over the Elkhorn Slough and adjacent Moro Cojo watersheds over time.
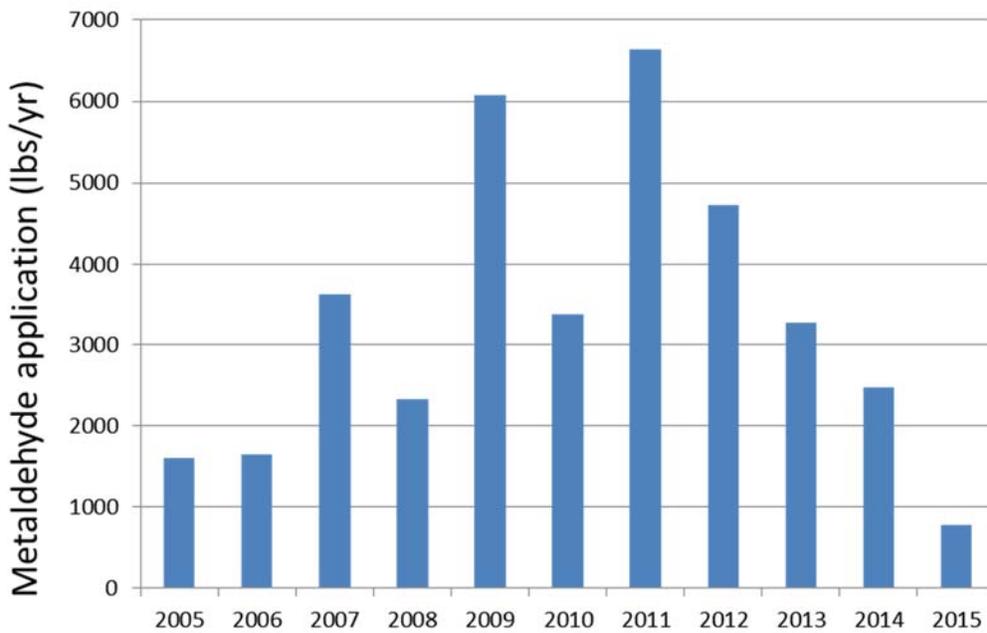
Figure S17. Spatial and temporal patterns of metaldehyde application. Data are shown for alternate years in the years before and during the *Batillaria* decline. The locations of the *Batillaria* sites, including 7-JN (stable/abundant mudsnails), 4-BH (declined/absent), 2-AP (declined/abundant), and 1-HL (declined/very rare), are shown on the 2005 panel. Amount of metaldehyde application is color coded; transparent boxes indicate that zero metaldehyde was applied to an area.
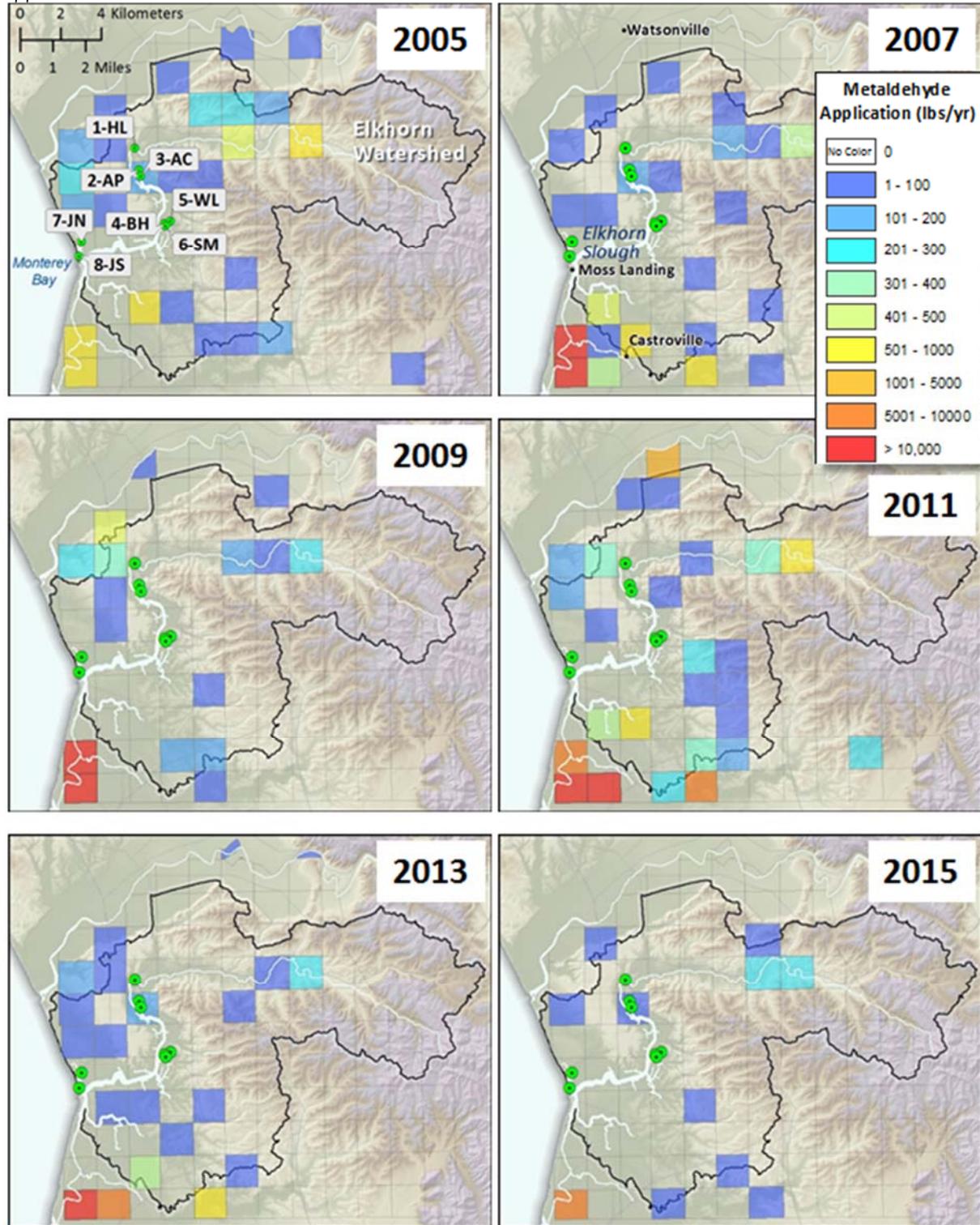
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