

Causes and consequences of historical multi-trophic diversity change in an intertidal seagrass bed

Tanya L. Rogers^{1,*}, David L. Kimbro^{1,2}

¹Northeastern University Marine Science Center, 430 Nahant Road, Nahant, MA 01908, USA

²Florida State University Coastal and Marine Laboratory, 3618 Highway 98, Saint Teresa, FL 32358, USA

ABSTRACT: To understand and predict patterns of diversity loss in response to global changes, combining historical ecological datasets with modern experimentation can be a valuable approach. In an intertidal seagrass community in northern Florida, USA, we used current and historical quantitative data and a factorial field experiment to investigate diversity changes within and across trophic levels from 1959 to 2013. Over this time period, an 80% reduction in habitat area due to changes in sediment deposition coincided with the disappearance of 2 of 6 large predatory gastropod species and a decline in the relative abundance of specialist relative to generalist gastropods. To investigate the effects of these compositional changes, we experimentally examined trophic interactions among community members in mimicked current and historical food webs. We found that the top predator (horse conch *Triplofusus giganteus*) had the greatest predatory effect on the 2 species that had disappeared from the community (lace murex *Chicoreus florifer* and true tulip *Fasciolaria tulipa*) and that lace murex had the greatest predatory effect on basal resources (bivalves). Therefore, the community not only experienced a reduction in the number of interaction pathways due to species loss, but also loss of the strongest top-down effects among trophic levels. Given that strong interactions are often unstable, this result may reflect loss of the least stable interactions in response to habitat loss. Whether interaction strength can predict extirpation vulnerability in ecosystems merits further empirical and theoretical investigation.

KEY WORDS: Biodiversity · Historical ecology · Non-random diversity change · Predator–prey interactions · Seagrass · Gastropods

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INTRODUCTION

Understanding the maintenance of species diversity has been and remains one of the foremost challenges in ecology, particularly as human activities are resulting in the rapid extinction, extirpation, and introduction of species worldwide (Paine 1966, Connell 1978, Cohen & Carlton 1998, McKinney & Lockwood 1999, Post et al. 2000, Sax & Gaines 2003). Research suggests that biodiversity is being changed non-randomly in 2 general ways. First, habitat fragmentation, habitat loss, and over-harvesting often decrease the vertical diversity of communities through the shortening of food chains, as species at middle and upper trophic levels often

face a disproportionate risk of extirpation (McKinney 1997, Post et al. 2000, Jackson et al. 2001, Duffy 2003, Henle et al. 2004, Layman et al. 2007). Second, horizontal diversity within trophic levels may be altered interactively through changes in top-down (e.g. predation pressure) and bottom-up controls (e.g. resource productivity), as well as through species introductions and disturbance, which tend to replace specialists with native and non-native generalists (Worm et al. 2002, Byrnes et al. 2007, Clavel et al. 2011). Because both vertical and horizontal diversity can influence key ecosystem processes and are changing simultaneously (Duffy et al. 2007, Stachowicz et al. 2007, Reynolds & Bruno 2012), both components must be consid-

ered simultaneously to fully understand the causes and consequences of community changes.

Marine seagrass systems have experienced considerable loss of habitat, and historical records suggest declines in the abundance of key, large grazers such as green turtles and dugongs (Jackson 2001, Orth et al. 2006, Waycott et al. 2009). Modern studies of seagrass subwebs (portions of the community composed of highly interacting, trophically related species; Paine 1966) indicate that top-down effects are important in these systems and depend on both the vertical and horizontal diversity of consumers (Duffy et al. 2003, 2005, Duffy 2006, Heck & Valentine 2007). For instance, higher diversity of invertebrate mesograzers increased consumer pressure on epiphytic algae only in the presence of a predatory crab, and higher grazer diversity also weakened top-down predator effects (Duffy et al. 2005). Thus, historical changes in consumer diversity and food chain length could have a profound impact on species interactions and ecosystem function, including top-down effects and trophic cascades.

Although the subweb involving seagrass epiphytes, invertebrate mesograzers, and their predators has been well studied, we know less about patterns and potential consequences of multi-trophic diversity change in the concurrent and equally diverse seagrass subweb involving carnivorous gastropods and infaunal bivalves. Infaunal bivalves, which form strong, mutualistic associations with seagrasses (Peterson 1982, Peterson & Heck 2001, van der Heide et al. 2012), form the base of this subweb and are prey to a variety of predatory gastropods (Wells 1958, Paine 1963a, Goshima & Peterson 2012). In tropical and sub-tropical environments, these food webs can have high vertical and horizontal diversity. For instance, Bay Mouth Bar (BMB) is an intertidal seagrass bed on the northern Gulf Coast of Florida, USA, with an especially diverse assemblage of large predatory gastropods. The ecologist Robert T. Paine conducted a study of this system in the late 1950s, in which he described the predatory behavior and trophic relationships of the resident gastropod species (Paine 1963a). In this gastropod-bivalve subweb, the apex predator is the large (up to 40 cm shell length) horse conch *Triplofusus giganteus*, which preys on 5 other gastropod species as well as pen shells *Atrina* spp. The other gastropods, which include both specialists and omnivorous generalists, prey on the variety of bivalves, smaller gastropods, and other infaunal species which inhabit the seagrass bed.

Since Paine (1963a), little research has been done in this system, and over the past 50 yr, there has been an 80% reduction in the area of BMB due to changes

in sediment deposition. This habitat loss suggests high potential for changes in community composition. In this study, we first investigated changes in vertical and horizontal diversity on BMB by surveying the current gastropod and bivalve community and by compiling historical quantitative and qualitative data on the BMB system. To better understand the causes and consequences of observed multi-trophic level changes, we examined current and historical environmental data for any abrupt changes in temperature and salinity that could account for these changes in addition to habitat reduction. We then experimentally examined trophic interactions among community members in mimicked current and historical food webs to determine the effects of observed compositional changes on top-down interactions. We manipulated gastropod diversity at both the top and intermediate trophic level to determine (1) how the current and historical mid-level consumer assemblages (MLCAs) compared in their top-down effects on infaunal prey, and (2) how strongly the horse conch directly and indirectly affected mid-level consumers and infaunal prey, respectively, in the presence and absence of extirpated species.

MATERIALS AND METHODS

Study site

BMB is a low intertidal seagrass bed located at the entrance to Alligator Harbor, Franklin County, Florida (29.922664° N, 84.447597° W; Fig. 1A). The harbor experiences regular and complete tidal exchange and has no major freshwater input (Olson 1955). Tides are semi-diurnal mixed (maximum tidal range ~1 m) and heavily wind driven. BMB is typically exposed ~8 d mo⁻¹ during the lower lows of the spring tides, except when strong winds (>10 knots) blow from the south. Currently, the bar is covered predominantly by the seagrass *Halodule wrightii* (hereafter 'Halodule'), with *Thalassia testudinum* (hereafter 'Thalassia') patches and mixed *Halodule/Thalassia* patches on the western side of the bar, patches of *Syringodium filiforme* along the deeper edges, and sandy pools scattered across the bar that remain filled at low tide. The above-ground seagrass dies back in winter and regrows seasonally.

Due to changes in sediment deposition, there has been a substantial reduction in the intertidal area of BMB and in *Thalassia* coverage over the last 50 yr (Fig. 2). Since at least 1860 (Olson 1955) BMB was separated from the western tip of Alligator Point by a

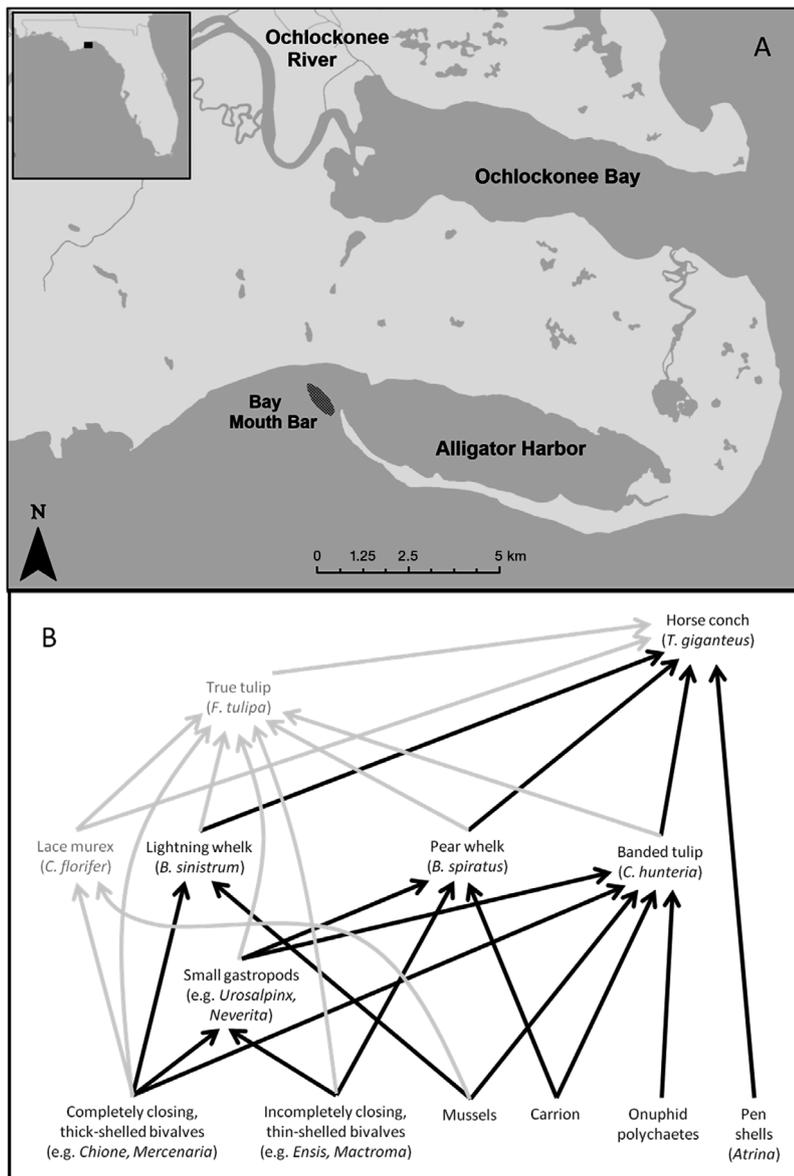


Fig. 1. (A) Study region showing location of Bay Mouth Bar (BMB), Florida, USA. (B) Interaction web depicting main trophic relations among the 6 large gastropods found on BMB and their prey. Full species names given in Table 2. Web is based on descriptions and data from Paine (1962, 1963a), Wells (1970), Kent (1983), and the current study. Gray lines connect species present in 1959–1960 but that were not observed in 2012–2013

natural narrow channel. In June 1972, Hurricane Agnes struck this region, which redistributed sediments and closed off the channel. Following this event, longshore sediment transport rapidly elongated the tip of Alligator Point, eliminating 80% of the area of BMB by 2010, along with the 'extensive meadows' of *Thalassia* reported growing on the bar's flanks (Paine 1963a). Adjacent subtidal areas may also have been affected that are beyond the scope of this study.

Field surveys

To assess the current composition of the gastropod-bivalve subweb, the BMB study area (306 × 155 m; 39 763 m²) was divided into 12 plots following the contours of the bar (Fig. 2), and each plot (average area: 3313 m²) was then divided into 6 subplots. Each month from January 2012 to February 2013 during the lower lows of a spring tide series, we surveyed all large gastropods (>20 mm shell length) on the surface of the bar within 1–2 randomly selected subplots per plot. Due to great difficulty in locating snails when the bar was submerged, surveys were only conducted when the bar was completely exposed or covered in <0.3 m of water. Because weather conditions prevented water levels from receding below this depth, we lack survey data from April and August 2012, and in September 2012 only 9 of the 12 plots were surveyed. For each snail found, we recorded species and shell length. We also recorded the habitat in which each snail was found (see the Supplement section 'Preferential habitat use by gastropods' for methods and results). In addition, we recorded the prey species if the snail was found feeding, as was done by Paine (1963a). The relative abundance, size, and diet of gastropods were compared to data extracted from Paine (1963a).

To survey the current infaunal community, we excavated 6 quadrats per plot (1 quadrat per subplot) in January 2013 (winter) and 3–6 quadrats per plot in July 2013 (summer) at pre-selected GPS locations. The quadrats (0.35 × 0.35 m, 5 cm depth) were dug up and sieved through a 5 mm mesh sieve in

the field. Bivalves, small gastropods, and small crabs were collected and then counted and identified to species in the lab. Other animals (e.g. echinoderms, ascidians, tubes of large polychaetes) were enumerated in the field. We also recorded the habitat type (sand, *Thalassia*, *Halodule*, or mixed *Thalassia*/*Halodule*) from which each quadrat was taken. After sampling all pre-selected locations, we focused additional sampling in specific habitats in order to get

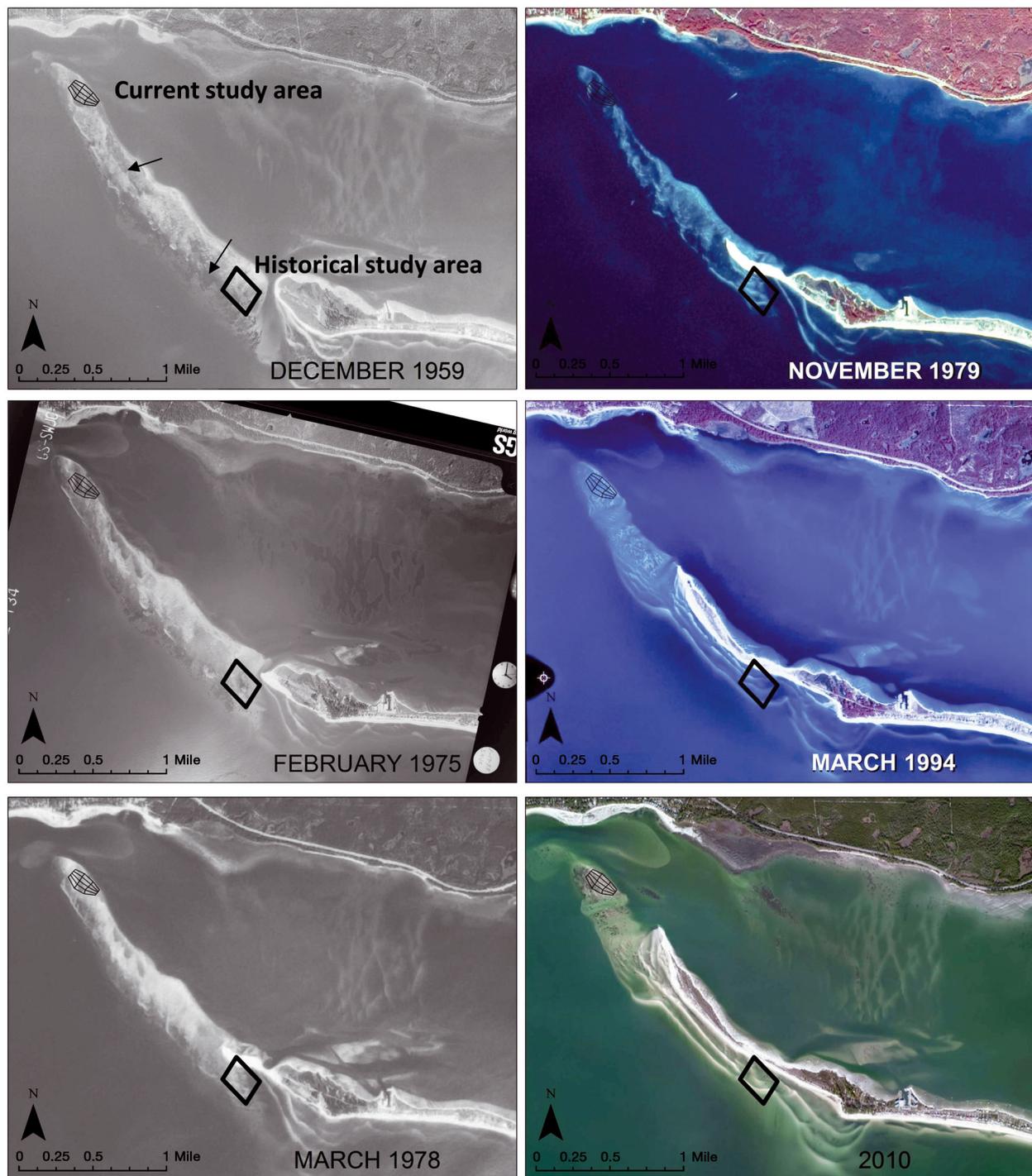


Fig. 2. Changes in sediment deposition, intertidal area, and *Thalassia* coverage of Bay Mouth Bar over 50 yr. Locations of the current (2012-2013) and historical (1959-1960) study areas are shown. Current study area displays the 12 plots used in field surveys. Darker patches indicated by arrows are likely *Thalassia* meadows. Image source: USGS

adequate representation from all habitat types. The effect of season (summer vs. winter) and habitat type on infaunal community composition was analyzed with PERMANOVA using the vegan package in R.

As a source of historical infaunal data, we obtained copies of unpublished data from R. T. Paine from 1959 and 1960. This included quantitative infaunal data from 11 excavated quadrats (330 cm²) taken

from *Halodule* during September 1959, and qualitative lists of infaunal species observed in excavated samples from *Halodule* during the spring of 1960, annotated with comments on abundance. Accounting for habitat type, season, and sampling area, we compared current and historical bivalve density using a Welch *t*-test and compiled a list of the 9 most common bivalve species currently and historically.

Environmental changes

To investigate whether changes in water quality may have altered the food web at BMB over the past 50 yr, we searched the literature and located 3 historical studies which contained water temperature and salinity data from BMB (Olson 1955, Paine 1963b, Pierce 1971). We extracted and graphically compared these data to monthly average water temperature and salinity data from 2003, 2008, and 2011. We also compared mean annual salinity between the recent and historical period using a Welch *t*-test. The recent data were extracted from archived water quality monitoring data collected by federal and state agencies using a YSI 6600-V2 sonde located near the Alligator Harbor shellfish lease (29.9175° N, 84.40992° W; data available online: http://shellfish.ifas.ufl.edu/alligator_harbor.html). The sonde was located ~3.5 km east of BMB, near the center of Alligator Harbor, but temperature and salinity vary relatively little between the entrance and center of the harbor due to tidal flushing (Olson 1955), and sonde measurements were within 1 ppt and 2°C of concurrent point measurements taken in 2012 on BMB proper.

Field experiment

In 1959, 6 large gastropod species were abundant on BMB (Fig. 1B, see the section 'Other carnivorous gastropods' in the Supplement at www.int-res.com/articles/suppl/m540p013_supp.pdf for treatment of 2 smaller gastropods discussed in Paine 1963a). The lace murex *Chicoreus florifer* (formerly *Murex florifer*) and lightning whelk *Busycon sinistrum* (formerly *B. contrarium*) are specialist consumers of bivalves, while the pear whelk *Busycotypus spiratus* (formerly *Busycon spiratum*) and banded tulip *Cinctura hunteria* (formerly *Fascio-*

laria hunteria) are omnivorous mid-level consumers. The true tulip *Fasciolaria tulipa* is an omnivorous top-level consumer, while the horse conch *Triplofusus giganteus* (formerly *Pleuroploca gigantea*) is a top-level consumer specializing on large gastropods and pen shells (all common names from Ruppert & Fox 1988). During our surveys, we found that lace murex and true tulips were rare in the community and that the relative abundance of the remaining 4 species had changed.

Paine (1963a) described distinct summer and winter gastropod communities on BMB, with 3 of the 6 species (horse conch, true tulip, and lace murex) only abundant during the summer months (April–November). In the current surveys, horse conchs still showed this seasonal change in abundance. As a result, we used the summer food web as a basis for a field experiment investigating differences in trophic interactions between the current and historical gastropod communities. We investigated (1) how the mid-level consumer assemblages (MLCA) present currently and in 1959 (hereafter, the 'current' and 'historical' MLCA) differed in their top-down effects on infaunal prey, and (2) how 2 top-level consumers (TLCs), only one of which is abundant currently, directly and indirectly affected mid-level consumers and their consumption of bivalve prey. We timed our experiment to coincide with the period when these species are (or would have been) present.

A total of 9 treatments were used (Table 1). The current and historic MLCA treatments each consisted of 6 snails, with compositions mimicking the relative abundances of each species found on the bar during the summer months, as determined using the current and historical survey data. The current MLCA consisted of 3 banded tulips, 2 pear whelks, and 1 lightning whelk. The historical MLCA consisted of 3 lace murex, 1 banded tulip, 1 pear whelk, and 1 light-

Table 1. Treatments used in field experiment. MLCA: mid-level consumer assemblage. Reps: number of replicate experimental plots

Treatment	Reps in <i>Halodule</i>	Reps in <i>Thalassia</i>	Total reps
(1) No snails (control)	4	4	8
(2) Current MLCA	4	3	7
(3) Current MLCA+ true tulip	4	3	7
(4) Current MLCA+ horse conch	4	3	7
(5) Current MLCA+ true tulip + horse conch	4	3	7
(6) Historic MLCA	4	3	7
(7) Historic MLCA+ true tulip	4	3	7
(8) Historic MLCA+ horse conch	0	3	3
(9) Historic MLCA+ true tulip + horse conch	0	3	3

ning whelk. For TLC treatments, we added either no TLC, 1 horse conch, 1 true tulip, or 1 individual of both species.

Experimental plots (56 total) were spread as evenly as possible across the BMB study area. To account for possible differences in interactions between seagrass habitat types, replicate plots were divided between the 2 dominant seagrass species and placed in areas with 100% cover of either *Halodule* or *Thalassia* (Table 1). Each experimental plot consisted of 6 PVC posts arranged in a hexagon 1.5 m across. The posts (~35 cm long) were driven into the sediment so that 3–4 cm extended aboveground. One mid-level consumer was tethered to each post by a 0.75 m length of 50 lb (~23 kg) fishing line. Positions of these snails were randomized. For treatments with TLCs, the horse conch and/or true tulip were tethered to a seventh post placed in the center of the hexagon. This setup allowed the TLCs to interact with each other (if both were present) and with all mid-level consumers, while minimizing the potential for tether entanglement. Control (no snail) plots were marked only with one long post.

For the experiment, lightning whelks (shell length: 80–130 mm), pear whelks (60–102 mm), and banded tulips (52–67 mm) were collected from BMB. Lace murex (45–60 mm) were collected from another seagrass bed ~6.5 km west of BMB, where this species is still abundant. True tulips (88–134 mm) were collected from subtidal seagrass beds in St. Joseph Bay, FL. Most of the horse conchs found on BMB were large individuals (>300 mm), which proved impractical for tethering, so smaller horse conchs (134–240 mm) were collected for the experiment from St. Joseph Bay and BMB. These smaller horse conchs were still observed to regularly consume the other gastropod species, both in the field and lab. The snails were tethered by embedding a fishing swivel in marine epoxy (Z-spar A-788 Splash Zone Compound) and affixing it to the top of the shell. Tethers did not inhibit the natural feeding or burying behavior of the snails.

Snails were deployed in late March 2013 and were checked for survivorship during the spring tides every 2 wk until late July 2013 (4 mo duration). If an empty shell was found attached to the tether, the snail was considered dead and the shell was removed. If the shell was no longer attached to the tether, the snail's disappearance was considered tether failure. During the first 3 checks, most dead or missing snails (87% per check, on average) were replaced with new live snails to prevent prey depletion and increase confidence in our estimates of snail mortality rates. In

the subsequent 4 checks, most dead snails (76% per check, on average) were not replaced to allow for potential development of indirect predator effects. During all checks, which plots did vs. did not receive replacement snails was haphazard.

Two weeks after deployment of the snails, 6 tethered clams (3 *Mercenaria mercenaria* [12–18 mm] and 3 *Macrocallista nimbosa* [19–27 mm], obtained from a hatchery) were placed within each central hexagon in order to assess consumption of bivalves. Clams were tethered to garden staples via thin monofilament, the end of which was embedded in marine epoxy and affixed to one valve of the clam. Placement of the tether on the shell did not inhibit the clams' natural burying behavior, and upon deployment, clams were manually inserted into the sediment in a natural position to facilitate burial. At the end of the experiment, we quantified clam survivorship and any features on the dead shells that might indicate the source of mortality (e.g. drill holes, chipping). We also assessed naturally occurring bivalves in the experimental plots, finding no differences among treatments (see 'Naturally occurring bivalves in experimental plots' in the Supplement for methods and results).

Because logistical constraints resulted in an unbalanced distribution of habitat types among plots (Table 1), we partitioned the data into multiple balanced subsets, which consistently revealed no effect of habitat type and no interaction between habitat type and other factors. Therefore, we assumed no effect of habitat or interactions with habitat in the full dataset, and we pooled replicates across habitat types for analysis. The survivorship of each gastropod species was analyzed using a right-censored Cox proportional hazards survival model (Cox 1972) allowing for non-independence of snails within the same plot. In this analysis, the length of time survived in the field by each individual snail since its deployment was fit to a hazard function, and the effect of horse conch presence, true tulip presence, MLCA treatment, and their interactions were statistically evaluated. This analysis allowed us incorporate data from all snails (initial and replacement) in all replicate plots, in spite of irregular replacement over time. Right-censoring accounted for individuals that survived the entire length of the study or that went missing prior to the end of the study due to tether failure. Analyses were done using the survival package in R (Fox 2002, Therneau 2014).

We analyzed the number of live and drilled tethered clams present at the end of the experiment using generalized linear models (GLMs) with Poisson

error and log link functions (O'Hara & Kotze 2010). We first analyzed the effect of MLCA-only treatments (Treatments 2 and 6; Table 1) on live and drilled clams relative to the control (no snail) plots. We then analyzed the effect of TLC and MLCA treatments together, which required us to exclude the control plots in order to obtain a balanced dataset.

RESULTS

Field surveys

Gastropod community composition on BMB has changed noticeably from 1959–1960 to 2012–2013. Seasonal patterns documented by Paine (1963a) for 2 species were still evident in the current study, with horse conchs showing greater abundance in the summer months (April–November), and lightning whelks showing greater abundance in the winter months (December–March), although the change in lightning whelk abundance was not as dramatic in 2012–2013 as in 1959–1960 (Fig. 3A; Fig. S1). However, lace murex and true tulips, which had formerly shown peak abundances in the summer months, were nearly absent from the present-day community at all times of year, comprising only 0.1 and 0.3%, respectively, of all snails observed. These species were equally rare in our pilot surveys, which began in 2010. In addition to this effective reduction in species diversity, the relative abundance of the remaining large gastropods had shifted (Fig. 3A). In Paine (1963a), lightning whelks were the most commonly observed snail species and the 2 bivalve specialists (lightning whelks and lace murex) comprised 54% of all snails observed. In the current study, the banded tulip was most commonly observed, and the 2 omnivorous species (banded tulips and pear whelks) comprised 77% of all snails observed. Since Paine (1963a) quantified gastropod abundance in snails h^{-1} , we could not directly compare the absolute abundance of gastropods in 1959–1960 to our data, which was measured in snails per area. We thus relied on qualitative accounts for estimating changes in absolute abundance (see 'Qualitative historical changes in the BMB community' in the Supplement).

Shell lengths of the 4 large gastropods currently found on the bar overlapped in range with the shell

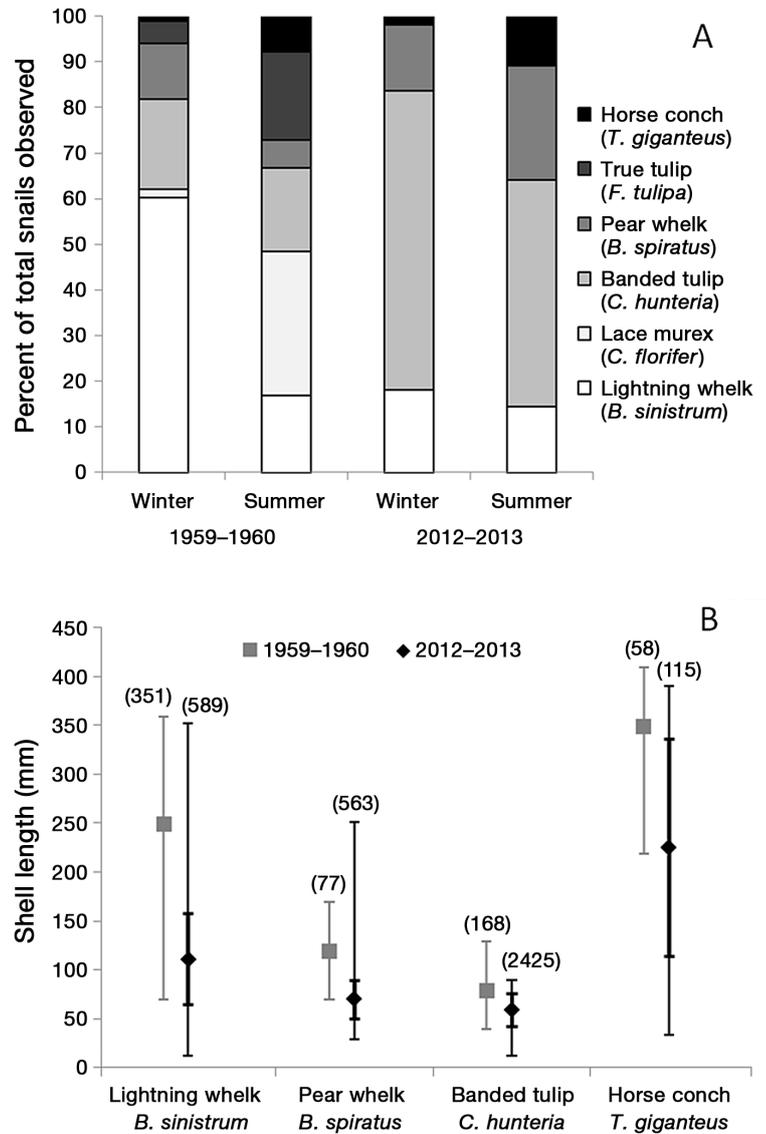


Fig. 3. (A) Relative abundance of 6 large gastropods in summer (April–November) and winter (December–March) in 1959–1960 and in 2012–2013. Full species names given in Table 2. (B) Shell length of 4 large gastropod species in 1959–1960 and in 2012–2013. Points are means, thin lines indicate range, and thick lines are ± 1 SD. Numbers in parentheses are sample sizes (no. of snails)

lengths recorded in 1959–1960; however, mean shell length was lower for all species when compared to historical means (Fig. 3B). Gastropod diet in the current study was similar to that observed in 1959; however, there appears to have been an increase in the proportion of mussels relative to clams in the diets of lightning whelks and banded tulips, and in small gastropods relative to clams in the diet of pear whelks (Table S1).

In the current infaunal samples, we identified 32 species of bivalves, 11 species of gastropods (not including the 6 focal species), the brachiopod *Glott-*

tidia pyramidata, and several crab, echinoderm, polychaete, and ascidian species. Infaunal community composition varied significantly both seasonally and with habitat type (PERMANOVA; season \times habitat, $F_{3,120} = 2.11$, $p = 0.001$, $R^2 = 0.039$; season, $F_{1,120} = 12.46$, $p = 0.001$, $R^2 = 0.078$; habitat, $F_{3,120} = 7.23$, $p = 0.001$, $R^2 = 0.14$). Bivalve abundance was generally higher in summer than in winter. The mussel *Modiolus squamosus* was generally associated with *Thalassia*, whereas the clam *Chione elevata* and various thin-shelled clams were generally associated with *Halodule* (Fig. S2).

After accounting for habitat type, season, and sampling area, there was no difference in density of bivalves between 1959 and 2013 (Welch t -test, $t = 0.467$, $df = 22.40$, $p = 0.645$; Fig. S3). However, the total area sampled in both datasets was quite small (0.36 m² in 1959 and 3.55 m² in summer 2013) and would not have captured changes in the community of larger bivalves. Nevertheless, a comparison of the 2013 results to the quantitative and qualitative 1959 datasets suggests a shift in the composition of the most common bivalve species in *Halodule* (Table S2). Qualitative accounts also suggested a noticeable decline in number of large bivalves over the last 30 yr, including *Stewartia floridana*, *Chione elevata*, *Modiolus squamosus*, *Mercenaria campechiensis*, *Macrocallista nimbosa*, and *Agropecten irradians* (see 'Qualitative historical changes in the BMB community' in the Supplement).

Environmental changes

Examination of available historical environmental data revealed no evidence of dramatic changes in temperature and salinity from the mid 1900s to the 2000s (Fig. 4). Salinity was highly variable from year to year and appeared equivalently variable in both time periods. Mean annual salinity was highest in the 2 most recent time series; however, there was no significant difference in mean annual salinity between the 2 time periods (Welch t -test, $t = 0.60$, $df = 4.0$, $p = 0.58$).

Field experiment

True tulips showed lower survival when paired with horse conchs than when alone, irrespective of the MLCA present (Fig. 5A; Table 2). Lace murex, which were only present in the historical MLCA, also showed lower survival when horse conchs were pres-

ent (the greatest reduction in survival of all species used) and were unaffected by the presence of true tulips (Fig. 5B; Table 2). Survival of lightning whelks was weakly but non-significantly lowered by horse conch presence and was not affected by true tulip presence or MLCA (Fig. 5C; Table 2). Survival of pear whelks was not affected by any of the experimental treatments. Survival of banded tulips showed a significant 3-way interaction between horse conch presence, true tulip presence, and MLCA; in the historical MLCA, horse conchs lowered survival of banded tulips, but only when true tulips were absent. However, the total number of banded tulips in historic MLCA treatments with horse conchs was extremely low (1 snail per plot, $n = 3$ plots with true tulips and $n = 3$ plots without true tulips), so care should be taken in interpreting these results. Considering only the current MLCA, where replication was higher, there was no effect of horse conchs on the survival of banded tulips. Of the 20 horse conchs used in the experiment, only 1 individual was observed to have died, although 5 individuals had gone missing by the end of the experiment due to tether failure.

During the first 3 checks of the experiment (when dead snails were replaced to prevent prey depletion), 1.9 dead snails per check, on average, were found in plots containing horse conchs, whereas 1.0 dead snails per check, on average, were found in plots lacking horse conchs. In outdoor cage experiments, Paine (1963c) calculated that horse conchs consume ~ 0.22 snails d⁻¹, which would equate to 3 snails per 2 wk period (the time between checks).

During checks, tethered horse conchs were observed consuming tethered snails within the same plot. In contrast, tethered true tulips were never observed consuming other tethered snails. Since the experimental plots were uncaged, we did observe large, resident, untethered horse conchs consuming tethered snails within the plots on 5 occasions, and we observed 1 untethered true tulip consuming a tethered banded tulip on 1 occasion.

Relative to the control (no snail) plots, tethered clam survivorship was reduced in the MLCA-only treatments (GLM, $\chi^2 = 13.57$, $df = 19$, $p = 0.018$; Fig. 6A). The number of live clams recovered was 62% less than controls in historical MLCA plots (Tukey contrasts, $p = 0.022$), and 31% less than controls in current MLCA plots (Tukey contrasts, $p = 0.41$). The number of drilled clams recovered in each plot was also substantially higher in historical MLCA plots compared to current MLCA and control plots (GLM, $\chi^2 = 15.885$, $df = 19$, $p = 0.0009$; Fig. 6B). Lace murex were the only snails used in the experiment

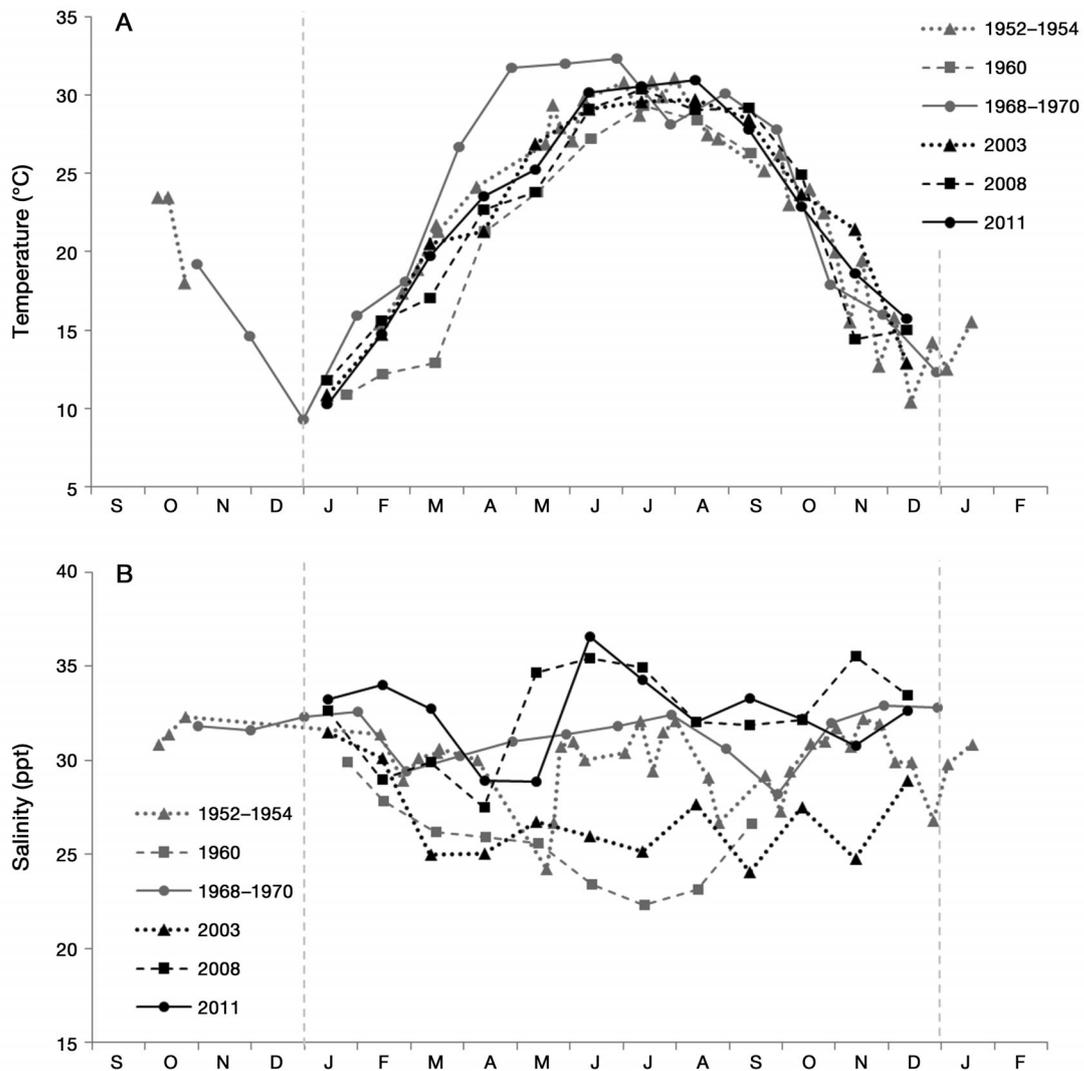


Fig. 4. (A) Water temperature and (B) salinity on Bay Mouth Bar. The 1952–1954 data are from Olson (1955), the 1960 data from Paine (1963b), and the 1968–1970 data from Pierce (1971). The 1968–1970 temperature data are daily maximum temperatures (mean temperatures were not reported). The 2003, 2008, and 2011 data are monthly means from a sonde near the Alligator Harbor shellfish lease. Vertical dashed lines mark January 1

that feed by drilling holes in clams (Paine 1963a). Although other species found on BMB also prey on bivalves by drilling (e.g. *Neverita duplicata*), the near absence of drilled clams in control and current MLCA plots suggests that the observed drilling was due to the experimentally manipulated lace murex.

Across all experimental treatments (excluding controls) the only factor with a significant effect on clam survival was MLCA treatment (GLM, MLCA treatment, $\chi^2 = 35.27$, $df = 40$, $p = 0.011$). Clam survival in historical MLCA plots was 40% lower than in current MLCA plots. Horse conch and true tulip presence had no effect on clam survivorship, and there were no significant interactions (all p -values > 0.2 ; Fig. 6C).

DISCUSSION

Habitat fragmentation and decreases in ecosystem area are generally associated with reductions in vertical species diversity and resultant reductions in species niche breadth and trophic position, particularly at higher trophic levels (Holt et al. 1999, Post et al. 2000, Duffy 2003, Layman et al. 2007). In the BMB seagrass bed, which has declined in area by 80% since a historical study (Paine 1963a), we observed this expected simplification of the food web, as well as a shifts in the horizontal diversity and composition of gastropods and infaunal bivalves. This included a shift from specialists to generalists similar to that ob-

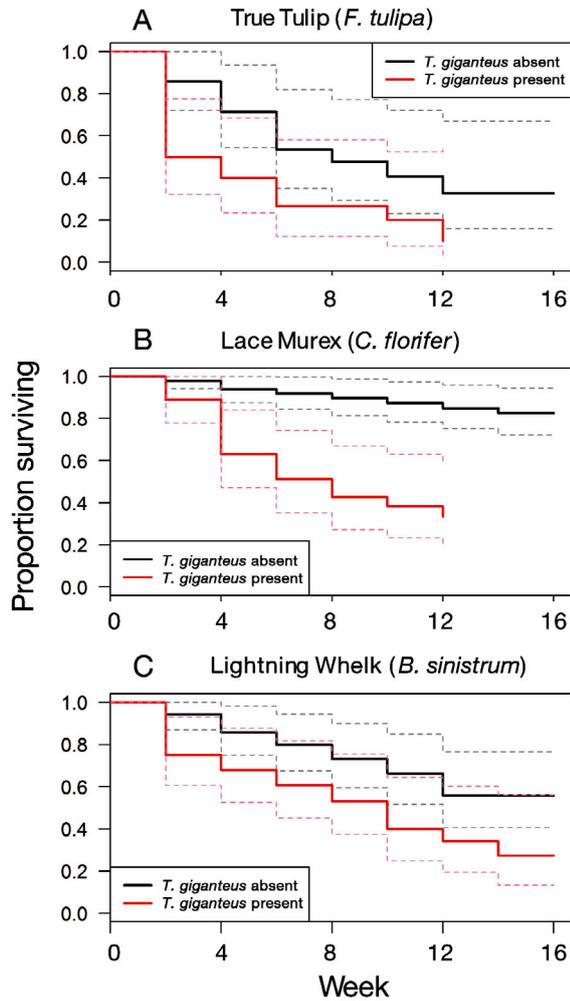


Fig. 5. Fitted survival curves for (A) true tulip *Fasciolaria tulipa*, (B) lace murex *Chicoreus florifer*, and (C) lightning whelk *Busycon sinistrum* as a function of horse conch *Triplofusus giganteus* presence in the field experiment. Dashed lines are 95% confidence intervals

served in other systems (Jackson et al. 2001, Clavel et al. 2011). Interestingly, our experiments revealed that the 2 species which had become rare in the system (the lace murex and true tulip) were the 2 species most greatly affected by the top predator (horse conch) and that lace murex had the greatest predatory effects on bivalves. Therefore, when transitioning from the historical to the current community, we see not only a reduction in the number of interaction pathways, but also the loss of what were the strongest top-down interactions in the food web.

One would predict that in response to a permanent perturbation such as a reduction in ecosystem area, the least stable interactions (which are likely to be strong interactions; McCann et al. 1998, Neutel et al. 2002) would be the first lost as the community re-

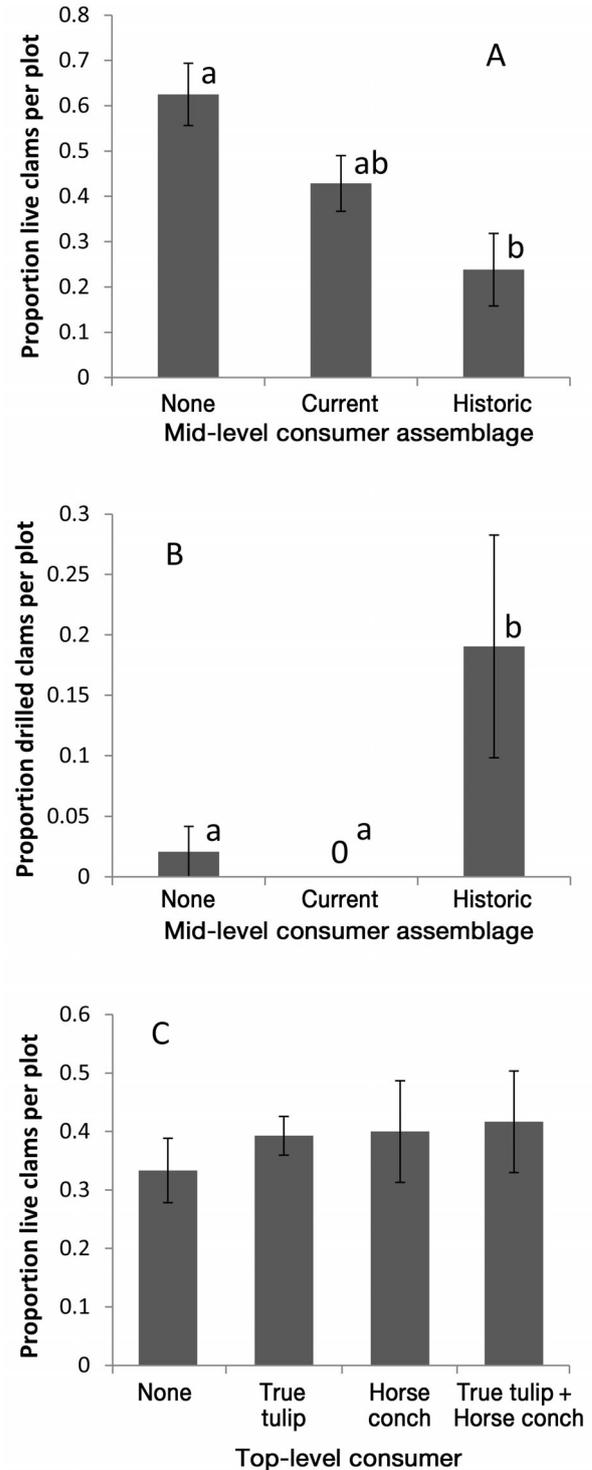


Fig. 6. Recovery of tethered clams in experimental plots as a function of mid-level consumer assemblage (MLCA) and top-level consumer (TLC) treatments. (A) Proportion of clams that were alive in control and MLCA-only treatments. (B) Proportion of clams that were drilled in control and MLCA-only treatments. (C) Proportion of clams that were alive in all treatments excluding controls as function of TLC treatment. All data are means and ± 1 SE. Bars with different letters are different at the $\alpha = 0.05$ level

Table 2. Cox proportional hazard models testing the effect of mid and top-level consumer treatments on the survival of gastropod species used in the field experiment. Results are corrected to allow for non-independence of snails in the same plot. Tg: horse conch *Triplofusus giganteus* presence/absence. Ft: true tulip *Fasciolaria tulipa* presence/absence. MLCA: current/historical mid-level consumer assemblage; HR: hazard ratio. *significant at the $\alpha = 0.05$ level

Species	Source	Snails (n)	Deaths (n)	HR	HR 95% CI		z	p
True tulip <i>Fasciolaria tulipa</i>	Tg	41	28	2.535	1.120	5.737	2.233	0.026*
	MLCA			0.996	0.429	2.313	-0.009	0.993
	Tg × MLCA			0.577	0.089	3.737	-0.576	0.564
Lace murex <i>Chicoreus florifer</i>	Tg	76	25	4.159	1.533	11.283	2.799	0.005*
	Ft			0.615	0.146	2.587	-0.663	0.507
	Tg × Ft			2.749	0.489	15.466	1.148	0.251
Lightning whelk <i>Busycon sinistrum</i>	MLCA	63	32	1.699	0.366	7.889	0.676	0.499
	Tg			2.914	0.633	13.422	1.372	0.170
	Ft			1.911	0.411	8.892	0.826	0.409
	MLCA × Tg			0.489	0.068	3.504	-0.712	0.477
	MLCA × Ft			0.483	0.069	3.369	-0.735	0.463
	Tg × Ft			0.757	0.114	5.033	0.288	0.773
	MLCA × Tg × Ft			2.257	0.090	56.892	0.494	0.621
Pear whelk <i>Busycotypus spiratus</i>	MLCA	113	96	0.830	0.409	1.684	-0.517	0.605
	Tg			0.990	0.412	2.376	-0.022	0.982
	Ft			1.296	0.649	2.586	0.735	0.462
	MLCA × Tg			0.867	0.134	5.591	-0.15	0.881
	MLCA × Ft			0.617	0.232	1.644	-0.966	0.334
	Tg × Ft			1.249	0.456	3.423	0.433	0.665
Banded tulip <i>Cinctura hunteria</i>	MLCA	212	191	0.577	0.246	1.354	-1.264	0.206
	Tg			1.284	0.675	2.443	0.762	0.446
	Ft			1.163	0.596	2.268	0.443	0.658
	MLCA × Tg			6.737	2.606	17.416	3.937	<0.001*
	MLCA × Ft			2.969	1.019	8.653	1.994	0.046*
	Tg × Ft			0.881	0.373	2.080	-0.288	0.773
	MLCA × Tg × Ft			0.074	0.019	0.283	-3.798	<0.001*

equilibrates to a new state of reduced complexity. Although many traits can influence species' vulnerability to extirpation following habitat fragmentation (McKinney 1997, Henle et al. 2004), whether the strength of top-down or other interactions can predict extirpation vulnerability is worthy of investigation in other systems and in theoretical studies on food web stability.

Several studies have shown that predator identity, whether through differences in consumption rate or hunting mode, influences both direct and indirect predator effects (Straub & Snyder 2006, O'Connor et al. 2008, Schmitz 2008). In our experiment, we also found that differences in prey consumption were strongly linked to consumer identity. At the upper trophic level, horse conchs were the dominant predator driving top-down effects. At the mid trophic level, the historical consumer assemblage had greater consumptive effects on bivalves because of the drilling lace murex. Behavioral differences between mid-level consumers, such as burrowing behavior and escape responses, may account for their differential susceptibil-

ity to horse conch predation (see the 'Gastropod burrowing behavior' in the Supplement). The effect of the overall decline in snail size is unknown, although it is perhaps important given known relationships between predator body size and prey consumption (Jochum et al. 2012, Toscano & Griffen 2012).

Horse conchs may have persisted on BMB due to their generalized diet of gastropods (Paine 1963a), and loss of the true tulip from the horse conch's diet would effectively decrease its trophic rank, as would be expected with decreasing habitat size (Holt et al. 1999). Concentration of the long-lived horse conchs into a smaller area may have contributed to lace murex and banded tulip declines and would be consistent with purported increases in horse conch density on BMB (see 'Qualitative historical changes in the BMB community' in the Supplement). The field experiment revealed that adult lace murex and true tulips were still capable of survival on the bar and are not excluded by environmental factors, so this top-down predation hypothesis appears plausible. However, it is important to recognize that declines in

bivalves as a food source and changes in bivalve species composition may have simultaneously contributed to the disappearance of lace murex, the increased proportion of generalist mid-level consumers on BMB, and the decline of clams in the diet of several snail species relative to other prey items.

Although we cannot make a causal link between observed community changes and the observed reduction in ecosystem area, results are consistent with expectations, and we can consider possible mechanisms and alternative explanations. Temperature and salinity have not changed significantly from the mid 1900s to the early 2000s, but higher mean annual salinities in the 2 most recent time series may reflect the increasing frequency of drought conditions in this region (USGCRP 2009), which may have affected metapopulation connectivity, physiological stress levels, or the influence of predators not included in this study such as stone crabs. Other environmental conditions may have also changed concurrently with ecosystem area, such as sedimentation rates, sediment properties, and water flow. These changes may have affected survival or recruitment directly or affected the suitability of the seagrass habitat. The reported declines and continued absence of large bivalves, which would have been expected to increase following the loss of the lace murex and declines in the lightning whelk, suggest that regional-scale processes, such as the disappearance and fragmentation of formerly extensive seagrass meadows, rather than top-down pressures, may underlie changes in the bivalve community. In terms of bivalve recruitment, we observed small recruits of some species, such as *Chione elevata* and *Modiolus squamosus*, in our excavated infaunal samples, but we very rarely observed any individuals of other formerly abundant species, such as *Stewartia floridana*, *Carditamera floridana*, and *Macrocallista nimbosa*. Given known mutualisms between bivalves and seagrasses (Peterson 1982, Irlandi 1997, Peterson & Heck 2001, van der Heide et al. 2012), it is possible that the fragmentation and decrease in seagrass area is linked to bivalve declines.

Although the direct interactions between top and mid-level consumers and between mid-level consumers and bivalves suggest the potential for a trophic cascade, it is worth considering why we failed to detect a cascade in our experiment. In tropical and subtropical systems with diverse, reticulate food webs and high amounts of omnivory, top-down consumer control and trophic cascades have been postulated and found to be weaker than in temperate, low-diversity environments (Strong 1992, Boyce et al. 2015). In the BMB system, which is diverse in both

consumer and prey species, there may likewise be suppression or buffering of trophic cascades due to this high complexity and connectivity. However, since the additional clam mortality in historical mid-level consumer treatments could be attributed entirely to lace murex, and lace murex had greater mortality in horse conch treatments, we would still have expected to see cascading effects. The absence of such effects may have been due to depletion of clams by lace murex before the snails were consumed by horse conchs. 'Immigration' of new lace murex through replacement would have further weakened consumptive effects. Non-consumptive effects (NCEs), which can be very strong and commonplace in systems (Preisser et al. 2005), would still have been present; however, our results suggest that these NCEs, if present, were relatively weak. Since horse conchs exhibited no indirect effects in our experiment, we also could not evaluate changes in these effects when true tulips, an omnivore subject to intraguild predation, was paired with horse conchs, which would be predicted to weaken indirect effects (Finke & Denno 2004, Stachowicz et al. 2007). This process is likely to be important in diverse food webs with high amounts of omnivory.

Since our field experiment lacked cages and used tethers, we must also consider the effects of these artifacts on interpretation of our results. When compared to estimates of horse conch consumption rates in outdoor cages (Paine 1963c), observed mortality in horse conch treatments was not higher than expected. However, the experimental setup likely produced overestimates of background mortality rates, particularly for banded tulips and pear whelks, which were not affected by experimental horse conchs but had the highest overall mortality rate of the species used (Fig. S4). This potentially prevented detection of predatory effects by tethered horse conchs and may be due to artifacts such as prey depletion or inability to move to more suitable microhabitats (such as sandy pools or the subtidal) during unfavorable environmental conditions. It is also possible that predation by the ambient horse conch population rapidly and uniformly depleted banded tulips and pear whelks, which left tethered horse conchs to prey upon the remaining species. Although this explanation seems less likely, we cannot completely exclude it, and additional caging or lab-based experiments are needed.

In summary, we used current and historical survey data to describe losses in horizontal and vertical diversity following a reduction in ecosystem area. We used experimental treatments mimicking the current and

historical community to provide insight into the mechanistic consequences of this multi-trophic diversity change. We found that the species with the greatest top-down effects were those lost from the system and showed multi-trophic identity effects linked to observed community changes. Although identifying the exact causes of such changes is difficult, particularly in the absence of an obvious human driver (e.g. harvesting), studies of past diversity change such as this one can help identify what may have been historically important species interactions and predict future diversity change. To understand and predict the patterns and consequences of diversity change, experimental and modeling studies based on actual, historical changes will be much more valuable than studies which randomly manipulate diversity.

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