Evaluating multiple stressor research in coastal wetlands: A systematic review

Andria Ostrowski *, Rod M. Connolly, Michael Sievers

Australian Rivers Institute – Coast and Estuaries, School of Environment and Science, Griffith University, Gold Coast, QLD, 4222, Australia

ARTICLE INFO

Keywords: Anthropogenic impact
Climate change
Ecosystem disturbance
Eutrophication
Experimental design
Human pressures
Interacting threats
Mechanistic
Multivariate statistics
Path analysis

ABSTRACT

Multiple stressors are ubiquitous in coastal ecosystems as a result of increased human activity and development along coastlines. Accurately assessing multiple stressor effects is essential for predicting stressor impacts and informing management to efficiently and effectively mitigate potentially complex ecological responses. Extracting relevant information on multiple stressor studies conducted specifically within coastal wetlands is not possible from existing reviews, posing challenges in highlighting knowledge gaps and guiding future research. Here, we systematically review manipulative studies that assess multiple anthropogenic stressors within saltmarsh, mangrove, and seagrass ecosystems. In the past decade, there has been a rapid increase in publications, with seagrasses receiving the most attention (76 out of a total of 143 studies). Across all studies, nutrient loading and temperature were tested most often (N = 64 and N = 48, respectively), while the most common stressor combination was temperature with salinity (N = 12). Stressor application and study design varied across ecosystems. Studies are mostly conducted in highly controlled environments, without considering how natural variations in the physicochemical environment of coastal ecosystems may influence stressor intensity and timing under these conditions. This may result in vastly different ecological responses across levels of biological organisation. Shifting focus from univariate analytical approaches to multivariate, particularly path analysis, will help elucidate complex ecological relationships and highlight direct and indirect effects of multiple stressors in coastal ecosystems. There is a solid foundation of multiple stressor research in coastal wetlands. However, we recommend future research enhance ecological realism in experimental design by studying the effects of stressor combinations whilst accounting for spatiotemporal variability that reflects natural conditions of coastal ecosystems.

1. Introduction

Coastal wetlands are amongst the most productive and important ecosystems in the world (Millennium Ecosystem Assessment 2005). Saltmarsh, mangrove and seagrass ecosystems provide critical habitat for a huge diversity of wildlife, including important fisheries species (Carrasquilla-Henao and Juanes 2017), threatened marine megafauna (Sievers et al., 2019), as well as birds, insects, and reptiles (Nagelkerken et al., 2008). Collectively referred to as ‘blue carbon ecosystems’, saltmarsh, mangrove, and seagrass ecosystems sequester enormous amounts of carbon relative to their extent (Duarte et al., 2013), and protect coastlines from erosion, storm surges, and wave action, which may be exacerbated by global climate change (Duarte et al., 2013; Silliman et al., 2019). Coastal wetlands occur within highly dynamic environments where hydrodynamics and physicochemical properties (e.g. salinity, pH) are in a constant state of flux and influenced by, for example, variation in tidal cycles, biological activities, and freshwater inflow (Cloern et al., 2016; Muduli and Pattanaik 2020). Despite their ecological and economic value, coastal wetlands face substantial anthropogenic threats (He and Silliman 2019) and have undergone significant global declines in both extent and condition (Waycott et al., 2009; Davidson 2014; Gu et al., 2018).

At the land-sea interface, coastal wetlands are vulnerable to impacts related to human population growth and coastal development. These threats introduce diverse stressors to the natural environment, such as nutrients, contaminants, sediments, hydrological modifications, and erosion, that adversely impact ecosystem productivity and the provision of services (Heckbert et al., 2012; Kennish et al., 2014; He and Silliman 2019; Gilby et al., 2020). Anthropogenic global climate change is compounding these impacts, creating additional stressors that affect
coastal ecosystems including increasing temperatures, ocean acidification, sea-level rise, and salinity changes (Doney et al., 2012; He and Silliman 2019). A stressor is a variable that exceeds natural variation and can drive changes in individuals, populations, communities, or ecosystem processes. Recognition that anthropogenic stressors rarely occur in isolation, and that interactions between multiple stressors can impose synergistic impacts, has spurred interest in studying the effects of multiple stressors (Cote et al., 2016; Orr et al., 2020).

The highly variable, complex, and dynamic nature of coastal wetlands makes accurate assessment of the impacts of multiple stressors particularly challenging (Gunderson et al., 2016). For example, Vieira et al. (2020) identified complex responses of the seagrass, Zostera noltii, to sediment, nutrients, and invasive macrophytes that varied by stressor intensity, response measured, combination of stressors, and species interactions within the community. Additionally, Poormahdi et al. (2018) found nitrogen and phosphorous enrichment ameliorated the otherwise adverse impacts of sulfate loading on saltmarsh plant growth. Perry et al. (2019) discovered no significant effects of ocean acidification, heat shock, or storm occurrence on Zostera marina populations when introduced independently, but observed deleterious effects on seagrass growth when all three stressors were applied concomitantly. Given the potential for complex and unexpected ecological outcomes to multiple stressors, it is vital that we accurately assess responses in order to predict stressor impacts and inform management to mitigate adverse effects in coastal wetlands. It is equally important that time and resources are used most efficiently and effectively to investigate these responses. To help achieve this, we systematically review previous research on vegetated coastal wetlands to document the present state of knowledge and highlight key gaps within multiple stressor research.

Existing reviews of multiple stressors in marine environments provide some insights into the general strengths and shortcomings of multiple stressor research. For instance, previous work has elucidated the potential for complex ‘ecological surprises’ that result from strong synergistic interactions among multiple stressors (e.g. Crain et al., 2008; Stockbridge et al., 2020); findings that shifted research focus away from single stressors and toward examining the interactive effects of multiple stressors (Cote et al., 2016). Additional work has led to development of frameworks intended for universal application in multiple stressor experiments to enhance understanding of complex stressor interactions (Griffen et al., 2016; Orr et al., 2020). Increased efforts in multiple stressor research have since led to improved predictions, conservation efforts, and adaptive management strategies for ecosystems affected by multiple interacting stressors (Boerema and Meire 2017; He and Silliman 2019). However, previous research also highlights the overreliance of applying stressors at fixed levels (Gunderson et al., 2016) and ignoring natural environmental fluctuations (Przeslawski et al., 2015; Gunderson et al., 2016). Additionally, failing to examine responses at higher levels of biological organisation (e.g. populations, communities, ecosystem processes; Harvey et al., 2013; Griffen et al., 2016), and lacking mechanistic understanding of multiple stressor impacts (Przeslawski et al., 2015; Griffen et al., 2016) was identified.

Despite a substantial knowledge base on multiple stressors within marine environments, there is currently no comprehensive overview of what research has been done on multiple stressors within the three key vegetated coastal wetland ecosystems. Several habitat-specific, qualitative reviews describe the key threats facing these ecosystems and are valuable for guiding future research (e.g. Gedan et al., 2009; Friess et al., 2019; Uehara et al., 2019). However, the lack of systematic, quantitative evaluations of multiple stressor research in any of the three focal ecosystems makes it difficult to identify understudied areas or stressors, or to highlight important knowledge gaps. Here, we systematically review the literature on multiple stressor research conducted in saltmarsh, mangrove, and seagrass ecosystems to identify, summarise, and describe how experiments are being designed and conducted. Our focus is on determining the ecosystem components and the types of response variables being measured.

2. Methods

2.1. Literature search

We conducted a search to capture studies that examined the effects of multiple stressors within saltmarsh, mangrove, or seagrass ecosystems. On March 3, 2020 we performed a literature search using the ISI Web of Knowledge (www.webofknowledge.com) and Scopus (www.scopus.com) databases, and the following search term: (mangrove* OR “salt marsh” OR “saltmarsh” OR “tidal marsh” OR seagrass* OR “manatee grass” OR “turtle grass” OR corgdgrass OR “shoal grass” OR elgrass OR Spartina) AND (“stressor” OR “combined effect” OR “interactive effect”) OR synergy OR synergism OR synergistic OR antagonism OR non-additive OR antagonistic OR additivity). We had no restrictions on publication date, but limited our search to papers published in English (which is unlikely to influence the final dataset; Morrison et al., 2012). Excluding duplicates, we were left with 1410 articles. We determined the relevance of these articles in an iterative process following the PRISMA protocol (Figure A1 in the supplementary material, Appendix A). We first screened articles for relevance by evaluating titles. Those with potentially relevant titles then had their abstracts evaluated, erring on the side of caution (i.e. inclusion). Any articles that did not consider at least one of the three ecosystems of interest were excluded. Once a comprehensive list of potentially relevant articles was gathered, we assessed each remaining paper for relevance at the whole-paper level based on a set of eligibility criteria outlined below. Articles that were not immediately identifiable as meeting or not meeting our criteria were checked by two of the author team before a final decision was made.

Our focus was on peer-reviewed studies of multiple stressor experiments under the assumption that a peer-review process is preferred before data should be used to develop hypotheses or incorporated into broader studies. Although some studies in the grey literature might have been missed, we regard peer-review as a gold standard for quality control and a standard for reviews because it provides systematic criteria for literature searches.

2.2. Data extraction and classification

To summarise multiple stressor research in saltmarsh, mangrove, and seagrass ecosystems, we established a set of criteria to assess the eligibility of articles. We only included original research that conducted manipulative experiments, thereby excluding reviews, modelling, and observational correlative studies from this review. Modelling or correlative (i.e. mensurative, observational) studies can provide useful and unique insights into the impacts of multiple stressors, sometimes at spatial scales larger than possible in manipulative experiments. However, we did not include modelling studies as we were interested only in experimental work that directly manipulated and quantified the impact of multiple stressors in our focal ecosystems. We excluded correlative studies as it can be difficult to: accurately document which stressors were examined (e.g. along an urban-rural gradient where a suite of stressors exists, many stressors are unmeasured, and stressor intensities vary); determine whether stressors were natural or anthropogenic (e.g. measuring salinity along a stress gradient within an estuary, which could exist as a result of natural variation, anthropogenic climate change, or water control structures), and; extract important metrics of interest (e.g. levels of stressor examined and duration of exposure).

To be considered relevant, a study must assess, at minimum, the effects of two anthropogenic abiotic stressors in combination. We define a stressor as any anthropogenically influenced environmental variable that extends beyond natural variation. We thus did not consider natural variations or gradients in environmental factors (e.g. tidally influenced hydroperiods, natural salinity gradients) as stressors here but noted whether studies considered such natural variation in the experimental design or how this may influence introduced stressors. Additionally, as our focus was on abiotic stressors, invasive species were not considered
as a stressor here. Biotic factors that may influence stressor effects were
documented only if assessed concomitantly with two or more abiotic
stressors. Therefore, studies that assessed the effects of a single abiotic
stressor (e.g. nutrient input) paired with a biotic interaction (e.g. grazing
pressure) were not included.

There are likely to be studies not captured in our search that examine
the effects of multiple stressors on animals that generally utilise our
focal ecosystems. These, for example, might not explicitly mention
mangroves, saltmarsh, or seagrass (such as animal-focused physiological
studies) and, thus, were not retrieved by our literature search. Here, we
included studies that sampled from, or conducted experiments within,
one or more of our focal ecosystems, including studies that solely
measured responses from affiliated animal and microbial organisms.
Additionally, studies that considered effects of multiple stressors within
a broad ecosystem context such as “estuarine” or “coastal” systems were
also initially captured by our search. These studies might have assessed
sites including one or more of our focal ecosystems but did not detail
habitat-specific responses and, thus, were excluded from this review.
From the 143 studies remaining (Figure A1), we extracted a range of
information types (Table 1). It is important to note that for most types of
information extracted, a single study may be counted multiple times,
and so the sum of percentages is typically greater than 100.

Given recent calls to shift the focus of multiple stressor studies to
incorporate variation in stressor intensity and timing to reflect natural
conditions (Gunderson et al., 2016), we were interested in identifying
changes in the way stressors are introduced in manipulative studies. We
calculated differences in proportion of studies that used various methods
of stressor application (i.e. static, fluctuating, simultaneous, consecu-

2.3. Statistical analysis

Duration of stressor application was extracted from each study,
which was the only non-count variable recorded, and the differences in
duration between ecosystem and study type were assessed using a two-
way analysis of variance (ANOVA). Before analysis, data were tested for
normality (Q-Q plot) and homogeneity of variance (residual plot). Data
were log transformed and significance levels were set at $\alpha = 0.05$. Sta-
tistical analyses were performed using R (version 3.6.3).

3. Results

3.1. Database overview

A total of 143 papers met the criteria and were included in this re-
view. Studies were conducted across 23 countries, most in the USA
(36%), followed by China (12%), Australia (10%), and Spain (8%) and
Portugal (8%; Fig. 1A). Multiple stressors were most studied in seagrass
ecosystems (52%), followed by saltmarsh (35%) and mangrove (13%)
ecosystems. Most saltmarsh and seagrass studies were conducted in the
USA (55% and 28%, respectively), whereas most mangrove studies were
conducted in China (44%). Where possible, we ideally identified the
location of sample collection. Where this was not possible, we relied on
the location where the experimental work was conducted. This was not
the case for nearly all studies, we relied on the location where the experi-
mental work was conducted as the location. It is possible that lab studies used translocated specimens, but in
most cases, we expect that the article would still describe the collection
site.

Proportionally, the number of publications documenting manipula-
tive multiple stressor experiments has increased over time (Fig. 1B). For
approximately two decades, the rate of publication increase generally


**3.2. Experimental design**

Although experimental designs differed across ecosystems, many similarities existed between saltmarsh and mangrove experiments. Control-impact designs were more commonly used in saltmarsh (59%) and mangrove (72%) studies, with before-after-control-impact designs most common in seagrass studies (54%). Additionally, laboratory studies made up a greater portion of seagrass studies (54%) relative to mangrove (39%) and saltmarsh (28%; Fig. 2A). The reverse was true for studies conducted in a greenhouse; common in saltmarsh (47%) and mangrove (50%) experiments relative to seagrass (8%). Field studies were less common across all ecosystems (saltmarsh 26%, mangrove 17%, and seagrass 17%), as were outdoor mesocosm experiments (saltmarsh 8%, mangrove 11%, and seagrass 21%; Fig. 2A).

**3.3. Common stressors studied**

A variety of stressors were tested, differing across ecosystem type. Overall, the effects of nutrient loading (44%) and temperature (33%) were tested most often. Nutrient loading was also the most common stressor tested in all three ecosystems (saltmarsh 41%, mangrove 33%, and seagrass 50%; Figure A3). Most studies tested the effects of stressors at no more than two levels (i.e. control and one stressor level; 54%), with fewer applying stressors at three (26%), four (8%), and five or more.

**Table 1**

<table>
<thead>
<tr>
<th>Information type</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Geographic location</td>
<td>Country, state and specific location the study took place</td>
</tr>
<tr>
<td>Study type</td>
<td>Laboratory, greenhouse, outdoor mesocosm or field experiments</td>
</tr>
<tr>
<td>Ecosystem type</td>
<td>Saltmarsh, mangrove or seagrass ecosystems</td>
</tr>
<tr>
<td>Duration of stressor application</td>
<td>The length of time (days) the stressor was applied</td>
</tr>
<tr>
<td>Plant species</td>
<td>Species of all plants studied</td>
</tr>
<tr>
<td>Plant life stage</td>
<td>Seed, seedling or adult (including young adult plants)</td>
</tr>
<tr>
<td>Animal taxa</td>
<td>Species of animals studied, if any</td>
</tr>
<tr>
<td>Additional organisms considered in study</td>
<td>Bacteria, fungi, or protists</td>
</tr>
<tr>
<td>Type of stressor</td>
<td>Abiotic or a combination of abiotic and biotic factors</td>
</tr>
<tr>
<td>Biotic factors assessed</td>
<td>Biotic factor considered concomitantly with abiotic stressors (e.g. competition, pathogens, herbivory, predation)</td>
</tr>
<tr>
<td>Stressors studied (general)</td>
<td>General classification of stressors (e.g. nutrients, sediments, metal)</td>
</tr>
<tr>
<td>Stressors studied (specific)</td>
<td>Specific stressor classification (e.g. nitrogen, lead)</td>
</tr>
<tr>
<td>Levels of stressors</td>
<td>Number of levels applied (including control) of each stressor</td>
</tr>
<tr>
<td>Application of stressors</td>
<td>Specific values established (e.g. °C, pH) or concentrations applied (e.g. nM, μM)</td>
</tr>
<tr>
<td>Method of stressor introduction</td>
<td>Whether stressor intensity remains static or fluctuates and whether stressors are introduced simultaneously or consecutively</td>
</tr>
<tr>
<td>Spatiotemporal variability considered</td>
<td>Natural variation in environmental variables over time and space (e.g. tidal cycle, seasonal variations) included in experimental design</td>
</tr>
<tr>
<td>Response variable measured</td>
<td>Variable assessed following stressor introduction (e.g. survival, photosynthesis, respiration)</td>
</tr>
<tr>
<td>Response variable category</td>
<td>Responses categorised as fitness (e.g. growth, survival), structure (e.g. biomass, biodiversity), or function (e.g. production, nutrient cycling)</td>
</tr>
<tr>
<td>Response level considered</td>
<td>Molecular, individual, population, community, ecosystem process</td>
</tr>
<tr>
<td>Statistical analysis method</td>
<td>Statistical test used (e.g. ANOVA, structural equation modelling)</td>
</tr>
</tbody>
</table>

The duration of stressor application varied by the ecosystem in which studies were conducted and by study type (interaction term: p = 0.048; ecosystem type p < 0.001; study type p < 0.001). In seagrass experiments, exposure duration was typically shorter than for saltmarsh or mangrove, and varied little across study type, with slightly longer time frames in field studies (Fig. 2B). Saltmarsh and mangrove studies applied stressors similarly in duration, which were on average longer than in seagrass studies. Duration increased from lab studies, to greenhouse studies, to outdoor mesocosms, and finally to field studies, apart from similar duration observed between mangrove outdoor mesocosm and field studies (Fig. 2B).

The effects of multiple stressors were examined on a large diversity of plant species and across various life stages, which differed by ecosystem type (Table 2). Notably, 67% of mangrove studies observed stressor effects on seedlings, while no studies tested effects on seeds. In seagrass experiments, nutrient loading was typically shorter than for saltmarsh or mangrove, and varied little across study type, with slightly longer time frames in field studies (Fig. 2B). Saltmarsh and mangrove studies applied stressors similarly in duration, which were on average longer than in seagrass studies. Duration increased from lab studies, to greenhouse studies, to outdoor mesocosms, and finally to field studies, apart from similar duration observed between mangrove outdoor mesocosm and field studies (Fig. 2B).

The effects of multiple stressors were examined on a large diversity of plant species and across various life stages, which differed by ecosystem type (Table 2). Notably, 67% of mangrove studies observed stressor effects on seedlings, while no studies tested effects on seeds. In seagrass experiments, nutrient loading was typically shorter than for saltmarsh or mangrove, and varied little across study type, with slightly longer time frames in field studies (Fig. 2B). Saltmarsh and mangrove studies applied stressors similarly in duration, which were on average longer than in seagrass studies. Duration increased from lab studies, to greenhouse studies, to outdoor mesocosms, and finally to field studies, apart from similar duration observed between mangrove outdoor mesocosm and field studies (Fig. 2B).

The effects of multiple stressors were examined on a large diversity of plant species and across various life stages, which differed by ecosystem type (Table 2). Notably, 67% of mangrove studies observed stressor effects on seedlings, while no studies tested effects on seeds. In seagrass experiments, nutrient loading was typically shorter than for saltmarsh or mangrove, and varied little across study type, with slightly longer time frames in field studies (Fig. 2B). Saltmarsh and mangrove studies applied stressors similarly in duration, which were on average longer than in seagrass studies. Duration increased from lab studies, to greenhouse studies, to outdoor mesocosms, and finally to field studies, apart from similar duration observed between mangrove outdoor mesocosm and field studies (Fig. 2B).
Most studies considered the effects of no more than two stressors in combination (81%). Temperature and salinity (8%) was the most common stressor combination tested across all ecosystems, followed by temperature and nutrient loading (8%), temperature and acidification (7%), and nutrient loading and light (7%; Fig. 3). Nutrient loading was tested in combination with each of the other top ten stressors observed across all studies, whereas other common stressors tested, such as metals, were not (Fig. 3). Common stressor combinations also varied by ecosystem type (Figure A4). The stressor combination most studied in saltmarsh ecosystems was atmospheric CO₂ enrichment and salinity (12% of total saltmarsh studies); in mangrove ecosystems, sea-level rise and salinity (17%), and two or more metals in combination (17%) were the most common stressor combinations, and finally, light and nutrient loading (12%), temperature and nutrient loading (12%), and temperature and acidification (12%) were the most common stressor combinations in seagrass ecosystems (Figure A4).

Few studies considered the effects of three or more stressors in combination (19%) and the combinations of stressors differed greatly across ecosystems. Of these studies (N = 28), 53% were in seagrass, 36% in saltmarsh, and 11% in mangrove ecosystems. The most common three-stressor combinations were three different herbicides and three different metals (Table A1). Additionally, six studies tested the combined effects of four stressors (one saltmarsh and five seagrass studies), all of which assessed unique combinations except two seagrass studies that observed four different herbicides in combination (Table A1). Finally, one saltmarsh experiment tested the cumulative effects of five stressors, which consisted of four types of metals combined with nutrient loading. Refer to Table A1 for a complete list of combinations of three or more stressors observed across ecosystems.

3.4. Stressor application

Consideration of natural environmental variation in multiple stressor experimental design was similar across ecosystems. Environmental spatiotemporal variability (e.g. tidal flux, seasonal variation, multiple site locations) was incorporated in approximately half of all studies (52%). However, few incorporated the effects of natural environmental variability into how they applied and manipulated the stressors (i.e. changes in intensity and timing of stressors). Based on our objective, our exclusion of correlative studies that include natural variation is warranted, however it does mean that there are studies outside of those reviewed here that will have incorporated natural variation. The method of stressor application, as well as the magnitude and timing at which stressors were introduced, was similar across ecosystems (Fig. 4A). The dominant way to apply stressors was static (i.e. stressor intensity applied at a constant rate for the duration of the experiment; 80%) and simultaneous (i.e. multiple stressors applied at the same time; 90%; Fig. 4A). Comparatively few studies applied stressors as fluctuating (i.e. stressor intensity changes over time; 36%) or consecutively (i.e. stressors introduced one after the other; 16%; Fig. 4A). Additionally, 16% of studies applied stressors at both static and fluctuating levels of intensity, while 6% applied stressors both simultaneously and consecutively. Following the call by Gunderson et al. (2016) that fluctuating and consecutive methods of stressor application are important to consider in experimental design, there has been little increase in their use (Fig. 4B).
3.5. Biological responses to stressors

Numerous response variables were measured following stressor introduction, which differed by ecosystem type. We categorised variables into different orders of hierarchy including: molecular (N = 174), whole individual (N = 328), population (N = 319), community responses (N = 84), ecosystem processes (N = 24) and sediment properties (N = 29; for specific variables that fall under these categories, see Fig. 5). Most studies measured multiple variables (mean ± SE; 6.7 ± 0.3) across multiple levels of biological organisation (2.6 ± 0.1). Across all categories, biomass and growth were the most common response variables measured (Figure A.7). Biomass in saltmarsh and mangrove ecosystems, and growth in seagrass ecosystems were the most commonly measured response variables per ecosystem (Fig. 5). Despite differences among ecosystems in terms of the absolute number of studies to assess various responses, there is consistency in the proportion of common response variables measured per level of biological organisation (Fig. 5). Plant tissue biochemistry (e.g. concentration of nutrients, metals, or contaminants within tissue; 36%) was the most common response variable measured at the molecular level. Growth (52%) at the individual level, as well as biomass at the population (54%) and community (17%) levels were the most commonly measured responses for these levels of biological organisation. Additionally, herbivory (6%) was the most commonly measured ecosystem process (Fig. 5).

4. Discussion

4.1. Overview

Our review of how manipulative multiple stressor experiments are conducted in saltmarsh, mangrove, and seagrass ecosystems highlights the substantial and recent surge in this field. This is likely driven by several seminal reports highlighting the importance of understanding the effects of multiple interacting stressors on ecosystems (Cote et al., 2016; Gunderson et al., 2016; Orr et al., 2020). Of the 143 studies we reviewed, most were conducted in North America and Europe, with few studies from areas with high levels of environmental impact (e.g. Brazil, Singapore, India, Bangladesh, Thailand; Bradshaw et al., 2010). Many of these regions also contain substantial areas of diverse coastal wetland ecosystems (Jayathilake and Costello 2018; Romaniach et al., 2018). To investigate potential differences in multiple stressor impacts geographically, it would be helpful to increase research effort in less well-studied regions and compare findings to existing research.

4.2. Experimental design

Most research was conducted in seagrass ecosystems, while mangroves received the least attention. Additionally, most mangrove studies focused at the seedling life stage, which is likely due to the fact that this is the initial growth stage of the plant in which it is most vulnerable to stress, as well as the comparative ease of experimentation relative to adult trees. Manipulative multiple stressor experiments are mostly conducted in highly controlled environments; primarily greenhouses for mangrove and saltmarsh studies, and in the laboratory for seagrass studies. Although similar, greenhouse experiments were often exposed to ambient light and temperature conditions in contrast to artificial lighting and regulated temperatures in the laboratory. These experimental designs are ideal for accurately manipulating factors of interest and aim to limit confounding variables that can influence outcomes and make it difficult to evaluate responses caused solely by stressors. However, in reducing environmental realism, these studies can produce conflicting results compared to more realistic field experiments (Crain et al., 2008; Wernberg et al., 2012). Additionally, stronger stressor effects are generally measured in controlled environments but are also more likely to include spurious effects less applicable for management (Crain et al., 2008). Conversely, field studies often measure overall weaker stressor effects but provide more relevant results by incorporating natural environmental conditions. The paucity of field studies is likely due to the difficulty associated with experimental design (e.g. sufficient replication), costs associated with large-scale manipulations and monitoring, and ethics related to the disruption of entire ecosystems. The ability to study effects of stressors at higher levels of
organisation and trophic levels is more difficult under a laboratory setting (Boyd and Brown 2015). This poses a challenge when extrapolating findings from studies on individual species to predict effects on populations or entire communities (Darling and Cote 2008; Griffen et al., 2016; Hodgson and Halpern 2019).

Duration of stressor application varied by ecosystem and study type, with highly controlled experiments shorter and field studies longer. Stressor duration may influence the effects on an organism or system because the cumulative effects of, or interactions among, stressors may vary over time, where measurable changes only occur over longer durations or overall effects may be mediated through time (Cote et al., 2016; Griffen et al., 2016; de los Santos et al., 2019). For example, a three-year field experiment identified changes in microbial community composition of a Spartina patens-dominated saltmarsh after annual nutrient treatments. Interannual and interseasonal variability, as well as nutrient addition, significantly altered microbial community composition. Results indicated the microbial communities within this system, although affected by nutrient addition, naturally shifted over time (Lage et al., 2010), illustrating the importance of considering natural long-term variability to predict effects of multiple stressors. Notably, the length of experiments may be longer than the duration of stressor application recorded in this review, such as studies that incorporated a prolonged period of observation following stressor application (e.g. Martone and Wasson 2008). This approach is beneficial for detecting long-term effects and identifying consistent trends in stressor responses (O’Gorman et al., 2012).

4.3. Common stressors studied

Studies evaluated ecologically relevant and important stressor combinations operating within saltmarsh, mangrove, and seagrass ecosystems. Nutrient loading and temperature were the most common stressors tested, with the combination of temperature and salinity the most common stressor pair tested. These results are consistent with more generalised findings from previous reviews assessing multiple stressor research conducted across all marine environments (e.g. Crain et al., 2008; Przeslawski et al., 2015; Gunderson et al., 2016). Nutrient loading was the only stressor that was combined with all the other top ten stressors identified across habitats. This may be explained by the relative ease at which this stressor can be manipulated. Additionally, the

---

Fig. 5. The total number of studies that measured response variables at molecular (A), individual (B), population (C), and community (D) levels of biological organisation, as well as measures of ecosystem processes (E) and sediment properties (F) in response to multiple stressors. Some response variables can be measured across more than one level and therefore are represented in multiple panels in this figure (e.g., biomass of the individual or entire population). Only the top response variables measured are shown. Note that studies measured more than one response variable and across more than one response level.
accelerated increase of nutrient loading in coastal environments due to human activities, which contributes to a significant decline in coastal water quality, may spur increased interest in understanding the environmental consequences associated with these events (Rabalais et al., 2009). Many studies also examined the effects of one or more climate variables (e.g. increased temperature, acidification, sea-level rise) concomitantly with a local stressor (e.g. nutrient loading, metal); most often temperature or acidification was paired with nutrient loading (e.g. Jiménez-Ramos et al., 2017; Gillis et al., 2019). Local stressors may affect ecosystem resilience to global change; therefore, elucidating the combined effects of global and local stressors may provide valuable insights for improved management of coastal ecosystems under changing global conditions (Brown et al., 2013; Falkenberg et al., 2013; He and Silliman 2019). Ultimately, a better understanding of various stressor interactions across ecosystems will inform management of the most effective strategies for removing target stressors to benefit impacted ecosystems (Stockbridge et al., 2020).

Our findings highlight stressor combinations that require attention in future research. Climate change, contaminants, nutrient loading, and sediment input are among the greatest threats to coastal ecosystems worldwide (Lu et al., 2018; He and Silliman 2019) and may naturally co-occur in the environment. Therefore, potentially environmentally important stressor combinations not analysed across our three focal habitats include: temperature and metal; acidification and herbicide; and sea-level rise and metal or herbicide or acidification. Furthermore, sedimentation was only considered in combination with nutrient loading; however, additional relevant combinations may include sediment paired with sea-level rise or contaminants. Increased application of novel stressor combinations highly relevant to coastal ecosystems will improve our understanding of anthropogenic impacts to these environments.

Designing and conducting multiple stressor experiments can become increasingly complex depending on the variables of interest within the study. Manipulating three or more combined stressors increases the number of possible interactions and the resources required to conduct the experiment. Additionally, application of a range of levels (i.e. intensities or concentrations) for each stressor further increases the complexity of the experiment. Such complexity may explain the paucity of studies that applied three or more stressors in combination or applied four or more levels (including the control) of stressors. Suggestions for realistically increasing experimental complexity are to generalise stressor interactions and responses (Cote et al., 2016), as well as use a collapsed or reduced design to assess only the most relevant levels and combinations of stressors to the organism or ecosystem of interest, rather than implement a fully factorial design (Boyd et al., 2018).

4.4. Stressor application

We found a lack of consideration for how spatiotemporal variation in coastal environments may interact with stressors to influence changes in stressor intensity and timing (Przeslawski et al., 2015; Gunderson et al., 2016). The physicochemical environment of coastal ecosystems is highly dynamic and constantly changing either predictably (e.g. tidal cycles) or randomly (e.g. storm disturbance; Wernberg et al., 2012), which may impact the nature of stressor interactions. However, many studies did not explicitly consider how stressor intensity and timing may influence ecosystem response to stressor exposure. Despite the important review by Gunderson et al. (2016) calling for consideration of spatiotemporal variation existing within natural environments in multiple stressor study design, the proportion of studies incorporating variability in stressor intensity and timing observed in coastal ecosystems has not increased. We acknowledge, however, that not much time has passed since publication, so a change in how stressors are applied might still be on its way.

Manipulating stressor intensity, whether fluctuating or static, might better reflect reality and thus better inform how ecosystems will respond to multiple stressors (Przeslawski et al., 2015; Gunderson et al., 2016; Klein et al., 2019). Variable stressor intensity may influence the timing of interactions with other stressors, which may impact the overall effects on organisms and ecological processes (Gunderson et al., 2016). For example, Ontoria et al. (2019) identified no significant interaction between increased temperature and ammonium on the seagrass, Cymodocea nodosa, when nutrient levels fluctuated throughout the experiment as a result of pulsed inputs. Alternatively, Egea et al. (2018) showed that the interactive effects of increased temperature and ammonium input significantly increased C. nodosa net production rates when nutrients remained at static levels for the duration of the experiment. Of the studies that varied stressor intensities, nutrient loading was the most common stressor applied at fluctuating levels. Nutrient addition simulated pulse inputs that may result from hydrologic factors such as tidal cycles and river discharge (Hale et al., 2015), seasonal storm disturbances and heavy rainfall (Xing et al., 2017), or agricultural effluent or sewage discharge (Xing et al., 2017; Wang et al., 2019). Other stressors (e.g. contaminants, temperature, pH) that are likely to vary considerably across both space and time were rarely considered in studies that incorporated variability in stressor application. Consequently, future research should include stressor fluctuations that represent natural spatiotemporal variations relevant to the study organism or system across all relevant stressor types (Wernberg et al., 2012; Gunderson et al., 2016).

The order in which stressors are introduced to a system – either consecutively or simultaneously – may also affect the impacts of multiple stressors (Breitburg et al., 2015; Gunderson et al., 2016; Orr et al., 2020). For example, exposure to one stressor may increase (e.g. Wizniewska et al., 2019) or decrease (e.g. Hanson et al., 2016) the tolerance of a system to subsequent stressors, whereas stressors that occur close in time are more likely to produce interactive, and often synergistic, effects (Gunderson et al., 2016). High variability within coastal ecosystems, such as diel variation in temperature and pH, may result in stressors that occur in or out of phase with one another. However, few studies introduced stressors consecutively. Furthermore, only one study investigated the potential for different responses due to the order in which each stressor was introduced by also assessing the effects of stressors applied in reverse order. This can have a strong effect on responses. For instance, Macinnis-Ng and Ralph (2004) observed greater toxicity when seagrass was exposed to metal first followed by herbicide, as opposed to herbicide followed by metal. These findings highlight the importance of investigating different stressor application methods.

4.5. Biological response to stressors

Studies measured multiple response variables across various levels of biological organisation, typically focusing on fitness, structural, and functional endpoints. Proportionally few studies measured effects at the community level or on ecosystem processes. Response to stressors may become increasingly complex at higher levels as some organisms within communities may be adversely affected while others benefit (Griffen et al., 2016; Orr et al., 2020). For example, Brustolin et al. (2019) discovered changes in metacommunity structure of seagrass ecosystems under the combined effects of increased temperature and acidification, resulting in reduced biodiversity but increased abundance of more tolerant, generalist species. Shifts in community structure may reduce functional diversity within ecosystems (Brustolin et al., 2019) and affect production, energy flow, and other ecological processes within existing communities (Brustolin et al., 2019; Nagelkerken et al., 2020). Accurately predicting community shifts due to multiple stressors may pose challenges if extrapolating findings from individual or population levels that exclude fundamental biotic interactions that can lead to unexpected responses or impose greater impacts on ecosystems (Urban et al., 2016). Furthermore, Hillebrand et al. (2020) compiled data on a suite of ecological responses to environmental pressures, and identified a lack of clearly defined thresholds that lead to critical transitions. This could result in overstated ecological concerns if predicted thresholds to
pressures within ecosystems are not observed and highlights a need to quantify responses across multiple levels of biological organisation to fully evaluate outcomes from exposure to multiple stressors.

Biotic interactions may dampen or amplify physiological sensitivities of individual species to stressors identified in small-scale multiple stressor studies when assessed at the community level (Riebesell and Gattuso 2014; Boyd et al., 2018). However, few studies assessed the effects of biotic factors concomitantly with abiotic stressors, despite its importance (Wernberg et al., 2012). For example, top-down control by consumers can reduce the effects of increased temperature and nutrients in seagrass ecosystems by reducing epibenthic biomass and intensifying heat stress due to macroalgal shading (Brodeur et al., 2015). Kelso et al. (2020) identified extreme reductions in plant cover of the invasive species, Lepidium latifolium, under drought conditions within a tidal saltmarsh, which allowed native plants to out-compete the invader species under these extreme conditions. However, nutrient addition reduced the effects of drought on all plants, suggesting advantages of native plants over L. latifolium may be reversed under combined drought and nutrient stress (Kelso et al., 2020).

4.6. Multivariate statistics can enhance broader understanding

Most multiple stressor research used univariate rather than multivariate techniques to analyse results despite the benefits of multivariate analyses. Multivariate statistical methods allow for simultaneous consideration of multiple response variables (Stuber et al., 2019), which is appropriate for evaluating concurrent effects of numerous response variables measured in multiple stressor ecological research. One useful statistical method is advanced pathway analyses, such as structural equation modelling (SEM). This is a multivariate technique that assesses direct and indirect effects of all hypothesised causal relationships simultaneously (Lefcheck and Freckleton 2015; Stuber et al., 2019). This method can provide a holistic assessment of complex networks and relationships within ecosystems (Fan et al., 2016), as well as a mechanistic evaluation of multiple stressors (Adams 2005; Hodgson and Halpern 2019) that other more common methods (e.g. ANOVA) cannot (e.g. Cherry et al., 2009; Blake and Duffy 2012). For example, Alsterberg et al. (2013) investigated the effects of temperature, acidification, and mesograzers on a seagrass community. Use of SEM identified strong direct and indirect stressor effects on benthic microalgae when mesograzers were absent but revealed weak or absent effects when present, whereas an ANOVA identified only weak cumulative effects regardless of mesograzers presence. The possibility for mediated stressor effects due to biotic interactions were not otherwise identified with a standard ANOVA. Despite the benefits of using path analysis techniques in ecological research, only three studies in this review used this statistical method.

4.7. Recommendations for future research

A significant body of multiple stressor research conducted in saltmarsh, mangrove, and seagrass ecosystems has contributed to our current understanding of how stressors interact to affect coastal ecosystems. However, we still lack a comprehensive understanding of complex stressor interactions and various net impacts on coastal ecosystems under realistic conditions. Therefore, continued research efforts that assure conditions are representative of natural ecosystems are required to better predict outcomes of ecologically important stressor interactions and develop more effective, habitat-specific management strategies. Based on our review and appreciating the time and financial constraints that affect much research, we recommend future research should focus on increasing ecological realism in multiple stressor experiments by considering the following:

1. Incorporate spatiotemporal variability, particularly in laboratory, greenhouse, and mesocosm experiments, to reflect changes in stressor intensity and timing representative of complex, dynamic conditions within coastal ecosystems (Griffen et al., 2016; Gunderson et al., 2016).

2. Conduct experiments over longer durations to analyse how stressors and their effects may change over time in natural environments (Cote et al., 2016; Griffen et al., 2016).

3. Pair small-scale manipulative experiments for enhanced mechanistic understanding of stressor effects with broad-scale, manipulative field studies that reflect natural environmental variability within communities. This approach will bolster the ecological relevance of results, which is valuable for accurately predicting effects of multiple stressors in natural ecosystems (Boyd et al., 2017).

4. Investigate cumulative effects of novel, ecologically relevant stressor combinations that operate within coastal ecosystems (Gunderson et al., 2016), for example: sea-level rise and acidification or sediment, light and contaminants, and sediment and contaminants or temperature.

5. Consider the effects of species interactions within natural communities concurrently with abiotic stressors, and evaluate how these interactions may mediate or amplify cumulative stressor effects in coastal ecosystems (Wernberg et al., 2012).

6. Use multivariate statistical methods to simultaneously assess multiple lines of evidence for a holistic approach to understanding multiple stressor effects in coastal ecosystems (Stuber et al., 2019). Furthermore, application of path analyses will further enhance our mechanistic understanding of how stressors operate in coastal ecosystems through assessment of direct and indirect stressor effects (Lefcheck and Freckleton 2015).

5. Conclusion

Coastal wetlands are susceptible to impacts from expanding human populations and increased activity along coastlines, which introduce various co-occurring stressors to coastal environments. This review provides an overview of current multiple stressor research conducted in saltmarsh, mangrove, and seagrass ecosystems. We highlight significant gaps that limit our understanding of the cumulative effects of anthropogenic stressors on ecosystem structure and function. Most studies were conducted in highly controlled environments that reduce ecological realism and lack consideration of natural spatiotemporal variability that may influence the nature, and overall impact, of multiple stressors. Our results suggest that researchers should design experiments under conditions representative of natural ecosystems, elucidate mechanisms of multiple stressor impacts along ecologically relevant spatiotemporal scales, and investigate novel stressor combinations likely to affect coastal ecosystems. Continued research will improve predicted outcomes of multiple stressor effects within specific ecosystems and inform the development of effective management strategies to mitigate adverse human impacts on coastal ecosystems.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

Author contributions

All authors: Conceptualization; AO: Data curation; AO: Formal analysis; All authors: Methodology; All authors: Visualization; AO: Writing - original draft; All authors: Writing - review & editing.

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.
Acknowledgements

We acknowledge support from the Global Wetlands Project, supported by a charitable organisation which neither seeks nor permits publicity for its efforts. We thank Renee Piccolo and Dr Ryan Pearson for comments on earlier drafts of this manuscript.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.marenvres.2020.105239.

References


