



Stressor fluctuations alter mechanisms of seagrass community responses relative to static stressors

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ABSTRACT

Ecosystems are increasingly affected by multiple anthropogenic stressors that contribute to habitat degradation and loss. Natural ecosystems are highly dynamic, yet multiple stressor experiments often ignore variability in stressor intensity and do not consider how effects could be mediated across trophic levels, with implications for models that underpin stressor management. Here, we investigated the in situ effects of changes in stressor intensity (i.e., fluctuations) and synchronicity (i.e., timing of fluctuations) on a seagrass community, applying the stressors reduced light and physical disturbance to the sediment. We used structural equation models (SEMs) to identify causal effects of dynamic multiple stressors on seagrass shoot density and leaf surface area, and abundance of associated crustaceans. Responses depended on whether stressor intensities fluctuated or remained static. Relative to static stressor exposure at the end of the experiment, shoot density, leaf surface area, and crustacean abundance all declined under in-phase (synchronous; 17, 33, and 30 % less, respectively) and out-of-phase (asynchronous; 11, 28, and 39 % less, respectively) fluctuating treatments. Static treatment increased seagrass leaf surface area and crustacean abundance relative to the control group. We hypothesised that crustacean responses are mediated by changes in seagrass; however, causal analysis found only weak evidence for a mediation effect via leaf surface area. Changes in crustacean abundance, therefore, were primarily a direct response to stressors. Our results suggest that the mechanisms underpinning stress responses change when stressors fluctuate. For instance, increased leaf surface area under static stress could be caused by seagrass acclimating to low light, whereas no response under fluctuating stressors suggests an acclimation response was not triggered. The SEMs also revealed that community responses to the stressors can be independent of one another. Therefore, models based on static experiments may be representing ecological mechanisms not observed in natural ecosystems, and underestimating the impacts of stressors on ecosystems.

1. Introduction

Managing multiple stressors is increasingly important in the Anthropocene as habitats continue to be lost and degraded due to interactive stressors (Millennium Ecosystem Assessment, 2005; Geldmann et al., 2014; Halpern et al., 2019). To better inform management actions, it is imperative to advance our understanding of how multiple stressors impact ecosystems in a real-world context (Côté et al., 2016; Orr et al., 2020; Ostrowski et al., 2022). Much of our current understanding is derived from highly controlled laboratory experiments that exclude natural ecological complexities, such as abiotic (e.g., tidal cycles) and biotic (e.g., herbivory) processes (Gunderson et al., 2016; Orr et al., 2020; Ostrowski et al., 2021). The effects of multiple stressors, however, are highly variable across ecological contexts (Kefford et al.,

2023), and results obtained from the field can contradict findings from laboratory experiments. For example, metal uptake and antioxidative responses of bivalves exposed to contaminated sediments is lower in the laboratory than in the field, likely because of multiple exposure pathways observed in natural environments (Marasinghe Wadige et al., 2017). Additionally, the loss of kelp biomass under reduced light conditions is faster in the field, potentially due to hydrodynamics or variable microbial activity (Frontier et al., 2021; Frontier et al., 2022). Despite the complexity of natural environments, management frameworks still simplify this complexity due to data and knowledge constraints, potentially limiting the accuracy of analyses and effectiveness of management activities (e.g., cumulative stressor maps assume constant stressor intensity over time; Halpern and Fujita, 2013).

Transferring knowledge of stressor effects observed in highly

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controlled settings to predict impacts on ecological communities poses challenges. First, stressor intensity can vary over time in the environment (Gunderson et al., 2016), and variations in stressor intensity (i.e., fluctuations) and synchronicity (i.e., timing of fluctuations) can alter both stressor interactions and biological responses (e.g., Verheyen and Stoks, 2020; Carrier-Belleau et al., 2021; Ostrowski et al., 2022). However, in typical lab experiments, multiple stressors are often introduced simultaneously and evaluated only under static (i.e., constant) conditions. Second, outcomes from measuring stressor responses at the physiological and individual levels in the laboratory might not reflect impacts observed within ecosystems if stressor interactions and responses differ across higher levels of biological organisation (Galic et al., 2018; Gissi et al., 2020). Species interactions within communities can ameliorate (e.g., Kour et al., 2020) or amplify (e.g., Bray et al., 2019) stressor effects, altering stressor interactions and impacts (Kroeker and Sanford, 2022; Zhou and Wang, 2023). Third, stressors can indirectly affect biological responses, such as when effects are mediated by biotic interactions (e.g., herbivory) and environmental variability (Bruder et al., 2017; Uriarte et al., 2018; Benkwitt et al., 2020). Relying on how stressors affect lower response levels (e.g., plant physiology) in lab experiments to inform predictions of stressor effects at the ecosystem scale could therefore lead to over or underestimation of impacts (Orr et al., 2021).

A key knowledge gap in multiple stressor research is how fluctuating stressors – that more closely reflect reality – influence biological responses relative to static stressors, and how exposure to fluctuating stressors impacts ecological communities in the field. Despite the importance of conducting field experiments with stressor intensities that better reflect real-world conditions, few studies adopt this approach. This is likely due to the limitation posed by increased complexity in experimental designs, such as increased number of treatments that make experiments costly, unmanageable, and riskier (Boyd et al., 2018). However, to accurately understand stressor impacts to ecosystems and improve environmental management, we need to increase our efforts in the field, adopt clever and novel experimental designs that incorporate variability in stressor intensity and synchronicity, and measure responses across multiple levels of biological organisation within ecological communities (Gunderson et al., 2016; Boyd et al., 2018; Bruder et al., 2019; Ostrowski et al., 2022).

Here, we evaluated the in situ effects of variable stressor intensity and synchronicity on a seagrass (*Zostera muelleri*) community. Across three variations in stressor intensity and synchronicity, we tested the combined effects of reduced light paired with physical disturbance to the sediment on a seagrass meadow and the associated epifaunal crustacean community. Poor water quality that reduces light penetration (e.g., increased turbidity and eutrophication; Lefcheck et al., 2017) is among the greatest threats to seagrasses (Griffiths et al., 2020; Turschwell et al., 2021). Physical disturbance to the sediment, such as that imposed by dredging operations, commercial bivalve harvesting, and extreme storm events, also results in seagrass loss (e.g., Grech et al., 2012; Ferriss et al., 2019). We aimed to determine how biological responses to multiple stressors differed across variations in stressor intensity and synchronicity in situ, and identify the causal effects by which combined physical disturbance and reduced light availability affect seagrass communities over time. We used structural causal modelling (SCM) and structural equation models (SEMs) to identify both direct and mediation (i.e., indirect) treatment effects within the seagrass community, and generalised additive models (GAMs) to identify total treatment effects on individual responses. SEMs can reveal complex ecological relationships and mechanistic effects of multiple stressors that univariate analyses cannot (Adams, 2005; Lefcheck, 2016). Ultimately, our results will provide evidence for the importance of incorporating environmentally relevant changes in stressor intensity and synchronicity in multiple stressor experimental designs.

2. Methods

2.1. Experimental design

We conducted a manipulative field experiment where we evaluated the combined effects of reduced light availability and physical disturbance to the sediment within an estuarine subtidal seagrass meadow in southeast Queensland, Australia (−28.109708, 153.449075). We established open experimental plots (60 × 60 cm plots, 0.36 m²; $N = 24$) in a randomised block design within the meadow. A single replicate of each of four treatments (see Section 2.1.1) was randomly allocated within each of the six experimental blocks (Fig. 1). All plots were placed 2 m apart with each block separated by 5 m across the entire seagrass meadow to control for potential variability of environmental conditions and responses to stressors based on plot location (Fig. 1). We placed PVC plot frames 0.5 m above the seagrass canopy, and staked them into the sediment. We cut the seagrass rhizomes surrounding the edge of the plots (15 cm deep) prior to the start of the experiment to prevent the transfer of sugars via rhizomes from shoots outside of experimental plots, which can mitigate light stress effects (Fitzpatrick and Kirkman, 1995). We monitored light levels throughout the experiment (HOBO Pendant MX Temp/Light; Onset Computer Corporation, United States) and to manipulate light levels within each plot, we created a canopy using a gradient of percent UV-blocking shade cloth (30 to 70 % UV light penetration). Physical disturbance to the sediment was applied using hand trowels to till the sediment (i.e., “digging”) in random locations within each plot. Although we acknowledge the benefits of also assessing single stressor effects under variable stressor intensity and synchronicity, our intention was to evaluate the combined effects of our target stressors rather than to identify the type of interaction between the stressors and, therefore, individual stressor effects were not assessed. This “collapsed” design allowed us to maintain appropriate replicability of experimental treatments while increasing complexity in stressor delivery methods.

2.1.1. Variable stressor intensity and synchronicity

Physical disturbance and light reduction were applied across three variations in stressor intensity and synchronicity according to Ostrowski et al. (2022). Experimental treatments were static-static, in-phase, and out-of-phase stressor introduction methods, and we included a control where no stressors were applied (Fig. 1). Stressors were manipulated weekly at low tide to maintain target treatment levels. For static treatments, both the level of physical disturbance (i.e., number of holes dug; applied three times weekly, approximately every 48 h) and light reduction levels remained constant throughout the experiment. A strictly static physical disturbance treatment is not practicable (e.g., constant digging), so our treatment reflects a regime of frequent, unchanging disturbance intensities rather than the varying disturbance intensities of fluctuating treatments. In-phase and out-of-phase treatments consisted of weekly stressor fluctuations alternating between high and low stressor intensities to achieve average stressor levels equivalent to those applied under static treatments. In-phase treatments consisted of both physical disturbance (applied three times weekly) and light reduction stressors fluctuating synchronously, where both stressors alternated weekly between high and low stressor intensities simultaneously (Fig. 1). Conversely, out-of-phase treatments consisted of weekly asynchronous stressor fluctuations, where high physical disturbance (applied three times weekly) was paired with low light reduction, followed by the reverse (Fig. 1). The control consisted of exposure to full light (100 % of ambient light) without physical disturbance to the sediment, to ensure our assessment of stressor impacts was not influenced by additional factors. The entire experiment was conducted across six weeks.

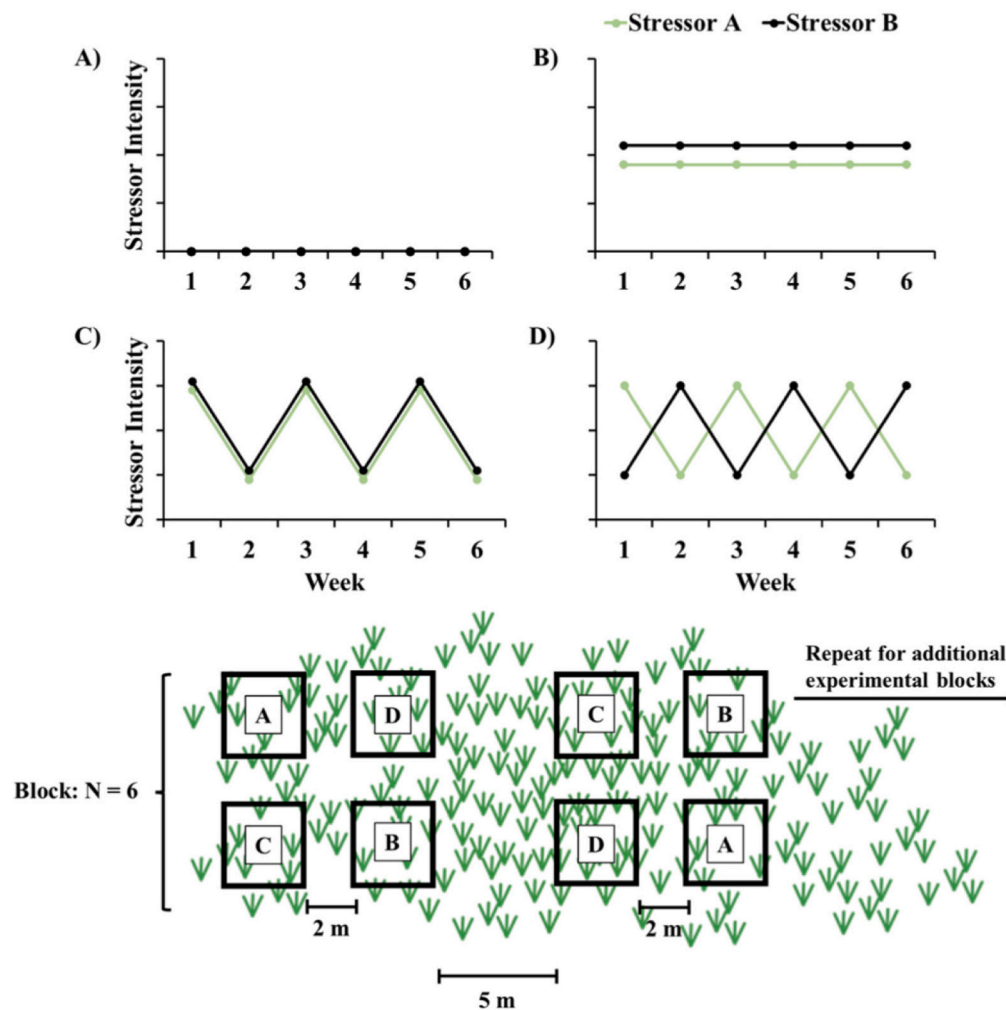


Fig. 1. Top: The treatments tested incorporating variation in stressor intensity and synchronicity including control (A), static-static (B), in-phase (C), and out-of-phase (D) methods of stressor introduction. Bottom: The (partial) experimental set-up of treatment plots within the seagrass meadow showing two of the six experimental blocks. Each treatment was replicated six times and experimental plots were arranged in randomised block design with each treatment represented once within each experimental block. Letters in the experimental plots correspond with the type of treatment tested indicated in the top figure.

2.1.2. Physical disturbance

To apply the physical disturbance stressor, a quadrat (30 × 30 cm) was placed at the center of each plot to create two sections: an inner (i.e., the quadrat) where shoot density was repeatedly counted, and an outer (i.e., the rest of the plot), where destructive seagrass collection methods occurred (thus avoiding disrupting subsequent density metrics). For static treatments, we tilled the sediment to a depth of 3 cm below the sediment surface in six places using a 7 cm wide trowel; two holes randomly within the quadrat, and one randomly along each edge of the plot outside of the quadrat ('medium disturbance'). For fluctuating treatments under high physical disturbance, we dug ten holes to a depth of 3 cm; two randomly within the quadrat and two randomly along each plot edge outside of the quadrat. Under low physical disturbance, we dug two holes; one randomly within the quadrat and one randomly along any plot edge outside of the quadrat.

2.1.3. Light reduction

Our average target light level across treatments was 25 % of total control light conditions (i.e., 75 % light reduction). We used a gradient of UV-blocking shade cloth to create a canopy to achieve target light levels. We arranged the shade canopy to the edge of the experimental plots, and avoided sample collections from the outer edge of plots (within 5 cm from edge) to avoid potential edge effects. For static treatments, we maintained 25 % of light relative to the control (mean ± SD; 23.07 ± 11.1 % of control light). For fluctuating treatments, we

targeted an average of 25 % of control light by alternating weekly between high intensity light stress (target 100 % reduction; impermeable tarpaulin) and low intensity light stress (50 % reduction; mean ± SD for in-phase = 20.87 ± 11.4 %, out-of-phase = 24.68 ± 12.26 % of control light). The intense light reduction under fluctuating treatments tested here is representative of extreme disturbance events, such as flood plumes, that prevent light penetration to autotrophs in coastal estuaries and can persist from days to weeks (Devlin et al., 2012; Waterhouse et al., 2017). As plots were open to full water exchange on all sides, water temperature did not differ among shading treatments (0.1 degree range; Table S1). Shade cloths and light loggers were cleaned every 48 h to remove any fouling. All stressor manipulations and sample collections were done at low tide.

2.2. Response variables

2.2.1. Shoot density

We measured seagrass shoot density at the start of the experiment, each week, and at the end of the experiment. We counted all individual shoots within a 30 × 30 cm quadrat placed at the center of each experimental plot and recorded density as total number of shoots per plot (extrapolated to 0.36 m²). As an approximation for survival, we also calculated the proportional change in shoot density between the final and initial counts (Fig. S1).

2.2.2. Leaf surface area

We estimated average seagrass leaf surface area per shoot at the start of the experiment, each week, and at the end of the experiment. Ten mature seagrass leaves were randomly collected from separate shoots outside of the density quadrat to avoid affecting subsequent shoot density measurements. We also measured the average number of leaves per shoot each week, and coupled with individual leaf length and width (cm), estimated the change in average leaf surface area (cm²) throughout the experiment, calculated as:

$$\text{average seagrass leaf length} \times \text{average seagrass leaf width} \times \text{average number of leaves per shoot.}$$

We also combined leaf surface area per shoot with density estimates to calculate change in total leaf surface area per plot as a proxy for aboveground biomass (Fig. S2).

2.2.3. Crustacean abundance

We measured epifaunal crustacean abundance within the seagrass canopy at the start of the experiment, each week, and at the end of the experiment. We used a hand net (60 μm mesh) to sweep the seagrass canopy at low tide in a grid-like pattern (i.e., four sweeps, one per each side of the experimental plot). We recorded the taxonomic group and abundance (the sum of all individuals caught in four sweeps per plot) of all animals before releasing them back into the plot from which they were sampled to minimise disturbance and avoid influencing future

measurements. >99 % of the invertebrates collected were crustaceans, so we excluded other taxa from the analysis. Caridean shrimp were the most frequently sampled crustacean (>98 %).

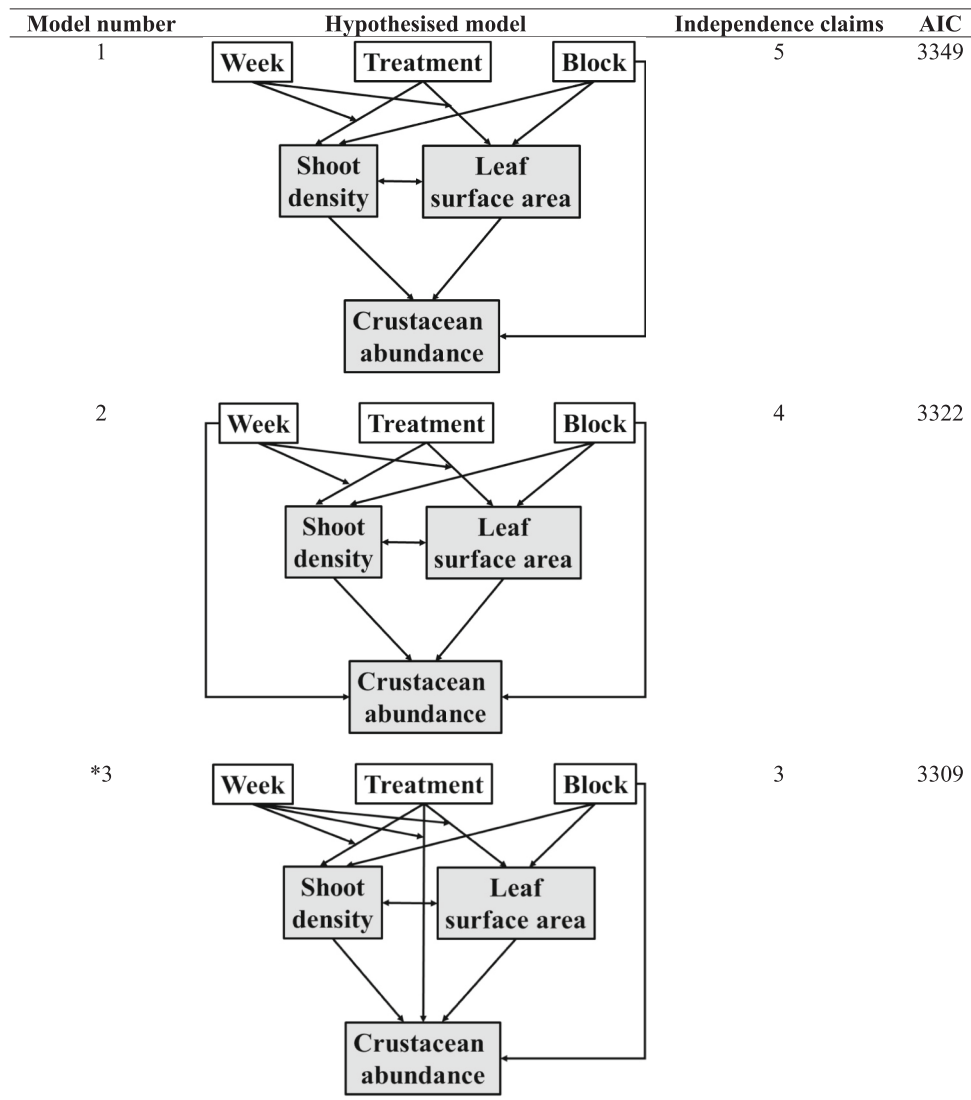


Fig. 2. Alternate hypothesised causal models and test statistics. Hypothesised causal relationships among treatment, week (i.e., time), shoot density, leaf surface area, and crustacean abundance. Shaded boxes represent the response variables, and an arrow extending from week and converging with an arrow extending from treatment indicates an interactive effect. Asterisk denotes the model with the lowest AIC that was selected for further evaluation of causal effects.

2.3. Statistical analysis

2.3.1. Testing alternate hypothesised causal relationships

We applied the logic of structural causal modelling (SCM) within a series of structural equation models (SEMs) to test for causal effects of week and treatment on seagrass and crustacean response variables (see Arif and MacNeil, 2023). We additionally accounted for random block effects in the SEMs. This approach allowed us to quantify hypothesised direct causal effects of treatments on the seagrass and crustacean response variables. We also tested a hypothesis that effects of stressors on crustaceans would be mediated by changes in the seagrass variables. We first constructed a series of alternate plausible causal path diagrams (i.e., directed acyclic graph, DAG; Fig. 2) that represented different hypothesised relationships between and among predictor and response variables (Pearl, 2009). We proposed three hypothesised causal relationships. In the first model (Model 1), we hypothesised direct effects of treatment and week (i.e., time) on seagrass and that crustaceans would respond to the treatment effect only via changes in their seagrass habitat. In the second (Model 2), we added a direct effect of week on crustaceans to determine whether crustacean abundances were highly variable over time rather than affected by treatments or changes in seagrass habitat. In the third (Model 3), we added a direct effect of treatment on crustaceans, hypothesising that crustaceans could respond both directly to the treatment and indirectly via changes to seagrass habitat (Fig. 2).

We then tested which hypothesis was most parsimonious given the observations using the piecewise SEM framework (Shipley, 2000, 2009). We chose to fit the data with generalised additive models (GAMs) to allow for a potentially non-linear effect of week. GAMs were fit with the R package *mgcv* (Wood, 2006) in the R programming language (version 4.2.2). In the piecewise SEM framework, each response variable in the hypothesised causal diagrams is modelled with a GAM (Fig. 2, GAM

formulation is given in Table S3). We then determined the Akaike information criterion (AIC) of the causal model as the sum of AICs from the sub-models (according to Shipley and Douma, 2020). Finally, the model with the lowest AIC was selected to test the strength of proposed relationships.

2.3.2. Identify total, direct, and mediating causal effects

Once the most parsimonious causal model was selected, we established the strength and statistical significance of each hypothesised cause (Fig. 3 – each arrow is a hypothesised cause). We applied the backdoor criteria to identify the necessary causal tests that account for confounders (Pearl, 2009). The backdoor criteria identifies, for each hypothesised causal effect, variables that should be conditioned upon. The criteria was implemented with the *dagitty* package (Textor et al., 2017). We used the canonical adjustment sets to increase our power to detect causal effects (Table S2). The models chosen with the backdoor criteria to test causal effects were the same models used in the SEM analysis (Tables S3, S4). For example, to test the causal effect of treatment on shoot density, we should condition on both block and week, while the effect of week on shoot density should be conditioned on block and treatment (Table S2). Therefore, for each response variable, all causal effects could be tested in a single GAM.

Causal tests were then performed with GAMs, using the same settings (i.e., block random effect, types of splines, degrees of freedom, and distribution family) that we used to calculate the overall model AICs. For the seagrass density and leaf surface area GAMs, we confirmed normality of residuals (Q-Q plot) and homogeneity of variance (residuals plot). For the crustacean abundance model, we used a rootogram to assess goodness of fit, because the data were fit with a negative binomial distribution. Median effects and credible intervals (CI) were calculated from the GAMs using the empirical Bayesian approach (Wood, 2006). In this approach we can draw samples from the posterior

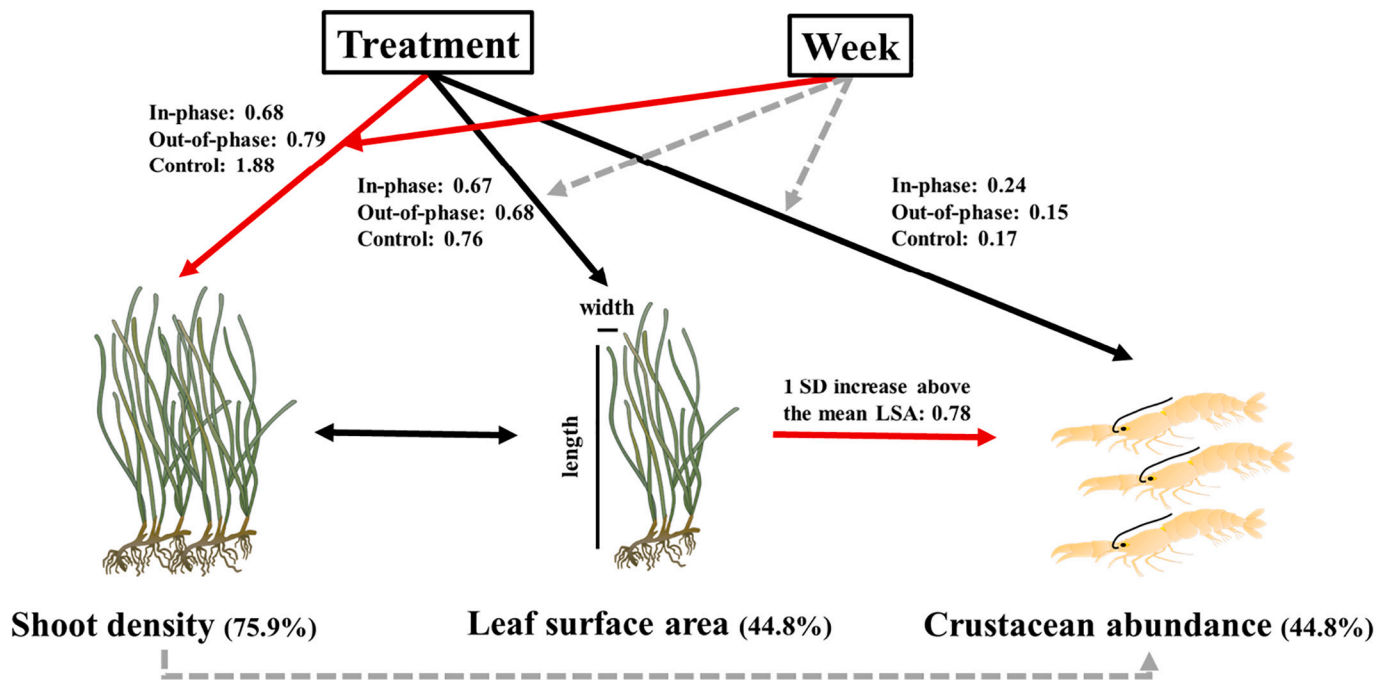


Fig. 3. The effects of treatment and week (i.e., time) on a seagrass community relative to control conditions. Solid arrows indicate a significant causal pathway between a predictor and a response variable ($\alpha = 0.05$). Dashed lines indicate insignificant paths. Black arrows indicate a positive effect of a predictor on a response variable, while red arrows indicate a negative effect. An arrow extending from week and converging with an arrow extending from treatment indicates an interactive effect. Median effect sizes for each causal effect in the SEM are shown for treatment effects (above the corresponding arrows), expressed as a multiple of the static treatment effect after six weeks (e.g., control effect/static effect). The median effect size for leaf surface area effect on crustacean abundance is shown as a one standard deviation (SD) increase above the mean leaf surface area across all treatments and the control. The deviance explained (%) is indicated for each sub model in the SEM that tests effects on each response variable. Note a correlation between shoot density and leaf surface area was assumed in our structural causal model.

distribution for each GAM, assuming the parameters follow a multivariate normal distribution.

We analysed changes over time for all response variables relative to initial measurements taken prior to stressor exposure (i.e., day 0 measurements used as an offset), using a week spline with the potential for independent time trends by block, and a random block effect. Mean and 95 % confidence intervals (CI) were calculated, and we interpreted statistically significant differences as where the CI of a treatment did not overlap with the mean of another treatment (see Payton et al., 2003). Effect sizes were calculated as multiples of the static-static treatment effect (e.g., median effect size of the in-phase response/median effect size of the static response). See Appendix A for details of each model used in the analyses (Table S4). These results represent the total effects of treatment and week on each response variable. For the seagrass variables, the direct effects are the same as their total effects. However, there are multiple pathways for treatment and week to affect crustacean abundance (directly, via leaf surface area, or via shoot density), so we did additional analyses to differentiate the direct and mediation (i.e., indirect) effects.

To quantify the strength of the direct and mediation effects, we estimated the average direct effect and average causal mediated effect sizes of each treatment on crustacean abundance (following Imai et al., 2010). These effects are expressed relative to the control group. We excluded shoot density from our causal mediated effect size calculation because it did not have a significant effect on crustacean abundance (i.e., we estimated the treatment effect on crustacean abundance mediated by leaf surface area only; Fig. 3, Table S5; <https://github.com/ostro2al/multi-stressor-field-stats>). See Appendix A for formal definitions of the causal effects and detailed methods for calculating direct and mediated effects.

3. Results

3.1. SEM data consistency, conditioning variables, and causal tests

Model 3, hypothesising both direct and mediation effects of treatment on crustacean abundance, had the lowest AIC and was thus selected to further test the strength of the causal effects (Fig. 2, Tables S5, S6). We found that treatment had direct effects on seagrass shoot density, leaf surface area, and crustacean abundance, but the

direction (i.e., positive or negative) of the effect varied by response variable (Fig. 3, Tables S5, S6). Additionally, we found that the effects of variable stressor intensity and synchronicity were mediated within the seagrass community (Fig. 3, Table S6).

3.2. Seagrass shoot density

We found a direct negative effect of all three treatments on shoot density relative to the control ($p < 0.001$) and an interactive effect between each treatment and week ($p < 0.001$; Fig. 3). Seagrass shoot density decreased over time across all stressor treatments relative to control conditions (which remained stable after an initial increase), with significant interactive effects between week and all stressor treatments ($p < 0.001$; Fig. 4). In-phase ($p < 0.001$; 17 % lower than static during week six) and out-of-phase ($p < 0.001$; 11 % lower than static during week six) treatments reduced density more than the static-static treatment, and this effect was observable by week three. By week five, in-phase and out-of-phase treatments diverged significantly, with in-phase causing the greatest reduction in density (Fig. 4). At the end of the experiment, shoot density in the in-phase and out-of-phase treatments was 0.68 (effect sizes; 0.61, $0.77 \pm 95\%$ CI) and 0.79 ($0.71, 0.87 \pm 95\%$ CI) times the effect of the static treatment, respectively (Table S5).

3.3. Seagrass leaf surface area

Seagrass leaf surface area was increased by the static-static treatment but decreased in all other treatments, while remaining relatively constant in the control (Fig. 5). Thus, all treatments had lower leaf surface area than the static-static treatment after six weeks (Fig. 3, Fig. 5). In-phase ($p = 0.003$; 33 % lower than static during week six) and out-of-phase ($p < 0.001$; 28 % lower than static during week six) treatments had reduced leaf surface area over time, and were significantly lower than the static-static treatment by week three (Fig. 5; note the mean for static treatment is outside the CI for all other treatments and the control). At the end of the experiment, leaf surface area in the in-phase and out-of-phase treatments were 0.67 (effect sizes; 0.47, $0.89 \pm 95\%$ CI) and 0.68 ($0.45, 0.90 \pm 95\%$ CI) times the value of the static treatment, respectively (Table S5). Leaf surface area under both fluctuating treatments did not differ from the control (Fig. 5).

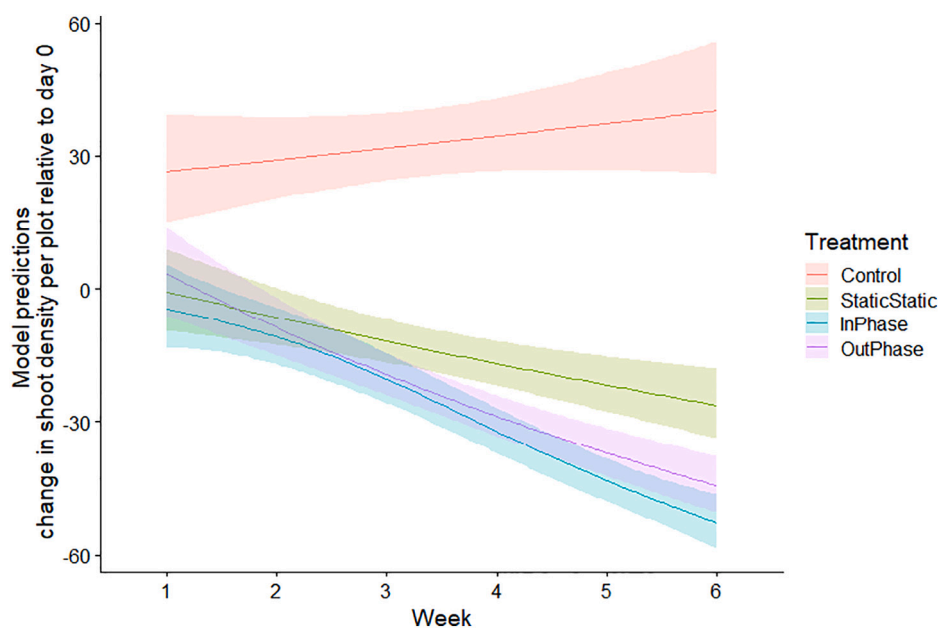


Fig. 4. Total effect of treatments on seagrass shoot density. Model predictions for mean ($\pm 95\%$ CI) change in shoot density (number of shoots per 0.36 m^2 plot) across all four treatments. The results are relative to initial measurements taken prior to stressor exposure. Each treatment was replicated six times.

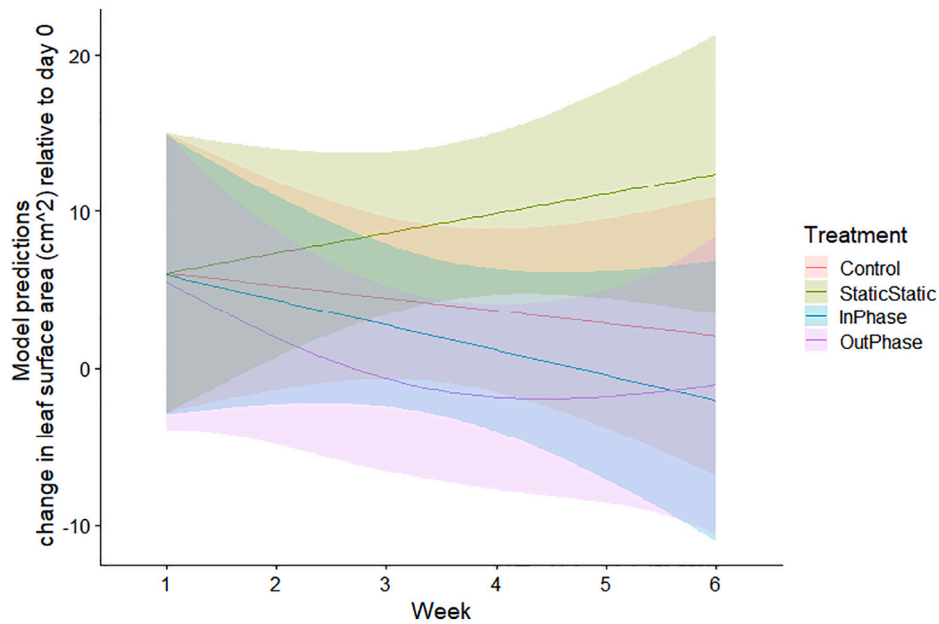


Fig. 5. Total effect of treatments on seagrass leaf surface area. Model predictions for mean ($\pm 95\%$ CI) change in leaf surface area (cm²) across all four treatments. The results are relative to initial measurements taken prior to stressor exposure. Each treatment was replicated six times.

3.4. Crustacean abundance

The SEM suggested that there was a direct effect of treatment on crustacean abundance, as well as a mediation effect by leaf surface area. First, we present the results for the total effect of treatment on crustacean abundance (i.e., the combined effect of all causal pathways), then we partition the contributions from the direct and mediation causal pathways.

3.4.1. Total effects

Following an initial increase during week one, crustacean abundance gradually decreased over time across all treatments and the control, with significant interactive effects between week and all stressor treatments ($p < 0.001$; Fig. 6) and significant variation in block effects over time (p

≤ 0.004). Crustacean abundance was highest in the static-static treatment. In-phase ($p < 0.001$; 30 % lower than static during week six) and out-of-phase ($p < 0.001$; 39 % lower than static during week six) treatments had lower abundances relative to the static-static treatment (Fig. 6). By the end of the experiment, crustacean abundance in the in-phase and out-of-phase treatments was 0.24 (effect sizes; 0.08, $0.74 \pm 95\%$ CI) and 0.15 (0.05, $0.54 \pm 95\%$ CI) times the effect of the static treatment, respectively (Table S5). There was no difference in crustacean abundance between the control and either of the fluctuating treatments (Fig. 6, Table S5).

3.4.2. Direct and mediating causal effects

We found a direct positive effect of static treatment on crustacean abundance, which was 3.72 (effect sizes; 2.05, $6.85 \pm 95\%$ CI) times the

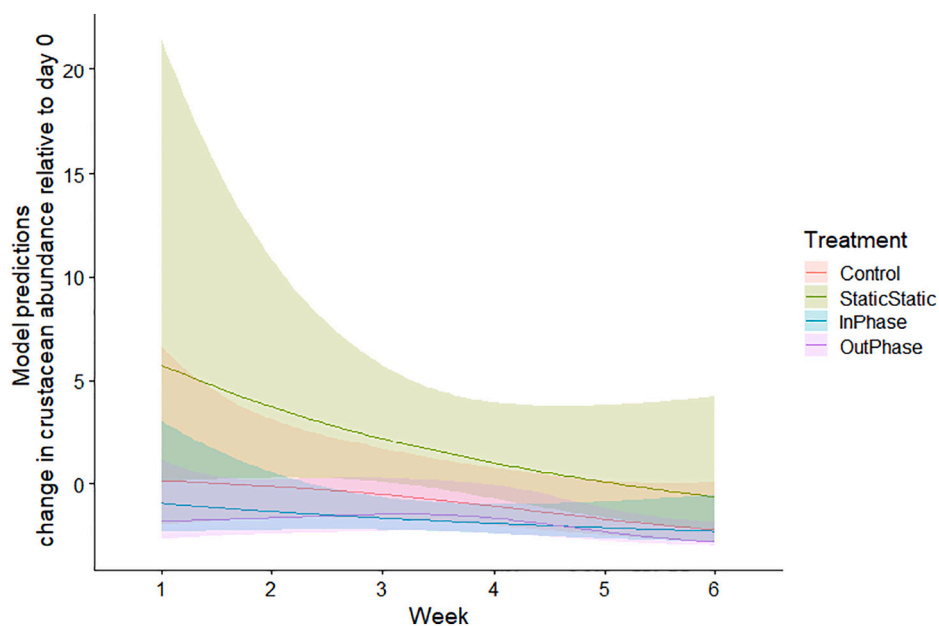


Fig. 6. Total effect of treatments on crustacean abundance. Model predictions for mean ($\pm 95\%$ CI) change in crustacean abundance across all four treatments. The results are relative to initial measurements taken prior to stressor exposure. Each treatment was replicated six times.

effect observed under control conditions (Table S6). In-phase and out-of-phase treatments had weak direct negative effects on crustacean abundance relative to the control (effect sizes; 0.79 [0.43, 1.49 ± 95 % CI] and 0.53 [0.29, 1.00 ± 95 % CI] times the control effect at week six, respectively; Table S6). Leaf surface area also had a direct negative effect on crustacean abundance ($p = 0.009$; Fig. 3). One standard deviation increase above the mean leaf surface area led to 0.78 (effect sizes; 0.61, 0.99 ± 95 % CI) times the effect on crustacean abundance (Fig. 3, Table S5). The direct effect of treatment on leaf surface area, and the direct effect of leaf surface area on crustacean abundance, revealed a mediation effect of treatment on crustacean abundance via seagrass leaf surface area (Fig. 3, Table S6).

Mediation effects of each treatment on crustacean abundance via leaf surface area were not as strong as the direct treatment effects (Fig. S3, Table S6). The mediation effects of in-phase and out-of-phase treatments were weakly positive (effect sizes; 1.05 [0.24, 4.66 ± 95 % CI] and 1.07 [0.26, 4.41 ± 95 % CI] times the control effect, respectively; Table S6), while the mediation effect of static treatment was the strongest and had a negative effect (effect sizes; 0.87 [0.21, 3.43 ± 95 % CI] times the control effect; Table S6). Due to the negative direct effect of leaf surface area on crustaceans (Fig. 3), the higher leaf surface area observed in the static treatment had a weak negative effect on crustaceans relative to all other treatments and the control (Fig. S3, Table S6). However, the direct positive effect of static treatment on crustacean abundance was much stronger than the negative mediation effect via leaf surface area, resulting in the total effect of static treatment on crustaceans being positive (Fig. 6, Table S6). The direct negative effects of in-phase and out-of-phase treatments on crustacean abundance were also stronger than the weak positive mediation effects via leaf surface area, thus resulting in total (weak and non-significant) negative effects on crustacean abundance under fluctuating conditions (Fig. 6, Table S6).

4. Discussion

4.1. Effects of stressor intensity and synchronicity

Variation in stressor intensity and synchronicity influenced how a seagrass community responded to multiple stressors, and we observed both direct effects of treatment, and evidence that effects on crustaceans are mediated by how seagrass plants respond to stressors. Stressor fluctuations – regardless of whether they were in- or out-of-phase – resulted in greater adverse impacts on seagrass communities than static stressors. While static stressors substantially reduced shoot density relative to the control, we found unexpected positive effects on seagrass leaf surface area and crustacean abundance. Furthermore, we found a weak mediation effect of the static treatment on crustacean abundance via seagrass leaf surface area, suggesting that different mechanisms are operating when seagrass responds to fluctuating rather than static stressors. Our results support recent evidence from laboratory experiments that suggest predictions of stressor impacts can differ based on how stressors are introduced (e.g., Cross et al., 2019; Pansch and Hiebenthal, 2019; Low and Micheli, 2020; Ostrowski et al., 2022). For stressors that fluctuate in nature, our findings suggest that future multiple stressor experiments could benefit from incorporating more environmentally relevant changes in stressor intensity and synchronicity to enhance the ecological relevance of experimental conclusions for ecosystem conservation and management.

Exposure to a highly variable environment might reduce rather than increase an organism's stressor tolerance relative to organisms that experience stress in a stable environment (e.g., Vargas et al., 2017; Klepac and Barshis, 2020). Under the constant low light conditions in the static treatment, the seagrass might have acclimated by increasing leaf length to enhance light absorption efficiency; a common compensatory response to low light (Bulthuis, 1983; Olesen et al., 2002). Additionally, under constant physical disturbance, nutrients released from the sediment could stimulate seagrass growth, thus increasing leaf

surface area. Since acute disturbances (i.e., fluctuating treatments) had greater adverse effects on seagrass shoot density and leaf surface area than chronic disturbance (i.e., static treatment), increased energy may have been required for seagrass to physiologically compensate for stressors under a continuously changing environment or during periods of more extreme stress intensity, (Mangan et al., 2017; Blewett et al., 2022; Vasquez et al., 2022). High intensity stress may also negatively affect species tolerance and adaptive potential to stressors (Vos et al., 2023). Seagrasses under fluctuating treatments were exposed to periods of higher intensity stress than static treatments to achieve the same average stressor intensity across all treatments. Fluctuations in acute stressors might require seagrass to invest increased energy to physiologically compensate for stress when compared to chronic stress at the same average magnitude, as higher intensities may surpass stress thresholds (Sokolova et al., 2012; Gunderson et al., 2016). Periods of higher stress intensity observed under fluctuating treatments may also have prevented seagrass acclimation via increased surface area, or intermittent periods of lower disturbance might not have released ample nutrients to stimulate growth.

In-phase, synchronous fluctuations had the overall greatest adverse effect on shoot density and leaf surface area by the end of the experiment. As both low light and physical disturbance were introduced at high intensities simultaneously, this treatment may have overwhelmed compensatory physiological responses and resulted in a synergistic impact (Gunderson et al., 2016). Enhanced physiological stress at the individual level can then have flow-on effects at higher levels of biological organisation, resulting in amplified stressor impacts on populations and communities (Petitjean et al., 2019), as observed for seagrass under fluctuating treatments. Our results suggest that the seagrass was more capable of acclimating to stressors under static conditions relative to a continuously changing environment (sensu Seebacher et al., 2015, Gobler et al., 2017).

4.2. Mechanistic effects of variable stressor intensity and synchronicity within seagrass communities

Biotic interactions can amplify or ameliorate multiple stressor effects, which can result in mediated stressor effects at higher levels of biological organisation and the over or underestimation of impacts on ecological communities (Beauchesne et al., 2021; Orr et al., 2021; Zhou and Wang, 2023). Across treatments, seagrass leaf surface area directly and negatively affected crustacean abundance, where higher leaf surface area caused lower crustacean abundance. While several studies have observed the opposite trend, whereby a positive effect of structural complexity on crustaceans has been observed (e.g., Main, 1987; Reynolds et al., 2018; Jinks et al., 2019), negative effects have also been observed where areas of lower structural complexity support higher epifauna abundance relative to areas of greater complexity (e.g., Cimon et al., 2021; Daudi et al., 2023). The weak negative mediation effect of static treatment on epifaunal crustaceans via increased seagrass leaf surface area, and the weak positive mediation effect of fluctuating treatments via reduced leaf surface area suggest that the mechanisms operating when seagrass respond to fluctuating stressors are different to those when stressors remain static (Fig. 7). Ultimately, our results suggest that structural metrics of seagrass meadows, which are highly susceptible to multiple stressors, can weakly influence the motile epifaunal crustaceans that utilise these habitats, and that the direction of this effect depends on whether the stressors fluctuate or remain static.

Total treatment effects on crustacean abundance (including all direct and indirect effect pathways measured and unmeasured here) were stronger than the mediation effects via leaf surface area, and again the impacts differed by method of stressor introduction. Static treatment had a strong, direct positive effect on crustacean abundance while fluctuating stressors had weak direct negative effects. While motile crustaceans are unlikely to move far beyond the bounds of the seagrass meadow, they can move throughout the water column and seagrass

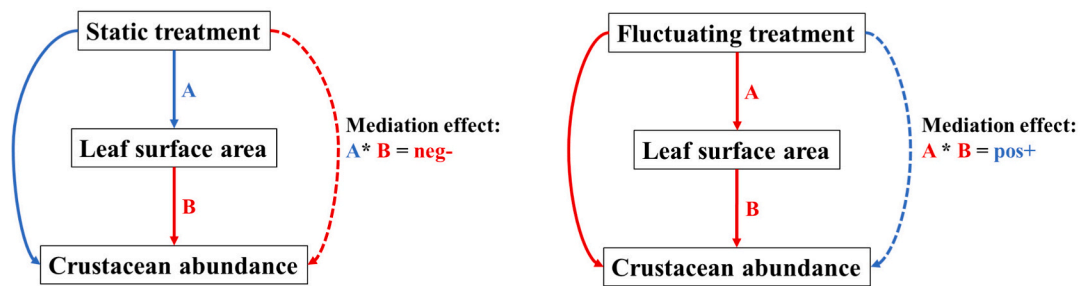


Fig. 7. Conceptual diagram outlining the weak negative mediation effect of static treatment on epifaunal crustaceans by increased seagrass leaf surface area, and the weak positive mediation effect of fluctuating treatments by reduced leaf surface area. Blue arrows indicate a positive effect and red arrows indicate a negative effect. Solid lines represent direct effects and dashed lines represent the total mediation effects.

canopy to actively select preferred microhabitats within the meadow (Bell and Westoby, 1986; Edgar and Robertson, 1992). One plausible reason is that the crustaceans were more strongly and positively influenced by other environmental factors also affected by the stressors, such as epiphytic algal biomass, than by structural habitat complexity and availability within the meadow (e.g., Daudi et al., 2023). Nutrients released when sediments are disturbed may not only spur seagrass growth but may also increase epiphytic algal biomass (Van Alstyne et al., 2011; Bourque et al., 2015). Constant, moderate intensity disturbance in the static treatments (compared to fluctuating treatments with alternating low and high intensities) may have resulted in greater algal biomass. Algae are a food source for crustaceans and associations between high algal biomass and high epifauna abundance within seagrass meadows are known (Hall and Bell, 1988; Gartner et al., 2013; Stark et al., 2020). Although not measured in this study (visually, epiphytic biomass was very low, and higher crustacean abundances can reduce epiphyte biomass via grazing), increased epiphyte biomass could be a factor influencing higher crustacean abundance within the static treatment plots (e.g., Navarro-Mayoral et al., 2023). Furthermore, constant light reduction under static conditions might provide more consistent refuge from predators than areas with higher light penetration (i.e., fluctuating treatment plots have periods of low intensity light reduction), thereby harbouring more crustaceans. Our SEM approach can be used in future experiments to enhance our understanding of these mechanistic treatment effects, for example, by measuring changes in epiphyte biomass and nutrient concentrations in the water column across each treatment to test for additional causal effects on crustacean abundance.

How seagrass structural complexity influences crustacean abundance, as previously mentioned, appears to be context dependent, with studies finding conflicting results. If we had not performed the causal mediation analysis, we might have concluded that there was a positive effect of leaf surface area on crustaceans because both crustacean abundance and leaf surface area were highest in the static treatment. However, the mediation analysis indicated that the static treatment effect via increased leaf surface area had a weak negative effect on crustaceans while the direct causal effect was strong and positive. This result highlights the importance of incorporating causal mediation analysis in multiple stressor experiments, which allowed us to control for confounding variables and avoid drawing inaccurate conclusions of causal stressor effects.

4.3. Management implications

Stressor fluctuations can amplify adverse impacts on biological responses and may alter the mechanisms by which organisms respond to stress, suggesting studies that only assess static stressors might lead to the underestimation of impacts within real-world ecosystems, reducing the efficacy of conservation and management. Given that high intensity fluctuating stressors can result in greater habitat loss (e.g., seagrass

shoot reduction), management strategies should consider environmentally relevant changes in stressor intensity and synchronicity. Management strategies would thus benefit from considering the timing of disturbance events in targeted stressor removal strategies (Wu et al., 2017). For example, restricting dredging operations near seagrass meadows to occur outside of peak flood plume events can allow the ecosystem to recover following the flood disturbance before subsequent stressor exposure via dredging. Localised stressors, such as physical disturbance to seagrass meadows via commercial bivalve harvesting and bait digging, are typically easier to manage than regional (e.g., flood plumes) or global (e.g., increased temperature due to global climate change) stressors. Therefore, targeted approaches to reduce compounding effects of high intensity local stressors, and inferring decisions based on the type of interaction between stressors of interest (Brown et al., 2013; Côté et al., 2016), can enhance positive management outcomes.

Understanding the mechanistic effects of stressors can also aid in more accurately predicting the effects of stressor removal on ecosystems, and can guide management efforts to achieve cascading positive impacts (Pirodda et al., 2022). Ultimately, the scaling-up of experimental results to predict responses of biodiversity to stressors is increasingly relying on representing ecological mechanisms in process-based models (Pilowsky et al., 2022). Our results provide an important caution for the integration of models and experiments. We found that the qualitative nature of the mechanisms for stress response depends on the environmental context; in our case, how stressor intensities vary through time. Static experiments might not explore mechanisms that are relevant in natural ecosystems and can misestimate stressor impacts, which may have limited relevance to the real world. Therefore, experiments should be conducted in an environmentally relevant context, otherwise process-based models might represent inappropriate mechanisms for stress responses. We suggest that future multiple stressor experiments incorporate environmentally relevant changes in stressor intensity and synchronicity, and measure responses across levels of biological organisation in situ over longer durations. Adopting this approach could provide more accurate experimental conclusions applicable to effective conservation and ecosystem management.

5. Conclusions

Dynamic environmental conditions can influence stressor intensity and synchronicity, yet our current understanding of multiple stressor effects is derived primarily from highly controlled laboratory studies assessing static stressor effects at lower levels of biological organisation. Our study is the first to identify causal effects of variable stressor intensity and synchronicity on seagrass communities in situ. We show that fluctuating stressors caused the greatest loss in seagrass shoot density and leaf surface area, and the lowest crustacean abundances. While static stressors gradually reduced shoot density, we observed increased leaf surface area and crustacean abundance in this treatment. We also

found evidence of mediation, where stressor effects on crustaceans are mediated by changes in seagrass habitat. Since the direction of this mediation depended on stressor intensity and synchronicity, our results suggest that different mechanisms are operating when seagrass respond to fluctuating stressors compared to static stressors. Static experiments thus might not explore mechanisms that are relevant in natural ecosystems and can misestimate stressor impacts, which may ultimately have limited relevance to the real world. Therefore, we suggest that future multiple stressor experiments incorporate environmentally relevant changes in stressor intensity and synchronicity, and measure responses across higher levels of biological organisation in situ. Adopting this approach will enhance our mechanistic understanding of stressor impacts to the environment and provide more accurate experimental conclusions applicable to effective conservation and ecosystem management.

CRedit authorship contribution statement

AO, RMC, and MS participated in conceptualisation and developed the methodology for the experiment. AO carried out field work and data curation. AO and CJB conducted the formal analysis. AO wrote the first draft of the manuscript. All authors contributed to visualisation, review, and editing of the manuscript.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

All data are available in the manuscript. All code will be freely available on the lead author's GitHub page, provided in the manuscript.

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Appendix A. Supplementary data

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