



OPEN Predation vulnerability of small invertebrates varies among and within major prey groups in eelgrass beds

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Small invertebrates in eelgrass beds form an important prey base for fish and larger invertebrates, thereby supporting trophic transfer in coastal food webs. Although previous studies indicate that mobile crustaceans generally experience higher predation pressure than gastropods in eelgrass ecosystems, it remains uncertain whether this broad taxonomic contrast sufficiently characterizes relative prey vulnerability, as vulnerability may also differ among species within the same broad prey group. We conducted standardized field tethering experiments at three eelgrass beds along the Pacific coast of Japan (approximately 34–43°N) to compare relative predation pressure among focal small benthic invertebrates. Predation pressure varied between major prey groups, with crustaceans generally more vulnerable than gastropods. Notably, considerable species-level variation was observed within these groups, particularly among crustaceans, indicating that biologically meaningful variation in prey vulnerability can occur within broad taxonomic categories. Body size alone did not account for this pattern, suggesting that species identity may capture additional aspects of prey biology not fully represented by size, such as morphology, behavior, microhabitat association, detectability, and defensive characteristics. These results indicate that incorporating species-level resolution, alongside broad prey-group identity, improves the characterization of relative prey vulnerability in eelgrass beds.

Keywords Seagrass, *Zostera*, Predation, Macrobenthos, Functional traits, Tethering

Small invertebrates represent a substantial component of animal diversity and often constitute a large share of abundance and prey availability in many ecosystems, serving as a crucial trophic link between primary producers and higher-level consumers^{1–3}. Variation in their vulnerability to predation can shape community structure and influence the transfer of production to higher trophic levels^{4,5}. Grouping prey into broad taxonomic categories, such as major prey groups or higher taxonomic levels, provides a useful way to compare predation patterns across ecosystems, regions, and environmental gradients, and to summarize prey assemblages across broad spatial and temporal scales. In terrestrial, freshwater, and marine systems, such comparative frameworks have helped explain variation in predation pressure and prey susceptibility through predator–prey size relationships⁶, prey traits⁷, food-web context⁸, temperature and latitude, predator community composition, habitat complexity, and patch context^{9–12}. However, multi-species prey assemblages can contain substantial variation in traits and vulnerability among species within the same broad prey group, and potentially also among individuals or life stages within species. Thus, contrasts among higher-level prey categories alone may be insufficient to identify the biological scale at which prey vulnerability is organized, leaving a more limited understanding of how prey vulnerability shapes prey availability to consumers.

Eelgrass (*Zostera marina*) is a foundation species that is widely distributed in shallow coastal areas of the temperate and subarctic Northern Hemisphere, where it forms structurally complex meadows known as eelgrass beds¹³. These habitats provide essential physical structure, substrate, and refuge for a wide variety of organisms, resulting in high biological productivity and biodiversity^{2,14–16}. Small invertebrates, including amphipods, gastropods, and polychaetes, colonize both the aboveground and belowground components of eelgrass beds and constitute an important prey base for fishes and larger invertebrates, thereby contributing substantially to

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trophic transfer in coastal food webs^{2,5,17,18}. Because eelgrass beds support habitat-specific prey assemblages within the meadow¹⁹, they represent an effective system for examining how variation in prey vulnerability may shape prey availability to consumers. However, despite this central trophic role, it remains unclear which small invertebrates are most vulnerable to predators, and whether this vulnerability is structured primarily by broad prey-group identity or by finer species-level differences within those groups.

A practical way to evaluate the importance of small invertebrates as prey in eelgrass beds is to compare their relative vulnerability to predation, hereafter termed relative predation pressure. Standardized large-scale studies have shown that predation pressure in marine systems, including eelgrass beds, varies predictably across broad geographic gradients, although these patterns are not always linear. Comparative studies spanning temperate to tropical coastal regions indicate that predator effects generally intensify under warmer conditions, whereas global assays of standardized consumption in shallow marine habitats suggest that feeding rates can peak at subtropical to warm-temperate latitudes rather than increasing monotonically toward the equator. This latter pattern appears to reflect shifts in predator community composition rather than temperature alone^{9,10}. Within eelgrass beds specifically, broad comparisons across the Northern Hemisphere have further shown that predation on common small invertebrate herbivores varies with latitude, long-term temperature regime, shoot density, and patch structure^{11,12}. At the same time, these studies also reveal a repeated broad taxonomic contrast: mobile crustacean prey generally experience higher relative predation pressure than gastropods, which often show uniformly low predation rates^{11,20}. Together, these findings suggest that predation pressure in eelgrass beds is shaped by both large-scale environmental variation and prey-related differences, but they do not yet show whether relative prey vulnerability is adequately characterized by broad taxonomic contrasts alone or also requires finer resolution among groups and species.

Beyond broad contrasts between distantly related taxa such as crustaceans and gastropods, differences in relative predation pressure may also occur among closely related taxa and even among species within the same taxonomic group. Previous studies focusing on specific prey taxa or species collectively suggest this possibility. For instance, among amphipods, which are common prey in eelgrass beds, relative predation pressure has often been reported to be high in non-caprellid forms but relatively low in caprellids^{11,21,22}. Such finer-scale variation may reflect differences in morphological and behavioral characteristics, such as body size, body form, mobility, and defensive capacity, because predator–prey interactions are fundamentally shaped by the relative functional traits of predators and prey²³. A trait-based perspective may therefore help interpret variation in predation vulnerability across species and taxonomic groups^{23,24}. However, because trait-based approaches to animals remain relatively recent and not yet fully generalized^{3,7}, applying this framework to eelgrass small-invertebrate communities remains challenging. Existing studies collectively suggest that relative predation pressure may vary systematically from broad taxonomic groups to finer taxonomic levels, yet few have directly compared multiple taxonomic groups and species within a unified experimental framework across eelgrass beds that differ in environmental conditions and community composition. Consequently, it remains unclear whether broad taxonomic contrasts alone sufficiently characterize relative prey vulnerability in eelgrass beds, or whether finer taxonomic resolution is needed to capture this variation.

In this study, we quantified relative predation pressure on multiple small benthic invertebrates inhabiting aboveground eelgrass beds using field tethering experiments. Tethering estimates relative predation pressure by restraining prey individuals and recording predation over a fixed period, and this approach has been widely applied in seagrass ecosystems and other coastal habitats^{11,12,25–28}. Although tethering restricts natural movement and escape behavior, it allows for comparisons of relative predation vulnerability among prey types under standardized, comparable conditions. To determine whether prey vulnerability exhibits repeatable structure across spatial gradients, experiments were conducted in three eelgrass beds along the Pacific coast of Japan, spanning temperate to subarctic environments (approximately 34–43°N).

The primary objective of this study was to identify the taxonomic scale at which predation vulnerability is structured among small invertebrates associated with eelgrass, thereby clarifying the relative importance of variation among major prey groups and species-level variation within those groups. Specifically, we tested whether pronounced interspecific differences in predation pressure were consistently observed within the same taxonomic groups across three eelgrass beds. Each site included multiple species within at least one major taxonomic group, enabling direct comparison of interspecific differences within a shared taxonomic framework while allowing local species composition to differ among eelgrass beds. We also evaluated whether coarse taxonomic categories were sufficient to characterize prey vulnerability in eelgrass beds or whether species-level resolution was necessary. The amphipod *Ampithoe lacertosa*, which is widely distributed along the Pacific coast^{29,30}, occurred at all three sites and therefore provided an additional point of comparison across eelgrass beds, particularly because it has been used in a hemispheric-scale tethering study across Pacific eelgrass beds and has consistently exhibited high predation pressure across regions¹¹. Additionally, local fish predators were surveyed, and stomach contents were analyzed to identify potential predators and the major prey categories represented in local diets, providing supplementary context for interpreting the tethering results (details in section 2 of the [Supplementary Information: Predator Survey](#)).

Materials and methods

Study site

We conducted field tethering experiments to evaluate predation on small invertebrates in three different eelgrass (*Zostera marina*) beds located off the coast of Shinryu in Akkeshi Bay, the coast of Kurihama in Kurihama Bay, and Oshima Tidal Flat in Kasado Bay (Fig. 1). Shinryu is located in subarctic waters, while Kurihama and Oshima Tidal Flat are found in temperate waters. In selecting the experimental sites, we avoided areas with undesirable conditions, including shallow zones where the experimental setup would dry out during low tide,

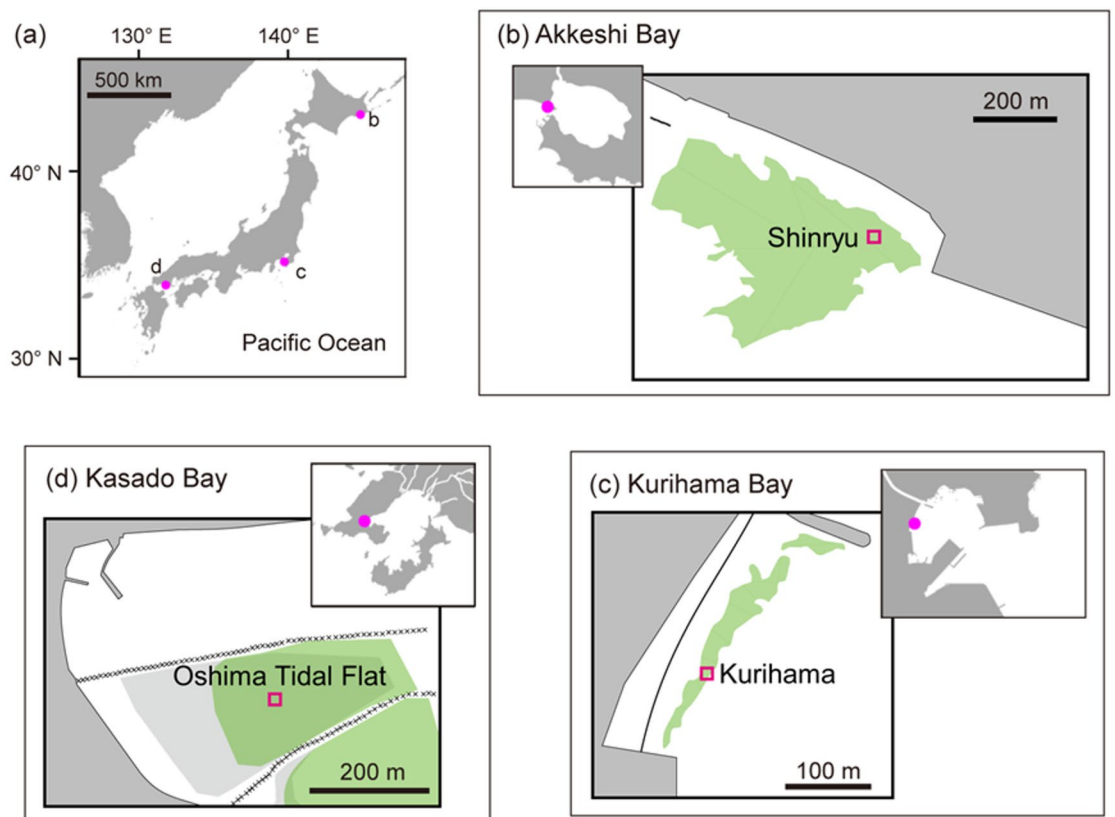


Fig. 1. (a) Locations of study sites (red circles). Detailed maps of the study sites are shown in (b) for *Shinryu* in Akkeshi Bay, (c) for *Kurihama* in Kurihama Bay and (d) for *Oshima Tidal Flat* in Kasado Bay. On the detailed maps, seagrass meadows (green areas) and experimental plot areas (red rectangles) are indicated.

locations with environmental heterogeneity (e.g., many gaps, rocks, and submerged objects) that could interfere with the experiments, and areas near the edges of eelgrass patches.

We established an experimental zone with an average depth of 1.5 m at Shinryu (Fig. 1b). The eelgrass bed consists of sandy and muddy sediment, with a dense meadow extending over areas shallower than 2 m³¹. As the water depth increases, *Z. marina* is replaced by *Z. asiatica*. During the summer, the water temperature in the eelgrass beds typically ranges from 16 to 19 °C and may exceed 20 °C³¹. The salinity in these areas is generally around 30 ± 2 psu³¹. Predators of small invertebrates include a variety of species, such as small fishes and crustaceans^{32,33}. As Reynolds et al.¹¹ conducted a study using the same experimental design at Shinryu and a site in the Akkeshi-ko estuary, we chose the other two experimental sites (i.e., Kurihama and Oshima Tidal Flat) with water conditions close to Shinryu^{34,35} to confirm the reproducibility among the experiments and to enhance the generality of our discussion.

An eelgrass bed at Kurihama extends along a sandy coastline, with water depths ranging from 0.2 to 2.3 m³⁶. We established an experimental zone with an average depth of 1.5 m. To avoid gaps and fragmented patches as much as possible, we excluded the zone near the mouth of the Hirasaku River (Fig. 1c). In summer, the water temperature typically ranges from 21 to 25 °C³⁶, while salinity is usually around 30 psu^{37,38}.

Oshima Tidal Flat is an artificial habitat created using dredged soil from a port project to support the Manila clam *Ruditapes philippinarum*. This habitat consists of three sections separated by submerged masonry breakwaters (Fig. 1d). We established an experimental zone in section II at an average depth of 2.5 m³⁵. *Z. marina* occupies more than 15 ha across the sections in the subtidal zone. The mean temperature and salinity were recorded during our experiment at 23.5 °C and 30.5 psu, respectively.

Prey species collection

Prey species were selected from candidate small invertebrates collected around each eelgrass bed during pre-experimental sampling (Table S1). Because several collection methods were used and their efficiency likely differed among prey types, the numbers of collected individuals were not used to estimate quantitative relative abundance. Instead, we used repeated pre-experimental collections to identify prey species that were consistently encountered across the eelgrass bed and could be obtained in sufficient numbers for replicated tethering trials. Among these candidates, final selection was also based on the feasibility of handling and tethering without severe damage. We focused on experimentally tractable prey groups commonly encountered in eelgrass beds, particularly small crustaceans and shelled gastropods, while excluding taxa unsuitable for tethering, such as soft-bodied polychaetes and shell-less gastropods. Among crustaceans, we focused on small benthic or epifaunal taxa

associated with eelgrass and excluded decapods, which can act as predators, and mysids, which are more mobile and less consistently associated with eelgrass habitats.

Because local prey assemblages differed among eelgrass beds, the prey species used in the tethering trials were not identical across sites. *Ampithoe lacertosa* was the only prey species included at all three sites, whereas *Alaba picta* was included at both temperate sites. Thus, the design was intended to compare locally available prey types among and within major prey groups, while allowing only limited direct cross-site comparisons of the same prey species.

Prey were collected from eelgrass meadows surrounding each experimental zone using dip nets or Hirota's sledge-net (mouth size: 60 cm width and 40 cm height; mesh size: 500 μ m). Individuals used in the experiments were selected from healthy, intact specimens suitable for tethering. We did not target a particular size class, although extremely small or visibly damaged individuals were avoided for practical reasons. Body size was not strictly standardized within or among species.

At Shinryu, the gastropod *Lacuna decorata*, the amphipod *A. lacertosa*, the ischyrocerid amphipod *Jassa marmorata*, and the caprellid amphipod *Caprella penantis* were collected. During pre-experimental sampling in the Shinryu eelgrass bed, *Jassa* spp. and *Caprella* spp. were identified as candidate prey taxa, and subsequent identification confirmed the presence of *J. marmorata* and *C. penantis*. However, targeted collection of sufficient individuals directly from eelgrass was inefficient, likely reflecting low encounter rates within the dense, structurally complex meadow and the difficulty of selectively collecting target species in the presence of closely related species. To avoid pooling congeners and preserve the interpretability of species-level comparisons, additional individuals identified as *J. marmorata* and *C. penantis* were collected from mooring ropes at oyster farming facilities in the Akkeshi-ko estuary³⁹.

At Kurihama, we collected *A. lacertosa*, *A. picta*, and the isopod *Synidotea laevidorsalis*. Prey were maintained in aquaria at the Port and Airport Research Institute.

At Oshima Tidal Flat, we collected *A. lacertosa* and three gastropods, *A. picta*, *Cantharidus japonicus*, and *Mitrella bicincta*. Because no aquarium facility was available near Oshima Tidal Flat, prey were held in mesh bags at the nearest fishing harbor until use in the experiments.

Predation assay

To assess relative survival rates, we conducted 24-hour tethering experiments in three trials at each eelgrass bed during peak productivity periods: late July to early August 2018 in Shinryu, early July 2019 in Kurihama, and late July to early August 2019 in Oshima Tidal Flat. The 24-hour duration was set to capture predator activity across day and night and to include at least one tidal cycle, thereby capturing cumulative predation pressure under natural diurnal and tidal rhythms. We applied the same experimental setup (Prey Tethering Unit [PTU]: Fig. 2) and procedure as Reynolds et al.¹¹. An experimental array comprising 20 plots (10 plots $\times$ 2 lines) was established in a selected zone. Plots were placed in vegetated areas, avoiding unvegetated gaps between eelgrass patches. We also avoided the edges of eelgrass patches because edge effects on predation pressure can be complex¹². Four PTUs were arranged as one plot-level unit to facilitate reliable deployment and retrieval under field conditions, particularly when underwater visibility was low (Fig. 2). The four-PTU unit was adopted because preliminary information indicated that the number of focal prey species required for the planned comparisons would not exceed four at any site. Within each unit, all focal prey species used at that site were represented, and the positions of prey species within the unit were randomized among trials. At sites with fewer than four focal prey species, the remaining PTU position in each unit was assigned to an additional individual of one focal species, with these additional assignments distributed as evenly as possible among species within each trial. Because we could not maintain the experimental array between trials in Kurihama and Oshima Tidal Flat, we set up and removed the arrays and checked eelgrass cover before each trial. We avoided periods of extreme tidal fluctuations and unstable conditions to minimize any unintended environmental impacts.

Each PTU consisted of a 10 cm fluorocarbon fishing line (diam.: 0.113 mm, Berkley, Pure Fishing Japan, Tokyo) and a transparent acrylic rod (diam.: 8 mm, length: 60 cm). Prey were carefully attached to the end of the fishing line using a small amount of liquid glue (LOCTITE, Henkel Japan, Yokohama) without damaging vital structures (e.g., mouth, gills, and joints). Before gluing, we measured the body size of each prey (Table 1). More than 12 h prior to each trial, prey were glued, and the PTUs were stored in large indoor aquariums or, at Oshima Tidal Flat, in mesh-covered buckets deployed in the sea to monitor for weakening, mortality, detachment, or abnormal behavior before deployment. Prey exhibiting any abnormalities were excluded from field placement. Preliminary aquarium trials confirmed that prey did not naturally detach from PTUs under water flows ranging from 5 to 15 cm s⁻¹ over 24 h.

Predation status was first checked underwater during PTU retrieval and then rechecked after recovery either on board or on land. Surviving prey were classified as "unconsumed", whereas missing prey were classified as "consumed" only when the PTU remained intact and there was no evidence of non-predatory attachment failure. Dead prey exhibiting predation marks (Fig. S1) were also classified as "consumed". Because crustaceans were observed molting after attachment to the PTU (Fig. S2), individuals with only their molted exoskeleton remaining were not classified as "consumed". Although the rate of false positives due to attachment failure was not independently quantified, pre-deployment checks and conservative scoring rules were implemented to minimize this source of misclassification.

All experimental procedures involved only small marine invertebrates and complied with institutional and national guidelines for the ethical treatment of invertebrates. No specific ethical approval was required under Japanese law. Sample collection and use were conducted in accordance with Japanese regulations, the Convention on Biological Diversity, and the Nagoya Protocol. Experimental design and reporting followed the principles of transparency and reproducibility recommended in the ARRIVE guidelines.

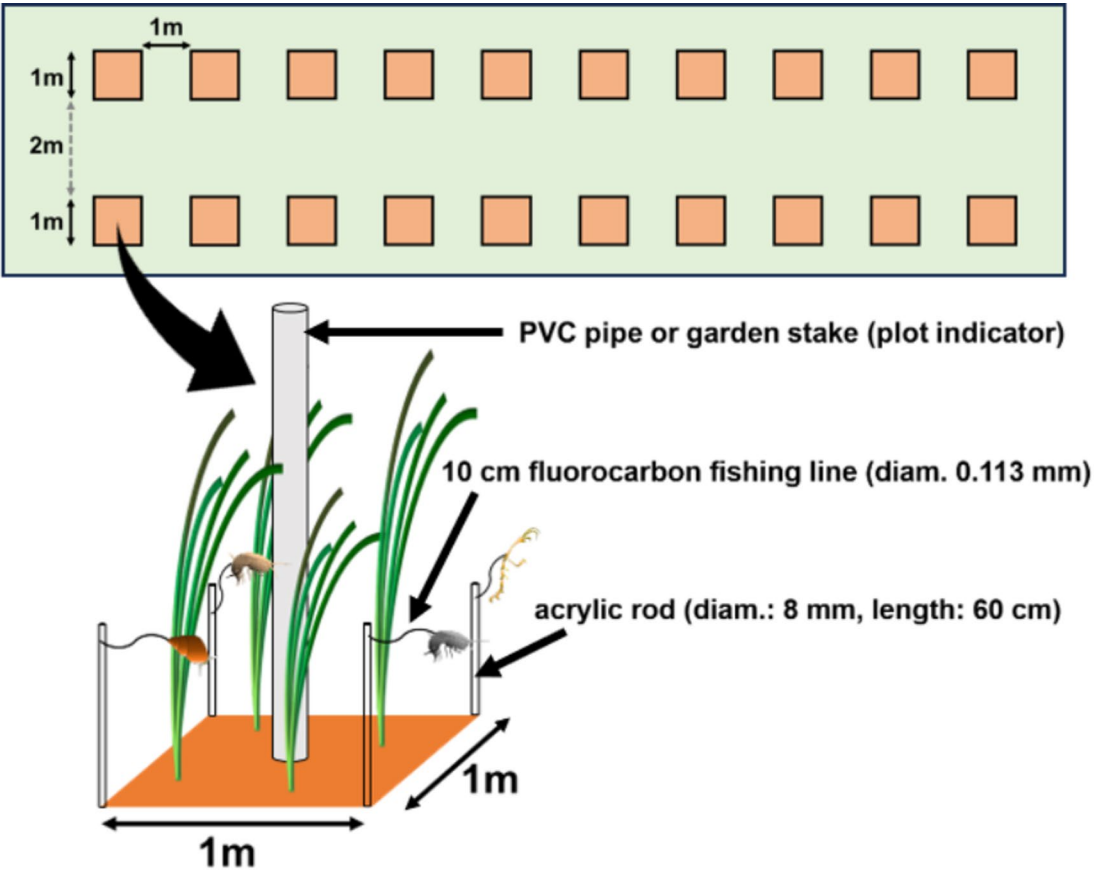


Fig. 2. Experimental plot arrangement and details.

Species	Body size (mm: Mean ± SD)	Body shape	Body defense	Escape response
Non-caprellid amphipod				
<i>Ampithoe lacertosa</i>	7.4 ± 1.0 (Shinryu) 7.4 ± 1.5 (Kurihama) 6.7 ± 0.7 (Oshima Tidal Flat)	Round	Soft Exoskeleton	Swimming
<i>Jassa marmorata</i>	5.5 ± 1.0 (Shinryu)	Round	Hard Exoskeleton	Clinging/Swimming
Caprellid amphipod				
<i>Caprella penantis</i>	15.3 ± 2.3 (Shinryu)	Elongated	Soft Exoskeleton	Clinging
Isopod				
<i>Synidotea laevidorsalis</i>	10.2 ± 2.3 (Kurihama)	Elongated	Hard Exoskeleton	Clinging/Swimming
Gastropod				
<i>Alaba picta</i>	4.7 ± 0.7 (Kurihama) 5.1 ± 0.4 (Oshima Tidal Flat)	Round	Soft Shell	Crawling/Falling
<i>Cantharidus japonicus</i>	5.5 ± 0.6 (Oshima Tidal Flat)	Round	Hard Shell	Crawling/Falling
<i>Lacuna decorata</i>	4.1 ± 0.8 (Shinryu)	Round	Hard Shell	Crawling/Falling
<i>Mitrella bicincta</i>	10.5 ± 0.6 (Oshima Tidal Flat)	Elongated	Hard Shell	Crawling/Falling

Table 1. Functional traits of employed prey species. Note that escape responses are reactions to external stimuli under the constrained state enforced by tethering.

Prey trait measurement

We categorized the morphological traits and responses to external stimuli (simulating predator encounters) of each prey species in the laboratory (Table 1). Although these evaluation results were not included in the statistical analyses, we used them as reference information to interpret differences in predation pressure detected between taxonomic groups or species.

Morphological traits were categorized based on body size, shape, and defense structure. For body shape, species curling up in response to external stimuli and species with body widths approximately equal to their body

lengths were categorized as “Round”. In contrast, species with body widths less than half of their body lengths were categorized as “Elongated”. Regarding the defense structure, we differentiated between “exoskeleton” and “shell” and further categorized them as “Soft” or “Hard” based on relative hardness, as determined by pinching with forceps. If an exoskeleton was deformed or the outer lips of a shell were easily chipped, we categorized it as “Soft”.

For stimulus-induced responses, we categorized each species ($N=20$ individuals) according to their escape responses when stimulated with forceps on eelgrass blades under natural conditions as follows: species immediately swimming frantically were categorized as “Swimming”, species firmly clinging to eelgrass blades as “Clinging”, species crawling on the blades as “Crawling”, and species falling from the blades as “Falling” (Table 1).

Data analysis

We conducted statistical analyses to evaluate whether relative predation pressure differed between major prey groups (crustaceans and gastropods) and to assess additional variation among species within these groups. All analyses were performed using *R* version 4.4.3⁴⁰.

Relative predation pressure was analyzed as a binary response variable, indicating whether each prey item was consumed within 24 h. We fitted binomial generalized linear models (GLMs) with a logit link to model the log-odds of predation. Candidate fixed effects included major prey group, species identity, and body size. Body size was included as a general biological characteristic that could influence predation pressure across taxa and species. Other functional traits were primarily used to aid the interpretation of species-level patterns rather than as main explanatory variables. This approach was adopted because the number of prey species was limited relative to the number of trait dimensions considered, several trait states were represented by only a small number of species, and some behavioral traits may have been constrained under tethering conditions. Individuals removed prior to deployment and those with ambiguous predation status, such as likely molting events, were excluded from all analyses.

Although site and trial effects were not the primary focus of the study, observations were structured by site, trial, and plot-level deployment units. Because trials were conducted separately within each eelgrass bed and did not follow a common temporal sequence across sites, site- and trial-level heterogeneity was represented using a combined site-trial term in all biologically structured candidate models. This term was treated as a fixed effect, as the sites and trial occasions were specific levels defined by the experimental design rather than random samples from broader populations. The combined site-trial term was intended to account for broad differences among eelgrass beds and trials, including unmeasured environmental heterogeneity and variation in predator assemblages or activity.

Individual prey items served as the response units because predation status was scored separately for each PTU. Plot-level structure was primarily addressed through the experimental design. Each plot-level unit included all focal prey species used at that site, with the positions of prey species within the unit randomized among trials. Additional PTU assignments at sites with fewer than four focal prey species were distributed as evenly as possible among species within each trial. This arrangement interspersed prey species within the same local plot environment and reduced the likelihood that plot-scale heterogeneity would generate apparent species differences. Plot identity was therefore not included as an additional model term, and any remaining plot-level variation was treated as residual variation. Inference focused on relative differences among prey groups and species after accounting for site-trial-level heterogeneity. Taxonomic group and species identity were not included simultaneously in the same model because species were nested within taxonomic groups.

Variation in predation pressure was evaluated by comparing candidate models using Akaike's Information Criterion corrected for small sample size (AICc)⁴¹. The candidate models included an intercept-only null model, a baseline model with the combined site-trial term, and biologically structured models incorporating taxonomic group, species identity, body size, or their permitted combinations. AICc values, Δ AICc, and model weights were calculated with the *aictab* function in the *AICcmodavg* package⁴².

For models including taxonomic group or species identity that were retained for interpretation after model comparison, back-transformed estimated marginal means and 95% confidence intervals were calculated on the response scale using the *emmeans* package⁴³. For models with the combined site-trial term, estimates were obtained by averaging across its levels. For models including body size, estimates were calculated with body size fixed at its mean value.

To complement the model-based analyses, functional similarity among prey species was summarized using a species-by-trait matrix that included mean body size, body shape, body defense, and escape response (see also Table 1). As the trait dataset contained both continuous and categorical variables, functional distances among species were calculated using Gower distance, and species were ordinated using principal coordinates analysis (PCoA). This exploratory analysis was conducted to interpret both taxonomic- and species-level patterns in predation vulnerability. Given the limited number of prey species relative to the number and structure of traits considered, and the potential constraint of some behavioral traits under tethering conditions, this ordination was used as a descriptive aid rather than as a primary inferential framework.

As supplementary analyses, we fitted separate binomial GLMs for each site to describe interspecific contrasts within sites, with species identity as the primary explanatory variable and trial as a fixed effect. The trial term was included to account for variation among repeated deployments within each site. Species effects in these within-site models were evaluated using Type II Wald χ^2 tests implemented in the *Anova* function of the *car* package⁴⁴. For further examination of species-level contrasts, pairwise comparisons were conducted using estimated marginal means with false discovery rate adjustment for multiple testing⁴⁵. Due to incomplete replication across trials at Oshima Tidal Flat, *Amphithoe lacertosa* was excluded from the site-specific interspecific comparison for that site. Because *A. lacertosa* was the only prey species deployed across all three sites, we also fitted a cross-site binomial GLM for this species with the combined site-trial term and body size as fixed effects.

Model	k	LogLik	AICc	Δ AICc	ω_i
Site-trial + Species + Body size	17	-347.02	728.95	0.00	0.55
Site-trial + Species	16	-348.28	729.38	0.42	0.45
Site-trial + Taxon + Body size	11	-382.48	787.36	58.40	0.00
Site-trial + Taxon	10	-391.85	804.03	75.08	0.00
Site-trial	9	-447.01	912.28	183.32	0.00
Site-trial + Body size	10	-446.20	912.72	183.76	0.00
Null (Intercept-only)	1	-467.59	937.18	208.22	0.00

Table 2. Results of model selection based on AICc to identify factors related to variation in predation pressure. The number of parameters (k), log-likelihood (LogLik), corrected Akaike information criterion (AICc), difference in AICc between the candidate models and the best-supported model (Δ AICc), and model weights (ω_i) are presented.

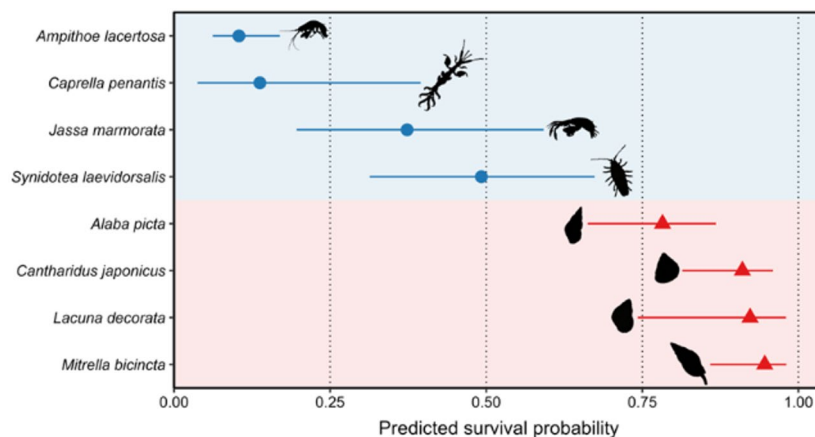


Fig. 3. Back-transformed estimated marginal means ($\pm$ 95% confidence intervals) of survival probability for each prey species from the best-supported binomial GLM, calculated by averaging over the remaining model terms.

This supplementary analysis provided the only direct species-level comparison across all eelgrass beds and was used to evaluate spatial consistency in relative predation pressure for this species.

Results

Model comparison strongly supported models including species identity (Table 2). The most strongly supported model included site-trial, species identity, and body size, whereas the next most strongly supported model included site-trial and species identity. In contrast, models including only major prey groups received substantially less support, and models lacking species identity performed poorly overall. These results indicate that, within the prey species examined, models including species identity captured variation in predation pressure more effectively than models based only on broad taxonomic group, while body size contributed only limited additional support.

Back-transformed estimated marginal means from the best-supported model showed clear variation in survival probability among prey species (Fig. 3). Survival probabilities were generally lower in crustaceans than in gastropods, indicating an overall contrast between the two major prey groups. At the same time, substantial variation was evident among crustaceans, with consistently low survival in *Amphithoe lacertosa* and lower to intermediate survival in *Caprella penantis*, *Jassa marmorata*, and *Synidotea laevidorsalis*, depending on the model and site-specific comparison. In contrast, gastropods generally showed higher survival probabilities than crustaceans, although some interspecific variation was also observed within gastropods. The corresponding estimates from the second best-supported model showed the same overall pattern (Fig. S3). Site-specific GLMs generally supported the presence of interspecific variation in predation pressure within individual eelgrass beds (Table S2; Fig. S4). In a supplementary cross-site GLM for *A. lacertosa*, the only prey species deployed at all three sites, neither the combined site-trial term ($\chi^2 = 8.29$, $df = 6$, $P = 0.22$) nor body size ($\chi^2 = 0.67$, $df = 1$, $P = 0.41$) showed a significant effect. Thus, within the limits of this single-species cross-site comparison, no clear site- or size-dependent variation in predation pressure was detected for *A. lacertosa*. Observed counts and raw survival proportions are provided in Table S3 for reference.

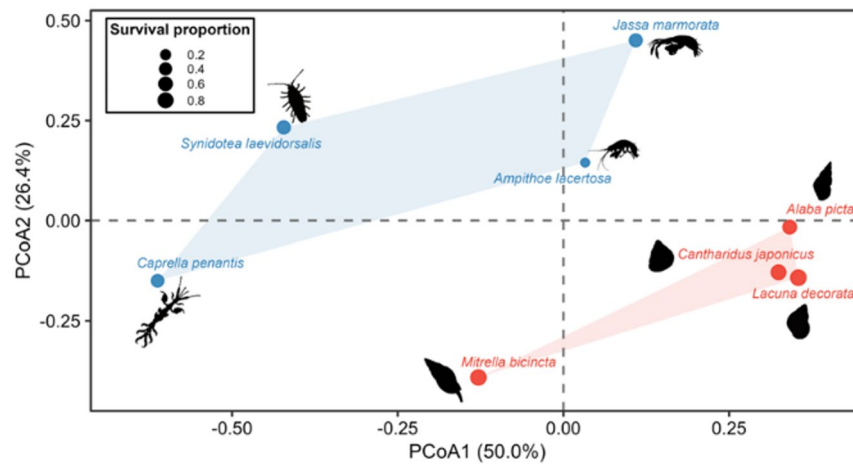


Fig. 4. PCoA plot illustrating prey species based on functional similarity. Ordination is based on Gower distances calculated from body size, body shape, body defense, and escape response. Each point corresponds to a single species, with point size indicating the mean survival proportion observed in tethering trials. Colors distinguish major prey groups, specifically crustaceans and gastropods. Convex hulls outline the functional trait space occupied by each taxonomic group within the two-dimensional ordination.

An exploratory trait-based analysis using ordination of prey species in multivariate trait space, based on Gower distance and principal coordinates analysis (PCoA), demonstrated a clear separation between crustaceans and gastropods, while also revealing substantial variation within each group (Fig. 4). The first two axes accounted for 76.4% of the total trait variation (PCoA1: 50.0%; PCoA2: 26.4%). Crustacean species occupied a broader region of trait space than gastropods, suggesting greater functional heterogeneity within this group. This finding aligns with the greater within-group variation in survival probability observed among crustaceans in the tethering experiment.

Although body size distributions differed both within and among species (Fig. S5), a linear model with species identity as the explanatory variable showed that species identity accounted for a substantial proportion of the total variation in body size (adjusted $R^2 = 0.86$, $F(7, 727) = 625.85$, $P = 0.00$). This indicates that much of the observed size variation was aligned with species-level differences, providing context for the limited improvement in model support when body size was added to the species-based predation model.

Discussion

In this study, we examined the taxonomic scale at which predation vulnerability is structured among small invertebrates inhabiting eelgrass beds, using standardized tethering experiments at three sites with contrasting environmental conditions and predator assemblages. The findings indicate that, while broad taxonomic contrasts are apparent, predation pressure is more strongly structured at finer taxonomic resolutions. Crustaceans generally experienced higher relative predation pressure than gastropods, consistent with previous eelgrass studies, but substantial variation was also observed among crustacean species. Therefore, coarse taxonomic categories captured only part of the biologically relevant structure in prey vulnerability. These results suggest that interpreting prey availability to consumers in eelgrass beds requires consideration of both differences between major prey groups and finer-scale variation within those groups. Although the number of focal species examined in this study is limited, the findings demonstrate that increasing taxonomic resolution can substantially alter the characterization of relative prey vulnerability in eelgrass beds.

Our results complement previous spatial studies of predation by showing that, within each eelgrass bed, locally available prey species differed in vulnerability, and that this species-level structure was apparent across sites with contrasting prey assemblages and predator communities. Broad-scale and comparative studies have shown that predation pressure in seagrass systems varies substantially across space, reflecting regional gradients, local habitat structure, patch context, and predator community composition^{9–12}. These studies provide an important spatial framework for understanding where predation pressure is likely to be strong, while the prey-species composition associated with that variation has received less direct attention. By comparing locally available prey species across sites that differed in environmental setting, predator assemblage, and prey composition, our study shows that small invertebrate taxa within eelgrass beds are not interchangeable as prey resources. Spatially, eelgrass beds with similar broad prey composition may differ in prey availability to consumers if the species composing those groups differ among local communities. Functionally, the contribution of small invertebrates to trophic transfer may depend not only on abundance or broad taxonomic identity, but also on species-specific vulnerability. This prey-identity perspective is consistent with experimental evidence that top-down effects in vegetated aquatic habitats often depend on which prey species or functional groups are both food-web important and vulnerable to dominant predators^{25,46–48}. By directly comparing relative predation pressure among tethered prey species, our study shows that substantial heterogeneity in prey vulnerability exists within crustaceans, with potential consequences for trophic transfer to higher consumers in eelgrass beds.

At the same time, the prey-identity perspective developed here should be interpreted within the limits of the species set used at each site. Because prey species were only partially shared across eelgrass beds, our multispecies comparisons do not constitute a fully crossed test of identical prey-species sets across sites. Accordingly, cross-site patterns should be interpreted primarily as comparisons among locally available prey assemblages, rather than as species-specific responses replicated across all sites. Nevertheless, because multiple species were included within at least one major prey group at each site, the design still allowed us to evaluate whether broad prey-group categories were sufficient to characterize local variation in relative prey vulnerability.

The mechanisms underlying this species-level structure can be cautiously framed by considering that tethering outcomes integrate multiple stages of the predation process, including detection, encounter, attack, capture, handling, and consumption²⁸. At early stages, traits related to conspicuousness and microhabitat use, such as movement, body form, crypsis, and association with eelgrass surfaces, may alter predator perception and encounter rates^{23,24,49}. At later stages, traits related to escape, attachment, and defense, including escape mode, tube-building, exoskeleton or shell properties, and structural or chemical defenses, may influence whether an encountered prey item is successfully captured and consumed^{28,50,51}. In this framework, the consistently high predation pressure on *Ampithoe lacertosa* may partly reflect its conspicuous escape-related responses. However, because this was the only prey species assigned to the swimming-response category, its vulnerability cannot be attributed specifically to swimming behavior and may also reflect other species-specific attributes. Variation among the other crustaceans, including *Caprella penantis*, *Jassa marmorata*, and *Synidotea laevidorsalis*, may reflect different combinations of microhabitat association, attachment or clinging behavior, refuge use, morphology, and defensive responses, rather than a simple contrast between caprellid and non-caprellid forms^{51–53}. Thus, species identity should be interpreted here as an integrative indicator of prey accessibility or susceptibility, rather than as evidence for any single behavioral, morphological, or chemical mechanism.

Some of the amphipod species examined in this study, including *A. lacertosa* and *J. marmorata*, are known to exhibit tube-building or other substrate-associated behaviors under natural conditions^{54–56}. Because microhabitat use and refuge-associated behavior can influence prey exposure and vulnerability⁵¹, our tethering protocol may not have fully captured natural differences among species that vary in tube-building or substrate-associated behavior. Our results should therefore be interpreted as relative vulnerability under a standardized tethering protocol, rather than as exact estimates of natural predation risk for each species. At the same time, the experiment still detected clear interspecific differences under those standardized conditions, suggesting that species-level differences in prey accessibility or susceptibility to predators remained biologically meaningful even when access to preferred microhabitats was constrained.

In contrast to crustaceans, the gastropod species examined exhibited relatively low overall predation pressure, although detectable interspecific differences persisted. Predator collections and stomach-content data suggest that predation on gastropods was likely associated with shell-crushing predators, such as pufferfish, or a limited number of abundant fish species (see Tables S4 and S5). This indicates that gastropods are not an important, universal prey resource for most predatory fish in eelgrass beds, but rather represent a specialized food source depending on the composition of the predator community. Within the limited range of interspecific comparisons available in this study, the magnitude of differences in predation pressure among gastropod species was smaller than that observed among crustaceans and could not be readily explained by simple shell morphological traits, such as shell shape or thickness (measured here as hardness). This pattern is consistent with previous studies suggesting that, in structurally complex habitats, the effects of shell morphology on predation risk are relatively weak or highly context-dependent^{57,58}. Although the number of gastropod species examined here was limited, the range of predation pressure observed in this study was comparable to that reported in previous eelgrass tethering experiments using different dominant prey species¹¹. Considered together, the gastropod prey examined here and in Reynolds et al.¹¹ may represent an important part of the common phylogenetic and functional variation among blade-associated epifaunal gastropods in *Zostera marina*. Our results may therefore reflect a recurring pattern of predator–prey interactions among common epifaunal gastropods in eelgrass beds. Nevertheless, some functionally distinct taxa remain poorly tested, including shell-less gastropods such as sea slugs, in which chemical defense is often important^{59,60}, and limpet-like taxa, for which anti-predator defense may depend not only on shell form but also on chemical deterrence^{61,62}. Expanding prey coverage to include such groups will therefore be important for developing a more comprehensive understanding of prey vulnerability in eelgrass beds.

Body size had only limited explanatory value relative to species identity. In model comparison, adding body size improved support only slightly beyond models including species identity, and in the cross-site analysis of *Ampithoe lacertosa*, body size showed no detectable effect. One likely explanation is that, although the prey species examined here differed in size, they still largely fell within a size range accessible to local fish predators. The predatory fishes recorded in these eelgrass beds ranged from approximately 20 to 200 mm in body length (Table S5), and prey up to about 20% of predator body length are generally known to be consumed^{63,64}. Under such conditions, strong size-based selection may have been limited within the prey domain represented here, even if body size is often important across broader prey assemblages. In addition, much of the observed size variation was aligned with species-level differences rather than acting as an independent axis of vulnerability. Thus, body size alone was insufficient to explain the observed structure in predation pressure, and other correlated aspects of prey biology likely contributed.

The present study focused on variation among major prey groups and among species within those groups, but the same logic may also extend to variation within species. Species-level comparisons can still obscure predictable variation among individuals, life stages, or local populations when such variation affects traits involved in predator–prey interactions^{65,66}. Within a prey species, ontogenetic or growth-related changes may alter prey size, behavior, habitat use, and defensive traits, thereby changing how individuals are exposed to, captured by, or handled by predators⁶⁷. In addition, environmentally induced plasticity may generate similar

variation in morphology or behavior in response to local conditions, including predation risk or habitat structure⁶⁸. Although these sources of within-species variation were not explicitly measured here, they may have contributed to unexplained residual variation, or, when consistently associated with particular species or local populations, to apparent species-level differences in vulnerability. Future studies combining tethering experiments with measurements of individual traits, life stage, environmental history, and population origin would help clarify how predation vulnerability is structured across individual, population, species, and broader prey-group scales.

Conclusions

Our study shows that prey vulnerability in eelgrass-associated small invertebrate assemblages is structured at a finer, species-level scale within major prey groups, particularly among crustaceans, and is not fully characterized by the broad contrast between crustaceans and gastropods alone. By comparing locally available prey species at three eelgrass beds with contrasting environmental conditions and predator assemblages, we found that biologically meaningful variation in predation pressure can occur within coarse taxonomic categories. This finding indicates that broad prey-group categories alone may overlook important variation in relative prey vulnerability, and that species-level resolution can improve local interpretations of prey availability to consumers.

The species-level differences detected here were not explained solely by body size. Rather, they likely reflect multiple correlated aspects of prey biology, including morphology, behavior, microhabitat association, detectability, attachment ability, and defensive traits, which may influence different stages of predator–prey interactions^{23,24,28}. Because individual traits were not experimentally isolated, species identity should be interpreted as an integrative indicator of prey accessibility or susceptibility rather than as evidence for a single causal mechanism. At the same time, tethering experiments characterize relative predation pressure rather than realized trophic transfer or ecosystem function itself. Actual trophic transfer will also depend on predator assemblages, predator preferences, prey abundance, habitat structure, and environmental conditions. Integrating these processes with species-level and potential intraspecific variation in prey vulnerability will therefore be important for clarifying how prey assemblage composition contributes to the trophic role of eelgrass ecosystems.

Data availability

Summary data for the predation assay are provided in Supplementary Table S3. Additional datasets from this study are available from the corresponding author upon reasonable request.

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Author contributions

KM and SH conceived the ideas and designed the experiment; KM and SH collected samples; KM analyzed the data; KM and SH interpreted the data; KM wrote the first draft of the manuscript. Both authors contributed critically to the revision of the manuscript.

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Competing interests

The authors declare no competing interests.

Additional information

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