


## Positive and negative interactions control a facilitation cascade

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**Abstract.** Facilitation cascades, which enhance the diversity of ecological communities in many ecosystems, have been viewed as the net outcome of positive species' interactions. The strength and direction of these interactions, and thus the realized biodiversity, however, are likely to vary with the density and traits of the habitat-formers and via negative interactions among interacting species. To test this, we manipulated the density and status (alive vs. dead) of a secondary habitat-former, the razor clam *Pinna* sp., and measured responses by the primary habitat-former, the seagrass *Zostera muelleri*, associated epifauna and infauna, and fish foraging behavior. At the plot level, for both live and dead clams, the total abundance of epifauna increased with clam density. However, for individual clams, the density of epifauna/cm<sup>2</sup> decreased with increasing clam density. Video image analysis showed higher fish predation of epifauna on dead compared to live clams at high but not low densities and path analysis indicated that these strong negative trophic interactions increased with dead clam density via both direct and indirect pathways. By contrast, an increasing density of live but not dead clams was negatively correlated with seagrass faunal densities. However, seagrass growth and standing biomass were unaffected by clam density or status. Our study illustrates that the realized facilitation cascade is a function of nested negative and positive interactions which change as a function of the density of clams and whether they were dead or alive, and therefore do not represent a collection of hierarchical positive interactions.

**Key words:** biodiversity; ecosystem engineer; facilitation; feedbacks; habitat-former; non-trophic interactions; razor clam; seagrass; trophic interactions.

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### INTRODUCTION

For most of the history of ecology, negative interactions such as predation and competition were assumed to be the predominant mechanisms structuring communities (Connell 1961, Paine 1966, Menge and Sutherland 1976). However, over the past two decades, many studies have demonstrated the positive effects of

habitat-forming or "foundation species" such as corals, trees, and seagrasses on associated organisms. Facilitation of entire communities by habitat-forming species commonly occurs via enhanced resource provisioning (e.g., surfaces for colonization for epibionts on algae and epiphytes in trees) or reducing abiotic (wind, temperature, wave action) and/or biotic (e.g., predation) stress (Wright and Jones 2004, Badano

and Cavieres 2006, Gribben and Wright 2006, Thomsen and Wernberg 2014). Because of the diverse food webs they support, management of foundation species is increasingly advocated as critical to conservation efforts (Bruno et al. 2003, Byers et al. 2006, Halpern et al. 2007).

More recently, research has shown that positive interactions among habitat-forming species can result in a facilitation cascade whereby primary habitat-forming species facilitate secondary habitat-forming species, creating a synergistic effect on the biodiversity of associated species (Ellwood and Foster 2004, Altieri et al. 2007, Gribben et al. 2009, Angelini and Silliman 2014, Hughes et al. 2014, Thomsen and Wernberg 2014). The communities associated with these facilitation cascades are thus considered hierarchically organized through a series of positive interactions (Bruno and Bertness 2001, Bruno et al. 2003). Recent reviews suggest that facilitation cascades may be far more important for driving patterns of global biodiversity than currently acknowledged (Thomsen et al. 2010, Angelini et al. 2011), and thus, cascading positive interactions need to be integrated in conservation/restoration strategies.

Given that communities associated with individual foundation species are often structured by both negative and positive interspecific interactions (Bruno and Bertness 2001, Stachowicz 2001), the mechanisms driving biodiversity in facilitation cascades are likely more complicated than currently acknowledged. To date, however, no studies have investigated how the addition of secondary habitat-formers may alter both positive and negative interactions within a facilitation cascade, although our expectation is that they do. For instance, while habitat-forming species can positively benefit prey by providing them with structural refuge from predators, they can also promote the abundance of predators and thus negatively impact prey (Gribben and Wright 2006, 2014, Miyashita and Takada 2007, Farina et al. 2014). Moreover, the colonization of one habitat-former by another can negatively feedback onto the growth and survivorship of primary habitat-formers by controlling limited resources (e.g., access to light or nutrients; Benzing and Seemann 1978, Flores-Palacios 2016, Zotz 2016), which, in turn, may have strong consequences for the entire cascade. If the loss or

removal of an associated community member alters the strength of negative interactions, then the community supported by a facilitation cascade may depart from predictions based solely on positive interactions. Thus, understanding when both positive (often non-trophic) and negative (trophic) interactions are important is necessary to develop the theory on facilitation cascades and provide predictions for conservation and restoration efforts (Wilby et al. 2001, Kéfi et al. 2012).

Predicting the outcome of facilitation cascades may also depend on the density and traits of the secondary habitat-forming species (Bruno and Bertness 2001, Gribben and Wright 2014). For example, positive effects on biodiversity may increase with the density of the secondary habitat-former (Bishop et al. 2012, 2013, Hughes et al. 2014), but densities beyond a threshold may then exert negative effects on the primary facilitator. In addition, increasing the density of secondary habitat-formers may have important trophic feedbacks if increasing structure decreases predator-prey encounter rates (Grabowski 2004, Griffen and Byers 2006) or if predators also benefit from increasing habitat structure (Leonard 2000, Trussell et al. 2006, Miyashita and Takada 2007, Pearson 2009). An important trait that may interact with density is the legacy effect that occurs when the structure (i.e., ecosystem engineering properties) of habitat-forming species persists long after they die (Lenihan 1999, Hastings et al. 2007). With the cessation of biological processes and changes in morphology following the mortality of habitat-formers, we should expect changes in associated communities, and in the strength and/or direction of interactions within a facilitation cascade. While Thomsen et al. (2010) highlighted the need for a better understanding of density-dependent feedbacks of focal organisms, how the density and traits of the secondary habitat-formers influence interactions within facilitation cascades remains unclear.

In this study, we use a field experiment to determine how the density and state (live or dead) of a secondary habitat-former (razor clams) influence a facilitation cascade within a seagrass meadow (the primary habitat-former) via positive and negative interactions. Seagrasses are globally important foundation species in coastal

ecosystems that support a broad diversity of organisms (e.g., fish, birds, and invertebrates), some of which are themselves habitat-providing species which further promote biodiversity. Razor clams (also called razor shells, razor fish, and pen shells) are often found associated with seagrass beds and harbor extensive epibiotic communities that colonize the large shells of these bivalves extending above the sediment (Munguia 2004, Munguia and Miller 2008). Community structure on the clams is also influenced by patterns of recruitment and competition (primarily for space) among the facilitated invertebrates and predation by fish (Keough 1984, Munguia 2004). Fish are also more often associated with dead than live clams (Macreadie et al. 2014). Although the influence of razor clams and their communities on seagrass infaunal communities is unknown, infaunal communities in unvegetated sediments have lower total abundance, richness, and different community structure in areas with razor clams than in areas without (Warwick et al. 1997, Cummings et al. 1998). Warwick et al. (1997) suggested that the presence of razor clams influences infaunal communities by their activity (increasing biodeposits) and their physical presence, resulting in reduced oxygen levels. Thus, we may predict razor clams will have similar negative effects on the abundance of fauna associated with seagrasses and potentially affect the health of seagrass itself. Alternatively, seagrasses or associated filter feeders or deposit feeders may benefit from increased organic input or nitrogenous waste from razor clams. When clams die, their shells provide a hard substrate in the habitat for extended periods. Dead bivalve shells commonly support higher abundance and diversity of organisms than live bivalves because of changes to or loss of the periostracum (a thin organic coating that protects the shell) that commonly inhibits epibiosis (Wahl et al. 1998, Scardino and de Nys 2004). Collectively, these disparate studies suggest that the seagrass/razor clam facilitation cascade is an ideal model system that may be controlled by a variety of positive and negative interactions and feedbacks among the habitat-formers and the different community components (e.g., clam communities, sediment communities, and fish communities).

To determine the existence, strength, and biotic components of a facilitation cascade, we

manipulated the density and status (alive or dead) of razor clams within a seagrass meadow and quantified changes in abundance of associated epifauna, infauna, seagrass, and fish communities. We further quantified the rates of two processes that influence species interactions in this system: seagrass productivity and fish predation pressure on epifauna. Specifically, we hypothesized that the abundance of epibionts and fish/quadrat would increase with increasing clam density, the abundance of epibionts would be higher on dead than on live clams, and the community structure of the seagrass-associated fauna and on clams would be different on live and dead clams. In addition, we predicted that seagrass biomass and productivity and predation pressure on epibionts on clams and seagrass-associated fauna would increase with clam density.

## MATERIALS AND METHODS

### *Study site and species*

This study was conducted at Point Wolstoncroft in Lake Macquarie, New South Wales, Australia (33°07' S, 151°35' E). Lake Macquarie is the largest coastal lake in Australia with extensive seagrass beds, including the native seagrass *Zostera muelleri* (Irmisch ex Asch.), with many seagrass beds housing razor clams (Macreadie et al. 2014). Point Wolstoncroft is a sheltered, shallow embayment containing the highest densities of razor clams in the genus *Pinna* (family Pinnidae) among *Z. muelleri* found in the lake (Macreadie et al. 2014). Preliminary surveys at this site indicated that razor clams do not occur outside seagrass beds: Razor clams appear reliant on seagrass as a recruitment habitat (which presumably provides a refuge from predation for vulnerable post-settlement clams; Orth et al. 1984, Irlandi and Peterson 1991), thus forming a facilitation cascade. The species present on the New South Wales coast has been referred to as *Pinna bicolor* (Gmelin), but a recent revision of the family (Schultz and Huber 2013) and a molecular phylogeny (Lemer et al. 2014) indicate that *P. bicolor* is restricted to the Indian Ocean, and that the SE Australian species is likely *Pinna madida* (Reeve). With this yet to be confirmed, we refer to the clams as *Pinna* sp.

*Pinna* spp. are habitat-forming bivalves that occur within many of the world's seagrass

meadows. *Pinna* sp. is a large species (max. length 500 mm) of razor clam often found in seagrass beds in tropical and temperate estuaries around Australia. They are fan-shaped with their ventral margin (i.e., the tip) buried in the sand, although most of the shell protrudes above the sediment surface, including their razor-sharp broad posterior margins from which the clam takes its name. They occur from 0 to 10 m in water depth but can be particularly abundant near the low tide mark (Waycott et al. 2014). The seagrass *Z. muelleri* is found from tropical to temperate regions in Australia and New Zealand. It commonly occurs as meadows in mud and sand from 0 to 4 m depth in estuaries and shallow lagoons, and has a maximum leaf length of approximately 600 mm (Edgar 2000).

#### *Effects of razor clam density and status on the seagrass/clam facilitation cascade*

We conducted an experiment in which we manipulated razor clam density (three levels: 0, 1, and 4 clams  $0.25 \text{ m}^{-2}$ ) and status (two levels: alive and dead) to determine their effects on different components of the facilitation cascade, including colonization of clams by mobile and sessile epibionts, fish community structure and feeding, seagrass-associated fauna (which included mobile and sessile fauna on seagrass and in the sediments within seagrass; hereafter referred to as “seagrass fauna”), and seagrass biomass and productivity. We established the experiment in a bed of 100% seagrass cover in approximately 1 m below low tide line. Within the bed, we marked out 50 plots ( $0.5 \times 0.5 \text{ m}$ ) in between existing razor clams such that all plots contained no razor clams and plots were separated by  $>1 \text{ m}$ . Live and dead razor clams were collected from nearby bays to limit disturbance to the area in which our experiment was conducted. Only dead razor clams still erect in seagrass were collected; dead clams lying on the seagrass surface appeared to have degraded shells (P. E. Gribben and A. G. B. Poore, *personal observation*).

Once collected, razor clams were gently scrubbed clean of all fouling organisms (no remnants of epibionts remained) and randomly allocated to plots resulting in 10 replicates for each combination of density and status. We did not observe any loss of the periostracum on live clams as a result of scrubbing clams, and no

residual epibiota was observed on clams. All clams were reburied to the depth from which they were collected, with the below-ground part of the shells easily visible due to darker coloration, presumably due to sediment anoxia, than the above-ground part of the shell. All clams survived transplantation for the duration of the experiment. The clams used had a mean  $\pm$  SE shell length of  $32.98 \text{ cm} \pm 0.71$  with a mean  $\pm$  SE surface area of  $254 \pm 7.93 \text{ cm}^2$ . The experiment was established on 19 December 2015 and ran until 19 February 2016 to capture key recruitment periods for invertebrates.

#### *Sampling of seagrass fauna and clam epibiota*

At the end of the experiment, seagrass fauna (on seagrass and in the sediment combined) were sampled by taking a single core (10 cm diameter  $\times$  4 cm depth) from the center of each plot and placing its contents in labeled plastic bags. Following this, all clams were retrieved from the plots and the mobile and sessile epibiota attached to the surface of individual clams was carefully scraped off onto a 500- $\mu\text{m}$  sieve, rinsed in seawater, and fixed with 5% formalin. We also took photographs of individual clams to later determine their total surface area and above-ground surface area. All samples were transported back to the laboratory where the seagrass fauna samples were frozen until organisms could be identified. Prior to identification, all samples were again washed on a 500- $\mu\text{m}$  sieve to remove particulate matter. Seagrass present from the cores was kept for further analyses (see *Sampling of seagrass biomass and productivity*). All seagrass fauna and epibiota were identified to morpho-species and counted.

The abundance, species richness, diversity (Shannon–Weaver index), and evenness of the seagrass fauna per plot were contrasted among treatments with ANOVA with clam density (one and four per plot) and status (alive and dead) as fixed, factorial factors. Following these analyses, all plots with clams were contrasted to those control plots lacking clams using a planned contrast following a one-way ANOVA that used all five unique treatments. The abundance, expressed as counts per plot and per  $\text{cm}^2$  of clam surface, species richness, diversity, and evenness of epifauna were contrasted among these treatments with a generalized linear model (with gamma error distribution) and factorial ANOVA, respectively.



For both epifauna and seagrass fauna, the composition of morpho-species was contrasted among the treatments with permutational multivariate analysis of variance in the R package *vegan* (Oksanen et al. 2013) with the community matrix standardized by total abundance. Differences in the composition of morpho-species among treatments were visualized by multi-dimensional scaling using the Bray–Curtis index as the measure of similarity among samples.

#### *Sampling of seagrass biomass and productivity*

To determine potential feedbacks of razor clams onto seagrass, we measured seagrass standing biomass and productivity. Seagrass biomass was determined for each of the infaunal sediment cores sampled above. For each core, we measured both above- and below-ground biomass by separating the seagrass roots and rhizomes from the leaves. We then oven-dried all material (at 60°C for 48 h) and determined dry weight of each component on a Mettler balance.

Because biomass does not always reflect seagrass turnover, we also determined seagrass productivity. We measured growth rate within five replicate plots from each treatment by marking five individual shoots with a loose cable tie and then puncturing each shoot with two needle holes just above the basal meristem. These punctures leave scars on all leaves within the leaf sheath and allow measurement of leaf elongation (Short and Duarte 2001). Seven days later, we removed all tagged shoots by hand, including the roots and rhizomes. Shoots were separated into individual leaves, and leaf length and width measurements were used to calculate the area of new growth per shoot.

Seagrass growth, expressed as area of new leaf produced, total biomass, above-ground biomass, and below-ground biomass per plot were contrasted among treatments with clam density (1 or 4) and clam status (alive or dead) as fixed factors in ANOVA. Subsequently, all plots with clams were compared to those control plots lacking clams using a planned contrast following a one-way ANOVA that used all five unique treatments.

#### *Sampling of fish communities*

To determine fish community structure and feeding rates, we deployed remote underwater

video cameras (GoPro Hero 4 Silver with a waterproof housing; GoPro Inc, San Mateo, California, USA) on four replicate days, one day/week for 4 weeks from the fourth week of the experiment. On each occasion, two replicates of each treatment combination (10 plots in total) were randomly selected and filmed for 1 h in the morning. Cameras were placed on steel frames (52 × 52 cm base with cameras set consistently to 30 cm height before each deployment) and were positioned approximately 30 cm from each plot, angled slightly downwards in order to capture the entire plot including any clams present.

We analyzed 17 min from each one-hour segment of footage (between the 18- and 34-min marks), to minimize the potential effect of human disturbance on fish behavior. We used *EventMeasure* software (SeaGIS Pty Ltd, Bacchus March, Victoria, Australia) to record MaxN values, a conservative measure of relative abundance that quantifies the maximum number of each fish species in the frame at any one time (Cappo et al. 2003). All fish were identified to species. We also recorded fish feeding rates on both seagrass and clam surfaces by quantifying the total number of bites made by each species of fish over the course of the 17-min filming period.

The total abundance of fish per treatment (sum of the MaxN values for each species), the species richness of fish, and the total number of observed bites were contrasted among treatments using linear mixed models with clam density (one and four per plot) and status (alive and dead) as fixed factors and sampling date as a random factor. The analyses were run in the R package *lme4* (Bates et al. 2014) with likelihood ratio tests between full and reduced models used to test the significance of fixed factors. A subsequent analysis contrasted these same variables between plots that lacked clams and plots where clams were present.

#### *Path analysis methods*

We used path analysis to quantify the effects of variation in the density and traits of a secondary habitat-forming species on the presence and nature of a facilitation cascade (Grace and Bollen 2005, Grace et al. 2012). In this path analysis, we only explored pathways that were deemed biologically relevant in the analyses

above (see Fig. 4 for pathways included in the models). Path analysis can be thought of as a series of multiple regressions linking variables based on an a priori model (Wootton 1994, Petraitis et al. 1996). By allowing variables to serve as both endogenous (response) and exogenous (explanatory) variables, path analysis can be used to quantify direct and indirect relationships via path coefficients. The total effect of one variable on another is thus equal to the sum of (1) the product of the indirect path coefficients and (2) the direct path coefficient linking them together. The standardized coefficients in our models represent the predicted change in the response variable measured in standard deviations due to a shift in the explanatory variable of one standard deviation (Grace and Bollen 2005). Although more abstract than regression slopes, standardized coefficients facilitate comparisons and make it possible to compare the effects of explanatory variables with inherently different ranges.

We used the classical likelihood approach to fit the model covariance matrix to the observed covariance matrix (Grace et al. 2012). This means that our path analysis shares the same assumptions as general linear models, namely normality and independence of residuals, homoscedasticity, linear relationships between endogenous and exogenous variables, and lack of collinearity between the variables. All variables were log-transformed when necessary in order to adhere to these assumptions. We generated both bottom-up and top-down models to investigate different relationships among the variables. However, we present only the bottom-up model (although it allows for both positive and negative interactions) because its Akaike's information criterion (AIC), corrected for sample sizes value was substantially lower than that of the top-down model (AIC weight >0.99).

## RESULTS

### *Seagrass fauna and clam epifauna*

A total of 50 species of seagrass fauna were collected from the experimental plots, dominated by gastropods (24% of species and 35% of all individuals collected) and bivalves (14% of species and 15% of individuals). The abundance of seagrass fauna per plot did not differ among

treatments of clam density, status, or their interaction (Fig. 1a; Appendix S1: Table S1). The species richness and diversity, but not evenness, of seagrass fauna were significantly lower in plots with dead clams in contrast to plots with live clams and those lacking clams (Fig. 1b; Appendix S1: Table S1). The composition of morpho-species in the seagrass also differed between live and dead clams, but not between plots of varying clam density (Appendix S1: Fig. S1, Table S1). There were no significant interactions between clam density and status for the abundance, richness, diversity, evenness, or composition of seagrass fauna (Appendix S1: Fig. S1, Table S1). Of the most abundant major taxa,

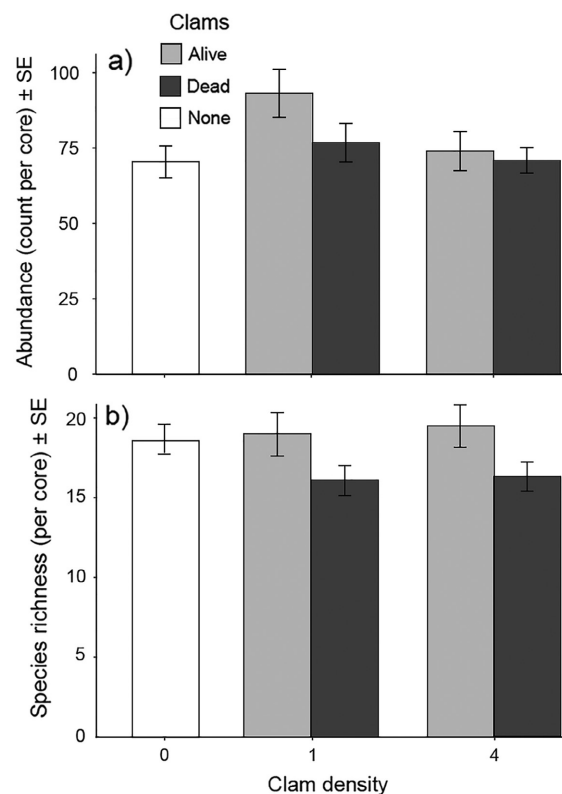


Fig. 1. Abundance (a) and species richness (b) of seagrass fauna inhabiting the seagrass *Zostera muelleri* in experimental plots with live and dead clams at two densities (one and four per 0.25 m<sup>-2</sup> plot) and control plots with no clams. The data are counts of individuals and species per 10 cm diameter cores in each plot. Data are means ± SE with  $n = 9-10$  plots per treatment.

gastropods were more abundant in the plots with one clam, than those with four clams and did not vary with clam status, while the abundance of amphipods and bivalves did not vary among any treatments (Appendix S1: Fig. S2, Table S1).

The clams supported 60 species of epifaunal invertebrates, dominated by gastropods (17% of species and 21% of all individuals collected), polychaetes (18% of species and 13% of individuals), and amphipods (13% of species and 13% of individuals). Abundance of epifauna per plot (deviance = 3.5411,  $df = 1$ , 36,  $P < 0.001$ ) and per unit area of clam surface was higher on live vs. dead clams (Fig. 2a; Appendix S2: Fig. S1). Abundance of epifauna per plot was higher (approximately 2 $\times$ ; deviance = 2.7577,  $df = 1$ , 38,  $P < 0.001$ ) in the high-density (four clams per plot) than the low-density (one clam per plot) plots, but the opposite pattern was observed per unit area of clam surface (Fig. 2a; Appendix S2: Fig. S1, Table S1). The species richness and diversity of epifauna, but not evenness, were significantly higher on clams in the high-density plots than the low-density plots, but the magnitude of these differences was small (8% on average; Fig. 2b; Appendix S2: Table S1). The composition of morpho-species inhabiting the clam surface differed between live and dead clams, but not between plots of varying clam density (Appendix S2: Fig. S2, Table S1). Of the most abundant major taxa, amphipods and polychaetes were more abundant on live than on dead clams, and more abundant in the plots with one clam, while gastropod abundance varied with clam density but not between live and dead clams (Appendix S2: Fig. S3, Table S1).

#### Seagrass biomass and production

Neither the growth of seagrass nor the standing biomass of seagrass (mean  $\pm$  SE =  $15.8 \pm 0.89$  g dry weight per plot) varied among experimental treatments (clam density and status or their interaction, Appendix S3: Fig. S1, Table S1). We detected no differences among treatments for either above- or below-ground biomass (Appendix S3: Table S1).

#### Fish communities and feeding activity

Twelve species of fish were associated with the experimental plots, with five of these actively

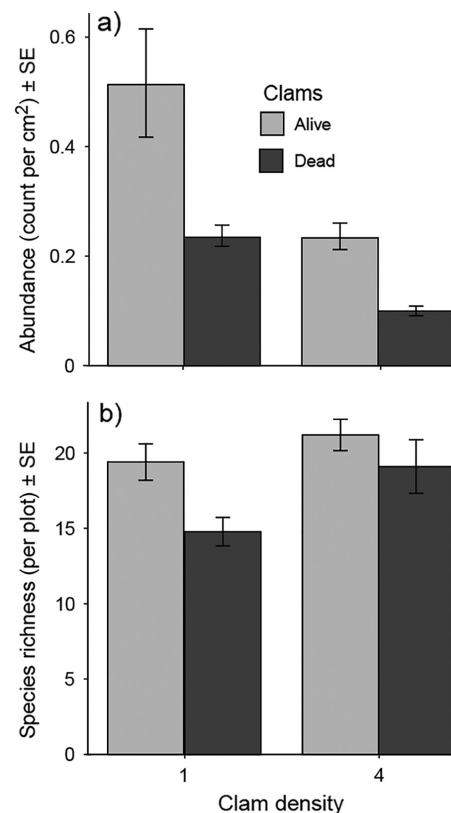


Fig. 2. Abundance (a) and species richness (b) of mobile epifauna inhabiting the surface of the razor clams in experimental plots with live and dead clams at two densities (one and four per 0.25 m<sup>-2</sup> plot). The abundance data are counts per cm<sup>2</sup> of surface area, and species richness is the number of species in each plot. Data are means  $\pm$  SE with  $n = 9$ –10 plots per treatment.

feeding on the surface of clams: *Acanthopagrus australis* (Sparidae), *Gerres subfasciatus* (Gerreidae), *Monacanthus chinensis* (Monacanthidae), *Pelates sexlineatus* (Terapontidae), and *Petroscirtes lupus* (Blennidae). The feeding activity of fish was largely concentrated in the plots containing clams, in particular the high-density plots (Fig. 3). Clam density and status interacted to determine the total number of bites per observation period (Appendix S4: Table S1), with more bites observed on dead clams than on live clams in the high-density plots, but more bites observed on live clams than on dead clams in the low-density plots (Fig. 3). The abundance of fish, conservatively estimated as the sum of MaxN for

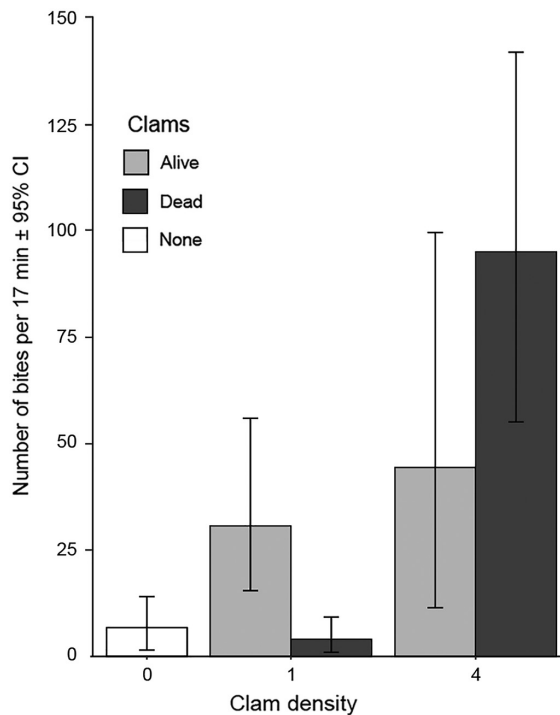


Fig. 3. The number of fish bites observed in video surveys of the experimental plots with no clams, and with live and dead clams at two densities (one and four per 0.25 m<sup>-2</sup> plot). Data are means  $\pm$  95% confidence intervals, with sampling dates pooled ( $n = 7-8$  videos per treatment).

each fish species, and the species richness of fish per observation period did not differ among treatments (clam density, status, or their interaction, Appendix S4: Table S1).

#### Path analysis results

*Live clams.*—The bottom-up model generated a covariance matrix that was not statistically different than the covariance matrix observed for the live clam dataset ( $\chi^2 = 4.18$ ,  $df = 5$ ,  $P = 0.52$ ). The model explained 53% of the variance in seagrass faunal abundance, 47% of the variance in the epifaunal abundance, but only 2% of the variance in the total number of fish bites (Fig. 4a; Appendix S4: Table S2). Live clam density was negatively related to both seagrass faunal and epifaunal abundance whereas seagrass density was positively related to seagrass faunal abundance (Fig. 4; Appendix S4: Table S2). Live clam

density also had a weak and non-significant negative effect on the number of fish bites.

*Dead clams.*—The predicted covariance matrix under the bottom-up model was not statistically different than the covariance matrix observed for the dead clam dataset ( $\chi^2 = 3.39$ ,  $df = 5$ ,  $P = 0.64$ ). The model explained 1% of the variance in seagrass faunal abundance, 94% of the variance in the epifaunal abundance and 46% of the variance in the total number of fish bites (Fig. 4b; Appendix S4: Table S2). Here, seagrass density was not significantly related to seagrass faunal abundance. Dead clam density was more strongly negatively related to epifaunal abundance than live clam density. Additionally, dead clam density was more strongly (although not significantly) related to fish bites via direct and indirect pathways. Here, the lack of significance for both sets of pathways was due to the strong collinearity between the direct and epifaunal-mediated indirect effects of clam density on the total number of fish bites. Indeed, dropping either the direct or indirect effect of dead clam density on total fish bites yielded a significant positive relationship between dead clam density and total fish bites and a significant negative relationship between epifaunal abundance and total fish bites (Fig. 4c, d). Hence, the direct and indirect effects of dead clam density on total fish bites are positive (Fig. 4c, d; Appendix S4: Table S3).

## DISCUSSION

Our research demonstrated that large habitat-forming bivalves within a meadow of seagrass create a facilitation cascade but the strength and, importantly, the direction of the interactions within the cascade were mediated by both the density and the status (alive vs. dead) of the secondary habitat-former. At the plot scale, increasing clam density clearly increased the total abundance of epifauna for both live and dead clams. However, at the scale of individual clams, the density of epifauna per unit area of clam surface decreased with increasing clam density. Moreover, our results show that when clams die, their positive effect on epifaunal densities weakens, because dead clams facilitate fish predation on the epifauna at high densities. Although the response of seagrass fauna was weaker, an



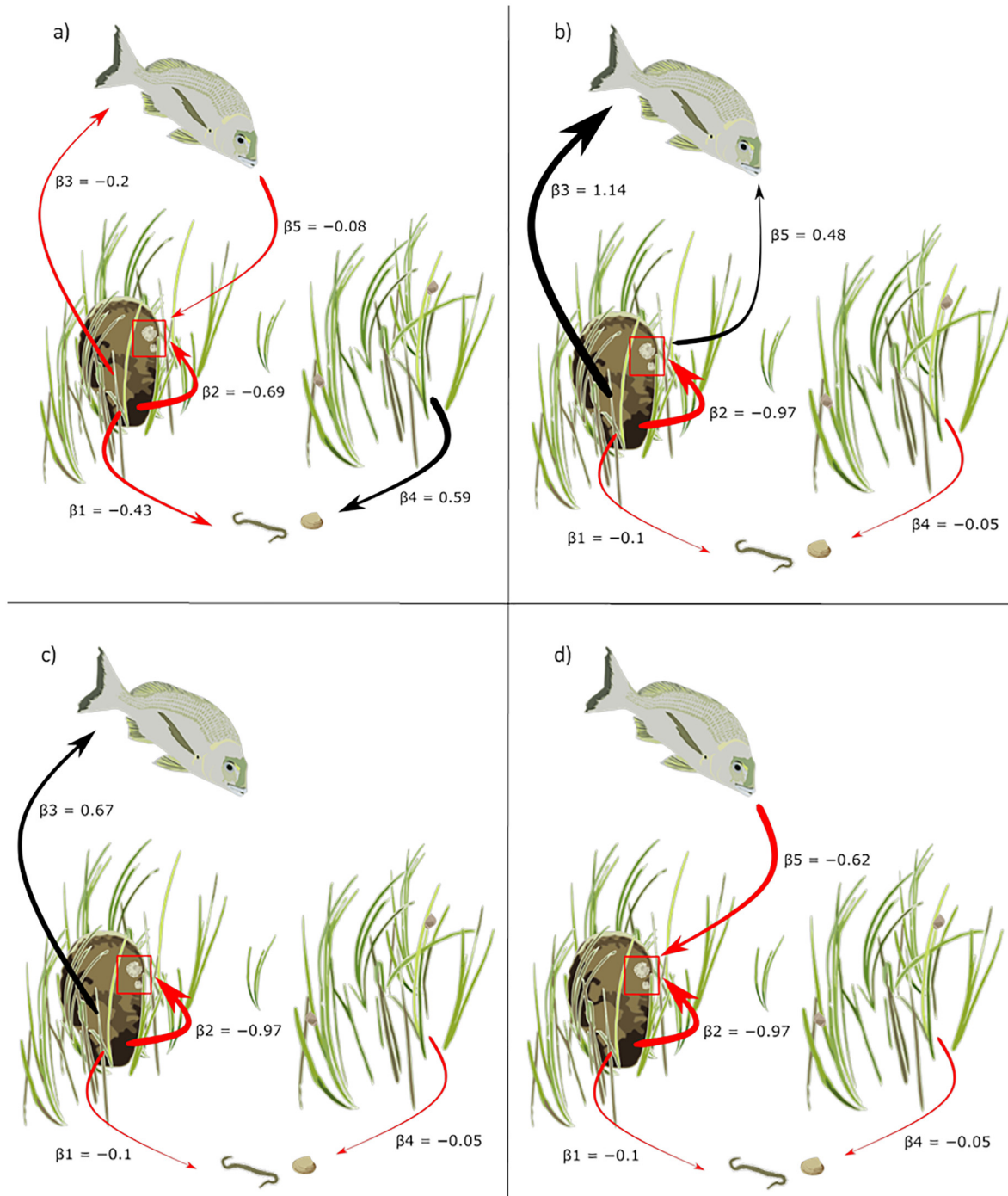


Fig. 4. Path diagram of interactions among clams, clam epifauna, seagrass fauna, seagrass biomass and fish bites. (a) The bottom-up model fit to the live clam dataset, (b) the bottom-up model fit to the dead clam dataset, (c) the bottom-up model fit to the dead clam dataset with no indirect pathway between clams and fish bites, and (d) the dead clam dataset with no direct pathway between clams and fish bites. Red and black arrows indicate negative and positive correlations, respectively.  $\beta$ -values are standardized path coefficients. Arrows to the front and back end of fish represent fish bites rates and abundance, respectively. Closed (a) and open (b–d) clams indicate live and dead clams, respectively.

increasing density of live clams, but not dead clams, was negatively correlated with density. While investigations of facilitation cascades have focused on the pervasive positive effect of the secondary habitat-formers on biodiversity, our study illustrates that the realized facilitation cascade is a function of both negative and positive interactions, as well as direct and indirect pathways, which change with the density and traits of habitat-formers.

Theory predicts that the overall effects of foundation species on community diversity depend on the density of the foundation species (Bruno and Bertness 2001). Consequently, at large spatial scales, it seems reasonable to predict that the degree to which positive interactions of foundation species cascade throughout the community will scale positively with increasing population size of secondary habitat-forming species. For example, increasing biomass of a secondary habitat-former, the alga *Hormosira banksii*, on mangrove roots was positively correlated with the abundance of the facilitated mollusk community (Bishop et al. 2012, 2013, Hughes et al. 2014). Alternatively, competition theory predicts that the per capita contribution of secondary facilitators may decrease with increasing population size if the pool of associated species able to benefit from facilitation is limited by resources (Harper and White 1970, Tilman and Cowan 1989). Indeed, that is what occurred in our study; increasing clam density resulted in a higher total abundance of epifauna per unit area of seafloor, but a lower density of epifauna per unit area of hard substrate provided by the clams (Fig. 2). This may be due to the pool of recruiting organisms, many of whom are highly mobile (particularly gastropods and amphipods), coming from the surrounding habitat matrix. A finite pool of colonizers would have more clam surface area to colonize in plots with four clams than with single clams. Similarly, Roberts and Poore (2006) found lower densities of mobile amphipods in large algal patches than small, also likely due to the concentration of dispersing animals from the matrix. The difference between our study and those of Bishop et al. (2012, 2013) and Hughes et al. (2014) on *Hormosira* may reflect the patchiness of the secondary habitat-former. For example, *H. banksii* forms continuous beds

among the mangrove roots, whereas razor clams are discrete habitats and thus may represent a more limited resource. Clearly, understanding how the density of the secondary habitat-formers influences the per capita density of associated species, and where and when the relationships may be generally positive or negative will be important for integrating facilitation cascades into conservation strategies.

In addition to density, the literature on facilitation cascades has rarely considered another cornerstone in the theory describing positive interactions, namely that the associated community will consist of hierarchically nested interactions that are positive as well as negative (Bruno 2000, Stachowicz 2001, Bruno et al. 2003). If present, negative interactions such as competition and predation may result in realized facilitation cascades that are only a subset of the potential (or fundamental) cascade. Indeed, in our study reduced epifauna density on dead clams correlated with the stronger trophic feedbacks on dead vs. live clams; dead and live clams have different epibiont communities which may differ in quality as a food source for fish. The results were consistent for two of the three most abundant taxa (mobile polychaetes and amphipods) but not for mollusks. Similarly, Keough (1984) found that predation by fish had little influence on most species of sessile epibionts on live razor clams, except for tunicates for which the effects of predation were variable in time and space. Moreover, the negative feedback onto epifauna strengthened with increasing density of dead clams via both direct and indirect pathways (Fig. 4c, d). Increasing density of dead clams likely increased food supply per unit area of seafloor, attracting more fish bites, and subsequent indirect negative feedbacks on associated epifauna. These effects were not caused by the structural attributes of the clams as both live and dead clams provided similar spatial refuges. Supporting this, we found no differences in MaxN or species richness among plots with no, live, or dead clams, indicating that these fish were routinely foraging across the entire experimental area, on scales of 10–100s of meters. Regardless, our results clearly show that the secondary habitat-formers also foster strong positive and negative interactions, and, in particular, top-down processes are important components

in explaining realized biodiversity in facilitation cascades.

In contrast to the strong negative feedbacks of fish onto epifauna, clam density and status had much weaker effects on seagrass fauna—there was a weak negative relationship between clam density and seagrass fauna for the structural equation model only. Biodeposits from razor clams can facilitate benthic communities compared to where they are absent, dependent on levels of suspended sediment (Norkko et al. 2006). Given that our study was shallow (<1.5 m water depth), prone to sediment resuspension when afternoon breezes strengthened (P. E. Gribben and A. G. B. Poore, *personal observation*) and seagrass beds have high organic loads within them (Williams and Heck 2001), the addition of further material from clams probably has limited influence on benthic communities. However, plots with dead clams did support lower species richness and altered composition, in contrast to plots with live clams, indicating that they alter communities in different ways. The higher frequency of fish bites on dead clams did not result in fish also targeting specific prey in nearby seagrass, as our video analyses had fish almost exclusively feeding on the surface of clams themselves. Benthic community patterns may be influenced by dispersal between these communities and those on clam surfaces, which did differ between live and dead clams.

While our focus was on determining the effects of the density and status of a secondary habitat-former on a facilitation cascade, primary habitat-formers also vary in their density and traits with significant effects on associated biodiversity. Indeed, there was a positive relationship between seagrass biomass and seagrass fauna. Thus, interactions between the density and traits both within and between habitat-formers may possibly have additional consequences for the facilitation cascade. Although the density or status of clams did not affect seagrass biomass or productivity, this may not always be the case. Moreover, communities on razor clams, recruitment of epibionts to razor clams, and predation on those epibionts can vary spatially and temporally (Keough 1984, Munguia 2007) and may vary with the successional stage investigated (Munguia 2004). Thus, at the landscape level, community structure will likely be a function of

the environmental heterogeneity (Hughes et al. 2014), variation in the density and traits of the habitat-formers, as well as external recruitment processes.

Here, we have demonstrated that facilitation cascades contain positive and negative interactions that are modified by both the density and the traits of the secondary habitat-former. Currently, there is public concern because of the injuries suffered by swimmers who step on razor clams and there is increasing public pressure to remove razor clams from Lake Macquarie (Macreadie et al. 2014). Given that razor clams are common in seagrass beds throughout Lake Macquarie (Macreadie et al. 2014), Australia's largest coastal lake, our research suggests that removal of razor clams may have flow-on effects, not just for associated biodiversity, but also for the trophic transfer of energy from the benthos to mobile fishes, especially if fish are food limited and clam epibionts form a significant component of their diet. Further research is needed to establish whether this is the case. Regardless, understanding both the positive and the negative mechanisms that underpin facilitation cascades is critical for facilitation theory to develop and for predicting where and when facilitation cascades can be integrated into management strategies.

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