A Bioenergetics Framework for Integrating the Effects of Multiple Stressors: Opening a 'Black Box' in Climate Change Research

A bioenergetics framework for integrating the effects of multiple stressors: Opening a 'black box' in climate change research*

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Abstract: Climate change is already impacting marine ecosystems across a range of scales, from individual physiology, to changes in species interactions and community structure, and ultimately to patterns in geographic distribution. Predicting how marine ecosystems will respond to environmental change is a significant challenge because vulnerability to climatic and non-climatic stressors is highly variable, and depends on an organism's functional traits, tolerance to stressors, and the environment in which it lives. We present a mechanistic approach based on biophysical and dynamic energy budget models that integrates the cumulative effects of multiple environmental stressors (temperature and food) and stress associated with the presence of predators (the "fear of being eaten"), with the functional traits of an organism. We describe how multiple factors such as feeding time, food availability, and weather can be combined into a few simple metrics and explore how the physiological and behavioral impacts of predation risk can be included in this framework by altering prey feeding time and performance. Importantly, we highlight several critical gaps in our basic understanding of the fundamental mechanisms that drive responses to multiple stressors in natural systems. The framework presented here is, thus, intended to serve as a guide for the formulation of explicit, testable hypotheses and further controlled experimentation.

Key words: Intertidal, Dynamic Energy Budget model, Biophysical model, Ecophysiology, *Mytilus californianus*

Rocky intertidal ecosystems have long served as a model system for exploring the interactive effects of abiotic and biotic stressors on natural ecosystems. The more or less two-dimensional structure of many rocky shores enables experimental manipulation of space as a limiting resource (Connell 1972, Paine 1994), and the steep gradients of physiological stress that result from the alternate exposure to aquatic and terrestrial environments create an ideal model for studying the physiological and ecological impacts of environmental stressors (Somero 2002). Finally, most rocky intertidal organisms are sessile or slow moving, facilitating studies of the role of behavior (Williams and Morritt 1995), vulnerability to environmental stressors (Petes *et al.* 2007), competition (Wethey 1983), facilitation (Bertness *et al.* 1999, Bruno *et al.* 2003), and predator-prey interactions (Pincebourde *et al.* 2008, Kordas *et al.* 2011, Matassa and Trussell 2011). Molluscs have played a central role in many of these studies as primary occupiers of space (Paine and Levin 1981) and providers of habitat (Smith *et al.* 2006) for mobile consumers (Yamane and Gilman 2009).

In recent decades this model system has served as a test bed for investigating the impacts of environmental change over multiple scales. The general assumption is that the distributions of intertidal invertebrates such as mussels and gastropods are driven in large part by physical stress, and that differential susceptibility between competing species has strong implications for zonation patterns (Somero 2002, Davenport and Davenport 2005). For example, Tomanek and Sanford (2003) found that heat shock protein induction in two congeneric gastropods was consistent with their observed distribution on the shore. Studies such as these have suggested that as environmental stressors such as air temperature increase, the upper zonation limits of some species may shift downwards, making intertidal ecosystems a model for exploring how climate change and other stressors will affect natural communities (Harley and Paine 2009, Somero 2011). Similar studies on broader geographic scales have matched the environmental tolerances of invertebrates to past, current and future environmental predictions to explain observed and projected shifts in geographic distribution (Mieszkowska *et al.* 2006, Jones *et al.* 2009).

However, a number of studies highlight why this issue may be far more complicated than previously appreciated.
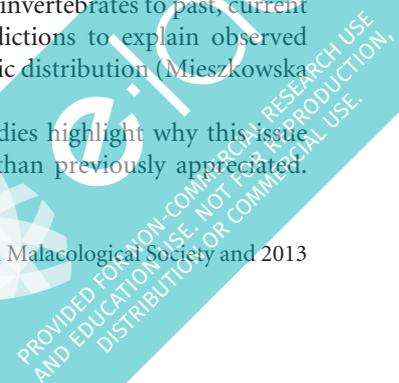
First, environmental conditions in intertidal systems can be highly variable over a range of temporal and spatial scales, especially during low tide when body temperatures are driven by several environmental parameters including air temperature, solar radiation, and wind speed (Helmuth 2002). For example, body temperatures over the scale of cm between shaded and unshaded microhabitats can exceed those observed over thousands of km of shoreline (Helmuth and Hofmann 2001, Denny et al. 2011, Seabra et al. 2011). Additionally, local conditions such as the timing of low tide and wave splash can supersede larger-scale gradients so that thermal stress does not always increase with decreasing latitude (Helmuth et al. 2006, Pearson et al. 2009, Mislan et al. 2011). While temperature is among the most commonly studied variables, evidence suggests that other environmental stressors such as salinity (Garza and Robles 2010), food availability (Place et al. 2012, Dowd et al. 2013, Sarà, Palmeri, Montalto et al. 2013), and the interaction of the two are also important (Schneider et al. 2010, Williams et al. 2011, Howard et al. 2013), and can change over small scales.

Second, responses to environmental stressors in nature can be difficult to interpret because seldom do these factors act in isolation. Indeed, understanding the potential impacts of multiple stressors on ecosystems has emerged as a major knowledge gap in predicting the likely impacts of climate change (Howard et al. 2013). The complexities of multiple stressors arise from multiple levels of biological organization. Interacting stressors can affect individual organisms at a physiological level in complex ways (Pörtner et al. 1999, Pörtner 2010, Sokolova et al. 2012, 2013). For example, several empirical and modeling approaches have shown that reduced food levels can potentially exacerbate some of the effects of both thermal (Schneider et al. 2010, Sarà et al. 2012) and CO_2 stress (Pansch et al. 2013). Williams et al. (2011) showed that the timing of exposure to elevated temperatures and decreased salinity levels impacted limpet survival. Similarly, Pincebourde and colleagues (2012) showed that the timing of elevated temperatures during high tide (in water) and low tide (in air) had important implications for foraging by seastars. Multiple stressors can also influence species interactions and ecosystem functioning (Breitburg et al. 1998, Vinebrooke et al. 2004, Halpern et al. 2007, Crain et al. 2008), both through effects on species interactions such as rates of predation (Pincebourde et al. 2008) and competition (Wethey 1983), and as a result of differential susceptibility of interacting species such as predators and competitors to environmental stress (Darling and Côté 2008, Crain et al. 2008). For example, multiple stressors acting on trophic levels have been shown to cause additive, synergistic and antagonistic effects at the level of communities (Crain et al. 2008). Thus, the cumulative effects of combined stressors may not be additive, and as a result may not always be accurately predicted based on knowledge of the effects of single stressors acting alone (Breitburg et al. 1998, Crain et al. 2008, Darling and Côté 2008).

Third, local and geographic distributions can be set by different aspects of "environmental signals" including both rare but extreme events as well as chronic exposure to sublethal conditions (Helmuth et al. 2010, Woodin et al. 2013). A recent modeling study by Mislan et al. (2014) found that lethal temperatures were associated with only 2 of 15 upper limits in mussel beds along the west coast of the United States, suggesting that stressors other than rare temperatures determined these limits. Other studies have shown that while lethal exposures are well correlated with the equatorial distribution of Mytilus edulis (Linnaeus, 1758) on the east coast of the United States (Jones et al. 2010), geographic limits in Europe could only be explained when chronic exposure to high but sublethal temperatures was considered (Woodin et al. 2013). Similarly, modeling by Sarà et al. (2011) suggested that while lethally high temperatures explained the absence of Mytilus galloprovincialis (Lamarck, 1819) in the Mediterranean, energetic limitation best explained absences at other sites. Factors other than temperature such as salinity (Garza and Robles 2010) and desiccation stress can also play important roles in setting distributional limits, and all of these drivers may alternate in their relative importance. Thus, whereas exposure to rare but extreme events certainly can play a role in setting the local and geographic distributions of species (Harley and Paine 2009), it is highly likely that energetic limitations resulting from a combination of various physical stressors may play an even more important role in many locations.

Finally, the effects of environmental change can also occur indirectly through effects on an organism's predators and competitors. For example, increasing temperatures have been shown to both increase (in water, Sanford 2002) and decrease (when aerially exposed, Pincebourde et al. 2008) seastar predation rates on mussels. Moreover, the simple presence of predators can have surprisingly strong effects on prey species. Indeed, the effects of the "fear of being eaten" can be so strong that these nonconsumptive effects (NCEs) can rival the direct impacts of predation or thermal stress in driving physiological performance (Trussell et al. 2006) and shape the abundance and distribution of basal resources. For example, Matassa and Trussell (2011) recently showed that the presence of predatory crabs limited the foraging rate and behavior of Nucella lapillus (Linnaeus, 1758), which subsequently altered patterns of distribution in their barnacle prey. These impacts occurred for two reasons. First, the presence of predators increased refuge use by N. lapillus, resulting in lower rates of food intake. Second, crab risk cues had a direct physiological effect on N. lapillus, potentially through increased respiratory demand, so that snail growth efficiency was

dramatically lowered due to an increase of the metabolic requirement. Hence, not only did *N. lapillus* forage less in the presence of crab risk cues, they were also less efficient in metabolizing the limited food they ingested (Trussell *et al.* 2011).

Taken together, these studies emphasize the interactive nature of multiple physiological stressors, species interactions, and food availability in driving local and geographic patterns of abundance and distribution. They further highlight the complexities inherent in quantitatively predicting how changes in the environment will likely alter intertidal ecosystems, especially considering that many of the first observable effects of climate change may lie in nonlethal responses. Here we develop a generic framework based on energetics theory (*e.g.*, Sokolova 2013) that permits a quantitative examination of how submergence (feeding) time, environmental conditions (local weather, food availability), and the presence of predators (which affect the feeding behavior and physiological performance of prey) drive growth, reproduction, and survival of intertidal mussels (Fig. 1). Our approach is based on Dynamic Energy Budget theory (DEB;

Kooijman 2010) and thermal physiological modeling, and additionally modifies parameters to account for the influence of predation risk on prey feeding rates and metabolic demands. We focus on the physiological impacts of body temperature and food intake in order to develop a generic framework for exploring the interaction of biotic and abiotic stressors, with the full realization that other abiotic factors such as salinity can also play important roles under field conditions.

Bioenergetic models offer a mechanistic approach to predicting growth rate, ultimate body size, and reproductive output of individuals as a function of their environment (Widdows and Johnson 1988, Nisbet *et al.* 2000, Sarà *et al.* 2008, Kearney *et al.* 2010) by applying empirical and theoretical approaches to describe the flow of energy through an organism (Sarà, Palmeri, Montalto *et al.* 2013). DEB models have been particularly successful for this application because they offer a whole-organism approach to explaining how factors in the physical environment translate into biologically and ecologically relevant responses (Kearney *et al.* 2010, Sarà *et al.* 2012). Central to DEB theory is the concept that food and body temperature are the primary drivers of an individual's metabolic machinery (Sarà, Palmeri, Montalto *et al.* 2013, Montalto *et al.* 2014); both of which are a direct function of (and often extremely sensitive to) the environment. This is especially true for sessile intertidal ectotherms such as mussels that have little or no behavioral control of their body temperatures. When combined with biophysical models that calculate body temperature as a function of multiple environmental parameters (Helmuth 1998, Denny and Harley 2006), DEB models provide powerful tools for exploring how environmental change will likely affect physiological performance (Kearney *et al.* 2010, Sarà *et al.* 2011).

Using the mussel *Mytilus californianus* (Conrad, 1837) as an example, we explore how this approach can be used to quantitatively explore the coupled effects of feeding time, predation risk, food availability, and body temperature on mussel physiology.
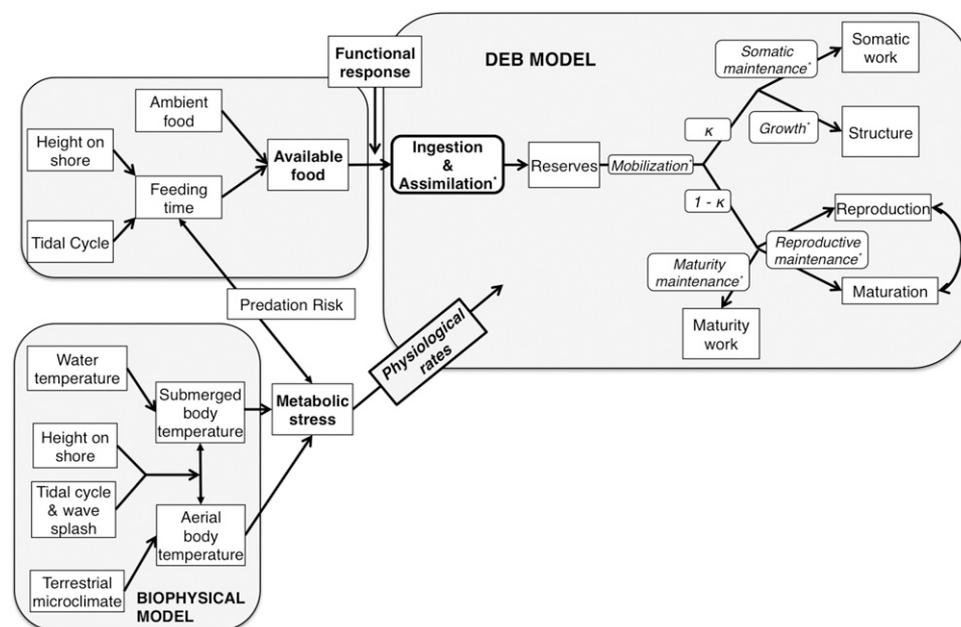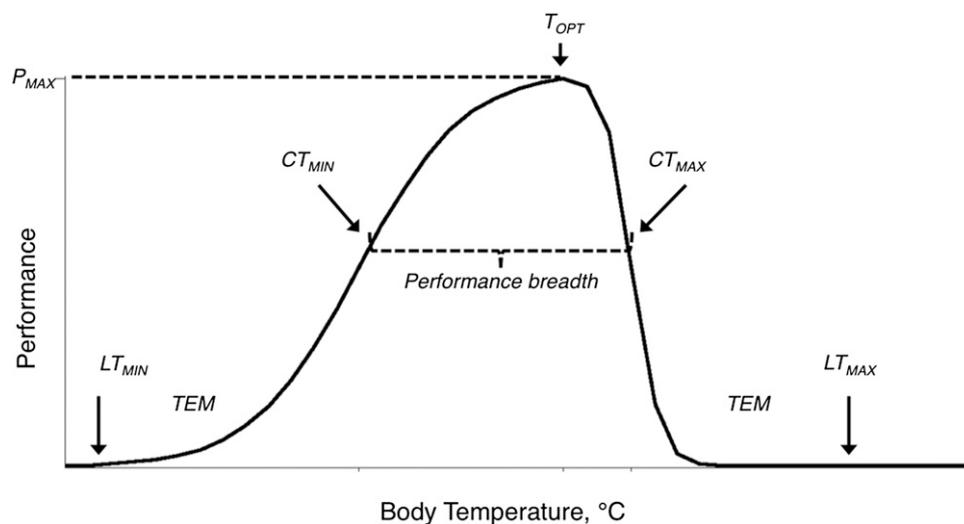

Figure 1. A conceptual framework for studying multiple stressors in intertidal systems on the basis of bioenergetics by combining biophysical modeling with habitat characteristics and species interactions within the DEB model. Energy acquisition is limited by resource density and feeding time, which is in turn impacted by tidal characteristics and behavioral responses to predation risk. The biophysical model predicts body temperatures as a function of the environment, which in turn impacts metabolism and drives rates in the DEB model. For sessile intertidal invertebrates, the relative exposure to both sources of stress is a function of intertidal height and tidal cycle. Non-lethal effects of predators impact feeding time as well as impose metabolic stress. The culmination of these stressors drives relative energy allocation to growth, maintenance, development and reproduction in the DEB model. Rates in the model that are affected by temperature and predation risk are marked with an asterisk (*).

Our model incorporates sublethal stress based on performance curves (Fig 2; Freitas *et al.* 2007), and provides a potential method for predicting cumulative impacts of environmental stress over intertidal stress gradients in the presence and absence of predators. While we make an attempt to use ecologically and physiologically relevant parameters wherever possible, one of the major take-home messages of this study is that our understanding of many components of this system is still poor. Thus, it is critical to note that while the ultimate goal of the approach presented here is to develop a predictive model that is reflective of actual field conditions, there are still major knowledge gaps that preclude these predictions. Thus, our goal is to present a framework to help guide further experimentation, with the hope that eventually such modeling may be possible.

METHODS

Temperature sensitivity, risk, and performance

Performance curves have long served as a standard means of describing levels of performance associated with fitness [for example growth efficiency (Angilletta and Dunham 2003); assimilation, fecundity, and survivorship (Angilletta 2009) and feeding rate (Bayne *et al.* 1976)], as a function of environmental factors such as temperature and salinity (Sokolova *et al.* 2012). A number of curve shapes have been reported (Angilletta 2009), often as a function of body temperature.
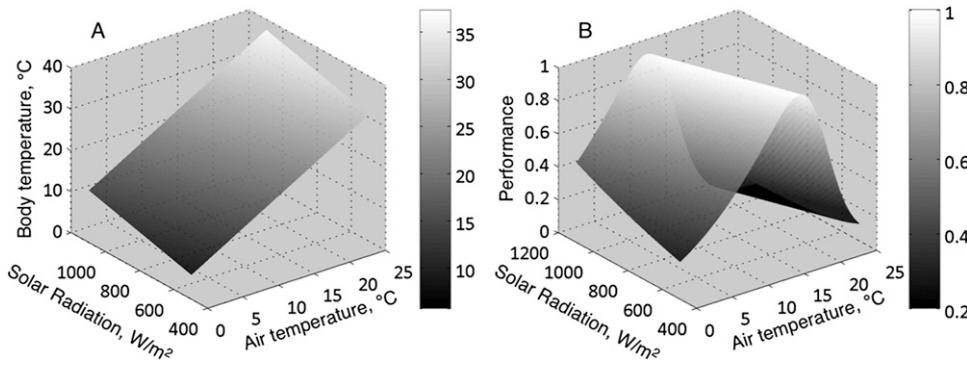
One of the most common curves (Fig. 2) exhibits a left skew where, at conditions below optimum, fairly substantial increases in temperature lead to modest increases in performance. At temperatures higher than optimum, small increases in temperature can lead to rapid reductions in growth, reproductive failure, and mortality. These curves can either be defined based on some direct measure such as sprint speed or feeding rate (and, thus, "performance" is always greater than or equal to 0) or can be used to represent net energy balance (in which case values can drop below zero). For example, in the latter case a "Transient Event Margin" (Woodin *et al.* 2013) represents the often narrow range of nonlethal temperatures that organisms can tolerate for only short periods of time because they are in a negative energy balance, and in some cases are within only a few degrees of lethality (Fig. 2). In both instances a common descriptor of these curves--and one which we capitalize on here--is performance breadth, defined as the optimal range of temperatures over which physiological performance is at least 69% of the maximum value (van der Veer *et al.* 2006, Freitas *et al.* 2007). We base our curves on rates of feeding described by Bayne (1976) and Bayne *et al.* (1976), and, thus, values of performance are always positive.

An important consideration is that performance curves are based not on habitat temperature *per se* (Kearney 2006) but rather on body temperature, which can in some cases be significantly different from the temperature of the surrounding air (Helmuth 2002) or even water (Fabricius 2006). To this end, biophysical models are increasingly being used to estimate body temperatures under different environmental conditions (Fig. 3A). When combined with thermal performance curves these models, thus, provide a means of predicting physiological performance from multiple environmental conditions, for example air temperature and solar radiation (Fig. 3B). While this combination of thermal modeling and performance curves allows quantification of multiple niche axes, the additional step of using energetics models permits an assessment of the cumulative effects of multiple sources of physiological stress over time (Kearney *et al.* 2013). A particularly powerful approach that has emerged is, thus, the combination of biophysical modeling

Figure 2. Standard performance curve for ectotherms depicting critical temperatures (CT_{MIN}, CT_{MAX}), performance breadth, lethal limits (LT_{MIN}, LT_{MAX}), optimum temperature (T_{OPT}) at maximum performance (P_{MAX}), and the Transient Event Margin (*TEM*). Performance is defined here as any positive measurement that describes an organism's ability to function, including growth, fecundity, and survivorship (Angilletta 2009).

Figure 3. A. Body temperature as a function of solar radiation and wind speed using a biophysical model (adapted from Helmuth 1998). **B.** Thermal performance of *Mytilus californianus* plotted as a function of air temperature and solar radiation. Thermal performance was estimated based on Arrhenius temperature relationships (see text).

and energetics modeling (Kearney *et al.* 2010; 2012, Sarà *et al.* 2011, Sarà, Palmeri, Rinaldi *et al.* 2013) for predicting patterns of growth and reproduction in the field.

Dynamic Energy Budget Models

Dynamic Energy Budget modeling has served as an effective means of evaluating the physiological sensitivity of species to environmental change (Kearney *et al.* 2010; 2013, Sarà, Palmeri, Rinaldi *et al.* 2013). An important aspect of DEB theory is that apart from food, an organism's body temperature is the primary driver of metabolism. The dependence of metabolic rates on temperature can be described in the model by the Arrhenius relationship (Freitas *et al.* 2010, Kooijman 2010), which is a good indicator of temperature sensitivity of a species across its entire geographic range. The Arrhenius temperature relationship describes the boundaries of sensitivity (the range between the upper and lower body temperatures that support organismal metabolic functioning) and implicitly sets the limits of the fundamental thermal niche of a species (Kearney and Porter 2009). While typical DEB theory uses species-specific values for this function, it can also be adopted to account for variability amongst individuals and populations (Freitas *et al.* 2010). The performance breadth is defined as the range of body temperatures where performance is above a particular level (Angilletta 2009). In this study, we define the performance breadth as the range of temperatures where performance is at least 69% of optimum (Fig. 4; Freitas *et al.* 2010), which can be estimated from measurements of physiological rates over an organism's thermal tolerance range. At body temperatures above and below the performance breadth, performance quickly decreases until the organism can no longer survive, which defines the tolerance range.

We estimated temperature-dependent parameters using feeding rates of *Mytilus californianus* reported in the literature (Bayne 1976) following the procedure described in van

der Veer *et al.* (2006). The lower (T_L) and upper (T_H) temperatures define where 69% [= ln(2)] of enzymes are active, and T_{AL} and T_{AH} are the Arrhenius temperatures that describe the rate of decrease at T_L and T_H. The approach we use here is similar to that used by Freitas *et al.* (2007) for describing the dependence of physiological rates on temperature by interacting species (see also van der Veer *et al.* 2006, Ren and Schiel 2008).

All rates in the DEB model are corrected for temperature by a temperature correction factor T_c by incorporating Sharpe and DeMichele's (1977) concepts of enzyme inactivation at high and low body temperatures with Arrhenius temperature relationships:

$$\dot{k}(T_b) = \dot{k}_{OPT} T_c$$

$$T_c = \frac{exp\left(\dfrac{T_A}{T_{OPT}} - \dfrac{T_A}{T_b}\right)\left[1 + exp\left\{\dfrac{T_{AL}}{T_{OPT}} - \dfrac{T_{AL}}{T_L}\right\} + exp\left\{\dfrac{T_{AH}}{T_H} - \dfrac{T_{AH}}{T_{OPT}}\right\}\right]}{\left(1 + exp\left\{\dfrac{T_{AL}}{T_b} - \dfrac{T_{AL}}{T_L}\right\} + exp\left\{\dfrac{T_{AH}}{T_H} - \dfrac{T_{AH}}{T_b}\right\}\right)}$$

Where $\dot{k}(T_b)$ is the rate at body temperature T_b and $\dot{k}_{OPT}$ is the rate at optimum temperature T_{OPT}. In this way, metabolic rates are maximal when $T_b = T_{OPT}$.

Performance curves also offer a mechanism for accounting for the physiological impacts of predation risk. Empirical studies have shown that predation risk can have a surprisingly strong influence on prey thermal sensitivity, and the effects of risk can even exceed the effects of temperature (Miller *et al.* 2014). However, the precise nature of the interaction between predation risk and temperature is unknown for mussels. In the absence of data we, thus, adopt a simple relationship in order to simplify our explanation of the model, and assume that the presence of predators leads to a 20% reduction in performance (Fig. 4), and adjust thermal performance curves accordingly. The modeling results shown here are, therefore, purely theoretical, and intended only to offer a framework that can be used as actual parameter values are measured empirically. In reality the shapes of the temperature/predator exposure response surface could be highly nonlinear. For example, Trussell *et al.* (2011) found that high-risk scenarios were as energetically favorable as low-risk scenarios for *Nucella lapillus*, and the effect of risk on growth was strongest
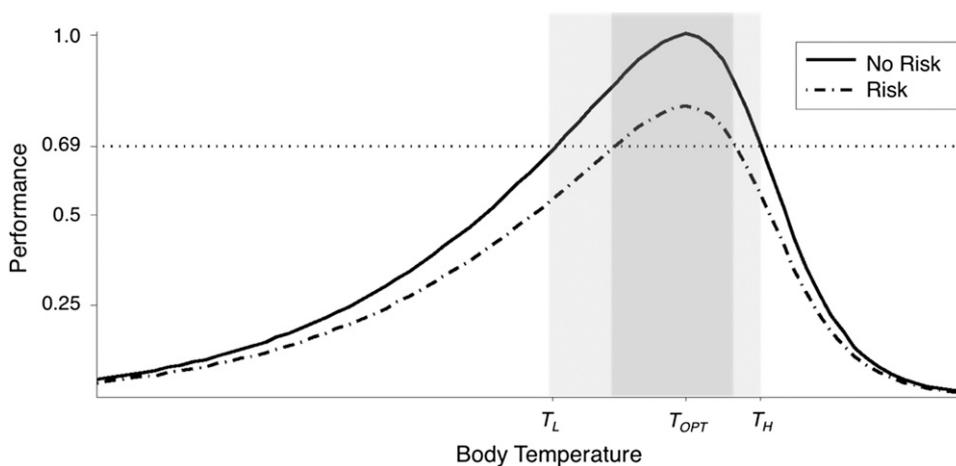
Figure 4. Performance curve for *Mytilus californianus* from measurements of feeding rate (Bayne 1976) using Arrhenius temperature relationships (solid line). T_{OPT} is the temperature where performance is optimal and the performance breadth (grey shaded regions) is the range of temperatures where performance is 69% of optimum. The dashed line represents the performance curve after applying a 20% reduction in metabolic efficiency, and the dark grey shading represents the resultant decrease in performance breadth.

at intermediate levels of risk cue exposure. Comparatively, the effect of risk on foraging increased with increasing risk exposure, emphasizing the importance of decoupling physiology and food acquisition when describing the relative importance of predators in natural systems. *Thus, empirical measurements of the response surface describing the influence of predation risk on metabolic efficiency and feeding rates (described below) to a range of risk levels are critical, but as yet have not been measured.*

Food acquisition and the functional response

The total food available to intertidal bivalves is dependent on both ambient food density as well as the amount of time spent feeding. The former metric is commonly based on measurements of chlorophyll-a concentration (*chl*-a), although more sophisticated methods account for a much wider diversity of food particles including detritus (*e.g.*, Shumway *et al.* 1985). For sessile and slow moving intertidal organisms, tidal elevation is a major determinant of the amount of time filter feeders can feed during submersion, which is reflected in observations that growth rate and size at first sexual maturity of intertidal mussels varies with tidal elevation (Suchanek 1981). The risk of predation (NCEs) can further reduce feeding time; for example, bivalves close their valves as a first line of defense from predators, thereby reducing feeding by preventing water flow to the gills (Robson *et al.* 2010). As a result, it is theoretically possible to combine the effects of feeding time (modified by tidal elevation and predation risk) and food density with a single parameter that describes ingestion and assimilation rates.

In standard DEB models, the functional response *f* is used to describe ingestion rate $\dot{P}_X$ and assimilation rate $\dot{P}_A$ as a function of scaled food density:

$$\dot{p}_X = f \cdot T_c \left\{ \dot{p}_{Xm} \right\} \cdot L^2$$

$$\dot{p}_A = f \cdot T_c \left\{ \dot{p}_{Am} \right\} \cdot L^2$$

$$\text{with } f = X/(K + X)$$

Both the surface area specific maximum ingestion rate $\left\{ \dot{p}_{Xm} \right\}$ and the surface area specific maximum assimilation rate $\left\{ \dot{p}_{Am} \right\}$ are corrected for temperature via T_c. Food density *X* is expressed relative to the maximum capacity for food intake through the half-saturation coefficient *K*, the food density where the ingestion rate is half the maximum. *L* is the volumetric length.

For this study, we extended the standard DEB model to examine the combined effects of food density, intertidal elevation and predation risk through the functional response (Fig. 5). Specifically, we calculated the functional response from the "actual food density": a product of the total ambient food density and the potential for feeding (a function of predation risk, intertidal height and tidal cycle). We expressed food density in terms of C-moles L^{-1} to be consistent with the estimated half saturation coefficient for *Mytilus californianus* (Matzelle *et al.* 2014). Food density ranged from 0.1 to 10 C-moles L^{-1}, which spanned the possible values for functional response (0-1).

Submersion time was estimated using tide estimates for Waldport, Oregon (mixed semidiurnal tide, range MLLW -0.7 m to +2.9 m) for the year 2013 using xtide (http://tbone.biol.sc.edu/tide/), which was then repeated for 10 years. We modeled 28 tidal heights ranging from -0.2 to +2.5 m above Mean Lower Low Water (MLLW) characteristic of mussel habitats common to the west coast of North America. For each tide height, hourly functional response was calculated during submersion based on ambient food density (values and equation described above). During emersion hours, feeding was assumed to stop, corresponding with a functional response value of 0. We then calculated daily functional response by averaging the hourly values for each day at each intertidal height. Finally, we accounted for the negative impacts of predation risk on feeding by applying an arbitrary (but realistic) 20% reduction in feeding time, which was reflected in a decreased functional response (Fig. 5). As with performance, we recognize that this likely is an overly simplistic
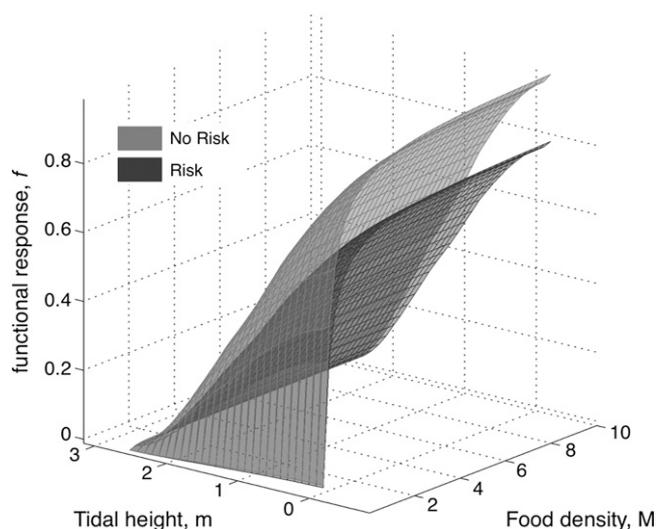
Figure 5. Average functional response *f* as a function of food density (C-moles L^{-1}) and intertidal height above MLLW (m), both in the presence and absence of risk. The effects of tidal height and risk on feeding time are described with the scaled functional response.

assumption, but use this as a starting point. Likewise, because we were estimating steady-state conditions as a means of presenting a generic modeling approach, temperatures during aerial exposure and during submersion by necessity are assumed to be equivalent since total feeding time and temperature necessarily refer to daily values (*e.g.*, percentage of time exposed per day). A non-steady state analysis would allow separation of the roles of aerial and submerged temperature, but for the purposes of this initial study would confound generality. "Tidal elevation" is, thus, used here as a means of estimating feeding time, and not aerial body temperature. Likewise, we make no attempt to distinguish between temperature sensitivity at low and high tide. However, given sufficient but as yet undetermined physiological data, more complex, realistic (non steady-state) simulations that include actual temperatures experienced during low tide can be generated using the approach put forth here.

RESULTS AND DISCUSSION

The approach that we describe (conceptually depicted in Fig. 1) provides a method of predicting the cumulative effects of multiple stressors on intertidal mussels as a function of tidal elevation, food availability, body temperature, and the presence of predators. During high tide, mussel temperature can generally be assumed to equal the temperature of the surrounding water. Determinants of body temperature during low tide however are more complex. As seen in Figure 3, the interaction between solar

radiation and air temperature sets the steady-state body temperature of mussels during aerial exposure at low tide, which in turn impacts scaled performance under a fixed level of food (relevant to feeding during high tide). Comparably, the combined effects of food density and location on the shore are depicted in Figure 5, where slight increases in food density can result in a substantial increase in the functional response (*i.e.*, Type II functional response curve; Holling 1959), and the effect of food density depends on tidal elevation.

Because of our assumptions regarding the effects of predation risk (Figs. 4 and 5), the effects of predators are greatest at optimal conditions of temperature and food density (*i.e.*, because the effect of predation risk is fixed at 20%, the absolute difference between predation/no predation is highest when performance is maximal). This is consistent with theoretical studies showing that the effects of predation risk on prey foraging behavior are strongest at high resource levels (Luttbeg *et al.* 2003). However, the effects of predators on sessile invertebrate feeding processes should be tested more extensively. Similarly, empirical studies have demonstrated predation risk reduces prey growth efficiency (Trussell *et al.* 2006), potentially by increasing metabolic demands (Rovero *et al.* 1999). However, little quantitative information exists describing the effect of predation risk on prey thermal performance, or for its effect over a range of risk levels (but see Trussell *et al.* 2011 for how growth efficiency is impacted at variable risk cue durations).

The ultimate goal of this modeling approach is to estimate ecologically relevant metrics that resulted from the cumulative influence of multiple stressors over time. Figure 6 describes cumulative growth (here, total wet weight calculated over ten years), but similar graphs can be generated for other life-history metrics such as lifetime reproductive output (Darwinian fitness; Bozinovic *et al.* 2011). Together, Figures 3–6 show the cumulative effects of the abiotic environment on performance during low tide (air temperature and solar radiation on body temperature; Fig. 3) as well as the effects of food availability and tidal elevation (through the functional response; Fig 5.) on growth (Fig. 6). The additional effect of predation risk on functional response (through feeding) and performance (by physiological stress; Fig. 5) is also depicted.

CONCLUSIONS

A number of recent studies have emphasized a critical need to understand the effects of multiple stressors (Howard *et al.* 2013, Sokolova 2013) and the indirect effects of predators (Kordas *et al.* 2011, Matassa and Trussell 2011) on ecologically and commercially important species. This study highlights the potential power of a coupled biophysical-DEB framework in exploring these effects through the currency of
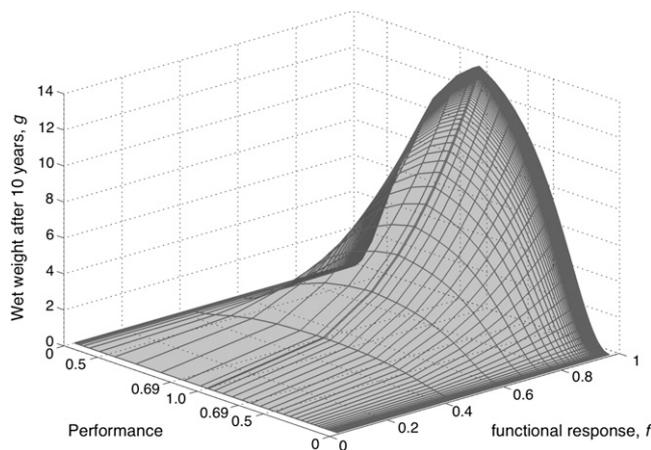
Figure 6. The cumulative effects of functional response and performance on growth (here wet weight, g). The effects of food limitation (potentially from decreased ambient food availability, predation, and/or upper tidal elevation) can be counteracted by body temperatures closer to optimum. Similarly, an increase in food acquisition (due to high ambient food availability, low predation, and/or low tidal elevation) can offset the negative impact of temperature and/or stress from risk on performance.

energetics. It also, however, highlights significant gaps in our knowledge that must be addressed. The strong influence of predation risk on gross measures of prey physiological performance have begun to be documented with controlled experiments that explore the effects of predator presence on patterns of growth and growth efficiency. A critical step is to construct performance curves that quantify the physiological effect of predators over a wide range of temperatures, akin to the theoretical curve shown in Figure 4. The advantage of this type of mechanistic approach is that by understanding basal processes by which organisms are affected by multiple stressors, we will be able to produce more robust predictive frameworks that better capture the often novel conditions represented by climate change. It also provides a means of generating explicit hypotheses regarding how some stressors may work synergistically or antagonistically with others in affecting ecological equilibria. For example, increases in food availability reduce the effects of limited feeding times as well as alleviate the effects of sublethal temperatures (Schneider *et al.* 2010). These results also suggest that, as shown by Sarà *et al.* (2012) for oligotrophic sites in the Mediterranean, slight increases in organic enrichment at nutrient poor locations may in fact increase performance, and, thus, may counteract some of the effects of warming. These observations are consistent with the functional relationship between food density, temperature, ingestion rate $\{\dot{p}_X\}$ and assimilation rate $\{\dot{p}_A\}$ described under a DEB theory framework (see equations above and Fig. 6 for effects on ultimate growth).

Bioenergetic models provide a mechanistic framework for studying multiple stressors based on the concept that energy balance is the common denominator that links an organism with all aspects of its environment (Sokolova 2013). While we present simplified, steady-state simulations as a means of exploring how different abiotic and biotic parameters can be combined in an energetics framework, our ultimate goal is to present a model that can be applied using site-specific information that can then be used to generate testable, explicit ecological hypotheses, lending confidence to predictions of ongoing and future changes due to global climate change.

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