

Effect of green macroalgal blooms on the behavior, growth, and survival of cockles *Clinocardium nuttallii* in Pacific NW estuaries

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ABSTRACT: Nutrient over-enrichment can produce adverse ecological effects within coastal ecosystems and negatively impact the production of ecosystem goods and services. In small estuaries of the US Pacific Northwest, seasonal blooms of green macroalgae (GMA; family Ulvaceae) are primarily associated with natural nutrient input, rather than anthropogenic sources. This provided us a unique opportunity to investigate the effects of naturally stimulated macroalgal blooms on intertidal bivalves. Heart cockles *Clinocardium nuttallii* are an important species for shellfisheries in the region. In summer population surveys, we found that cockles emerged from the sediment with greater frequency as GMA biomass increased. Experimental manipulation of GMA biomass in the field showed that GMA elicited emergence, evoked above-ground lateral movement, inhibited shell growth, and increased mortality (by $34.0 \pm 15.2\%$) in cockles. Laboratory experiments revealed that the interaction of a weighted barrier at the sediment surface and GMA presence elicited rapid emergence among cockles. Risk assessment of the emergence response in cockles showed that the *in situ* emergent population experienced $11.0 \pm 8.0\%$ mortality due to gull predation, while laboratory exposure to elevated temperatures ($\geq 34^\circ\text{C}$) slowed valve-closure, inhibited reburial, and increased mortality, which could have translated to $7.1 \pm 1.5\%$ *in situ* mortality. We found that cockles avoided mortality due to burial below GMA mats by emerging from the sediment, but that behavior consequently put them at risk of mortality due to heat stress or gull predation. Regardless of nutrient source, our research showed that GMA blooms pose a threat to the survival of intertidal bivalves.

KEY WORDS: Bivalves · Ulvoid · Ecosystem services · Nutrient enrichment · Behavior · Intertidal · Emergence · Eutrophication

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INTRODUCTION

Nutrient over-enrichment can produce adverse ecological effects within coastal ecosystems (Anderson et al. 2002, Bricker et al. 2008), which can negatively impact the functions and services provided by these productive environments. Estuaries naturally derive vital nutrients from sources such as geological weathering and oceanic upwelling (Boesch 2002, Hickey & Banas 2003, Bricker et al. 2008); however,

anthropogenic land-based input is the predominant source of nutrients in the majority of US estuaries, and has led to the over-enrichment of many estuaries worldwide (Boesch 2002, Howarth et al. 2002, Bricker et al. 2008). Consequences of nutrient pollution include hypoxia/anoxia, seagrass loss, fish/macrofauna die-offs, and macroalgal blooms (Boesch 2002, Breitburg 2002). Macroalgal blooms are often one of the first symptoms of eutrophication in shallow estuaries (Bricker et al. 2008) because some ephe-

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meral macroalgal taxa rapidly uptake and utilize nutrients from the water column for growth (Kennison et al. 2011). In small unurbanized estuaries of the US Pacific Northwest, seasonal blooms of green macroalgae (GMA; Family Ulvaceae) (Kentula & DeWitt 2003) are primarily associated with nutrient input from natural sources, such as oceanic upwelling and runoff from stands of nitrogen-fixing red alder *Alnus rubra* (Hickey & Banas 2003, Brown & Ozretich 2009), rather than anthropogenic sources. Low human population densities and forest-dominated land use in these watersheds have resulted in low rates of atmospheric deposition of nitrogen (Brown & Ozretich 2009) and generally low concentrations of toxic pollutants compared to urbanized areas (Baker et al. 1997, Brown et al. 1998, Nelson et al. 2004). This provided us with a unique opportunity to investigate the effects of naturally stimulated macroalgal blooms in the absence of confounding effects associated with substantial anthropogenic nutrient pollution, such as considerable surface runoff of chemical contaminants (e.g. pesticides), heavy metals (e.g. Pb, Zn, Cu), and polycyclic aromatic hydrocarbons (PAHs) that are frequently found in urbanized eutrophic estuaries (Hoffman et al. 1984, Davis et al. 2001, Joshi & Balasubramanian 2010), and adversely affect shellfish populations (Boening 1999, Fulton et al. 1999). This may allow for the establishment of natural-effect baselines of macroalgal blooms that could inform resource management in systems influenced by cultural eutrophication.

Macroalgal blooms can have disruptive and deleterious effects on infauna in soft-sediment habitats (Everett 1994, Norkko & Bonsdorff 1996a, Auffrey et al. 2004, Marsden & Bressington 2009). Although GMA may provide benthic organisms a buffer against insolation, protection from predators, and a settling surface for larvae (Bolam et al. 2000, Gribben et al. 2009), they can also significantly decrease dissolved oxygen (DO) within the underlying sediment and water (Norkko et al. 2000, Gribben et al. 2009), induce unfavorable biogeochemical conditions (Johnson & Welsh 1985, Bolam et al. 2000, Gribben et al. 2009), and create a physical barrier at the sediment surface (Bonsdorff 1992, Everett 1994, Norkko & Bonsdorff 1996b). Algal biomass, bloom duration, and species-specific tolerances influence the magnitude of GMA effects on specific benthic organisms (Norkko & Bonsdorff 1996a, Marsden & Maclaren 2010, Green et al. 2014, Hondula & Pace 2014). Specific responses of organisms to GMA seem largely dependent on mobility; sedentary infauna are more likely to experience negative effects than highly

mobile epifauna (Everett 1994, Norkko 1998). Bivalves, which are generally sedentary, are an important component of estuarine ecosystems as they contribute to the diet of many predators (Freitas et al. 2007) and provide other important ecological functions and ecosystem services, including bioturbation of the sediment (Pelegrini & Blackburn 1995, Nizzoli et al. 2006), filtration of the water column (Newell 2004, Dumbauld et al. 2009, Lonsdale et al. 2009), and cultural and provisioning services (e.g. food production for coastal communities) (Béné et al. 2011). Macroalgal accumulation has been shown to elicit an escape response (or emergence from the sediment) (Norkko & Bonsdorff 1996a,b, Auffrey et al. 2004), as well as increase mortality in several bivalve species (Everett 1994, Norkko & Bonsdorff 1996b, Norkko 1998, Thiel et al. 1998, Marsden & Bressington 2009). Research involving bivalve–macroalgae interactions has largely occurred in anthropogenically affected eutrophic regions outside of the US (Norkko & Bonsdorff 1996a, Norkko et al. 2000, Auffrey et al. 2004, Marsden & Bressington 2009). Studies of bivalve responses to naturally occurring macroalgal blooms provide a baseline for understanding the effects of anthropogenic nutrient-enrichment per se on these important fisheries species.

Within estuaries of the US Pacific Northwest (PNW), recreationally and commercially important bivalve species co-occur with accumulations of naturally stimulated GMA. *Clinocardium nuttallii* (heart cockle) is a short-siphoned, suspension-feeding bivalve that is an important ecosystem good for coastal communities as it is among the most sought bivalve species in these regional fisheries (Dethier 2006, Ainsworth et al. 2014). We investigated the effects of GMA on the behavior, growth, and survival of intertidal cockle populations in Yaquina Bay, Oregon, USA. This study offers several parallels to our international counterparts, however, it also provides a distinct perspective as it comprehensively evaluates the interactions between cockles indigenous to the PNW (Dethier 2006, Ainsworth et al. 2014) and natural, upwelling-driven blooms of macroalgae.

We hypothesized that the magnitude of GMA–cockle relationships may be greater late in the summer because GMA cover and biomass increase considerably during that period (Kentula & DeWitt 2003). Several studies have shown that infaunal organism densities differ between areas of differential macroalgal accumulation, so we anticipated that cockle densities would also vary with respect to GMA biomass. We predicted that cockles experimentally covered by GMA mats would have increased rates of

emergence from the sediment and decreased rates of growth and survival when compared to cockles in the absence of GMA, similar to other infaunal bivalves (Thiel et al. 1998, Wright et al. 2010). We also hypothesized that cockles' emergence behavior would be rapidly triggered by anoxic DO concentrations (as occur under GMA mats) (Johnson & Welsh 1985, Norkko & Bonsdorff 1996a) as a mechanism for the bivalves to move to normoxic conditions. Finally, we anticipated that during daytime low tides, cockles that emerged from within the sediment would experience increased mortality due to high temperatures or predation by gulls (Ellis et al. 2007), a pair of indirect yet lethal effects of nutrient enrichment on this important shellfishery species.

Pacific Ocean dominates seasonal nutrient input during the summer and drives blooms of ulvoid green macroalgae that accumulate on intertidal flats (Kentula & DeWitt 2003, Hickey & Banas 2003, Brown et al. 2007, Brown & Ozretich 2009). We focused our efforts on 'Idaho Flat' (~1.15 km²), an intertidal flat located on the south side of the bay that is a popular location for recreational harvest of cockles. The semi-protected flat is generally composed of silty sand sediment that contains low mean percentages (<20%) of combined fines (silt/clay; T. H. DeWitt unpubl. data), likely due to moderately low wave and tidal energy. The central and northeastern sections of Idaho Flat were chosen as study sites for our field research because of the co-occurrence of high cockle densities (A. F. D'Andrea unpubl. data) and extensive GMA mats (D. R. Young unpubl. data).

MATERIALS AND METHODS

Study area

Yaquina Bay, OR (Fig. 1), is a small (15.8 km²), drowned river basin subject to mixed semi-diurnal tides, typical of small estuaries in the PNW (Kentula & DeWitt 2003, Hickey & Banas 2003). Nutrient-rich upwelled water advected into the estuary from the

Field surveys

To quantify any spatial or temporal variability in cockle densities with respect to *in situ* GMA biomass, we conducted our field surveys during consecutive daytime low tides (<0.46 m mean lower low water [MLLW]) in both June and August 2014, which pro-

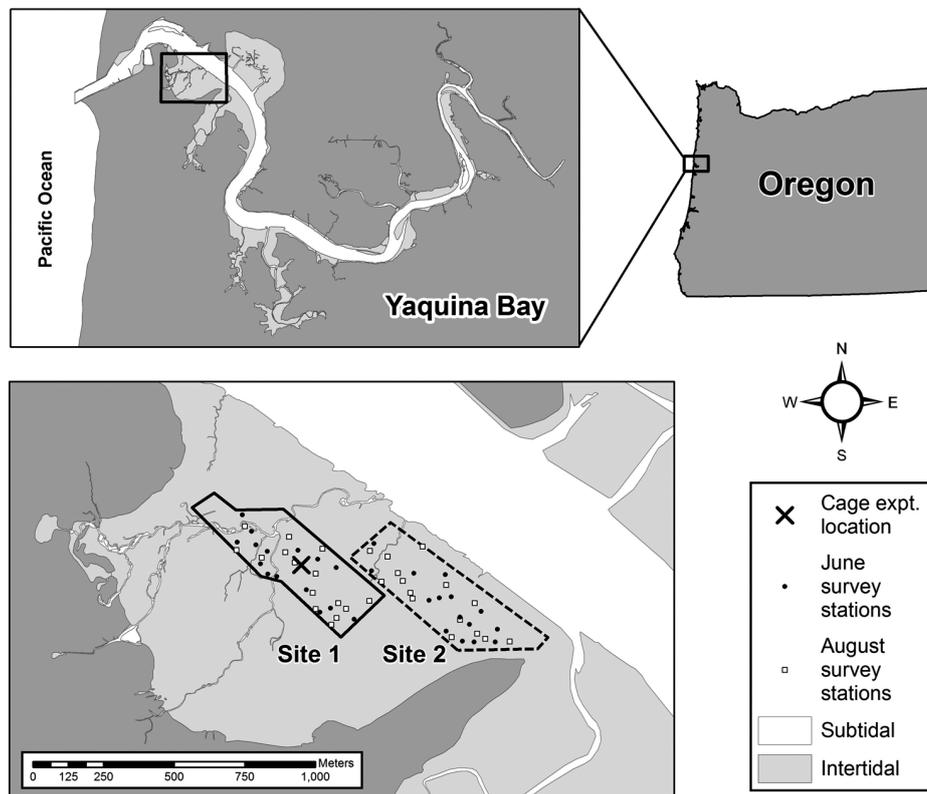


Fig. 1. Survey sites and stations within Yaquina Bay, Newport, Oregon

vided a comparison between early and late summer. During both monthly sampling surveys, 30 randomly established stations (via ArcGIS 10.1; ESRI) were sampled within two ~13 ha sites (15 stations site⁻¹) (Fig. 1), to also contrast potential differences based on 'historical' GMA biomass differences observed in time-series (1997 to 2007) GMA data (D. R. Young unpubl. data). Within a 10 m radius of each station, a 1 m² quadrat was haphazardly tossed within the patch of highest GMA biomass, as well as within the patch of lowest GMA biomass; patch selections were based on the visual determination of 2 samplers. The patches of lowest GMA biomass were established as those areas with the most sediment exposure, while the patches of highest representative GMA biomass were determined as the areas where GMA was most elevated above the sediment surface. Inside each quadrat, GMA biomass was removed with scissors from the southeastern-most quarter (0.25 m²) of the quadrat, then rinsed and measured volumetrically in a plastic 4000 ml graduated cylinder to the nearest 100 ml to estimate dry biomass (g dry wt [GDW] m⁻²) (Robbins & Boese 2002). We then extracted a 15 cm deep sediment core with a clear acrylic corer (5 cm diameter) from within the cleared 0.25 m² and measured the depth of the color change (oxic/anoxic) boundary in the sediment to estimate the apparent redox potential discontinuity (aRPD) depth (± 0.5 cm) (Gerwing et al. 2013). We counted the number of emergent cockles by removing the remaining GMA by hand from the quadrat, exposing the sediment surface. A 4-pronged garden rake (tines set to a 10 cm depth and 3 cm width) was used to rake sediment within the quadrat to a depth of 10 cm to extract buried cockles ≥ 3 cm in length (to avoid juvenile size classes); each plot was raked twice to ensure thorough collection. Qualitative burial depth (buried or emergent) and quantity of collected cockles were recorded upon completion of raking.

Field experiment

To determine whether differences in cockle density or qualitative burial depth observed during the field survey were affected by GMA mats, we conducted a field experiment whereby cockles were subjected to the presence or absence of simulated GMA mats for 12 wk (July to September 2014). At the center of Site 1 (Fig. 1), ten 1 m³ cages (PVC frame with 1.9 cm polypropylene mesh walls) were pressed 0.5 m into the sediment in 2 equidistant transects (distance between cages: 3.5 m; between transects: 8 m). The

sediment within each cage was raked to a depth of 10 cm and cleared of bivalves ≥ 3 cm in length. A 0.56 m² (75 × 75 cm) 'cockle corral' was then placed at the center of each cage to minimize an 'edge effect' near the cage sides and to contain the cockles under the center of the simulated GMA mat. Corrals were constructed from 4 rigid wire fence panels (total height 13 cm, mesh size 1 cm) and pressed 8 cm into the sediment, leaving the above-ground height at 5 cm. Cockles (n = 100, mean length [\pm SD] = 52.6 \pm 5.8 mm) were collected by raking large patches of bare sediment (GMA-free) on Idaho Flat 1 wk prior to the start of the experiment and kept in circulating seawater tables within our laboratory in Newport, OR. Prior to deployment in the cages, cockle shells were rapidly dried with compressed air, randomly numbered with black permanent marker, and measured for shell length, then cockles were placed back into the holding tank for 24 h. Prior to the start of the experiment, 10 cockles were pressed halfway into the sediment (anterior end down) within each corral to ensure no passive relocation occurred as the tide returned. After allowing the cockles to bury and acclimate for 24 h, approximately 16 000 ml of wet GMA (~1100 GDW m⁻²) was placed inside 5 of the 10 cages, while the remaining 5 cages were left as control cages without any GMA. An alternating pattern was used to assign cages to treatment or control groups. Cages were inspected 1 or 2 times wk⁻¹ to check for damage and to remove drift algae from the outside of the mesh. Cockles were monitored at 3 to 4 wk intervals (dependent upon suitable daytime low tides) throughout the experiment, during which the simulated GMA mats were removed and cockles were collected from within the cages; we recorded the relative burial depth, length, and abundance of cockles while in the field. After which, surviving cockles were placed back into their respective corrals and allowed to rebury, then fresh macroalgae (~1100 GDW m⁻²) was replaced in the GMA-treated cages as the flood tide returned.

Laboratory emergence experiment

To identify the factors of GMA accumulation that cause cockles to rapidly emerge from the sediment, we conducted a laboratory experiment to isolate the effects of anoxia, surficial pressure/barrier, GMA presence, and the interaction of GMA presence and a surficial pressure/barrier on the emergence behavior of buried cockles. We selected these as the factors of thick GMA biomass with the greatest potential to

impact infaunal organisms based on pertinent literature (Norkko & Bonsdorff 1996a, Auffrey et al. 2004) and preliminary observations (N. S. Lewis unpubl. data). Sandy sediment from Idaho Flat was sieved (<2 mm) to remove debris (macroinfauna and large organic matter) and was placed in 6 aquaria to a depth of 9 cm to allow for complete reburial of cockles. We added 12 l of pre-mixed seawater (Red Sea Salt and reverse osmosis [RO] water) at a salinity of 30.3 ± 0.5 psu to each aquarium. Submersible micro-pumps circulated water within each aquarium. Aquaria were placed in flowing water baths to maintain water temperatures of $14.88 \pm 0.28^\circ\text{C}$. The water temperature and DO within each aquarium were measured with a HOBOTM U26-001 dissolved oxygen logger (Onset Computer Corporation) at each examination interval (see Fig. S1 in Supplement 1 at www.int-res.com/articles/suppl/m582p105_supp/). Within each aquarium, 5 cockles were manually buried (anterior end down) and allowed to acclimate to conditions within the tank for 12 to 15 h prior to the start of the trial. Each aquarium was divided into 9×9 cm compartments with plexiglass dividers, and one cockle was manually buried within each compartment, which allowed us to exert treatments on each cockle independently. Any cockles that moved from their initial burial position were removed from the tank and excluded from analysis so that only responses from acclimated individuals were measured; only those tanks with 4 to 5 buried clams were included in the analyses. We quantified the emergence response of cockles in 5 treatments (control [no treatment], anoxia, surficial pressure, GMA, and GMA + surficial pressure) with 6 replicates (emergent proportion of cockles tank⁻¹) per treatment (n = 30 tanks). After the start of each trial, subjects were examined at 0.5, 1, 2, 4, 6, and 8 h for changes in burial depth; those that were partially or completely exposed at the sediment surface were considered to have exhibited an emergence response. After each trial, seawater and experimental subjects were replaced.

For the control treatment, we lightly bubbled aquaria with compressed air to maintain high mean (\pm SD) DO levels (8.21 ± 0.28 mg l⁻¹). Trials of the control treatment revealed the rate of emergence in the absence of other experimental influences. The anoxia treatment consisted of near-zero mean DO concentrations (0.05 ± 0.09 mg l⁻¹) generated by bubbling nitrogen gas into aquaria to purge DO from the seawater; this simulated DO levels observed beneath GMA mats in preliminary studies. Nitrogen gas was vigorously bubbled through an air stone in each aquarium to rapidly decrease DO to anoxic levels.

Bubble wrap was placed on the water surface and a foam cover was placed on top of the aquaria, to limit the diffusion of oxygen back into the seawater. The surficial pressure treatment was designed to simulate the impediment and physical pressure exerted by a macroalgal mat. Within each plexiglass compartment of the aquarium, a nylon cradle (made from 2 mm mesh netting) holding a PVC block (150 cm³, 218 g) was placed on top of the burial location of each cockle. A thin layer (~5 mm) of nylon netting was placed in the cradle under the PVC block to allow for siphon extension, water passage, and an even distribution of pressure, as would be experienced under a mat of macroalgae (see Fig. S2 in Supplement 1). Each block exerted approximately 109 N m⁻² of pressure at the sediment surface, which was comparable to the pressure exerted by the highest representative GMA biomass on the tidelflat at low tide (113 N m⁻²; calculated from survey GMA mass; N. S. Lewis unpubl. data). At each examination interval, the cradles were slowly raised and lowered back down to determine the qualitative burial depth (buried or emergent) of experimental subjects. The GMA presence treatment tested whether a small amount of GMA (11.7 ± 0.3 g wet wt; equivalent to ~196 GDW m⁻²; Green et al. 2014) stimulated the emergence response of cockles, perhaps due to natural algal exudates (Johnson & Welsh 1985). A layer of GMA (~5 mm; comparable to the layer of nylon netting used in the surficial pressure treatment) was placed in a nylon cradle and lightly pressed to the sediment surface within each compartment; a small plastic weight (4.8 g) was placed on top of each layer to keep the GMA neutrally buoyant (exerting no pressure) at the sediment surface. The 'GMA + pressure' treatment was conducted using the same method and setup as the surficial pressure treatment, except the nylon layer below the PVC block was replaced with a GMA layer (~5 mm; 11.7 ± 0.3 g wet wt); this treatment allowed us to test the cumulative effects of surface pressure and presence of GMA, which was our best attempt to simulate the most realistic combination of conditions that cockles experience on tideflats during GMA blooms.

Emergence vulnerability studies

We conducted vulnerability studies during 2015 and 2016 to determine whether emergence from the sediment affected cockle survival or physiological condition. We performed a field survey of the gull population from May to August 2015 in the same

low-mid intertidal range of Idaho Flat that other field experiments were conducted. Foraging gulls, predominantly *Larus* spp., were grouped only as 'gull spp.' because of their similar behavior within intertidal estuarine habitat (Frazier et al. 2014). The number of gulls within the site were counted (using a 65 mm Swarovski spotting scope, 20 to 60× magnification) from specified observation points adjacent to the survey sites. A subsample of gulls was observed to determine diet composition; starting at the tideline, individual gulls were selected for focal analysis and observed for 3 min. In similar studies, individual birds were observed for time periods ranging between 1 and 5 min (Baker 1974, Pienkowski 1983, Lewis & Kelly 2001); we selected an intermediate time within this range to observe a more comprehensive prey selection, increase our sample size, and decrease the probability of observing the same individuals more than once. Dietary selections and behavior were recorded. Depending on the size of the gull population within the survey areas, the observer rotated away from the tideline, selecting every second, third, or fourth gull as subsequent individuals to observe, until the entire population was subsampled or the flood tide completely inundated our survey sites (≥ 0.46 m MLLW). We subsampled $27.4 \pm 2.5\%$ of the foraging gull population within our survey areas on any given day, composing 23.5% of the total population counted during the study (319 gulls observed / 1358 gulls counted).

We carried out a lab experiment in July 2016, in which we exposed cockles to a range of air temperatures to simulate the physiological rigors of exposure to sunlight and air at the sediment surface during daytime low tides. Cockles were subjected to 12 different temperature (6, 14, 21, 30, 34, $38 \pm 1.0^\circ\text{C}$) \times time (2, 4 h) treatments within temperature-controlled chambers (monitored with TidbiT[®] temperature sensors; Onset Computer Corporation), representing the temperature and exposure durations regularly observed on Yaquina Bay mid-intertidal flats during daytime low tides in the summer (Kaldy et al. 2015; NOAA Stn 9435380, <http://tidesandcurrents.noaa.gov>). We corroborated these temperatures and established a temperature maximum during a preliminary deployment of TidbiT[®] sensors on the exposed surface of the tideflat (N. S. Lewis unpubl. data). All treatments were conducted in illuminated conditions. During each of the 12 treatments, 6 cockles (replicates) were placed in individual Petri dishes inside the environmental chamber ($n = 72$ cockles). Upon completion of each experimental treatment, we measured each cockle's

responsiveness to stimuli (fast, slow, none) by touching the mantle or siphon of each individual with a thin rod, then we placed the cockles on sediment within individual 1000 ml flasks (with flowing seawater) to record any reburial and mortality after 24 and 72 h.

Statistical analyses

Analyses were performed using the 'car', 'ez', and 'userfriendlyscience' packages within the statistical software R v.3.2.1 (R Development Core Team 2015). For the field survey, 3-factor analysis of variance (ANOVA) tests, followed by Tukey's multiple comparisons tests, were used to compare survey data (GMA biomass, aRPD depth, total cockle density, and cockle lengths) between sites, months, and qualitative GMA biomass levels. To meet the assumptions of our parametric analyses, we applied square-root transformations to GMA biomass and cockle densities. Heteroscedastic-corrected ANOVAs were conducted on those survey data that showed evidence of non-constant variance (burial densities). The ANOVAs corrected for non-constant variance utilized heteroscedasticity-corrected covariance matrices (HCCM; type HC3), often called 'White-Huber' covariance matrices, which allow for consistent tests of significance, unlike ordinary least squares estimates (White 1980, Long & Ervin 2000). To determine if the probability of cockle presence changed with differences in GMA biomass or aRPD depth, logistic regressions were applied.

Data from the field experiment were unbalanced due to a compromised control cage (excluded because of moderate GMA accumulation) and differential cockle loss from mortality in the 2 treatments. Therefore, cockle mortality, shell length, burial depth, and emigration were analyzed using Welch's 2-sample *t*-tests, which are robust against unequal variances of observations (Ruxton 2006).

For the laboratory experiment, we tested for the effect of treatment on cockle emergence at each time interval using univariate ANOVAs for heteroscedastic data, followed by Games-Howell post hoc tests to evaluate differences between treatments. We pooled data from replicate aquaria within each treatment to reach our target sample size.

To infer vulnerability to predation by gulls, we quantified the proportion of successful cockle depredations to unsuccessful attempts. We estimated the proportion of the cockle population consumed during the summer of 2015 by summing the daily cockle

consumption estimates throughout the survey, $\Sigma(C_g \times G \times D_e)$, and dividing the total consumption estimate by the study area population estimate, $[(C_{\text{site1}} \times A_{\text{site1}}) + (C_{\text{site2}} \times A_{\text{site2}})]$, where C_g is the daily cockle consumption rate (cockles consumed $\text{gull}^{-1} \text{min}^{-1}$), G is the daily foraging gull estimate, D_e is the daily day-light tideflat exposure time (min), C_{site1} and C_{site2} are site-specific cockle densities, and A_{site1} and A_{site2} are site-specific total area estimates (m^2).

We quantified the vulnerability to mortality from heat/desiccation using data from the laboratory chamber experiment, which we analyzed with ordinal logistic regression to determine the effect of temperature and time on response to stimulus in exposed cockles. Fisher's exact tests were used to examine any effects on reburial and mortality. We estimated the proportional mortality rate of the emergent cockle population (proportion dead per 2 h interval) due to temperature stress during the summer of 2015 by estimating tideflat temperatures using a regression of 2011 tideflat temperature (Kaldy et al. 2015) versus air temperature (NOAA Stn 9435380, <http://tidesandcurrents.noaa.gov>), $T = 3.7589 e^{0.028(x)}$, and applying those 2015 estimates to an experimentally derived cockle mortality versus temperature regression (2 h exposure time), $M_{2h} = 0.0833(T) - 2.5$, where x = the 2015 air temperatures measured at NOAA Stn 9435380. The total proportion of the emergent cockle population that expired due to temperature stress was then estimated by calculating the proportional mortality rate min^{-1} , $M_{2h} / 120 \text{ min}$ (proportion dead min^{-1}), and summing the proportional mortality over the total minutes of exposure to lethal temperatures. The proportion of the population that experienced mortality was removed from subsequent population calculations.

RESULTS

Field survey

Mean (\pm SE) GMA biomass did not differ between June and August at either qualitative biomass level. High biomass plots at Site 2 contained 277.2 GDW m^{-2} more GMA than high biomass plots at Site 1 ($p < 0.001$), whereas mean GMA biomass did not differ in low biomass plots between the sites (Fig. 2a, Table 1). The lack of interaction between qualitative GMA biomass level and month, coupled with a $518.8 \pm 45.3 \text{ GDW m}^{-2}$ greater mean biomass at high-biomass plots, suggest that our qualitative plot determination was sufficiently consistent for the purposes of this study.

Mean aRPD depths were significantly different between early and late summer, decreasing by $1.70 \pm 0.31 \text{ cm}$ from June to August (Table 1). No significant differences were exhibited in aRPD depth among GMA biomass or study sites, nor were differences observed in cockle densities among aRPD depths.

The total mean density of cockles in low-biomass GMA plots was $1.57 \pm 0.56 \text{ ind. m}^{-2}$ greater than high-biomass GMA plots in August ($p = 0.01$), providing evidence of an interaction (Table 1) as no difference in mean cockle density was detected between qualitative biomass levels in June (Fig. 2b). Mean (\pm SE) cockle density of low-biomass GMA plots within Site 1 was $1.4 \pm 0.6 \text{ ind. m}^{-2}$ greater than that of Site 2 ($p = 0.027$). The presence of high GMA biomass corresponded to high densities of emergent cockles relative to areas of low GMA biomass, particularly during the early summer ($p = 0.001$). Mean (\pm SE) density of buried cockles was $1.32 \pm 0.38 \text{ ind. m}^{-2}$ greater in low-biomass GMA plots than high-biomass plots, and was $0.79 \pm 0.33 \text{ ind. m}^{-2}$ greater at Site 1 than Site 2 (Table 1). No difference was observed in buried cockle density between June and August.

Field experiment

Cockles exhibited a greater proportion of emergence from the sediment, less growth, and higher mortality in cages that contained simulated GMA mats than in the control (bare) cages with no GMA. At each observation during the experiment, the mean proportion of surviving cockles that partially or completely emerged from the sediment was significantly greater within the GMA treatment (Fig. 3a, Table 2). Throughout the course of the experiment, the mean proportion of cockles that escaped (actively emigrated) from within the corrals was also significantly higher within the GMA treatment (Fig. 3b). No cockles emerged from the sediment or emigrated from within the corrals of control cages at any point in time. Mean (\pm SD) increase in shell length in surviving cockles at the end of the experiment was $3.71 \pm 0.93 \text{ mm}$ greater in the bare treatment than in the GMA treatment ($p = 0.003$). The mean (\pm SD) proportion of cockle mortality was 0.34 ± 0.15 greater in GMA-treated cages than in bare (control) cages (Table 2). All mortalities observed during the experiment occurred within corrals of the GMA-treated cages, so the mean proportion of cockle mortality reported within GMA cages is likely an underestimate since all emigrants survived the GMA treatment.

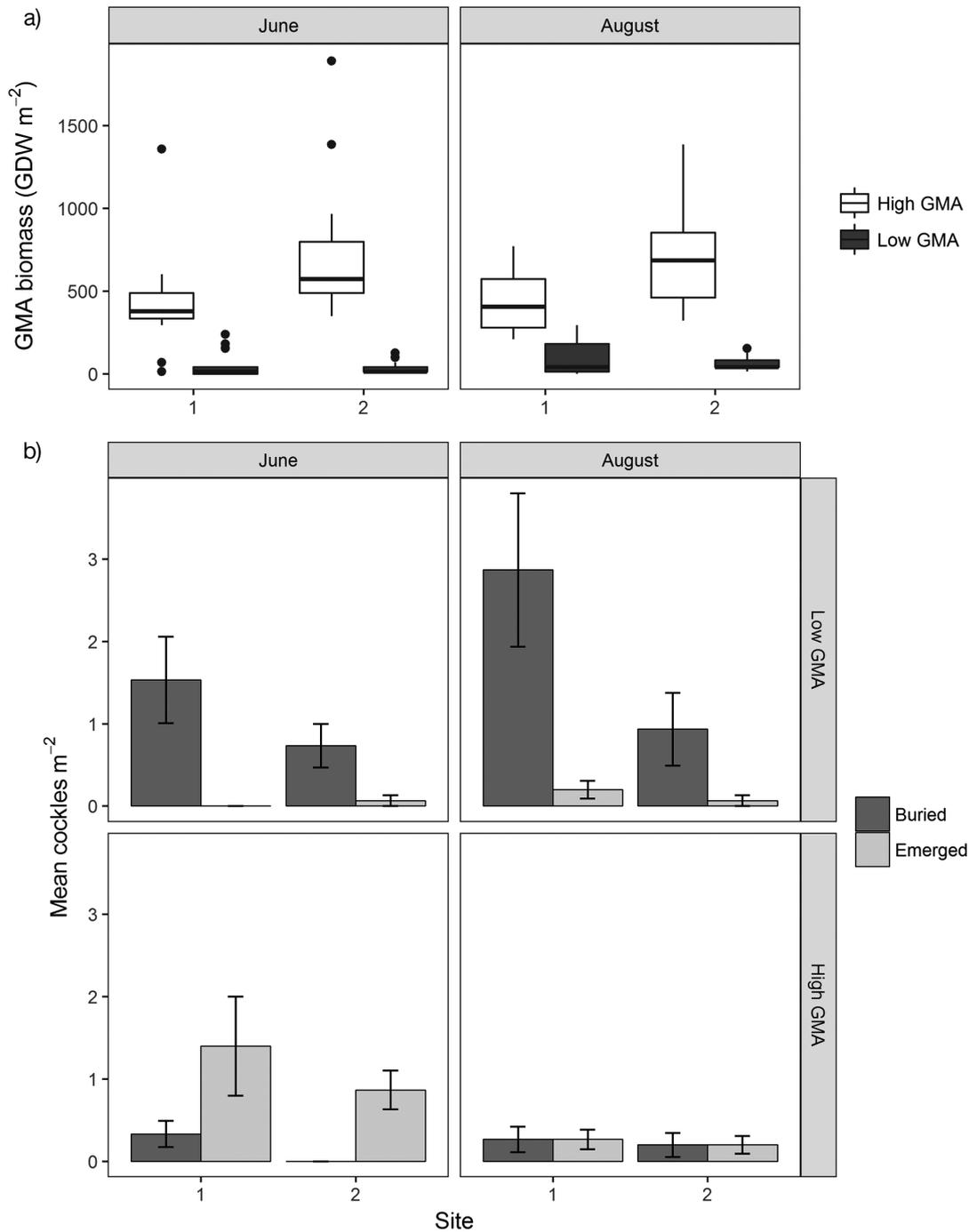


Fig. 2. (a) Comparisons in green macroalgal (GMA) biomass (g dry wt [GDW] m⁻²) between sites (1 vs. 2), qualitative GMA levels (high vs. low), and months (Jun vs. Aug). Boxes represent the interquartile range, with medians represented by dark bars within the boxes; whiskers extend to most extreme values < (1.5 × IQR), with extreme points plotted individually. (b) Corresponding comparisons in buried and emerged mean (±SE) cockles m⁻² between sites, qualitative GMA levels and months

Laboratory experiment

Cockles rapidly emerged from sediments in aquaria when subjected to mechanical pressure upon the sediment surface in the presence of GMA, but were

slower to emerge when subjected to pressure or GMA presence alone, and showed the slowest emergence response when exposed to anoxic conditions for 8 h. We examined differences among treatments by comparing the mean (±SD) proportion of cockles

Table 1. Output from 3-factor (site, month, qualitative green macroalgae [GMA] biomass level) ANOVAs performed on 2014 survey data. Heteroscedastic-corrected ANOVAs were utilized for emergent and buried cockle densities. Numerator degrees of freedom (df_N) = 1 for all response variables. Denominator degrees of freedom (df_D) = 107 for apparent redox potential discontinuity (aRPD) depth, and df_D = 112 for all other response variables. p-values in **bold** represent significance at $p \leq 0.05$

Response variables	Estimated GMA biomass		aRPD depth		Total cockle density		Emergent cockle density		Buried cockle density	
	F	p	F	p	F	p	F	p	F	p
Site	8.42	0.004	0.17	0.682	7.56	0.007	0.02	0.883	7.74	0.006
Month	3.47	0.065	28.26	<0.001	0.47	0.494	0.05	0.825	1.48	0.226
GMA	288.5	<0.001	0.28	0.6	3.85	0.052	13.06	<0.001	28.67	<0.001
Site × month	0.36	0.552	0.7	0.406	0.27	0.602	1.46	0.229	0.27	0.605
Site × GMA	9.48	0.003	0.02	0.904	1.6	0.209	0	0.966	1.84	0.177
Month × GMA	1.75	0.188	1.91	0.17	6.53	0.012	10.94	0.001	0.1	0.756
Site × month × GMA	0	0.995	0.14	0.706	1.14	0.288	0.38	0.538	1.67	0.198

tank⁻¹ that partially or completely emerged at the sediment surface. Differences among treatments became evident 0.5 h after the start of the experiment and remained at each subsequent observation throughout the course of the experiment (Table 3). Cockles within the control tanks remained predominantly buried throughout the duration of the experiment. Within the anoxic treatment (0.05 ± 0.09 mg l⁻¹),

cockles did not exhibit a significant response until 8 h into the experiment, at which point the mean emergent proportion of cockles was 0.72 ± 0.25 greater than the control treatment ($p < 0.001$). Cockles treated with surficial pressure showed a significant increase in emergence from control (0.84 ± 0.20 greater proportion), anoxic, and GMA treatments at 4 h (all comparisons, $p \leq 0.02$). The GMA treatment

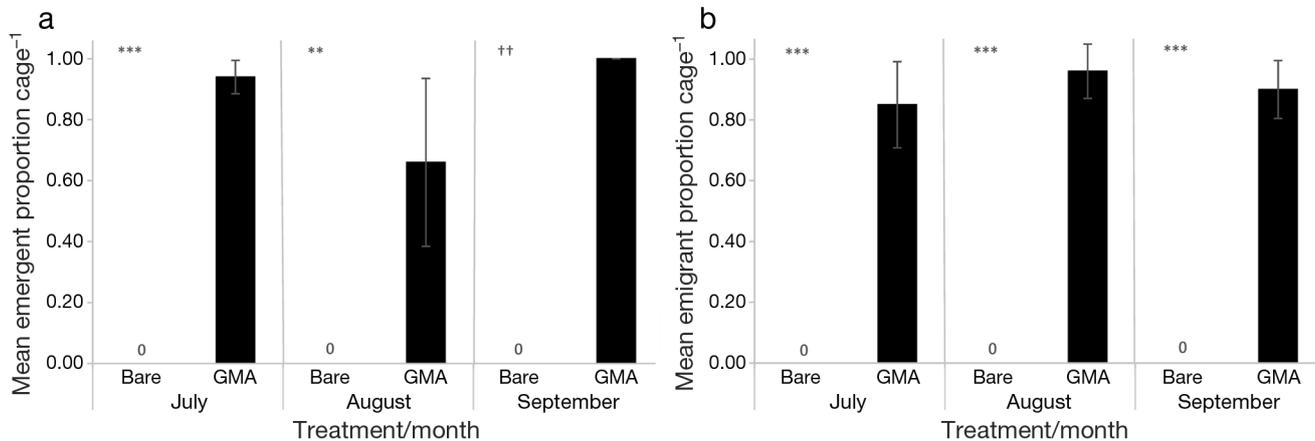


Fig. 3. Monthly mean (\pm SD) proportion of cockles within control (bare) and green macroalgae-treated (GMA) cages that (a) emerged to the point of partial or complete exposure at the sediment surface, and (b) escaped from the confines of the 5 cm corral fencing. No cockles emerged or emigrated from bare treatments; represented by '0'. Sample size (n) = 5 for each treatment/month combination, except for the bare treatment in September when $n = 4$. Significance levels from Welch's 2-sample t -tests of means between treatments: *** $p \leq 0.001$; ** $p \leq 0.01$; ††: data essentially constant

Table 2. Output from Welch's t -tests performed on cage experiment data with cockles *Clinocardium nuttallii*

Response variable	Between treatments					
	Observation 1		Observation 2		Observation 3	
	t -value	p	t -value	p	t -value	p
Mortality	$t_4 = -1.43$	0.226	$t_4 = -4.22$	0.013	$t_4 = -5.01$	0.007
Shell growth	$t_{6,5} = -4.87$	0.002	$t_{5,6} = -10.20$	0.001	$t_{3,2} = -7.82$	0.003
Emergence	$t_4 = 38.38$	0.001	$t_4 = 5.36$	0.006	Data essentially constant	
Emigration	$t_4 = 13.32$	0.001	$t_4 = 24$	0.001	$t_4 = 21.09$	0.001

Table 3. Mean proportion of emergent cockles per treatment at each time interval. For intervals in which the effect of treatment was significant (univariate ANOVA for heteroscedastic data), *F*- and *p*-values are provided in the far right columns. At all time intervals, sample size (*n*) = 30, numerator degrees of freedom (*df_N*) = 4, and denominator degrees of freedom (*df_D*) = 25. Shaded cells represent specific significant differences between treatment means at each time interval (Games-Howell post hoc comparisons). Light grey cells are only greater than the control; intermediate grey cells are greater than both control and green macroalgal (GMA) treatments; dark grey cells are greater than control, anoxia, and GMA treatments

Time (h)	Treatments					Effect of treatment	
	Control	Anoxia	GMA	Pressure	GMA + Pressure	<i>F</i>	<i>p</i>
0	0	0	0	0	0	–	–
0.5	0	0.04 ± 0.10	0.11 ± 0.17	0.5 ± 0.36	0.81 ± 0.22	19.31	<0.001
1	0	0.15 ± 0.23	0.22 ± 0.22	0.53 ± 0.36	0.84 ± 0.20	26.41	<0.001
2	0	0.15 ± 0.23	0.33 ± 0.15	0.58 ± 0.38	0.84 ± 0.20	31.14	<0.001
4	0.04 ± 0.10	0.15 ± 0.23	0.47 ± 0.21	0.88 ± 0.13	0.92 ± 0.20	42.39	<0.001
6	0.04 ± 0.10	0.43 ± 0.37	0.47 ± 0.17	0.96 ± 0.10	0.92 ± 0.13	61.19	<0.001
8	0.04 ± 0.10	0.76 ± 0.20	0.5 ± 0.22	0.96 ± 0.10	1 ± 0.0	118.97	<0.001

= mean greater than control;
 = mean greater than control & GMA;
 = mean greater than control, anoxia, & GMA

evoked a quicker emergence response in cockles than the pressure treatment, exhibiting a 0.33 ± 0.15 greater proportion of emergence than the control treatment after only 2 h ($p = 0.017$). The 'GMA + pressure' treatment elicited the most rapid response and increase in mean emergence proportion from control (0.81 ± 0.22 mean increase, $p = 0.002$), anoxia ($p < 0.001$), and GMA ($p = 0.001$) treatments, occurring within just 0.5 h.

Emergence vulnerability

Emergent cockles experienced lower mortality rates when exposed to gull predation and near-peak temperatures ($\geq 34^\circ\text{C}$) than the rate we observed during experimental smothering by GMA mats; however, the incidence of mortality was still greater than that observed on buried cockles in the absence of GMA.

We surveyed foraging gulls (June to August 2015) at low tide and found that $4.9 \pm 1.5\%$ of the mean daily consumed prey items were cockles. The mean daily success rate of foraging gulls on emergent cockles targeted on the surface was $36.8 \pm 9\%$. We obtained a rate of consumed cockles $\text{bird}^{-1} \text{min}^{-1}$

(0.016 ± 0.005), which was then applied to estimates of daily tideflat exposure (< 0.46 m MLLW) times during daylight hours and daily gull counts throughout the survey period (16 June to 4 Aug 2015) to provide a total estimate of $17\,136 \pm 5355$ cockles consumed within our survey area during that time period. Relative to our estimate of the number of cockles that emerged due to the macroalgal mats during 2014 ($155\,640 \pm 50\,747$), we estimate that approximately $11.0 \pm 8.0\%$ of the emergent cockles in the area could have been consumed by gulls (Table 4), assuming that emergence and predation rates were consistent between years.

When exposed cockles were subjected to temperature stress in the lab, their responsiveness to stimuli decreased with increasing temperature ($p = 0.045$; Fig. 4). Cockle reburial (24 h post-exposure) data provided convincing evidence of a difference in qualitative burial depth among treatment groups (including a control) (Table 5). All cockles exposed to the ($34^\circ\text{C} \times 4$ h), ($38^\circ\text{C} \times 2$ h), and ($38^\circ\text{C} \times 4$ h) treatments remained on the surface and did not rebury; these cockles, as well as the 2 in the ($34^\circ\text{C} \times 2$ h) treatment that did not rebury, were the only mortalities observed 72 h after completion of each treatment

Table 4. Mortality estimates of the 2015 cockle population within our study area exposed to direct and indirect effects of green macroalgal (GMA) accumulation during the summer months

	Bare sediment (buried)	GMA mats (below)	Birds (unburied)	High temps (unburied)
Estimation method	2014 cage Expt	2014 cage Expt	2015 survey	2016 chamber Expt
Estimated % mortality	0	34.0 ± 15.2 (SD)	11.0 ± 8.0 (SE)	7.1 ± 1.5 (SE)

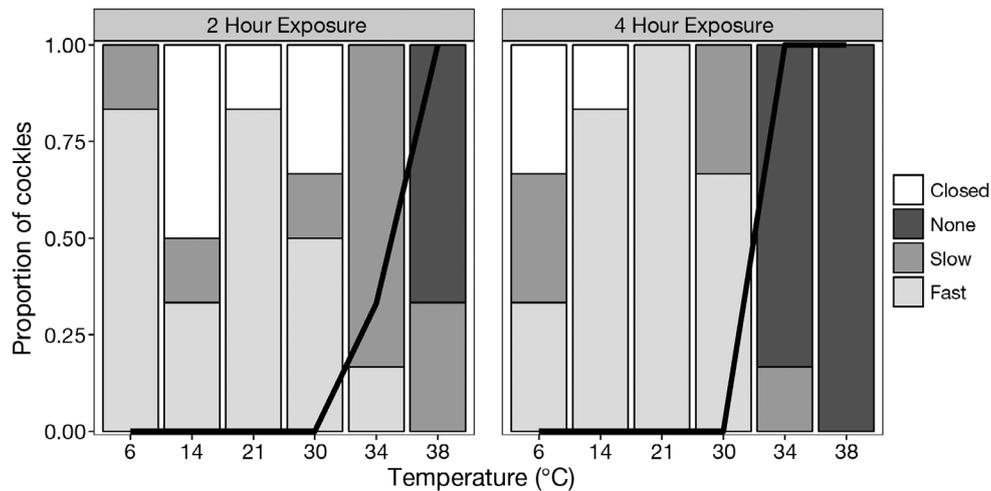


Fig. 4. Cockle valve-closure responses to stimuli within each temp (°C) × time (h) treatment in the laboratory chamber experiment. The valves of 'closed' cockles did not allow for a response to be measured. Also included is the mean mortality observed in circulating seawater chambers 72 h after experimental exposure, illustrated with the solid black line

Table 5. Output from ordinal logistic regression (stimulus response) and Fisher's exact test (24 h reburial and 72 h mortality) performed on temperature experiment data with cockles *Clinocardium nuttallii*

Response variable	Time	Temperature	Time × temperature
Stimulus response	$t_{66} = 0.39, p = 0.695$	$t_{66} = 2.01, p = 0.045$	$t_{66} = -0.48, p = 0.631$
24 h reburial	N/A	N/A	$t = N/A, p < 0.001$
72 h mortality	N/A	N/A	$t = N/A, p < 0.001$

($p < 0.001$). We developed mortality regressions between each temperature treatment and applied the (30 to 34°C × 2 h) regression to 2015 tideflat temperatures estimated from our 2011 tideflat temperature versus air temperature regression ($T = 3.7589 e^{0.028[x]}$), which allowed us to estimate that roughly $7.1 \pm 1.5\%$ of the emergent population in our study area could have experienced temperature-related mortality (Table 4). Application of mortality-temperature regressions between other treatments was not necessary, as potentially 'lethal' tideflat temperatures ranged between 30 to 33.1°C for <2 h.

DISCUSSION

In situ cockle densities in relation to GMA biomass

Our survey results showed that GMA accumulation throughout the summer significantly affected cockle *Clinocardium nuttallii* densities on the tideflat of a PNW estuary. Although the effect of GMA biomass in

early summer had no effect on the overall density of cockles, areas of high GMA biomass in late summer had significantly reduced local densities of cockles. Whereas the macroalgal biomass did not increase appreciably from June to August, the persistence of GMA mats on the tideflats throughout the summer might

have created conditions that fostered the cockles' emergence or death, as we found in the experiments. These results are similar to those of Thiel et al. (1998), who found that softshell clam *Mya arenaria* abundances decreased in a Maine estuary after several months of exposure to a thick GMA mat. Multiple studies in New Zealand found decreased native cockle *Austrovenus stutchburyi* densities within areas of macroalgal coverage during seasonal establishment of the seaweed (Mouritsen 2004, Marsden & Maclaren 2010). Our research suggests that the GMA-induced effect on cockle densities may not be limited to a small spatiotemporal scale, as observed differences in GMA and cockle densities between sites perhaps indicate the potential impacts that historical differences in GMA accumulation have on large-scale cockle distribution.

We initially expected any GMA-related impacts to cockle populations to predominantly stem from changes in oxygen saturation, which is one of the most significant chemical alterations caused by these thick mats (Hull 1987, Everett 1991, Bolam et al.

2000). Although we found no correlation between sediment aRPD depth and GMA biomass, the aRPD depth was shallower in late summer, perhaps due to the several month persistence of the GMA mat, increased temperatures, or increased bacterial activity. However, there was no correlation between aRPD depth and cockle density during summer 2014, suggesting that low DO might not be a direct driver for cockle mortality on Yaquina Bay tideflats.

***In situ* burial depths with respect to GMA biomass**

Within areas of low GMA biomass, significantly more cockles remained buried in the sediment, whereas most cockles in high-biomass areas emerged to the sediment surface. These findings are consistent with Auffrey et al. (2004) and Wright et al. (2010), who found that bivalve burial depths in southwest Canada and southeast Australia, respectively, decreased in the presence of macroalgal mats. The observed shift in total cockle density from areas of high GMA biomass to low GMA biomass through the summer possibly suggests that emergent cockles from high-biomass areas in June had moved to low-biomass areas by August. Such a relocation perhaps indicates that *C. nuttallii* alter their burial strategy when exposed to disturbance, vertically and laterally emigrating away from unfavorable conditions until sufficiently stable habitat is reached, as suggested with New Zealand cockles *A. stutchburyi* (Mouritsen 2004) and European cockles *Cerastoderma edule* (Richardson et al. 1993).

Experimental effects of GMA on behavior, growth, and survival of cockles

Macroalgal mats had a substantial effect on the burial depth, growth, and survival of cockles in our field experiment. In an experiment conducted by Wright et al. (2010), the Sydney cockle *Anadara trapezia* also emerged from within the sediment when subjected to dense macroalgal stands. Similar to our results, Thiel et al. (1998) found decreased growth and survival of *M. arenaria* under macroalgal mats. Contrary to our findings, Marsden & Maclaren (2010) demonstrated that macroalgal biomass had no influence on the growth or survival of *A. stutchburyi*, which illustrates that different species of intertidal bivalves, even as structurally similar as *C. nuttallii* and *A. stutchburyi*, may respond differently to burial by macroalgal mats. The results of our field caging

experiment showed that GMA affected the emergence behavior of *C. nuttallii*, consistent with our field survey observations.

An unexpected and novel finding during our field experiment was the above-ground lateral mobility that *C. nuttallii* demonstrated in response to thick GMA biomass. The majority of individuals subjected to GMA not only emerged from the sediment, but were able to 'jump' over the 5 cm corral and move to the edge of our experimental cages, where conditions were likely more favorable (i.e. near open sediment at the edge of the GMA mat). This behavior was also video recorded during preliminary tests in the laboratory, as some cockles were not only able to jump over a 9 cm plexiglass wall, but did so from beneath a weighted barrier (see Video S1 in Supplement 2 at www.int-res.com/articles/suppl/m582p105_supp/). All cockles that displayed this active emigration in the field experiment survived, while those cockles that remained in the corrals experienced a much higher mortality. The potential implications of this behavior on the tideflat include redistribution of the cockle population away from high GMA biomass to areas low in GMA biomass that are more conducive for survival, as suggested by our field survey findings. Though many previous studies acknowledge the vertical emergence of bivalves in response to unfavorable conditions, few (see Rygg 1970, Richardson et al. 1993, Mouritsen 2004) have reported active above-ground lateral movement in bivalves (e.g. crawling, creeping, locomotory behavior) that we refer to as 'active emigration'.

Active lateral movement has been acknowledged as a relocation mechanism in both New Zealand cockles (*A. stutchburyi*; Hewitt et al. 1996, Mouritsen 2004) and European cockles (*C. edule*; Rygg 1970, Richardson et al. 1993). Equipped with a strong muscular foot, heart cockles on the Pacific coast of the USA are credited with the capability to flip (Rudy et al. 2013) and escape seastar predation (Gallucci & Gallucci 1982); however, studies to date provide no evidence of active above-ground emigration in *C. nuttallii* and, in fact, suggest that this species does not migrate (Gallucci & Gallucci 1982). Conversely, passive lateral movement (by tides and currents) has been suggested as a mechanism for above-ground relocation for heart cockles (Ratti 1977), as well as European cockles (Sutherland 1982, Coffen-Smout & Rees 1999). Our research demonstrates that *C. nuttallii* also utilize active lateral movement (crawling and jumping), in addition to emergence, as a mode of relocation when subjected to GMA, and do so quite successfully.

Factors of GMA biomass affecting emergence in cockles

Contrary to our preliminary hypothesis, anoxic conditions did not evoke the rapid emergence that we observed in preliminary field tests. The most rapid response was elicited by the interaction of a weighted barrier at the sediment surface and GMA presence, simulating the mechanical effect of surface pressure and the presumed chemical cue of GMA. Cockles responded to both surface pressure and the presence of GMA individually — both more rapidly than to anoxic conditions. Individually, these findings are in agreement with much of the current literature; certain bivalves subjected to low-DO conditions in the laboratory decrease their burial depth (Long et al. 2008, Marsden & Bressington 2009, Wright et al. 2010), as do those subjected to macroalgal mats (Norkko et al. 2000, Auffrey et al. 2004, Marsden & Bressington 2009). Norkko & Bonsdorff (1996a), in a similar experiment, also demonstrated that algal mats had a more rapid and pronounced effect on emergence of *Macoma balthica* than hypoxia alone. Macroalgal mats create a physical barrier at the sediment surface, which not only exert pressure and restrict water circulation, but may also produce toxic exudates (Johnson & Welsh 1985)—our study strongly suggested that these were more influential cues than anoxia per se.

Seasonal development of macroalgal mats can have additional biogeochemical implications that potentially contribute to the condition and behavior of cockles on the tidelflat throughout the duration of the bloom, such as increased concentrations of H_2S and NH_4^+ , which occur under conditions of minimal oxygen saturation or anoxia (Morse et al. 1987, Kemp et al. 1990, Bagarinao 1992, Laudien et al. 2002). The production and upward diffusion of these compounds likely have a temporal component linked to the depth of the oxic boundary in the sediment, which was shown by our survey data to decrease throughout the summer. When subjected to H_2S , many bivalves have demonstrated the ability to mitigate impacts of toxicity through valve closure (Oeschger & Storey 1993, Jahn et al. 1997) and detoxification (Jahn & Theede 1997, Jahn et al. 1997, Laudien et al. 2002). We anticipate that potential effects of these compounds would be limited to the later portion of the summer under mats of high GMA biomass that present consistent anoxic conditions (i.e. where the sediment aRPD was near the sediment surface), perhaps only exacerbating the effects of anoxia (Bagarinao 1992, Oeschger & Storey 1993).

Vulnerability to mortality in emerged cockles

The process of emerging from within the sediment and emigrating on the tidelflat surface can subsequently expose cockles to increased predation and temperature stress (Thomas & Poulin 1998, Mouritsen 2004). We analyzed the vulnerability of emergent cockles to predation and temperature-related mortality to quantify this trade-off in mortality risk. Only one-third of the gull-cockle interactions ended in cockle mortality, which showed that the bivalves' valve-closure behavior was a successful defensive strategy to survive probing or pecking attempts by gulls. In a clam predation survey, Ward (1991) found that kelp gulls *Larus dominicanus* dropped closed clams on hard substrate to break the shells and access edible flesh. We did not observe any successful depredations on closed cockles; however, when the valves of a cockle were open, gulls aggressively targeted the unprotected muscular foot (see Video S2 in Supplement 2), particularly upon movement of the cockle. During successful depredations, healthy cockles with a rapid response were typically able to close their valves and sacrifice only a portion of their muscular foot (sublethal predation), while cockles with a slow valve-closure response were usually consumed entirely. The implications for *C. nuttallii* survival without a foot are unknown, but we assumed eventual mortality because of the reliance on this muscular foot for burial and escape (Richardson et al. 1993, Thomas & Poulin 1998, Mouritsen 2004). This assumption likely contributed to a predation mortality overestimate, as similarly foot-cropped *A. stutchburyi* are capable of surviving (Mouritsen & Poulin 2003). Our survey revealed that emergent cockles were able to mediate the risk of mortality due to gull predation better than the mortality risk associated with remaining below the thick GMA mats.

The physiological condition of cockles can also be affected by emerging from within the sediment, primarily due to temperature stress. The results from our heat stress experiment in the laboratory demonstrated that exposure to high temperatures decreased the response, reburial capability, and survival rate of cockles. A temperature threshold appeared to exist between 30 and 34°C; at which the valve-closure response slowed, reburial nearly ceased, and the mortality rate increased. This temperature-modulated effect presumably increased the likelihood of stress- and predator-induced mortality (Sobral & Widdows 1997), even if individuals survived until the return of the flood tide. In PNW estuaries, this threshold is not often reached annually, if at all (Kaldy et al. 2015).

Estimated tidelflat temperatures during 2015 surpassed 30°C for approximately 72 min. Our temperature mortality estimates showed that the emergent cockle population could better survive exposure to air temperatures on the tidelflat surface than burial below thick macroalgal mats.

Our studies suggest that emergence behavior may provide an escape from the physiological stress of being buried by GMA, but this behavior still imposed a substantial risk to cockle populations when compared to the observed negligible mortality rate of cockles buried in sediment without overlying GMA mats. Although our population mortality estimates are based on some simplistic assumptions (including constant cockle and gull population sizes across years, and seasonally consistent predation rates by gulls), the estimates demonstrate that GMA biomass on tidelflats can have both direct and indirect adverse effects on cockle populations. Effects of macroalgal mats on less-mobile bivalves are likely greater than for cockles because of their reduced capacity to emerge from under GMA mats or to move to more favorable microhabitats.

Finally, we hypothesize that the effects of macroalgal blooms on bivalve populations in culturally eutrophic estuaries are likely to be higher than those reported in our study of Yaquina Bay, OR, because of additional stress to the bivalves due to co-contaminants such as pesticides, heavy metals, or PAHs that often accompany nutrient enrichment. Studies such as ours, conducted under conditions where those co-contaminants are negligible, are critically important for parsing the direct effects of toxic chemical contamination from the indirect effects of nutrient pollution on estuarine fauna, particularly those that are of direct economic value such as harvested species of bivalves. Regardless of nutrient source, our research demonstrates that macroalgal mats pose a threat to the growth and survival of these intertidal bivalves.

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