Multiple Stressors in a Changing World: The Need for an Improved Perspective on Physiological Responses to the Dynamic Marine Environment

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Keywords

Abstract

Abiotic conditions (e.g., temperature and pH) fluctuate through time in most
marine environments, sometimes passing intensity thresholds that induce
physiological stress. Depending on habitat and season, the peak intensity of
different abiotic stressors can occur in or out of phase with one another. Thus,
some organisms are exposed to multiple stressors simultaneously, whereas
others experience them sequentially. Understanding these physicochemical
dynamics is critical because how organisms respond to multiple stressors de-
pends on the magnitude and relative timing of each stressor. Here, we first
discuss broad patterns of covariation between stressors in marine systems at
various temporal scales. We then describe how these dynamics will influence
physiological responses to multi-stressor exposures. Finally, we summarize
how multi-stressor effects are currently assessed. We find that multi-stressor
experiments have rarely incorporated naturalistic physicochemical variation
into their designs, and emphasize the importance of doing so to make eco-
logically relevant inferences about physiological responses to global change.

INTRODUCTION

Marine habitats are under increasing threat from human activity (Doney et al. 2012), with pronounced ecological consequences. These consequences include shifts in geographical ranges (Beaugrand et al. 2002, Perry et al. 2005, Sunday et al. 2012); changes in the strength and types of ecological interactions (Milazzo et al. 2012, Pincebourde et al. 2012, Sanford 1999); and, in the worst case scenarios, population collapse and species extinction (McCauley et al. 2015). A fundamental goal within the field of global change biology is to diagnose the causes of ecological changes that have occurred and, perhaps most importantly, to predict what species or populations are under threat from future anthropogenic change (Carpenter et al. 2008, Cheung et al. 2009). Once we have a predictive understanding of responses to climate change, we can begin to make informed decisions about how to manage marine habitats to minimize climate change's effects (Bernhardt & Leslie 2013, Green et al. 2014).

A major obstacle to developing robust predictions about biological responses to climate change in marine habitats is the multifarious nature of environmental change. Depending on location, organisms are experiencing changes such as increased water temperatures (Burrows et al. 2011, Gleckler et al. 2012), decreased pH (Hofmann et al. 2011), increased pollution such as dissolved heavy metals (Walker & Livingstone 1992), changes in salinity caused by altered freshwater inputs (Korhonen et al. 2013), and lowered oxygen availability (Keeling et al. 2010). Until recently, environmental physiologists have relied primarily on carefully controlled laboratory experiments in which they manipulate a single environmental variable in order to assess organismal performance under changing conditions (Todgham & Stillman 2013). However, investigators are increasingly realizing that single-stressor experiments may not be appropriate in assessing the effects of climate change in marine habitats (Wernberg et al. 2012). Experimental marine biologists now often incorporate two or more environmental factors into their experimental designs, with the hope of generating more realistic inferences about the effects of global change (e.g., Benner et al. 2013, Boyd et al. 2015, Dorts et al. 2014, Feidantsis et al. 2015, Harms et al. 2014, Lefebvre et al. 2012, McBryan et al. 2013, Melzner et al. 2013, Paganini et al. 2014, Przeslawski et al. 2015, Schalkhausser et al. 2014, Schluter et al. 2014). For example, studies on phytoplankton have examined the combination of warming and acidification (e.g., Benner et al. 2013, Schluter et al. 2014), acidification and nutrients (e.g., Lefebvre et al. 2012), and many other stressor combinations (Boyd et al. 2015).

Multi-stressor studies typically involve subjecting a set of organisms to baseline levels of all stressors, subjecting additional sets of organisms to elevated levels of each stressor individually (often based on projected future conditions), and subjecting another set of organisms to elevated levels of all stressors. Conceptually, organisms subjected to multiple stressors exhibit one of three types of responses: additive, antagonistic, or synergistic (Todgham & Stillman 2013) (**Figure 1**). An additive effect occurs when the combined effect of multiple stressors equals the sum of the effects of each stressor in isolation. For example, in a study of the reef-building coral *Porites panamensis*, increased temperature and reduced pH in isolation decreased coral polyp mass by 21% and 24% respectively; in combination, they reduced mass by 45% (Anlauf et al. 2011). An antagonistic effect occurs when the combined effect of multiple stressors is less than the expected additive effect in isolation. Rautenberger & Bischof's (2006) study of the Antarctic macroalgae *Ulva bulbosa* provides an example of this effect; the authors found that increased exposure to UV radiation inhibited photosynthesis, reducing F_v/F_m by 17%, and that this inhibition was ameliorated by increased temperature, with F_v/F_m values under simultaneous exposure equivalent to those under control conditions (Rautenberger & Bischof 2006). A synergistic effect occurs when the combined effect of multiple stressors is greater than the expected additive effect of the stressors in isolation. For example, increased acidity reduced fertilization success in the coral *Acropora tenuis* by approximately 7%, and
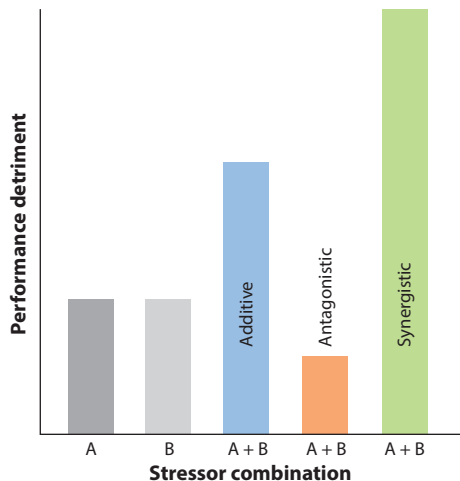
Figure 1

Conceptual diagram of possible effects of multiple stressors on physiological performance. Modified from Todgham & Stillman (2013) with permission from Oxford University Press.

increased temperature reduced fertilization success by 15%; however, the combination of increased acidity and increased temperature reduced fertilization success by 39% (Albright & Mason 2013).

The increased attention to multiple stressors has paralleled an increased focus on the temporal dynamics of environmental stress (Denny & Gaylord 2010, Harley & Paine 2009, Thompson et al. 2013). Experimental biologists have traditionally subjected organisms to constant conditions within a given treatment, usually based on summaries of environmental measurements such as mean or maximum values of a given stressor. However, environmental parameters fluctuate on multiple timescales, from hours to months to years. These fluctuations are likely to be extremely important for dictating how organisms respond to prevailing conditions and to human-induced environmental change (Vincenzi 2014). For example, mean environmental conditions ignore spikes in environmental stress that may have enormous consequences for population dynamics (Denny et al. 2011, Wethey et al. 2011). Similarly, exposure to constant extreme stress ignores reprieves from stress that are likely important in allowing organisms to cope with these conditions.

In this review, we consider multiple stressors in marine habitats from a physiological perspective while highlighting the importance of the timing and magnitude of stress events in mediating multi-stressor effects. We begin by discussing patterns of fluctuation in physicochemical conditions within a variety of marine habitats on multiple timescales. We then discuss the dynamics of the physiological mechanisms by which organisms respond to environmental stress, and in doing so highlight why the timing of environmental stress events and fluctuations in the magnitude of stress are so critical in understanding organismal responses to changing conditions. Finally, we discuss the degree to which multi-stressor studies reflect observed patterns of environmental variation in the field. Our goal is to provide a conceptual framework for understanding the effects of multiple stressors that can be used to facilitate ecologically relevant experimental designs and the interpretation of biological responses to multiple stressors. Although our review focuses on multiple stressors from a physiological perspective, we do not go into great detail about how specific physiological mechanisms promote stress tolerance, as many excellent reviews have already been written on that topic (Hochachka & Somero 2002, Hofmann & Todgham 2010, Kültz 2005, Schulte 2014, Sokolova 2013).
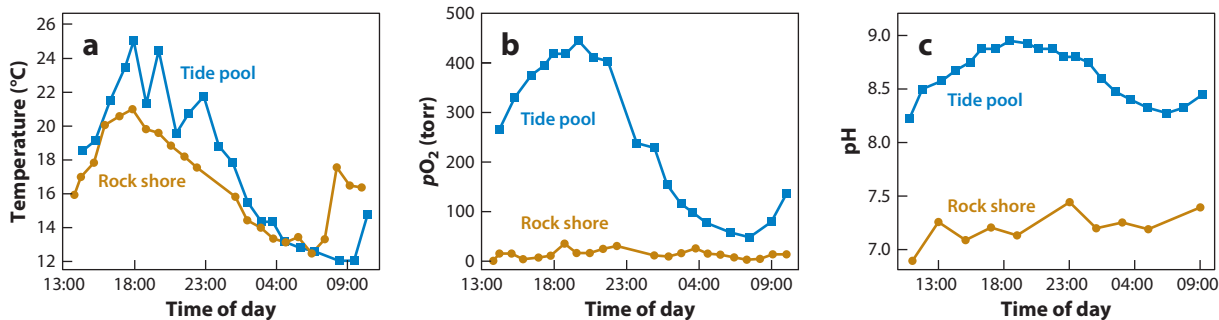
Figure 2

Physicochemical conditions of tide pool and rock shore habitats during summer on the Isle of Cumbrae, Scotland. The thermal conditions (panel *a*) in each type of habitat are similar, but there are large differences in dissolved O_2 (panel *b*) and pH (panel *c*), demonstrating the complex and phasic nature of the intertidal zone habitat. Tide pool data are from air- and sun-exposed pools containing algae and were taken from Morris & Taylor (1983) with permission from Elsevier; rock shore data are from pooled water under stones on a cobble shore and were taken from Taylor (1986) with permission from Taylor & Francis Ltd.

THE DYNAMICS OF ENVIRONMENTAL VARIABILITY

Shoreline and Coastal Habitats

The complex interplay of terrestrial and freshwater inputs and high connectivity between deep- and surface-water reservoirs in shallow coastal systems cause dynamic shifts in environmental conditions on semidiurnal (e.g., tidal influences), diurnal (biological productivity), and seasonal (wind strength, rainwater influx, and solar irradiance) timescales. Furthermore, these dynamics can differ substantially among different habitat types that are in close proximity to one another. This is exemplified by two studies of intertidal habitats on the Isle of Cumbrae, Scotland: one that investigated physicochemical conditions in tide pools (Morris & Taylor 1983) and one that did so for water under stones on a cobble shore (Taylor 1986). During summer months, the tide pools undergo a stereotypical diel pattern of change in dissolved O_2 (DO), temperature, and pH (**Figure 2**). During the day, water temperatures in tide pools rise owing to increased solar radiation and air temperatures, while photosynthetic activity by algae simultaneously increases pH and DO via CO_2 fixation and O_2 production (Ganning 1971, Huggett & Griffiths 1986, Morris & Taylor 1983, Truchot & Duhamel-Jouve 1980). At night, temperatures drop and photosynthesis ceases, so acidity increases and DO decreases owing to respiration (**Figure 2**). The overall result is that organisms in tide pools during the summer experience the greatest thermal stress at times when there is the least acidity and hypoxia stress, but high acidity and hypoxia stress occur simultaneously under cooler conditions (**Figure 2**). Such diel fluctuations can be important in other nearshore habitats as well; for example, diel fluctuations in temperature, acidity, and oxygen similar to those seen in tide pools have been observed in several coastal habitats, such as coral reefs (Le Campion-Alsumard et al. 1993, Ohde & van Woesik 1999, Shaw et al. 2013), kelp forests (Cornwall et al. 2013), and estuaries (Pinkster & Broodbakker 1980).

In contrast to the tide pools, water found under stones on the cobble shores of the Isle of Cumbrae do not contain photosynthetic algae. Thus, daily increases in under-rock water temperature are not accompanied by concomitant increases in DO, causing high temperature and hypoxic conditions to occur simultaneously (Taylor 1986). In addition, the lack of algae reduces diel fluctuations in pH. Diel patterns of environmental stress within these habitats are also overlain by seasonal variation. In the tide pools during the winter, both photosynthesis and respiration are

greatly reduced; as a result, fluctuations in temperature, acidity, and DO are minimized and diel patterns are muted (Morris & Taylor 1983). Within the cobble habitat, under-rock acidity was greatest in the summer months, when temperatures are highest and DO is lowest. In other words, on this rocky shore, diel and seasonal patterns each create simultaneous spikes in temperature, DO, and pH on different timescales (Taylor 1986).

Seasonal dynamics are important in coastal habitats beyond tide pools. Seasonal peaks of temperature and acidity stress are coincident in waters surrounding coral reefs in Bermuda, with the highest levels of pCO_2 occurring in the summer, when water temperatures are also highest (Bates 2002). Similarly, water temperature and UV stress are correlated but peaked during different months on a rocky Australian shore (Przeslawski & Davis 2007). Differences in seasonal relationships between stressors sometimes occur within broadly similar habitats. For example, a physicochemical analysis of two lagoons in the Mediterranean (one in the Adriatic Sea and one in the Tyrrhenian Sea) found seasonal patterns of physical variation that differed between sites (Specchiulli et al. 2008). In one lagoon, monthly means of temperature were positively correlated with salinity but negatively correlated with DO; in the other, mean monthly temperatures were positively related to DO and had no relationship to salinity. Thus, in one lagoon, high temperatures and salinity occur simultaneously with oxygen stress, whereas in the other, temperature and oxygen stress are out of phase and have no relationship to salinity.

Similarly, a recent analysis of the relationships between carbonate chemistry parameters (pCO_2), temperature, oxygen, and salinity in Puget Sound, Washington, found that acidity is strongly correlated with temperature and oxygen, but the direction and strength of the correlations changed seasonally and across geographic locations as a result of changes in prevailing wind direction and differences in the extent of benthic-pelagic mixing (Reum et al. 2014). In addition, there were strong seasonal shifts in the magnitude of environmental variability, with periods of upwelling (typically mid-to-late summer on the US West Coast) displaying greater physicochemical variability than other times of the year. Similar shifts in environmental variability occur in many open-ocean habitats where the strength of physical drivers (wind stress, rainfall, etc.) changes with season (Takahashi et al. 1993).

Perhaps the most well-studied coastal examples of patterns of physicochemical covariation are in eastern boundary current systems (e.g., the Benguela, California, Canary, and Humboldt Currents), where seasonal upwelling causes a suite of changes in surface-water chemistry (Bednarsek et al. 2014, Feely et al. 2008, Hauri et al. 2013, Mohrholz et al. 2014). Deep water that has been out of communication with the surface mixed layer tends to be enriched in CO_2 (lower pH) and depleted in O_2 relative to surface waters as a result of water column and benthic respiration. Therefore, during periods of upwelling, organisms experience the greatest acidity stress in phase with low DO (high hypoxic stress), whereas at relatively low temperatures, conditions are analogous to those experienced by tide pool organisms at night (**Figure 2**). However, the strength and duration of upwelling events vary seasonally, and upwelling systems often fluctuate between periods of low variability in pCO_2 (driven primarily by biological activity) and periods of high variability (driven by seasonally strong physical processes) (Frieder et al. 2012).

Open-Ocean Habitats

In comparison to nearshore and coastal environments, open-ocean habitats are characterized by low variability in physicochemical conditions, with any changes driven by factors that occur over longer temporal (e.g., seasonal) or larger spatial (e.g., oceanic gyres) scales. Here, we discuss the salient physicochemical features of a range of oceanic regions (gyres), focusing primarily on upper-ocean (surface mixed layer) habitats and on perturbations driven by the interplay between

deep- and surface-water reservoirs (upwelling, tidal mixing, etc.) and the influence of biological processes.

Along the equator, wind-driven Ekman transport can initiate upwelling, resulting in the co-incidence of high-$p\mathrm{CO_2}$, low-oxygen waters with relatively low temperatures occurring at the surface (Sweeney et al. 2005). However, this phenomenon is largely restricted to the central and eastern equatorial Pacific, where strong easterly trade winds blow unimpeded by continental land-masses (England et al. 2014). When equatorial Ekman pumping is particularly pronounced, such as during La Niña years, sea surface temperatures can vary significantly in both space and time (anomalies of $-3°C$; McPhaden & Hayes 1990). At other times, the magnitude and duration of the physicochemical fluctuations along the equator tend to be small (typical anomalies of $<1°C$; McPhaden & Hayes 1990) compared with other upwelling regimes (see description of coastal habitats above).

To either side of the equator lie large regions of circulating water masses called the subtropical gyres. Collectively, these gyres comprise 40% of the Earth's surface and include the largest con-tiguous biome on the planet (Karl & Church 2014). The vast majority of subtropical hydrographic data come from two long-monitored time-series stations: the Bermuda Atlantic Time-Series Study site and station ALOHA of the Hawaii Ocean Time-Series. Analysis of daily fluctuations in phys-icochemical conditions at both of these sites revealed a diel pattern similar to but several orders of magnitude smaller than that of nearshore environments, with a drawdown of $p\mathrm{CO_2}$ during the day, when temperatures and irradiance are high (Bates et al. 1996, Dore et al. 2009). The same pattern holds on seasonal timescales, with fluctuations in $p\mathrm{CO_2}$ on the order of ± 50 μatm gen-erally occurring tightly in phase with moderate excursions in temperature (± 4–$5°C$; Bates et al. 1998), suggesting that seasonal changes in surface $p\mathrm{CO_2}$ are driven largely by seasonal temperature change (**Figure 3**). This means that pelagic organisms in the subtropical Atlantic and Pacific tend to experience periods of high $p\mathrm{CO_2}$ (low pH) simultaneously with high temperatures (**Figure 3**).

In the subpolar gyres at higher latitudes (approximately 60°N and 60°S), seasonal fluctuations in $p\mathrm{CO_2}$ tend to be out of phase with temperature (Hauri et al. 2002, Takahashi et al. 2002). Thus, organisms in these locations are unlikely to experience maximum temperatures at the same time of year that they experience maximum acidity (see data for the subpolar North Atlantic shown in **Figure 3**). However, when biological influences are strong (e.g., during the spring bloom in the North Atlantic or at the transition-zone chlorophyll front in the North Pacific), the typical negative correlation between sea surface temperature and $p\mathrm{CO_2}$ can be disrupted, causing them to vary in phase (Thomas 2002) or to become relatively decoupled, as they often are in the North Pacific (Takahashi et al. 2002) (**Figure 3**).

Like the subpolar gyres, polar regions generally exhibit low seasonal variability in temperature, with $p\mathrm{CO_2}$ varying on the order of ± 130 μatm (Barnes et al. 2006, Peck et al. 2014, Takahashi et al. 2002). In these regions, the variability in surface pH is driven primarily by biological processes, with drawdown of $p\mathrm{CO_2}$ in the summer resulting in increased pH under more or less constant temperature (Takahashi et al. 2002). Seasonal influx of freshwater caused by sea or glacial ice melt can also be significant at the poles (Dierssen et al. 2002, Hauri et al. 2015), potentially leading to osmotic challenges for pelagic organisms related to physiological avoidance of ice formation (Aarset & Aunaas 1987, Dierssen et al. 2002), which will occur in phase with increases in temperature and UV radiation.

Behavior and Exposure to Abiotic Stress

In addition to environmental variability, it is important to consider that many organisms can utilize behavioral adjustments to modulate the abiotic conditions that they experience. As a result,
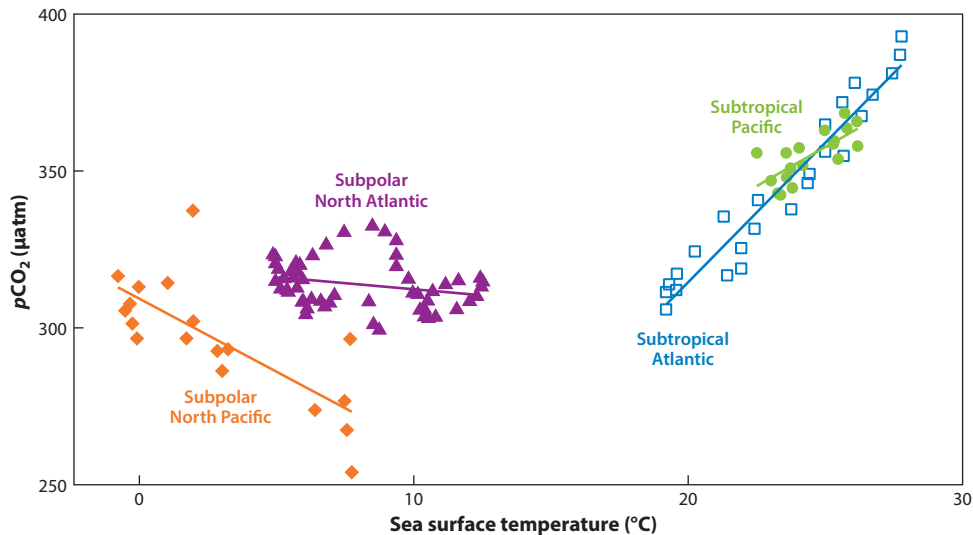
Figure 3

Patterns of covariation between sea surface temperature and pCO_2 for four major oceanic regions. All data are from surface mixed-layer measurements taken over a typical annual cycle. The strength and direction of the correlation vary across regions, with a strong positive correlation between temperature and pCO_2 in the subtropics, a negative correlation in the subpolar North Pacific, and a lack of correlation in the subpolar North Atlantic. Data for the subtropical Atlantic (Bermuda Atlantic Time-Series Study site) and subpolar North Pacific are from Takahashi et al. (2002), data for the subpolar North Atlantic are from Takahashi et al. (1993), and data for the subtropical Pacific (station ALOHA of the Hawaii Ocean Time-Series) are from Keeling et al. (2004).

measurements of environmental conditions at a particular location or averaged over a certain spatial extent may not represent the abiotic stress on a given taxa. Indeed, when the potential exists, behavioral avoidance of abiotic stress appears to be common. For example, many marine organisms move in response to changes in thermal conditions (Crossin et al. 1998, McGaw 2003), and fish avoid areas of low oxygen concentration during periodic hypoxic episodes (Eby & Crowder 2002, Wannamaker & Rice 2000). The ability to employ behavioral adjustments depends on the traits of the organism and the specific environment in which they occur. Organisms with greater motility (relative to the scale of spatial variability) will be better able to employ behavioral regulation. In addition, habitats with more spatial heterogeneity in environmental conditions provide greater potential to make effective behavioral adjustments (Huey et al. 2009). Thus, in many cases the behavior of the organism in question must be taken into account to generate a realistic picture of the abiotic stress dynamics experienced.

Physicochemical Conclusions

Marine organisms inhabit a world of multiple stressors, with intensities and durations that vary substantially across a wide range of temporal and spatial scales. In some cases, the fluctuations of different environmental stressors will be in phase, and in others they will be out of phase. Even within the same habitat, the temporal relationship between stressors can change depending on the prevailing physicochemical drivers. As discussed below, adequate consideration of environmental covariation among abiotic stressors will be essential for assessing organismal performance under

prevailing conditions and under human-induced environmental change (Bopp et al. 2013, Boyd et al. 2015, Byrne & Przeslawski 2013, Reum et al. 2014). In some habitats, it is difficult to generalize about patterns of physical stress because of unpredictable spatiotemporal complexity in environmental fluctuations (Reum et al. 2014), but this is another aspect of environments that needs to be considered.

WHY THE TIMING AND MAGNITUDE OF STRESS EVENTS MATTER FOR MARINE ORGANISMS

The Dynamics of Stress Responses

Abiotic factors fluctuate through time, and the temporal relationships among different factors can be in or out of phase. This is important because how organisms respond physiologically to multiple environmental stressors depends greatly on the intensity and relative timing of each stressor. Intensity is important because there are threshold levels at which a given environmental parameter becomes stressful (generally associated with the point at which macromolecular damage occurs, requiring energetic expenditures to ameliorate). Once the stress threshold is passed, the energy required to overcome the stress event increases with increasing stress intensity (Sokolova et al. 2012). Timing is important because the type and strength of interactive effects of multiple stressors may depend on when those stressors occur. For example, when organisms respond to different stressors using the same mechanisms of cellular protection, exposure to one stressor may prime the system to better handle exposure to a subsequent different stressor, an effect known as cross-tolerance or cross-protection (Todgham & Stillman 2013). Studies in bony fish have demonstrated that exposure to an acute sublethal heat stress can lead to increased tolerance to subsequent osmotic (DuBeau et al. 1998, Niu et al. 2008, Pan et al. 2000, Todgham et al. 2005), chemical (Brown et al. 1992, Dorts et al. 2014), and acidification (Feidantsis et al. 2015) challenges. Similar heat-induced cross-tolerance has also been demonstrated in a diverse array of aquatic/marine invertebrate taxa, including molluscs (Tedengren et al. 1999), arthropods (Baruah et al. 2010, 2011; Bond & Bradley 1995; Chen & Stillman 2012; de la Vega et al. 2006; Paganini et al. 2014; Sung et al. 2007, 2008), and freshwater poriferans (Müller et al. 1995). Cross-tolerance also results from priming stresses other than sublethal heat shock, including chemical stress (Baruah et al. 2010, 2011) and hypoxia exposure (Burleson & Silva 2011). Because pathways of resistance to many forms of abiotic stress remain highly conserved, cross-tolerance could be a common and ecologically important phenomenon (Hochachka & Somero 2002, Kültz 2005).

The consequences of different patterns of stressor timing can be related to the three forms of multi-stressor effects discussed above (additive, antagonistic, and synergistic) (**Figure 4**). Assuming that organisms are exposed to two stressors intense enough to exceed the stress threshold, the additive effects of multiple stressors are more likely to occur when stressors occur relatively far apart in time (**Figure 4**, scenario ①). In this situation, there is adequate time for physiological adjustments to the first stressor to be completed and for organisms to return to homeostasis before the second stress occurs. Because there is no overlap in the physiological responses to the two stressors, there is no opportunity for interactive effects. However, if the stressors occur close enough in time that exposure to the second stressor occurs either while the physiological response to the first stressor is still being mounted (**Figure 4**, scenarios ② and ③) or simultaneously with the first stressor (**Figure 4**, scenario ④), then interactive effects are more likely. Cross-tolerance (an antagonistic effect) will occur when the physiological response to the first stressor is still ongoing but there has been a reprieve such that much of the macromolecular damage or disequilibrium has already been repaired or removed (**Figure 4**, scenario ②). Synergistic effects are more likely
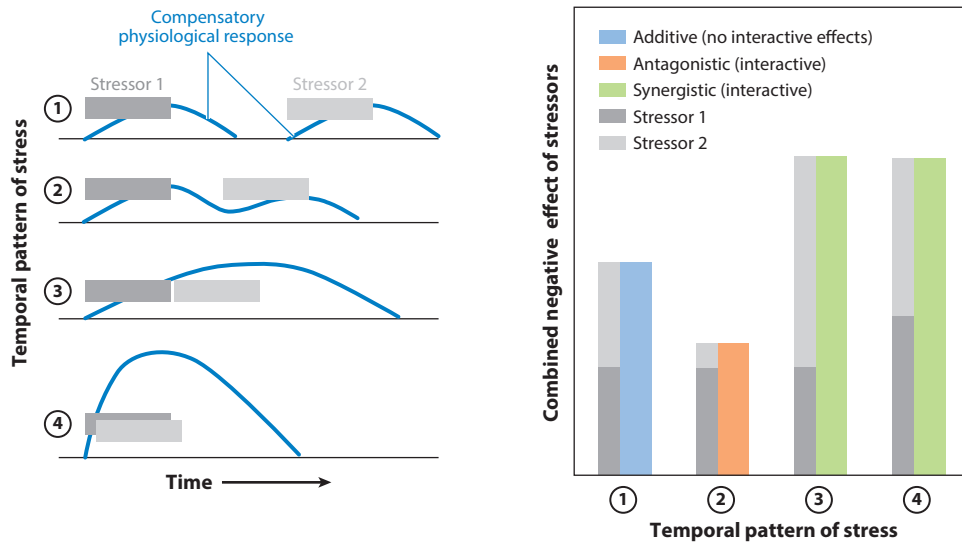
Figure 4

A model of time-dependent multi-stressor effects that predicts situations in which additive, antagonistic, and synergistic physiological responses are most likely to occur. The figure illustrates the simplest case, with only two stressors (*dark gray* and *light gray bars*). (*Left*) Four hypothetical scenarios of the temporal juxtaposition of exposure to two environmental variables (stressors). The curved lines denote the compensatory physiological response to the stressors. The areas under the physiological response curves represent the total physiological impact of stress in terms of the energetic expenditure required to return to homeostasis (*black horizontal lines*) (Sokolova et al. 2012). (*Right*) The expected consequence of each temporal pattern of multi-stressor exposure. The dark gray and light gray bars represent the magnitude of the response to each stressor (i.e., the area under the curves depicted in the left panels), and the blue, orange, and green bars represent the sums of those responses. Additive effects of two stressors are more likely to occur when physiological responses to the first stress do not coincide with the presence of a subsequent stress, as in scenario ①, but could also occur if the mechanistic bases of the physiological responses to the two stressors are entirely unrelated (Sinclair et al. 2013). Under this scenario, homeostasis has been reestablished prior to exposure to the second stressor, so there is no potential for physiological processes to mediate an interaction between the two stressors. Cross-tolerance, an antagonistic response, is more likely to occur when the physiological response to the first stress is still present when the second stress occurs, as in scenario ②. Here, the organism is primed to experience a physiological disturbance, and the effect of the second stressor is reduced. However, if the second stressor occurs too soon after the initial stressor ends, or if the second stressor overlaps temporally with the initial stressor, then a synergistic effect is more likely, as in scenarios ③ and ④. In this situation, the two stressors act as a single intense (in terms of magnitude, duration, or both) stress event that overwhelms the compensatory physiological response.

to occur when the second stressor occurs very soon after or simultaneously with the first stressor. In both cases, the second stressor is acting to increase the intensity of the first stress, in terms of either duration (**Figure 4**, scenario ③) or magnitude (**Figure 4**, scenario ④). Increased intensity makes it more likely that the stresses will overwhelm the physiological compensatory mechanisms, increasing the chances of a synergistic response.

Multi-stressor experiments that incorporate different intensities and temporal patterns of stress are rare, especially in marine systems, although the available examples are informative and generally support the temporal model described above (**Figure 4**). For example, Todgham et al. (2005) measured thresholds and temporal patterns of cross-tolerance to osmotic stress in the intertidal tide pool sculpin fish (*Oligocottus maculosus*) after 2 h of heat shock at different temperatures.
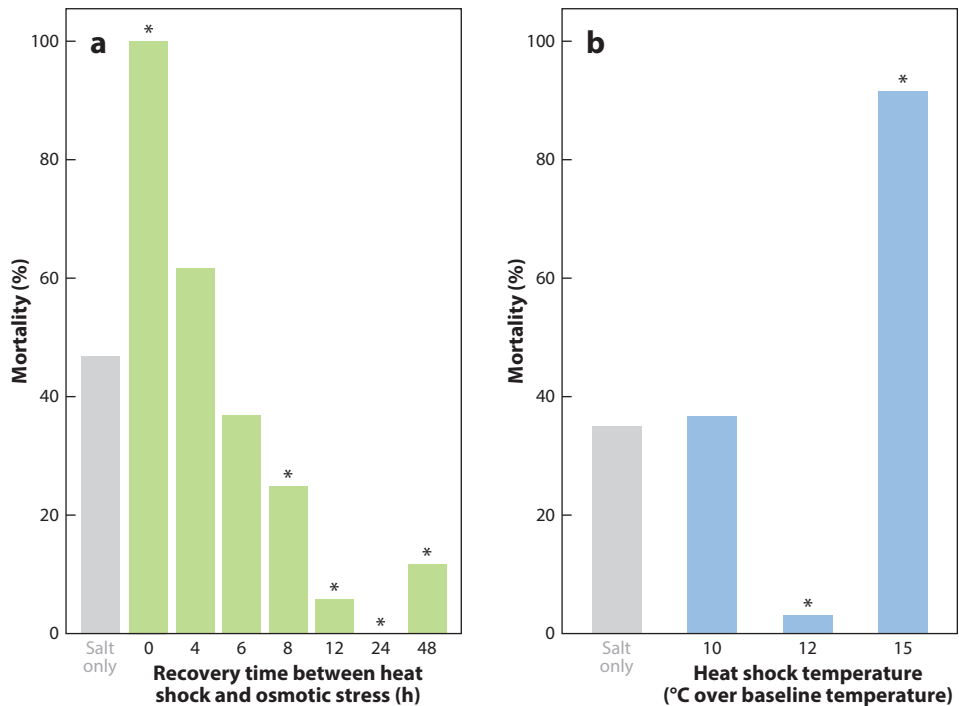
Figure 5

Timing and thresholds of cross-tolerance between thermal and osmotic stress in the tide pool sculpin
(*Oligocottus maculosus*). (*a*) Mortality in response to osmotic stress after different amounts of recovery time
following an initial 2-h heat shock. (*b*) Mortality in response to osmotic stress following 2-h heat shocks of
different temperatures above the baseline. Asterisks indicate a significant difference from a salt-only
treatment (i.e., no initial heat shock; *gray bars*). Figure and data adapted from Todgham et al. (2005) with
permission from the University of Chicago Press.

They found a strong synergistic interaction between thermal and osmotic stress if the osmotic
stress occurred immediately after the thermal stress (**Figure 5a**). However, an antagonistic cross-
tolerance effect occurred when the osmotic stress took place after 8 h of recovery from heat shock
(or 10 h from the beginning of heat shock). The strength of the antagonistic cross-tolerance effect
subsequently peaked at 24 h of recovery and remained high but was potentially decreasing at
48 h (**Figure 5a**). The time lag required for cross-tolerance suggests that a mechanism like that
depicted by scenario ② in **Figure 4** may be involved in sculpin response to salinity and temperature
stressors. Threshold stress effects were also apparent in Todgham et al.'s (2005) analysis. They
found that a heat shock of at least +12°C relative to the baseline temperature was required to
induce cross-tolerance for osmotic stress (**Figure 5b**). They also found that a +15°C heat shock
resulted in higher mortality under osmotic stress (**Figure 5b**). This highlights the fact that the
initial stress can be so intense that it overwhelms the cross-tolerance effect and makes animals
more sensitive to subsequent challenges.

It would be useful to have approximations of the timescales over which various multi-stressor
effects typically emerge when stressors do not occur simultaneously. For example, how much of a
time lag is required after the first stressor ends for an antagonistic cross-tolerance effect to occur?
Similarly, how long after the first stressor ends do additive effects typically appear? As mentioned
above, data on the dynamics of physiological responses to multiple stressors in marine systems are

rare and thus difficult to generalize. However, there are ample data on the expression dynamics of heat shock proteins (HSPs), which are thought to drive (or at least correlate with) cross-tolerance effects and overall compensatory physiological responses to multiple stressors (Kültz 2005). The temporal dynamics of HSP expression tend to follow a stereotypical pattern, with the specifics varying depending on the magnitude and duration of the initial stress, the particular taxa and tissue being investigated, and the HSP being assayed. Following exposure to a stress of sufficient intensity, significant HSP upregulation usually takes place within 0.5–6 h (Brun et al. 2008; Cellura et al. 2006; Cheng et al. 2007; Cottin et al. 2010; Dong et al. 2006; DuBeau et al. 1998; Lund et al. 2003, 2006; Manchado et al. 2008; Ming et al. 2010; Tomanek & Somero 2000; Zhenyu et al. 2004), although longer times have also been reported (Anestis et al. 2008). A peak expression level then follows, typically within 15 h of the initial exposure but sometimes after a longer period (e.g., Brun et al. 2008, Cellura et al. 2006). Finally, expression decreases and usually is back to control levels by 24 h after the exposure, although significant (but modest) upregulation sometimes continues for 48 h or more (Cheng et al. 2007, Dong et al. 2006).

It is also important that the threshold stress level that will induce the heat shock response can change with acclimation to different conditions (Barua & Heckathorn 2004, Buckley et al. 2001, Dietz & Somero 1992). For example, when Atlantic ditch shrimp (*Palaemonetes varians*) are acclimated to 10°C, upregulation of HSP70 occurs after a 1-h exposure to 28°C (Ravaux et al. 2012); when these shrimp are acclimated to 20°C, however, upregulation of HSP70 to the same level requires exposure to 32°C (Ravaux et al. 2012). Thus, the threshold stress level necessary to induce physiological responses that mediate multi-stressor effects is not necessarily constant and changes with recent environmental history.
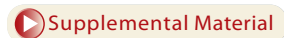
Physiological Conclusions

The impact of multiple stressors depends critically on the intensity and timing of each stressor. Synergistic effects are most likely to occur when the stress events occur simultaneously or in quick succession; antagonistic cross-tolerance is most likely to be important when the stress events occur asynchronously and over relatively short timescales (from hours to days). Such patterns occur in many habitat types for several stressor combinations, such as the diel asynchrony between temperature and hypoxia stress in tide pools (**Figure 2**). However, when stressors occur asynchronously over long timescales, such as seasonal changes in temperature and the hypercapnia common in some regions of the open ocean (**Figure 3**), interactive synergistic and antagonistic effects are unlikely to play a role, and additive effects should be expected.

HOW ARE INVESTIGATORS CURRENTLY TESTING FOR MULTI-STRESSOR EFFECTS?

Stressor Combinations Most Often Investigated

The dynamic and multifactorial nature of marine habitats means that there are many potential combinations of stress types that can affect marine organisms (see above). We attempted to quantify the number of studies that have assessed responses to different stressor combinations in marine systems by conducting systematic literature searches in Web of Science; the results of these searches are given in the **Supplemental Appendix** (follow the **Supplemental Materials link** from the Annual Reviews home page at **http://www.annualreviews.org**). Our goal in conducting these searches was to identify which stressor combinations are receiving the most attention and which potentially important stressor combinations might require more consideration. Our

▶ Supplemental Material

Figure 6

Results of Web of Science literature searches to identify the number of studies conducted on various pairs of abiotic stressors in marine habitats. References are provided in the **Supplemental Appendix**.

targets were studies that measured responses to experimentally manipulated abiotic conditions, and we therefore excluded correlational field studies from the final lists. Our lists of studies are almost certainly not exhaustive for any given stressor combination, but they should provide a broadly accurate picture of the relative focus of multi-stressor studies in marine habitats.

Six of the top seven stressor combinations investigated included temperature as one of the factors (**Figure 6**). The most commonly investigated combination was temperature and salinity, followed by temperature and acidification and then temperature and pO_2. The lone stressor combination with a relatively large number of studies that did not include temperature was salinity and heavy metals, which is an aggregate of studies on environmental salinity and several different metal stressors (e.g., lead, mercury, or cadmium).

Many potentially important stressor combinations have received very little attention. For instance, we found only one study that investigated the combined effects of heavy metals and acidification. It is worth noting that several studies have investigated how ocean acidification influences the bioavailability of metal nutrients such as iron (Breitbarth et al. 2010, Millero et al. 2009, Shi et al. 2010), but because the metals are not heavy metals and are not acting as pollutants in such cases, they are not included in our analysis. We found no studies that investigated the combination of UV and pO_2; similarly, relatively few studies have investigated the interaction between salinity and pO_2 or acidification and pO_2. Our understanding of the impacts of marine stressors would benefit from increasing attention to these less-studied stressor combinations, some of which are predicted to be very important in certain marine habitat types. For example, in many coastal surface waters, hypoxia is likely to co-occur with low pH under climate change, a situation few studies have investigated (Melzner et al. 2013).

Effects Most Commonly Observed in Multi-Stressor Studies

Several meta-analyses and reviews have been conducted with the goal of assessing the type of impact (i.e., additive, antagonistic, or synergistic) multi-stressor exposures have on marine organisms. A meta-analysis of multi-stressor effects in marine habitats across several types of stressors (including biotic interactions such as predation and disease) found that most multi-stressor effects were synergistic, that synergistic effects were most common in population-level studies of heterotrophic

species, and that the addition of a third stressor made synergism more likely (Crain et al. 2008). A more recent meta-analysis of multi-stressor effects that focused on embryos and larvae also found that synergistic effects were the most commonly observed type (Przeslawski et al. 2015). Several reviews have investigated the effects of specific stressor combinations and, again, tend to find that synergism is the most common outcome. For example, a review of the literature on the combined effects of temperature and pO_2 in fish (McBryan et al. 2013) and a meta-analysis of the combined effects of temperature and acidification on marine organisms generally (Harvey et al. 2013) both found synergism to be the most common response. Although not restricted to marine organisms, a review of interactions between chemical toxins and other stress types also found synergism to be the most common result of multi-stressor exposure (Holmstrup et al. 2010).

The pattern emerging from these reviews is that interactive effects among stressors are common, meaning that extrapolation of multi-stressor effects from single-stressor studies will often be misleading. In addition, synergism appears to be the most common interactive effect observed in multi-stressor studies, suggesting that exposure to multiple stressors will be extremely detrimental to marine organisms under changing climatic conditions.

How Are Multi-Stressor Studies Designed?

As discussed above, environmental stressors vary in time and magnitude, and, in turn, the physiological responses organisms mount when challenged with environmental stressors are time and magnitude dependent. With this in mind, the important question becomes, are researchers capturing relevant environmental variability in multi-stressor experimental designs? To address this question, we summarized the experimental designs employed in 166 experiments from 126 papers included in two of the recent reviews of multi-stressor effects on marine organisms discussed above (Crain et al. 2008, Przeslawski et al. 2015). We counted the number of experiments in which stressors were applied simultaneously versus sequentially (i.e., were stressors in phase or out of phase?) and under constant versus variable stress intensity. We included only studies in which experimental manipulation of environmental conditions occurred, and excluded studies that relied only on field measurements of stressor levels and/or included biotic interactions as a stressor. The studies were biased toward organisms from coastal habitats, and most focused on two or more of the following environmental variables: temperature, salinity, pH, UV, and DO (**Table 1**; for full data sets, see **Supplemental Tables 1** and **2**).

▶ Supplemental Material

The vast majority of experiments (96%) exposed organisms to stressors simultaneously (**Figure 7***a*). In addition, most experiments (80%) exposed organisms to constant levels of each stressor (**Figure 7***b*). Of the minority of studies that had fluctuations in environmental conditions, the variable that fluctuated was most often UV intensity (67% of experiments with environmental fluctuation). Thus, despite the prevalence of asynchronous stress exposure in many marine environments and the dynamic fluctuations observed in most stressors through time, the performance consequences of two or more fluctuating stressors applied sequentially are rarely assessed. This is not to say that exposing organisms to stressors simultaneously and with no fluctuation is inherently incorrect; such studies are representative of certain habitats at certain times when stressors are occurring in phase, and they have the added benefit of being relatively easy to implement. Nonetheless, they may hold limited potential for understanding multi-stressor effects generally and could in fact lead to erroneous generalizations. For example, the repeated observation that synergism is the most common outcome of multi-stressor exposure could result from our collective bias for experimental designs that favor synergistic interactive effects (**Figure 4**). Simultaneous, constant stress exposure is not the norm for most habitats and most stressor combinations, and it is the pattern of exposure expected to yield the most negative results.

Table 1   Summary of multivariate studies of the physiological effects of environmental drivers included in two recent reviews and used in the analyses presented in Figure 7 (for complete data sets, see Supplemental Tables 1 and 2)

Habitat	Stressor 1	Stressor 2	Number of studies	
			Crain et al. (2008)	Przeslawski et al. (2015)
Estuarine	Temperature	Salinity	10	15
	Salinity	pH	0	1
		UV	1	1
		Pollutant	0	1
Intertidal	Temperature	Salinity	13	25
		pH	2	0
		UV	4	10
	Salinity	Dissolved O_2	1	0
Subtidal/shelf/reef	Temperature	Salinity	37	52
		pH	13	28
		UV	0	0
		Dissolved O_2	2	2
		Pollutant	5	1
	Salinity	pH	1	0
		Pollutant	4	1

To be ecologically relevant, the design of experiments aimed at uncovering the consequences of multiple stressors (and environmental stress in general) must consider the dynamics of environmental stress at the spatial and temporal scales appropriate for the organism under study. Researchers must ask several questions when designing such an experiment. For example, over what timescales do the stressors in question fluctuate? Do the stressors in question fluctuate synchronously or asynchronously? What is the magnitude of fluctuation for each stressor, and how much variability is there in magnitude? Answering these questions requires detailed measurements of environmental conditions, ideally within the specific habitat that the organism in question occupies (Helmuth et al. 2010, Reum et al. 2014) at the life stage being considered (Hofmann et al. 2014). Once this information is in hand, experiments can be designed that mimic the realistic patterns of abiotic stress that organisms experience, and those parameters can serve as a baseline to be modified to reflect expected future conditions. In many cases, the relevant experimental design that emerges from environmental data will not reflect the simultaneous, constant multi-stressor exposure regimes that most studies have followed to date (**Figure 7**). Furthermore, for some systems, such as those in which stressors are out of phase on a seasonal basis, it may not make sense to expose experimental organisms to peak levels of each stressor, even sequentially, unless the experiment is expected to run for several months.

A recent study of the intertidal porcelain crab *Petrolisthes cinctipes* by Paganini et al. (2014) provides an example of a multi-stressor experimental design driven by ecologically relevant variation in environmental conditions. In this study, crabs were exposed to different magnitudes of daily fluctuation in pH and temperature in a factorial design, with peak pH and temperature stress out of phase in treatments with both stressors, commensurate with the temporal stress pattern expected in the intertidal habitat of this species. A cross-tolerance effect was observed with respect to whole-organism thermal tolerance, as the crabs subjected to low pH had higher thermal tolerance than those under control pH conditions. The authors also found that exposure to low pH or high temperature in isolation had little or no effect on metabolic rates. However, an interactive
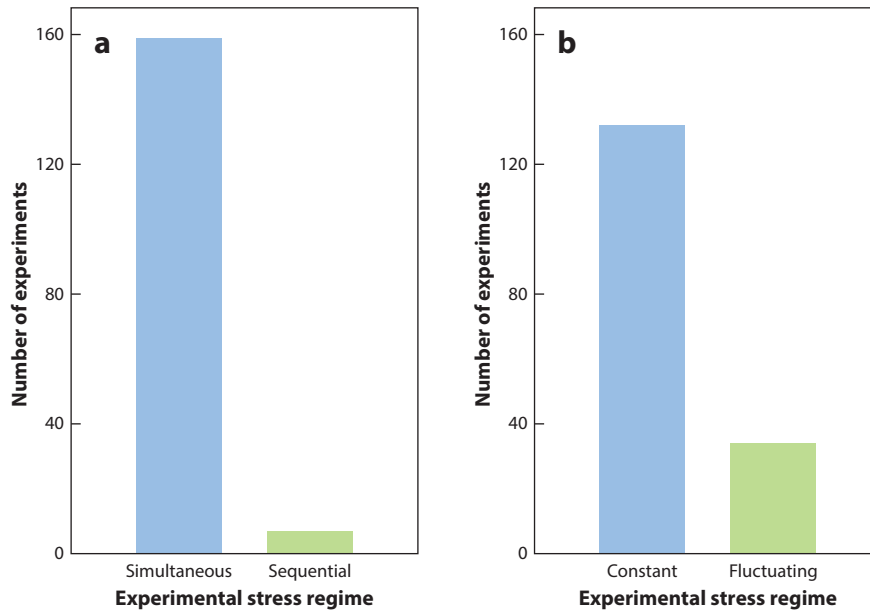
Figure 7

Literature survey of multivariate studies of the physiological effects of environmental drivers included in two recent reviews (Crain et al. 2008, Przeslawski et al. 2015), showing the number of experiments in which stressors were applied (*a*) simultaneously versus sequentially and (*b*) under constant versus variable stress intensity. Most studies have been performed under conditions where organisms are exposed to environmental drivers simultaneously at constant levels. Whether such exposures reflect the natural habitat (e.g., **Figure 2**) requires careful consideration when interpreting the results of the studies. References are provided in **Supplemental Tables 1** and **2**.

▶ Supplemental Material

effect was observed in animals that experienced both the lowest pH and the highest temperature, with metabolic rates decreasing significantly below control levels. The lack of a metabolic response to fluctuating pH alone is in contrast to previous work on *P. cinctipes* that found depressed metabolic and heart rates in animals exposed to unrealistic constant low pH (Carter et al. 2013, Ceballos-Osuna et al. 2013). This highlights the fact that very different responses to the same stressor combinations can occur in the same organism depending on how realistic the experimental conditions are, which carries subsequent implications for the ecological interpretation as well.

SUMMARY POINTS

1. Physicochemical characteristics of marine systems are dynamic, and peak levels of certain stressors can sometimes be in or out of phase with one another depending on the habitat and time of year.

2. Multi-stressor studies have focused primarily on the combined effects of temperature and/or pH with other stressors, and many important stressor combinations have received little attention.

3. How organisms respond to exposure to multiple stressors depends critically on the magnitude and relative timing of each stressor, with interactive effects of stressors more common when they occur close together in time.

4. Experimental studies have found that stressors most often act interactively as opposed to additively in their effects on organisms. Synergistic effects, in which the consequences of multi-stressor exposure are more negative than expected based on responses to each stressor in isolation, are the most common interactive response observed.

5. Despite the complex physicochemical variability found in marine systems, most multi-stressor studies have exposed organisms to the stressors simultaneously and at constant intensity levels. This bias in experimental design favors the observation of synergism between stressors.

6. Multi-stressor studies should strive to conduct more naturalistic exposure regimes based on the rigorous measurement of environmental parameters at spatial and temporal scales appropriate to the organisms and life stage under study.

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Contents

Errata

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