

Mussels as a Model System for Integrative Ecomechanics

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Abstract

Mussels form dense aggregations that dominate temperate rocky shores, and they are key aquaculture species worldwide. Coastal environments are dynamic across a broad range of spatial and temporal scales, and their changing abiotic conditions affect mussel populations in a variety of ways, including altering their investments in structures, physiological processes, growth, and reproduction. Here, we describe four categories of ecomechanical models (biochemical, mechanical, energetic, and population) that we have developed to describe specific aspects of mussel biology, ranging from byssal attachment to energetics, population growth, and fitness. This review highlights how recent advances in these mechanistic models now allow us to link them together across molecular, material, organismal, and population scales of organization. This integrated ecomechanical approach provides explicit and sometimes novel predictions about how natural and farmed mussel populations will fare in changing climatic conditions.

1. INTRODUCTION

Marine mussels have a rich history as a model system for ecological, physiological, and biomechanical studies, owing largely to their cosmopolitan distribution in temperate coastal waters and their ecological and economic importance. Just a few well-known examples include the development of disturbance theory (Paine & Levin 1981), ecological genetics (Hilbish & Koehn 1985), biophysical forecasting (Wetthey et al. 2011), environmental toxicity assays (Widdows & Donkin 1992), and bioinspired design of underwater adhesives (Lee et al. 2011). In this review, we present mussels as a model system for marine ecomechanics (*sensu* Denny & Gaylord 2010), an emergent field that calls for an explicit framework for evaluating interactions between organisms and their changing environment. Here, we describe four categories of ecomechanical models that we have developed, relatively independently, to describe specific aspects of mussel biology: biochemical, mechanical, energetic, and population. Our goal is to highlight recent advances in these approaches that enable their integration from molecules to populations as well as how they explicitly incorporate changing environmental conditions (**Figure 1**). This integrated ecomechanical perspective, which can be applied to other marine organisms, provides a powerful framework that is yielding exciting new insights and novel predictions about the structure, function, growth, and distribution of mussels under current and future environmental conditions.

We focus here on the physiological process of manufacturing and maintaining structural integrity, which often constrains organismal performance in natural environments. This mechanical constraint is clearly evident in coastal marine ecosystems, where shells and spines deter predators; body size, shape, and flexibility influence hydrodynamic stress; and the strength of tethers and

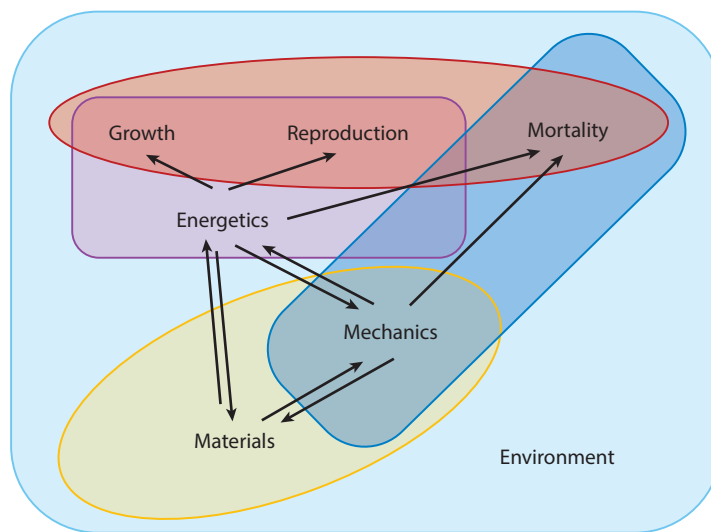


Figure 1

Schematic of an integrated ecomechanical model that links four mechanistic models describing different aspects of mussel biology and their interactions with the environment. This conceptual framework includes models of life history and fitness (*red*), the dynamic energy budget (*purple*), ecomaterials (*yellow*), and ecological biomechanics (*dark blue*). Importantly, the environment (*light blue background*) influences each component of the integrated model (growth, reproduction, mortality, energetics, mechanics, and materials) and all interactions (*arrows*). Aspects of the environment include climate, weather, and habitat (e.g., water temperature, pH, food supply, wave action, emersion time, and substrate characteristics). For simplicity, we have omitted interspecific interactions and other biotic influences.

adhesives secures attachment to benthic substrates. It is also well known that environmental conditions themselves can feed back to influence structural integrity, directly altering material composition, structure, and form or acting indirectly via energetic or developmental constraints. Introducing the multiple dimensions of this interplay between the environment, organisms, and the materials they make is an additional goal of this review. Although we emphasize the ecomechanics of the mussel byssus—the structure that anchors mussels to hard surfaces (see Section 3)—this approach can be adapted to other biomaterials and physiological processes.

2. MUSSELS IN CHANGING COASTAL ENVIRONMENTS

Mussels manufacture their structural materials in dynamic environments. Seawater conditions vary on timescales ranging from hours to years, and this variability can be large and frequent, especially in coastal environments, where mussels thrive. Waves, currents, upwelling, tidal mixing, weather, and local inputs influence water temperature, pH, salinity, nutrients, turbidity, oxygen concentration, and other environmental metrics. The directional effects of climate change on coastal conditions are already evident as well (Deutsch et al. 2005, Gilman et al. 2010, Barton et al. 2012, Wootton & Pfister 2012). Of recent concern is the accelerating release of anthropogenic CO₂ to the atmosphere, which over the past 200 years has made the oceans approximately 30% more acidic (Feely et al. 2004). This decline in pH and carbonate saturation levels, together loosely referred to as ocean acidification (OA), will affect biological processes and ecological interactions across multiple spatial and temporal scales (Pfister et al. 2014). In mussels, OA alters calcification rates as well as resource allocation to noncalcified tissues (e.g., Gaylord et al. 2011, Melzner et al. 2011, O'Donnell et al. 2013). The accumulated negative impacts of climate change therefore threaten the ability of mussels to serve as ecosystem engineers by providing habitats and benthic-pelagic coupling, filtering out sediments and pollutants, and providing food for higher trophic levels.

3. MUSSEL ATTACHMENT IN A WARMER, HIGH-CO₂ WORLD

3.1. Patterns in Wild and Farmed Mussel Populations

A key to the success of mussels in coastal habitats is strong byssal attachment. The byssus is an extracellular structure that mussels produce to withstand heavy wave action, resist mobile predators, and overgrow competitors for limited space. It is an assemblage of numerous collagenous fibers, each molded one at a time within a pedal groove, that collectively tether mussels to hard substrates and to one another (**Figure 2**; see also sidebar, The Living History of Sea Silk). Mussel tenacity, or the strength of the entire byssus, is determined by the number of threads present and their strength and extensibility (Bell & Gosline 1996, 1997; Qin & Buehler 2013). The thread number reflects the net balance of thread production and decay, both of which vary seasonally. Thread longevity is typically ~8 weeks but can be as low as 2 weeks in summer (Bell & Gosline 1996, Moeser & Carrington 2006).

Our understanding of the biomechanics of mussel attachment has advanced considerably in the past few decades. Research in this area was initially motivated by the development of ecomechanical models to predict wave disturbance of mussel beds (e.g., Denny et al. 1985, Bell & Gosline 1997, Carrington 2002b, Carrington et al. 2009). Although this process is dependent on wave action (an abiotic factor), the primary driver is mussel tenacity, which cycles seasonally and causes wild and commercially farmed populations to fall off, or dislodge during specific times of the year (**Figure 3**). For example, *Mytilus edulis* on the Northwest Atlantic coast of the United States and Canada is

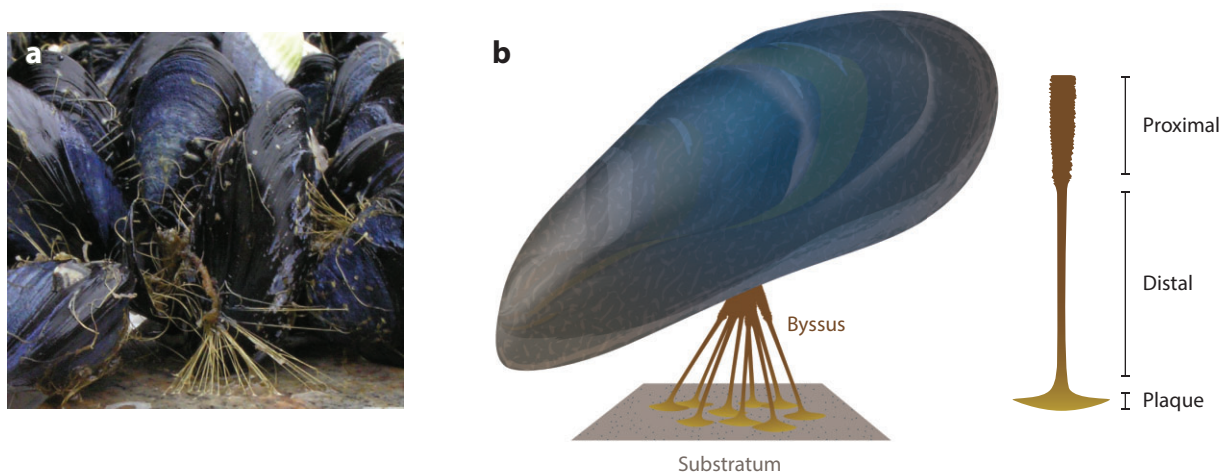


Figure 2

(a) Byssal attachment. Mussels tether themselves to hard substrates with many byssal threads, each molded in minutes by a protruding foot. (b) Schematic mussel showing the extracellular byssus. Each thread is a composite material comprising three distinct regions connected in series (proximal thread, distal thread, and adhesive plaque). Artwork in panel *b* by Meghan Rock.

prone to dislodgment in late summer and early fall, when weak attachment coincides with increased storms or harvest activities (Lachance et al. 2008, Carrington et al. 2009). By contrast, the same species on the Northeast Atlantic coast of the United Kingdom is at risk of dislodgment in late winter and early spring because the season for weak attachment occurs four months earlier (Price 1980, Carrington 2002a). These intraspecific differences in the timing of the attachment cycle make it difficult to pinpoint the aspect of the environment that triggers byssus weakening.

Natural cycles in *M. edulis* tenacity primarily reflect changes in the material properties of the byssal threads that mussels produce (Moeser & Carrington 2006) (Figure 3). When loaded in tension to failure, threads made by mussels in the Northwest Atlantic in winter and spring are twice as strong and extensible as they are in late summer and fall. This loss of thread performance is due to weakening in both the proximal thread region and adhesive plaques, which fail before the distal thread region can yield and extend (Figure 3). Byssal thread weakening leads to mussel dislodgment (killing approximately 33% of the population; Carrington et al. 2009), which in turn undermines the ability of dense aggregations of mussels to serve in their foundational role in coastal communities.

THE LIVING HISTORY OF SEA SILK

Scientists have studied the biochemistry, mechanics, and ecology of mussel byssal attachment for several decades, but there is a much longer history of using byssal threads to spin “sea silk” for weavings and needlework. This ancient craft may have originated as early as the second century; the oldest known fabric fragment is from Budapest and has been dated to the fourth century (Maeder 2008). The fine, long, golden byssal fibers are still harvested sustainably from the noble pen shell (*Pinna nobilis*) in the Mediterranean region. In the village of Sant’Atioco in Sardinia, Italy, the craft and its associated rituals and traditions have been passed down through the generations to the *maestro di bisso*, a title currently held by Chiara Vigo.

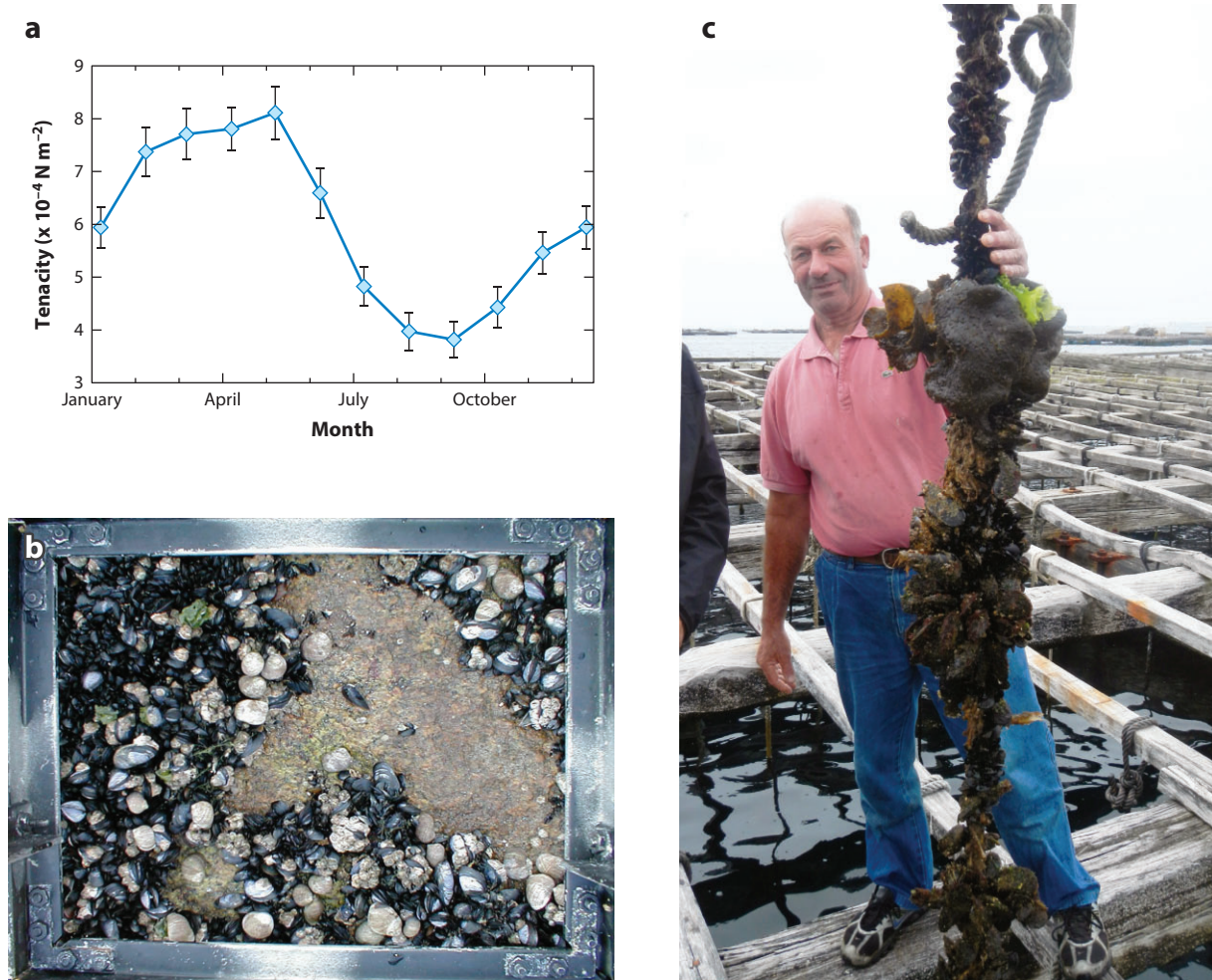


Figure 3

(a) Monthly mean *Mytilus edulis* tenacity in Rhode Island from 1998 to 2003. In this region, mussel tenacity in the winter and spring is twice that in the late summer and fall. (b) A gap in a natural mussel bed. The seasonal drop in tenacity renders populations prone to dislodgment mortality by waves, creating such gaps. (c) Seasonal fall-off (or sloughing) in Vigo, Spain. This fall-off is commonly seen in suspension rope cultures on commercial mussel farms during periods of low tenacity.

Seasonal fall-off (or sloughing) is also commonly seen in suspension rope cultures at commercial mussel farms (Lachance et al. 2008; I. Jefferds, personal communication). Mussel growers typically try to minimize sloughing by lowering mussel densities and inserting stakes in the ropes to support the weight of the mussels, a traditional Spanish method. Despite such efforts to improve retention on farms in Washington State, weak attachment commonly causes losses of *Mytilus trossulus* and *Mytilus galloprovincialis*. Thus, the loss of byssal thread integrity can negatively affect the economic sustainability of shellfish farms growing mussels in suspension culture via reduced yields at harvest and/or increased handling requirements. Farms in Quebec, Spain, and New Zealand have reported similar sloughing problems (Lachance et al. 2008; J. Babarro & J. Guinotte, personal communication).

The underlying mechanism by which byssal threads weaken seasonally in natural and farmed settings is unknown. Although field observations of natural populations have identified many potential causes (related to increased water temperature, reduced wave action, spawning cycles, and low food supply), their frequent co-occurrence makes it impossible to identify conclusively the trigger(s) for weak mussel attachment. We hypothesize that the energy available for byssus production will affect both the quality and number of threads produced, and that these factors will influence the probability of dislodgment and thus mortality. Measurements of energy needed to produce byssal threads are currently unavailable for most species, and the few that exist are not from realistic field conditions. We use existing estimates of energetic cost in both the dynamic energy budget (DEB) models and the fitness models presented here (Sections 4 and 5), but with the understanding that these are rough initial numbers. In particular, the link between the energy used to produce byssi and survivorship probability is purely theoretical in the fitness model, but we hope future research will fill this gap.

3.2. Insights from Laboratory Mesocosm Studies

Recent laboratory studies have begun to explore how changing seawater conditions alter the mechanical properties of the byssal threads mussels produce. O'Donnell et al. (2013) used custom laboratory mesocosms to expose the Pacific mussel *M. trossulus* to a broad range of OA (300–1,500 $\mu\text{atm } p\text{CO}_2$) and temperature (10–25°C) conditions and, after several weeks of exposure, collected the newly produced byssal threads and used a tensometer to quantify their material properties (**Figure 4**). Increasing OA (i.e., higher $p\text{CO}_2$ and lower pH) significantly reduced byssal thread strength: Attachment was 40% weaker when formed at a $p\text{CO}_2$ above 1,200 μatm (pH < 7.5) (O'Donnell et al. 2013). This nonlinear effect was due to weakening of the adhesive plaque; the mechanical behavior of the distal and proximal thread regions was unaffected by OA. In a similar assay, byssal threads formed in warmer seawater were also of poor quality (L. Newcomb, unpublished data) (**Figure 4**). Threads were ~60% weaker in mussels maintained at 25°C relative to those at 10–18°C. Unlike OA, however, the primary effect of elevated temperature was to weaken the proximal threads. This region was visibly altered at the higher temperature, apparently because of poor molding in the foot groove when it was made.

Together, these studies show that OA and high temperature weaken and reduce the extensibility of byssal threads but do so through different mechanisms: OA weakens adhesive plaques, whereas warming weakens the proximal threads. These results were the first to identify how specific environmental stressors, such as OA and increased temperatures, critically compromise the structural integrity of the mussel byssus. Because they act on separate regions of the same tensile element, their combined effects are not synergistic or additive; a chain is only as strong as its weakest link.

3.3. Applying Lessons from the Laboratory

An important next step will be to extend these laboratory findings to real-world conditions by considering the timing and magnitude of key environmental fluctuations encountered by mussels in nature. What aspects of the environment trigger episodes of weak attachment? Qualitative observations by mussel growers suggest that sloughing is exacerbated by several factors, all of which tend to increase in summer. As mussels grow larger, the diameter of the aggregation around the rope expands, and a greater fraction of the mussels are attached to one another rather than to the rope itself, creating instability. Summer is also a time of reduced wind-driven water motion, which is reduced even further in dense aggregations (Carrington et al. 2008). Reduced flow near

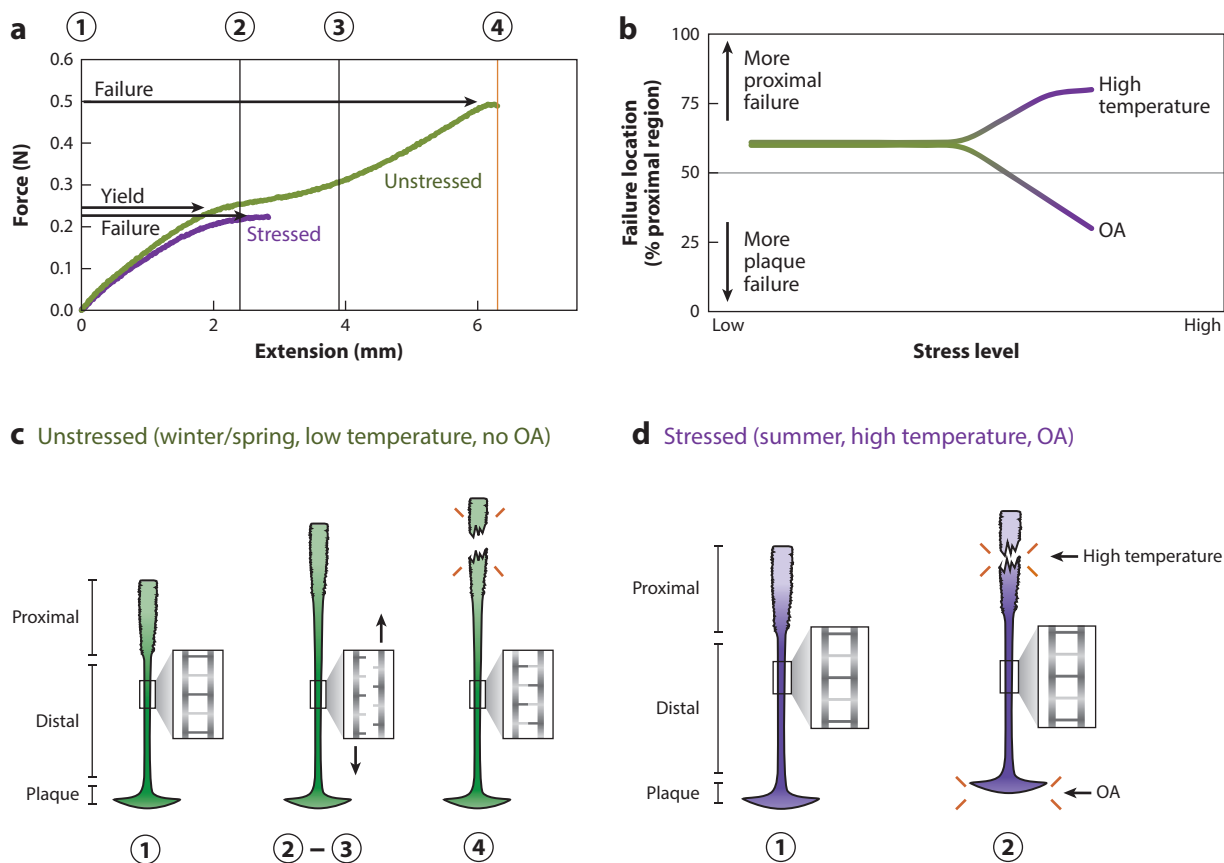


Figure 4

The effects of environmental stressors on byssal thread mechanics, based on studies by Moeser & Carrington (2006), O'Donnell et al. (2013), and L. Newcomb (unpublished data). (a) Mechanical failure in unstressed and stressed byssal threads. Under low-stress conditions (green), threads pulled in tension are initially stiff (①–②), then yield and extend in the distal thread (①–②) before the proximal thread or adhesive plaque fails (④). Stressed threads—i.e., those made in summer or in laboratory conditions of high temperature or ocean acidification (OA) (purple)—are weaker and less extensible, failing before the distal thread yields. (b) Points of failure in unstressed and stressed byssal threads. The failure location of the unstressed threads has a slight bias toward the proximal thread (60%). High-temperature stress disproportionately weakens the proximal thread, shifting the bias even higher (80%). OA targets the adhesive plaque, effectively shifting the failure location to this region. (c,d) Illustrations of these mechanical behaviors for unstressed (panel c) and stressed (panel d) byssal threads. The yield occurs only in the unstressed thread, where the tension becomes high enough to break cross-links connecting fibrils in the distal region, allowing the fibrils to slide relative to one another and then re-form to stabilize a new fibrillar alignment. This unstressed thread is therefore both stronger and more extensible than the stressed thread. Because high temperature and OA weaken different regions of the tensile structure, their effects are independent and cannot combine to weaken the thread further; the weakest link (region) determines the thread's structural integrity. Artwork in panels c and d by Meghan Rock.

the innermost layer of the mussel aggregation could limit food delivery and waste removal and/or increase sedimentation, which could potentially reduce mussel attachment and promote sloughing. Other field observations (Price 1980, Carrington 2002b, Lachance et al. 2008, Carrington et al. 2009) have not adequately quantified concurrent environmental or mussel physiological conditions, making it difficult to infer the likely trigger(s) of weak byssus production. These correlative studies do suggest, however, that both abiotic and biotic factors may be involved; weak attachment generally correlates with warmer water, low food supply, low mussel somatic and gonadic weight,

and high parasite prevalence. Thus, weak attachment could reflect energetic stress (e.g., owing to metabolic effects of pH and temperature) and/or direct effects on thread production, decay, and quality (**Figure 1**). Furthermore, different mechanisms may be involved for different mussel populations (e.g., different species or different locations). These scenarios and their potential implications are addressed further in the following sections.

A second important step will be to explore the underlying mechanism by which the environment alters the byssus manufacturing process. For example, the OA effect on byssus weakening has been isolated to a specific region, the adhesive plaque. This discovery will greatly focus efforts to describe the molecular underpinnings of weak adhesion, which in turn may reveal its cause (e.g., direct physiochemical effects versus indirect energetic constraints) and ultimately help to develop strategies that promote the manufacture of strong byssus material in both industry and nature. Understanding the cause of byssus weakening is of interest not only to marine ecologists and mussel growers but also to materials scientists designing composite fibers and underwater sealants that can withstand a broad range of environmental conditions (Lee et al. 2011). In this manner, the study of biomaterials in an ecological context—ecomaterials—can inform and inspire new biotechnological advances. This approach is reviewed in the following section.

4. MYTILUS BYSSUS BIOCHEMISTRY

Mytilus byssus biochemistry has been extensively reviewed from different perspectives (Silverman & Roberto 2007, Lee et al. 2011, Waite & Broomell 2012). Here, we divide byssus biochemistry into three interconnected themes: the molecular composition and construction of the *Mytilus* byssus, the role of the environment in normal byssus formation, and the byssus in changing environments.

4.1. The Molecular Composition and Construction of the Byssus

The *Mytilus* byssus resembles a bundle of fibers or threads that are ~95% protein by dry weight (Waite et al. 2002). A dozen or so distinct byssal proteins have been characterized, most of which are highly localized in their distribution within the byssus (**Table 1**). There are also small but important amounts of carbohydrates, metal ions, and perhaps lipid components (Waite et al. 2002). All known carbohydrates are glycosidically bound to one or two glycoproteins (Sun et al. 2002). The metal ions, notably Fe, Cu, Zn, and Ca ions (Holten-Andersen et al. 2009), are also bound to specific proteins and therefore have the same distributions as their protein ligands. Below, we summarize the protein distribution in the byssus. In addition to the three thread regions shown in **Figure 2**, we include here the interface with the substratum and the byssus stem, which connects all threads to the mussel body.

4.1.1. Plaque-substratum interface. Interfacial proteins are the first to be deposited onto the substratum by the foot, and therefore might be thought of as a base coat or primer proteins for byssal adhesion. These proteins are referred to as *Mytilus* foot proteins (Mfps). **Table 1** summarizes the four known Mfps in the plaque-substratum interface (Mfp-3f, Mfp-3s, Mfp-5, and Mfp-6), which are thought to mediate adhesion to targeted substrata (Lee et al. 2011) (**Figure 5a**). These proteins have low molecular weights (<12 kDa), a 3,4-dihydroxy-phenylalanine (Dopa) content ranging from 5 to 30 mol%, and high isoelectric points (i.e., they contain more positive than negative charges) and are generally unstructured in solution (Hwang & Waite 2012). Of the four proteins, only Mfp-6 exhibits poor adhesion to mica in a surface forces apparatus, but it provides excellent antioxidant properties to keep Dopa in other Mfps from being turned into Dopa-quinone

Table 1 Localization of proteins in the *Mytilus* byssus, along with their mass, Dopa content, metal binding, and sequence motifs

Byssus portion	Protein	Mass (kDa)	Dopa (mol%)	Metal binding	Sequence motifs (number of repeats)	Reference
Plaque-substratum interface	Mfp-3f	5–7	20	—	Dopa-Arg/Lys (6)	Lee et al. 2011
	Mfp-3s	5–7	10	—	Gly-Dopa (8)	Wei et al. 2013
	Mfp-5	10	30	Mg ²⁺ /Ca ²⁺	Dopa-Lys (16)	Lee et al. 2011
	Mfp-6	11	5	—	Cys (3), Tyr-Lys (5)	Zhao & Waite 2006
Adhesive plaque (noninterfacial)	Mfp-1	100	15	Fe ³⁺	Decapeptide (75)	Lee et al. 2011
	Mfp-2	45	5	Fe ³⁺ /Ca ²⁺	EGF domain (11)	Hwang et al. 2010
	Mfp-4	80	2	Cu ²⁺	Dodecapeptide (4)	Lee et al. 2011
Distal thread	Mfp-1	100	15	Fe ³⁺	Decapeptide (75)	Lee et al. 2011
	PreCOL-D	250	<1	Zn ²⁺ /Cu ²⁺	Collagen (1), silk (2)	Harrington & Waite 2007
	PreCOL-NG	250	10	Zn ²⁺ /Cu ²⁺	Collagen (1), polyGly (2)	Harrington & Waite 2007
	TMP	35	3	—	Asn-Gly (44)	Sagert & Waite 2009
Proximal thread	Mfp-1	100	15	Fe ³⁺	Decapeptide (75)	Lee et al. 2011
	PreCOL-P	250	<1	Zn ²⁺ /Cu ²⁺	Collagen (1), elastin (2)	Coyne et al. 1997
	PreCOL-NG	250	<1	Zn ²⁺ /Cu ²⁺	Collagen (1), polyGly (2)	Lee et al. 2011
	TMP	35	5	—	Asn-Gly (44)	Sagert & Waite 2009
	PTMP	250	3	Zn ²⁺ /Cu ²⁺	Von Willebrand (2)	Sun et al. 2002
Stem	PreCOL-P	250	<1	Zn ²⁺ /Cu ²⁺	Collagen (1), elastin (2)	Coyne et al. 1997
	PreCOL-NG	250	<1	Zn ²⁺ /Cu ²⁺	Collagen (1), polyGly (2)	Waite et al. 1998

Abbreviations: EGF, epidermal growth factor; Mfp, *Mytilus* foot protein; preCOL, prepolymerized collagen; PTMP, proximal thread matrix protein; TMP, thread matrix protein. In the preCOL protein names, D, NG, and P stand for distal, nongraded, and proximal, respectively. Dashes indicate that data were not reported.

by a two-electron oxidation that is usually coupled to O₂ reduction to water (Yu et al. 2011). Mfp-5 exhibits the greatest adhesion to mica in a surface forces apparatus. A study of the adhesion of these proteins to a variety of surface types showed that, in all known cases, keeping the Dopa reduced is necessary for the two-pronged or bidentate mode of chemisorption (Yu et al. 2011).

4.1.2. Adhesive plaque. The noninterfacial plaque proteins include Mfp-1, Mfp-2, Mfp-4, thread matrix protein (TMP), and prepolymerized collagens (preCOLs) (**Table 1**). These are larger proteins, ranging from 45 kDa for Mfp-2 to nearly 250 kDa for the preCOLs. As with the proteins in the interfacial group, all are positively charged at seawater pH and contain Dopa, albeit at lower levels [ranging from <1 mol% in preCOL-D (where D stands for distal) to 15 mol% in Mfp-1]. What sets these proteins apart from the interfacial set is their repetitive structure: All have at least one conserved consensus sequence motif that is tandemly repeated (**Table 1**). The shortest repeat motif is the Gly-X-Y tripeptide repeat in the collagen domain of the preCOLs, followed by the decapeptide of Mfp-1 and the dodecapeptide of Mfp-4. Mfp-2 and TMP both have repeat motifs >30 amino acids long (Lee et al. 2011). In the plaque, Mfp-2 interacts with itself in Ca- and Fe-mediated complexes and by H bonding with Mfp-3 and Mfp-5 (Hwang et al. 2010). Mfp-4 couples the preCOLs to Mfp-2. TMP helps to separate preCOL fiber bundles, and Mfp-1, which is usually complexed to Fe³⁺ in submicron granules (**Figure 5b**), coats the plaque surface facing the seawater (Holten-Andersen et al. 2007, Harrington et al. 2010).

4.1.3. Distal thread. The distal thread portion is dominated by a core of preCOL fibrils that are separated by the TMP matrix and coated by a thin, ~5-μm cuticle of Mfp-1 that is continuous

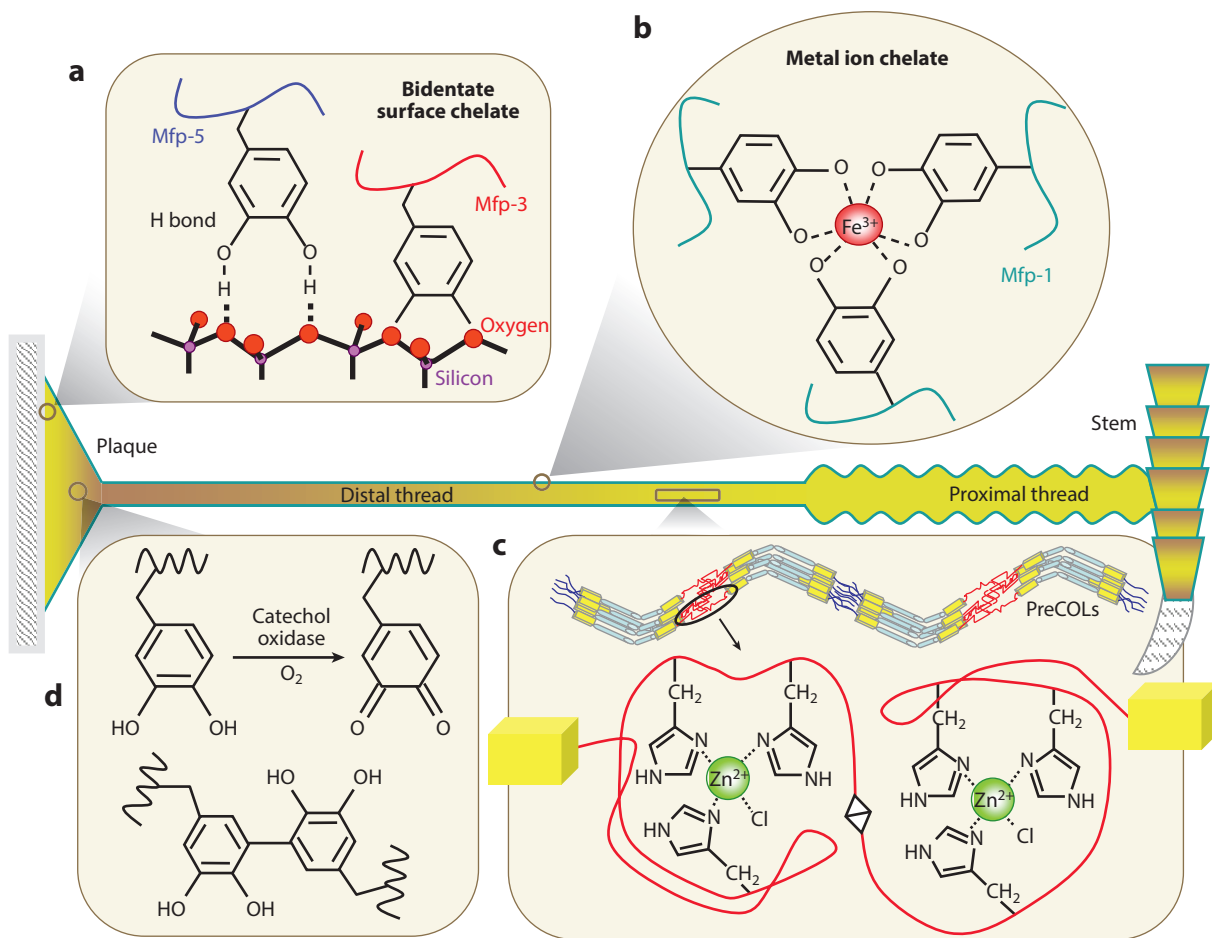


Figure 5

Metal ion-mediated interactions in the *Mytilus* byssus that are prone to perturbation. Dopa-mediated interactions between Mfp-3 and Mfp-5 and an oxide surface such as silica or hematite include (a) a bidentate H bond and bidentate coordination complex (oxygen shown in red, silicon shown in purple), (b) a triscatecholate chelate formed between three Dopa side chains from Mfp-1 and one Fe^{3+} ion, and (c) histidine-mediated interactions between preCOLs (zig-zag bundles) and Zn^{2+} or Cu^{2+} , showing how the two end sequences (red) ravel up around the Zn^{2+} ion. The double triangle represents a covalent cross-link that is likely similar to the diDopa shown in panel d. That panel shows the activity of catechol oxidase involving the O_2 -dependent two-electron oxidation of Dopa in certain proteins to Dopa-quinone. The coupling of one Dopa and one Dopa-quinone results in the formation of a 5,5'-diDopa cross-link. Abbreviations: Dopa, 3,4-dihydroxy-phenylalanine; Mfp, *Mytilus* foot protein; preCOL, prepolymerized collagen.

with the plaque (Sagert & Waite 2009, Harrington et al. 2010). The distal thread contains two distinct preCOLs. One of these, preCOL-D, is a penta-block copolymer consisting of a triple-helical kinked collagen midblock, two flanking domains that resemble spider ampullate silks, and two histidine-rich endblocks. By contrast, preCOL-NG (where NG stands for nongraded) has glycine-rich flanking domains but otherwise resembles preCOL-D (Waite et al. 1998). The assembly of preCOLs in the collagen gland is discussed in Section 4.2.

4.1.4. Proximal thread. The proximal thread portion is rarely exposed outside the shell enclosure in living mussels, and it is less stiff and more extensible than the distal portion. Visually, there

appears to be a sharp distinction between the distal and proximal portions, but this belies the subtle biochemical transitions that actually occur within the thread. Specifically, preCOL-D gradually gives way to preCOL-P (where P stands for proximal) (Waite et al. 2002), and there is a greater volume of matrix proteins, notably the glycosylated proximal TMP that binds and helps to separate the collagenous bundles (Sun & Waite 2005, Suhre et al. 2014). Only preCOL-NG remains the same throughout both the distal and proximal portions. Like preCOL-D, preCOL-P has a pentablock copolymer structure, but the flanking domains contain elastin-like XGXP repeats, in which X denotes glycine or a nonpolar amino acid. In other respects, the preCOLs are very similar. The proximal thread is also covered by an Mfp-1-containing cuticle, but its Dopa and Fe levels are significantly lower than those in the distal portion (Sun & Waite 2005).

4.1.5. Stem. The stem, like the thread, is covered by an Mfp-1-containing cuticle, and preCOL-P and preCOL-NG extend from the proximal thread into the stem (Coyne & Waite 2000). At the proximal end of the stem is a junction or anastomosis between fibrous byssal proteins (nonliving) and the byssal retractor muscles (living). This junction enables the retractor muscles to control the resting tension of the byssal threads. The stem/root proteins have not been characterized but merit attention as the site of autotomy when mussels voluntarily jettison their byssi.

4.2. The Role of the Environment in Normal Byssus Formation

Mussels depend on the properties (particularly the pH) of seawater as switching cues for byssal protein assembly and maturation. Most of the byssal proteins are partially assembled, indeed prefabricated, as metastable complex fluids (Hassenkam et al. 2004, Wei et al. 2014) in acidic compartments that are both intra- and extracellular (Yu et al. 2011). Precursor fluidity helps the foot mold a new thread, but at some point the protein assemblies need to be solidified.

The use of environmental pH as a solidification switch is exemplified in byssal preCOLs. PreCOLs are produced in the endoplasmic reticulum of the mussel foot's collagen gland and stored as liquid crystal assemblies in large (~1–2- μm diameter) secretory granules (Vitellaro-Zuccarello 1980). The secretory granule pH is typically between 5 and 6 (Casey et al. 2010), whereas the cytosolic pH is ~7 and seawater's pH is 8.2. This means that histidines (average pK_a of 6.5) clustered in preCOL endblocks are ~99% positively charged at pH 5 but ~99% uncharged at pH 8 (Harrington & Waite 2007). At pH 5, charge-charge repulsions between the imidazolium-rich ends of preCOLs assist the cells in preventing fiber formation and exploiting instead the lateral interactions between the smectic liquid crystals (i.e., molecules in lateral register, similar to books or bottles on shelves) (Hassenkam et al. 2004). The smectic rafts are secreted en masse into the ventral groove during thread formation, but the ambient pH is no longer regulated as it was by proton pumping in the secretory granules. Instead, preCOL pH reequilibrates with the seawater pH (8.2). As the histidines become uncharged, the repulsion between the smectic rafts disappears and is replaced by interactions with cosecreted fourth-row transition metal ions such as Cu, Zn, and Fe ions, which typically coordinate three or four different histidines per metal ion (Degtyar et al. 2014). Because of proton-metal ion repulsion, charged histidines are not capable of this coordination; however, at a pH above the pK_a , imidazole-metal binding is instantaneous. The first phase of preCOL cross-linking is therefore a pH-dependent formation of metal-mediated histidine bridges (**Figure 5c**). Histidine-metal interactions are not rendered superfluous by subsequent covalent cross-linking. Instead, these interactions remain at the ends of the preCOLs, providing critical sacrificial bonds (e.g., His-Zn) and unstretched loops of sequence (Harrington & Waite 2007, Degtyar et al. 2014), as shown in **Figure 5c**, that explain the reversible high strain, hysteresis, and toughness in the distal thread portion.

Another type of solidification switch involves Dopa and Fe^{3+} complexes in the mature cuticle of the byssus. The participating Dopa residues belong to Mfp-1 (**Table 1**), which is made and stored in acidic granules of the accessory gland prior to secretion (Vitellaro-Zuccarello 1981). Dopa is a catechol (*o*-dihydroxybenzene) and therefore has an extremely high Fe^{3+} binding affinity ($\log K_s$ as high as 10^{50}) (Taylor et al. 1994), with the Dopa- Fe^{3+} interaction achieving roughly half the bond energy of a covalent bond (Lee et al. 2006). In contrast to the histidine off/on, charged/uncharged switch, catechol-based switches are more tunable, in large part because of three incremental binding steps that are highly dependent on pH: (a) mono [$\text{Dopa} + \text{Fe}^{3+} \rightleftharpoons (\text{Dopa-Fe}^{3+})$], (b) bis [$(\text{Dopa-Fe}^{3+}) + \text{Dopa} \rightleftharpoons (\text{Dopa}_2\text{-Fe}^{3+})$], and (c) tris [$(\text{Dopa}_2\text{-Fe}^{3+}) + \text{Dopa} \rightleftharpoons (\text{Dopa}_3\text{-Fe}^{3+})$] (**Figure 5b**). At pH 5, the Dopa- Fe^{3+} complex is mononuclear bidentate—i.e., it binds each metal nucleus with two phenoxy prongs (bidentate) and therefore has no cross-linking effect. However, as the pH increases to that of seawater (8.2), the complex recruits more Dopa to form the bis- and triscatecholate modes (**Figure 5b**), which are capable of cross-linking two or three different protein chains (Harrington et al. 2010). Again, pH provides a switch for changing chemical and thus mechanical properties in threads, this time in the cuticle (Harrington et al. 2010).

A third, more permanent enzyme-dependent cross-linking occurs in the threads and plaques. This involves the enzyme catechol oxidase (EC 1.10.3.1), known to be present in mytilid byssi (Smyth 1954, Waite 1985) and recently identified in a transcriptome library of a *Perna viridis* foot (Guerette et al. 2013). Catechol oxidase catalyzes the two-electron oxidation of Dopa in target proteins to Dopa-quinone in a reaction that is stoichiometrically coupled with O_2 reduction to H_2O (**Figure 5d**). The reaction is fast (typical solution turnover $\sim 100\text{--}500\text{ s}^{-1}$; Tech. Univ. Braunsch. 2014), but the nonsolution state of the byssus and the need for tethered Dopa-quinones to find cross-linking partners likely slows the process. Suffice it to say that the pH optimum of this enzyme is narrowly limited to 7.5–8.5, with, for example, <10% activity remaining at pH 7 (Waite 1985).

The cross-linking chemistry of Dopa-quinones involves at least two well-known organic reactions: the Michael addition and aryl coupling. In the first, a nucleophile such as an amine or thiolate attacks an electron-poor carbon in the ring. Such cross-links have been described in *Mytilus* byssi, e.g., 5-cysteinyl-Dopa from plaques and histidinyl-Dopa from threads (Zhao & Waite 2006; M.J. Harrington & J.H. Waite, unpublished data). McDowell et al. (1999) detected 5,5'-diDopa, an example of aryl coupling, in situ in byssal plaques using solid-state NMR (**Figure 5d**). Indeed, diDopa formation rate is closely linked to ambient water flow around the threads—a reminder that a ready supply of the other substrate (O_2) is required for Dopa-quinone production. Which Dopa residues in the various byssal proteins (**Table 1**) are targeted for oxidation and cross-link formation remains unknown.

In summary, mussels appear to use a one-two punch to solidify their byssi, with the fast but weaker first punch holding the material together well and long enough to enable the slower but stronger second punch to form the covalent cohorts. The presence of two cross-linking pathways—one metal mediated and reversible, the other covalent and irreversible—may seem extravagant but is perfectly adapted to the needs of *Mytilus*, where each byssal thread is formed in approximately 5 min and then immediately recruited into load-bearing service. Metal-mediated cross-linking occurs extremely rapidly (indeed, too rapidly to be measured by available methods), whereas covalent cross-links between other amino acids and Dopa-quinones require more time to find one another in the byssal matrix (Danner et al. 2012).

4.3. The Byssus in Changing Environments

There have been no direct analyses of environment-induced changes in the byssal molecular structure, and the complex relationships between the byssal structure and its properties, and their

dependence on a mussel's energetic or nutritional state, are incompletely understood. Nevertheless, we can make certain predictions about how byssally attached mussels would respond to two scenarios of environmental insult. In the first, a mussel with an assemblage of normal threads is assaulted by a short-term change; in the second, a mussel is assaulted by a long-term change that is accompanied by a significant turnover of threads. At this time, confidence in predicting biochemical relationships between the structure and its properties is limited to how the performance of a normal byssus might be compromised by seawater with altered properties.

Because mussels depend on seawater to provide switching cues for byssal assembly and solidification, it is reasonable to predict that, as seawater pH decreases, (*a*) histidine-metal interactions will become weaker, leading to threads that are less stiff with lower hysteresis; (*b*) Dopa-Fe complexes in the cuticle and plaque will shift from being tris-functional to being bis- and mono-functional, which would be expected to decrease the hardness of the cuticle and therefore make it more prone to damage by abrasion and perhaps microbial attack; and (*c*) the activity of catechol oxidase will be sharply reduced, resulting in fewer quinones, which in combination with increasing levels of charged histidine and lysine will result in fewer covalent cross-links. Fewer covalent cross-links would then be expected to reduce stiffness, strength, and the reversibility of extension.

Some temperature-dependent changes in byssal thread chemistry are also predictable. A temperature increase to 34–38°C would be expected to melt most or all of the collagen triple helix in preCOLs (<http://compbio.cs.princeton.edu/csc>; Persikov et al. 2005). Increased temperatures will also enhance asparagine deamidation in TMP (Sagert & Waite 2009), but the effects that this would have on thread properties are unknown. Finally, temperature, pH, and ionic strength all perturb the stability of the complex fluids that serve as vehicles for various proteins during thread formation (Wei et al. 2014).

5. MUSSEL ENERGETICS AND DYNAMIC ENERGY BUDGETS

5.1. Dynamic Energy Budgets

Among the most critical desiderata of ecologists, wildlife managers, and stakeholders are tools to predict quantitatively how environmental variance affects the functioning of coastal animals and thereby the likelihood of losing their associated biodiversity (Harley 2011). The mechanistic framework presented here relies on energy and mass flows as the main currencies for all ecological processes, from individuals to ecosystems (*sensu* Webb et al. 2010). Thus, tracking mass and energy provides a means to unify individual processes with populations and communities through constraints controlling the acquisition, allocation, and disposal of materials and energy (Loreau 2010).

The mechanistic nature of this framework represents the functional core of quantitative predictions of population spatial persistence over time in cases where organisms are subjected to altered patterns of environmental variance. If we can parameterize species functional traits through energy and mass flows and then link the magnitude of functional traits to life history traits such as growth or number of eggs per spawning, we increase greatly the power of our predictions for population persistence.

The steps of this process are well captured by recent bioenergetics models based on the dynamic energy budget (DEB) theory of Kooijman (2010), which in the past decade has become one of the most powerful tools for predicting the fate of organisms across gradients of both natural and human-amplified environmental variance (Kearney et al. 2010). The most recent applications of DEB theory have been in both terrestrial (e.g., Kearney 2012) and aquatic habitats (e.g., Freitas et al. 2010; Jusup et al. 2011; Sarà et al. 2011, 2012, 2013a,b, 2014b; Nisbet et al. 2012; Teal et al. 2012; Maury & Poggiale 2013; Matzelle et al. 2014; Rinaldi et al. 2014), spatially contextualizing

the modeling effort by using a high-resolution series of local temperatures and food density (e.g., Montalto et al. 2014b). The DEB approach is currently used in a broad range of species and environmental contexts to answer basic ecological questions (e.g., Jager & Klok 2010). The power of the functional-trait-based DEB model lies in its utility as a practical solution for providing quantitative, accurate predictions of species abundance in a rapidly changing world (Araujo & Rahbek 2006, Sarà et al. 2014a). It is essentially based on ecological niche theory (Kearney & Porter 2009, Kearney et al. 2010, Matzelle et al. 2014) and exploits mechanistic rules to connect environmental variability to functional traits (Schoener 1986), which in turn inform species life history traits (Stearns 1992), thereby linking mass and energy flow from the organismal level to population dynamics and density (Cheung et al. 2011) and ultimately to community structure (Laughlin et al. 2012) (**Figure 6**).

5.2. Incorporating Costs for Byssus Production

In the sessile world of intertidal mussels, environmental variability can affect the energetic performance of individuals, which has direct effects at the population level (e.g., growth, reproduction, or mortality) (**Figure 6**). For example, chronic and severe wind and storms influence local hydrodynamics and increase the risk of mussel dislodgment (Denny et al. 1985, Hunt & Scheibling 2001, Carrington et al. 2009), which in turn can reduce lifetime growth and reproduction. Many mussel species sense and respond to increased wave action by producing stronger byssi, presumably via increased thread production (Van Winkle 1970, Lee et al. 1990, Dolmer & Svane 1994, Bell & Gosline 1997, Moeser et al. 2006, Carrington et al. 2008).

Although higher tenacity can reduce dislodgment risk for mussels at wave-exposed sites (e.g., Carrington 2002b, Zardi et al. 2007, Carrington et al. 2009), increased byssus production may come at an energetic cost. For example, Hawkins & Bayne (1985) estimated that byssus production can consume up to 8% of a mussel's monthly energy expenditure. In DEB theory, the cost of byssus production can be incorporated into the energy cost of somatic metabolic maintenance (Kooijman 2010) (**Figure 6**). To meet these extra costs, mussels must therefore divert energy from other vital processes, such as growth and reproduction, according to the κ rule (**Figure 6**); the resulting prediction is reduced body size (sensu Kooijman 2010).

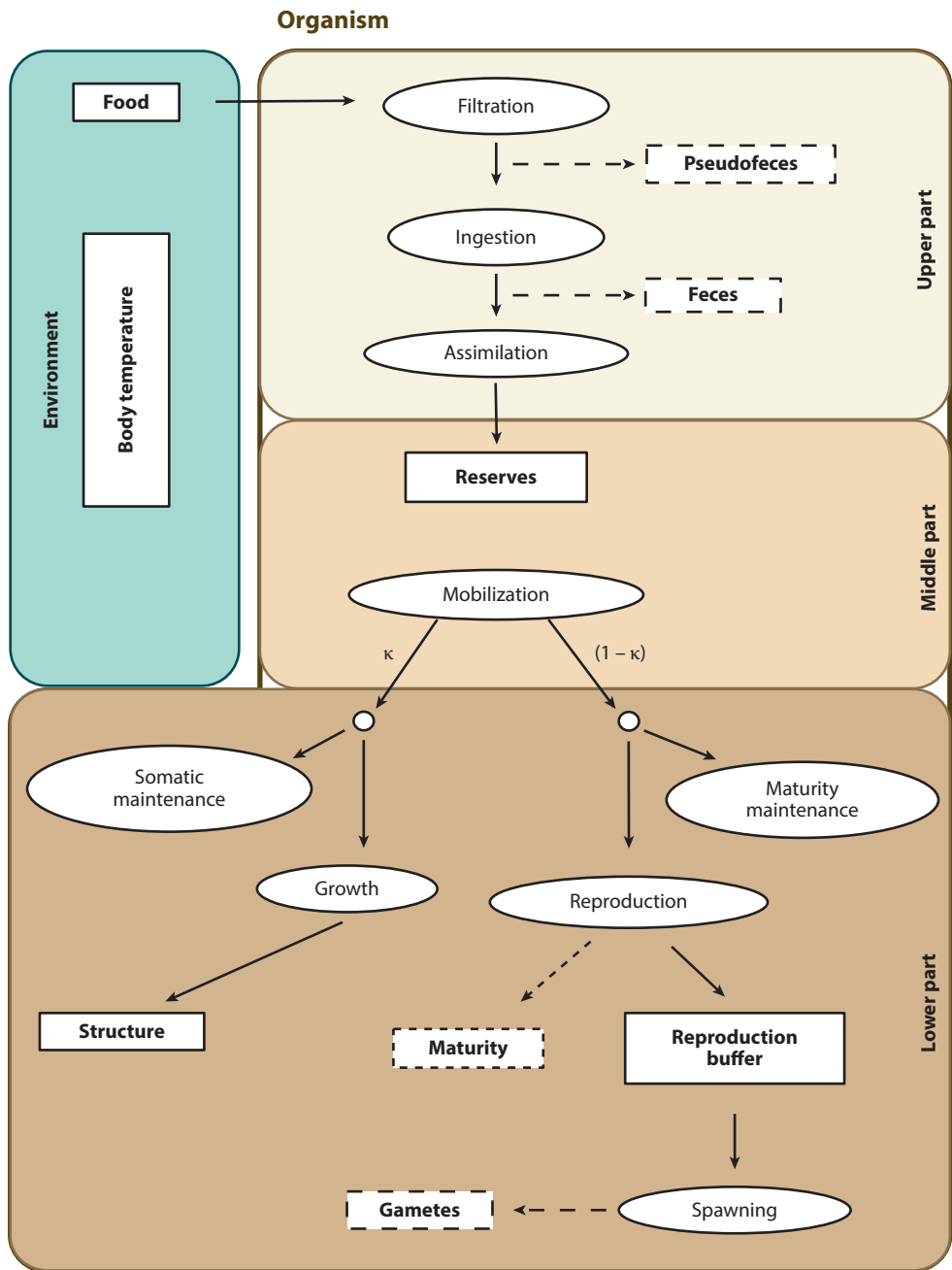
Note that every species follows a specific rule in partitioning energy among growth and reproduction and maintenance functions. Although there is recent ecotoxicological evidence that some levels of stressful conditions may also alter the entity of fraction κ , in general κ is fixed per species. What usually can change in stressful conditions is the energy mobilization rate from reserves. When the energy fixed into reserves is depleted (owing to poor food availability or acquisition), the amount of energy allocated to κ (and $1 - \kappa$) will be so low that organisms will stop the

Figure 6

The standard dynamic energy budget model for bivalves (Kooijman 2010), tracking the flux of energy coming from the environment through the organism to predict functional and life history traits. Metabolic processes are estimated from food availability and individual body temperature. In ectotherms such as mussels, body temperature must be estimated from environmental conditions. Neither the feeding process nor reserves accrue maintenance costs. The rate at which this energy is used from the reserve follows the dynamics of the κ rule, where a fixed fraction (κ) of assimilated energy is allocated to maintenance and somatic growth, and the remaining fraction ($1 - \kappa$) is available for maturity maintenance and reproduction. (In the example shown in **Figure 7**, the somatic maintenance—where we assume the cost of byssus production is paid—is expressed as the parameter pM , which is a good approximation of the organism's basal metabolism and is indirectly related to oxygen consumption.)

allocation according to the following—highly schematic—priority list before it dies: reproduction, growth, sexual maturity maintenance, and finally somatic metabolic maintenance.

In ectotherms with indeterminate growth, such as mussels, the gonad production potential (which is the base of the Darwinian fitness; Bozinovic et al. 2011, Sarà et al. 2014b) scales directly to body size: The larger the body size is, the larger the gonad mass is (Sebens 1982a, Strathmann &



Strathmann 1982). Such changes in reproductive effort can affect local population dynamics (see Section 6) and propagule spillover to adjacent populations (Simberloff 2009, Sarà et al. 2013b).

In the real world, however, biological responses are driven by the combination of multiple forces acting across gradients of intensity, frequency, and duration (Miller et al. 2011, Sokolova et al. 2012). The direct stochastic effect of wave forces on local mussels' performance, leading to smaller animals and lower fitness, combines with other potential forces driving the mussels' responses. For example, local food availability determines the amount of energy available to organisms (Sarà et al. 2014b) and thus the mussels' local ecological responses. When there are extra-energetic costs resulting from high hydrodynamic forces, the amount of food (Sarà et al. 2003, Sarà & Mazzola 2004) should play a crucial role in buffering the energetic costs resulting from extra byssus production. With adequate information, we should thus be able to predict the potential effects, in terms of body size and fitness, of an increasing wave climate under different conditions of temperature, food availability, and population density.

5.3. A Case Study for Intertidal Mussels

An extensive treatise on DEB mechanistic theory and its potential applications is beyond the scope of this review. We instead present an example for *M. galloprovincialis*, the native Mediterranean mussel now invading many coasts (e.g., Zardi et al. 2007). Using a spatially explicit DEB model (Montalto et al. 2014b), we estimated the lifetime reproductive output for intertidal *M. galloprovincialis* in Trieste (Italy, Northern Adriatic Sea) tidal conditions using an environmental data set similar to that used by Sarà et al. (2011) for 2006–2009. The basic modeling approach follows that of Sarà et al. (2011, 2012, 2013a, 2014a,b), Kearney (2012), Kearney et al. (2012), Matzelle et al. (2014), Montalto et al. (2014a,b), and Rinaldi et al. (2014). We predicted lifetime reproductive output for varying energetic costs paid on somatic metabolic maintenance resulting from extra byssus production and under different scenarios of local food density. We estimated body temperature hourly using the environmental time series as inputs into the biophysical model of Helmuth (1998) for mussels at +0.25 m on the shore, as in the study by Sarà et al. (2011).

The model generated the life history trait of lifetime reproductive output (total eggs produced over the four-year life span), an important component of fitness. Byssus costs were paid as percent increases in the somatic maintenance cost. We performed a sensitivity analysis using 6 levels of byssus cost and 11 levels of food accessibility, for a total of 66 scenarios (Figure 7). When food is scarce ($<1 \mu\text{g}$ chlorophyll *a* L^{-1}), intertidal *Mytilus* mussels do not have sufficient energy to reproduce, regardless of byssus production. At higher food levels, lifetime reproductive output depends on the cost of byssus investment: A 20% increase in byssus investment reduces reproduction by 6–30%, depending on food level. Altogether, this analysis shows quantitatively (a) how an environmentally induced increased investment in structural integrity (the byssus) places energetic constraints on life history traits and (b) how an increased food ration can buffer these detrimental energetic constraints. Such spatially explicit mechanistic models thus have the power to predict individual responses for a broad range of environmental conditions, which can in turn feed into population models, as described in the next section.

6. ESTIMATING FITNESS FROM ENERGETICS AND MECHANICS

6.1. Estimating Fitness

The performance, and thus the fitness, of any organism is affected by environmental variability, including unidirectional change, and by its own phylogenetic, genetic, physiological, and

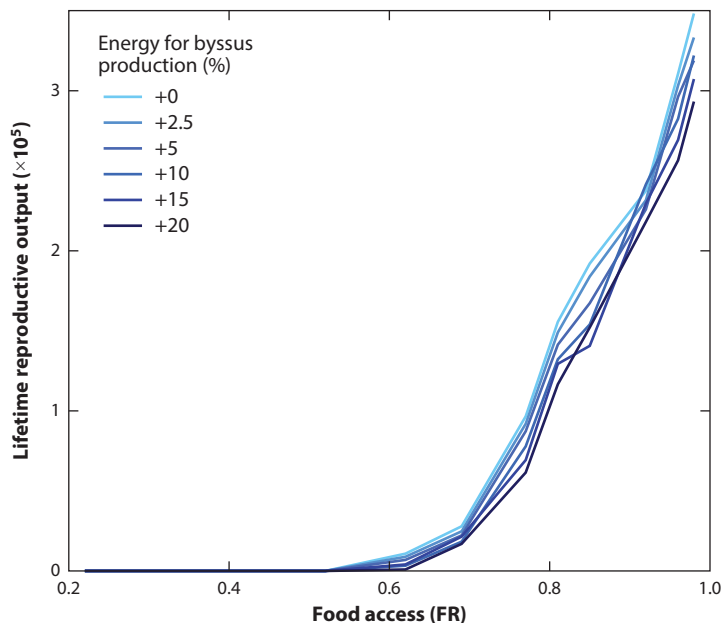


Figure 7

Output of a dynamic energy budget (DEB) model for *Mytilus galloprovincialis* in Trieste (Italy, Northern Adriatic Sea), incorporating the cost of byssus production. The lifetime reproductive output, a component of fitness, was estimated as a function of food density ($0.25\text{--}50\ \mu\text{g chlorophyll } a\ \text{L}^{-1}$) and the cost of byssus production. We used the Holling (1959) functional response (FR) to describe food accessibility: $\text{FR} = \text{food density}/(\text{food density} + X_k)$ where X_k is the half-saturation coefficient for this species (0.91; Montalto et al. 2014a). A lower FR implies that less energy from food is available for vital processes. We simulated the potential extra costs of byssus production as an increase in the somatic maintenance cost (pM in the context of DEB theory, in joules per hour; Sarà et al. 2013b, 2014b) by 2.5–20% above the typical value for this species ($0.834\ \text{J h}^{-1}$; Montalto et al. 2014a).

developmental constraints. The consequences of any set of adaptations in a given environment—whether morphological, physiological, or life history traits—are best evaluated by examining both performance and fitness (Endler 1986; Arnold & Wade 1984a,b; Kozłowski 1993). Short-term fitness components such as growth rate, energy balance, reproductive output per season, mortality rate, and lifetime egg/offspring production are common measures of performance (often referred to as fitness) (Thoday 1953, McGraw & Caswell 1996; for mussels, see Bierne et al. 2006, Shields et al. 2008), but population-level responses can be assessed only when all such response variables are incorporated into a multigeneration measure of fitness based on growth, reproduction, and survivorship (de Jong 1994).

To this end, researchers have used a combination of empirical measurements and mathematical models to generate comparative measures of population growth rate (i.e., r or λ , where $r = \ln\lambda$) (Metz et al. 1992, McGraw & Caswell 1996) as an estimation of individual (Darwinian) fitness. This can be done for each individual within a population (McGraw & Caswell 1996) or for a hypothetical population of identical individuals (genotypes or phenotypes) with a particular set of traits (Sebens 2002; Kozłowski et al. 2004; Buckley & Kingsolver 2012a,b). Comparing fitness estimates for a range of traits and conditions can then provide useful predictions about how well certain phenotypes will perform under existing or new environmental conditions (Lande &

Arnold 1983, Pelletier et al. 2007, Buckley & Kingsolver 2012b) and whether their geographic or altitudinal ranges might then shift with climate change (Buckley 2008).

One approach to estimating fitness is biophysical: Performance (e.g., egg production) is measured over a range of physical environmental conditions (e.g., temperatures), and offspring production rates as a function of body temperature are then used along with survivorship to calculate fitness (e.g., as λ) (Buckley & Kingsolver 2012a,b). Another approach uses energetics as a basis for estimating fitness. Recently, there have been improvements in the methodologies and computational power applied to whole-organism and population-level energetics, including the DEB approach. However, there have been few attempts to combine the fitness estimation approach (McGraw & Caswell 1996) based on life history data with the well-established energy budget models (Kooijman 2010, Nisbet et al. 2012). Sebens (2002) provided a general model of this type that—given sufficient information on energetic and life history parameters—can be applied to marine invertebrates or any other organism, and thus can be a useful theoretical tool independent of organism type or habitat.

6.2. A Model for Mussel Fitness

Here, we provide an example from our ongoing research (K. Sebens, G. Sarà & E. Carrington, manuscript in preparation) in which we have used this modeling approach with mussels. McGraw & Caswell (1996) noted that it is not always possible to follow individuals over their lifetimes, but fertility, growth, and survivorship data might be available for limited time periods and for a subset of individuals. They suggested that a useful modeling approach would take those limited empirical data and embed them in a model using simulated data for all other parameters, with the model then able to show the comparative effect of the measured components on overall fitness; our model uses this approach. This is particularly important for marine invertebrates such as mussels, which have open populations and long-range dispersal, making it nearly impossible to obtain some of the survivorship and recruitment data. In the energetics portion of our model, we used the basic energetic and growth equations from Sebens (1982b, 1987, 2002) for growth, energy surplus (intake minus cost), and asymptotic or final size, where surplus is equivalent to scope for growth (and reproduction). The allocation of energy to byssus production and to other nonliving structures is incorporated as an increase in metabolic cost during byssus production, as measured by Lurman et al. (2013). This is a simpler formulation than using the full DEB model, which will be incorporated in the next phase, and is more appropriate here for our general theoretical explorations.

Energy cost and intake are functions of food availability, temperature, and other environmental factors as well as trait variation (e.g., allocation within an organism) and are commonly modeled as power functions of mass. For this comparison, we modeled the temperature effects on metabolic cost and energy intake following the approach of Sarà et al. (2013b) and used values for energy intake, cost, growth rate, and egg production from their data for the mussel *Brachidontes pharaonis* at sites in Italy. For this comparison, we assumed that the temperature effects were greater on metabolic cost than on intake, and that the intake increases with temperature to some maximum and then decreases (e.g., as observed in sea stars; Sanford 2000, 2002). This assumption is based on the well-known effect of temperature increasing metabolic rate in poikilotherms (i.e., Q_{10} relationship; Kooijman 2010) and the general paucity of data on temperature effects on food acquisition and energy intake. For passive suspension feeders, where intake depends on the area of capture surfaces presented to moving water, there is likely to be a range of temperatures at which temperature has little or no effect. Active suspension feeders may increase pumping rates with temperature, over a certain range, but this also raises metabolic cost.

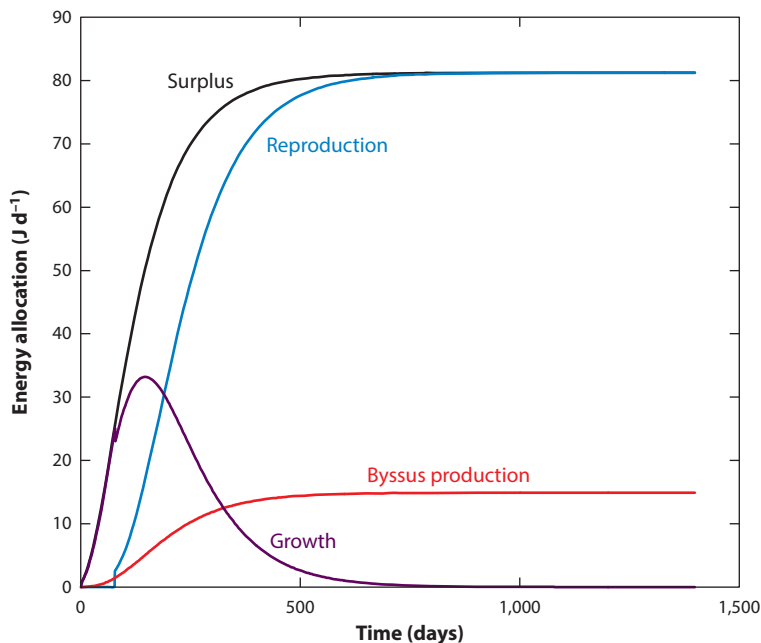


Figure 8

Daily energy allocation during the growth of an individual mussel in the energy budget model, calculated in daily time steps over a four-year life span with constant food availability and temperature. Energy surplus, or scope for growth (intake minus cost), is used for growth and reproduction. The amount of energy used for byssus production is modeled as an increase in metabolic cost.

We then used a life history model (an age-structured Leslie matrix; Leslie 1948, Caswell 1989) based on that of Sebens (2002) and McGraw & Caswell (1996) to model population growth over many generations. Once a stable age distribution was achieved in the modeled population, we calculated a per-capita (exponential) growth rate (r) that could be used as an estimate of multigeneration (overlapping) fitness. We then examined the effects on fitness and components of fitness for different environmental conditions, energy allocations, reproduction timings, and other energetic and life history traits. The mortality of larvae and of mussels at each growth stage was set artificially so that populations would be stable for a reference site at a 16°C mean annual temperature. Added mortality affected by byssus production was modeled as a logistic function, such that low production resulted in a high risk of mortality (up to 100%) and high production reached a plateau where mortality rate was constant.

Figure 8 shows the energy for somatic growth, reproduction, byssus formation, and energy surplus during growth for one of the model runs. Note that the energy used for growth peaks at approximately 300 days and then declines gradually to zero as the individual approaches a size asymptote at the energetic optimum (greatest surplus). Commonly measured components of fitness (**Figure 9a**) include scope for growth (summed over lifetime), reproduction (summed over lifetime), and asymptotic (final or maximum) size. The energy used for these components decreased as more energy was used for byssus production under the conditions of our model. However, fitness (measured as r) showed a very different response, first increasing because of greater survivorship and then decreasing because too much energy was being devoted to byssus production (**Figure 9b**). Examining only the separate components of fitness would not be enough

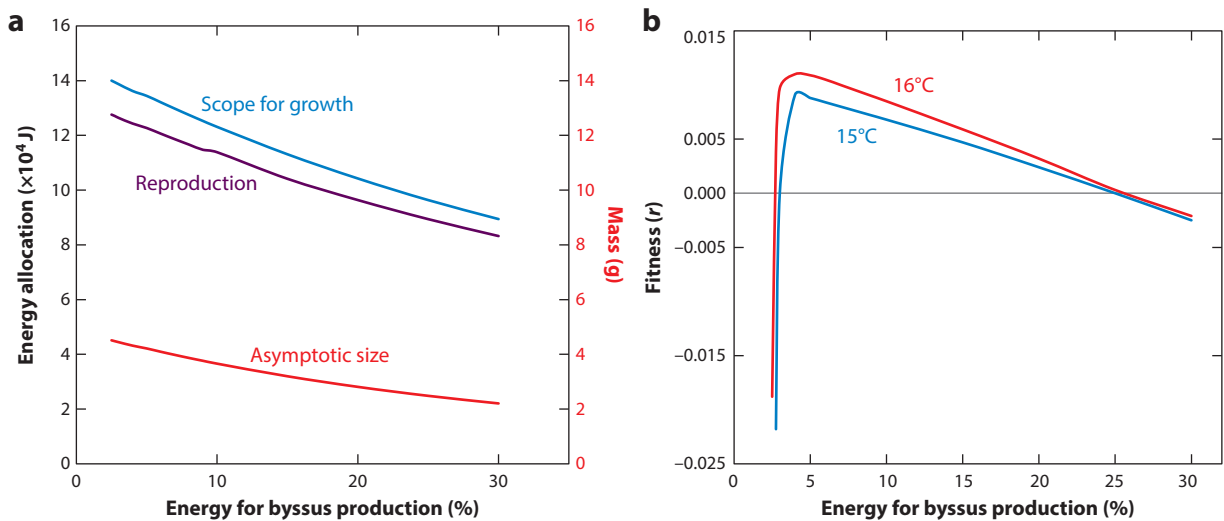


Figure 9

(a) Components of fitness: energy allocations to scope for growth and reproduction (*left y axis*, both summed over lifetime) and asymptotic (final or maximum) size (*right y axis*) for a range of byssus production rates (as percent increase in metabolic rate). (b) Fitness as a per-capita rate of increase (r). No population growth occurs when $r = 0$. Note that the shape of the fitness response curve for r is very different from the shapes for the other components of fitness.

to determine how energy should be allocated to byssus production under these conditions to produce the greatest fitness. Note that a 1°C change in annual mean temperature resulted in quite a large change in fitness, especially at the lower byssus allocation levels.

Models of this type can be used to examine trait variation in field populations of mussels or most any other organism. A wealth of energetics and life history data are available from mussel populations in diverse geographic regions (Grant 1996, Grant & Bacher 1998, Fly & Hilbish 2013, Sarà et al. 2013b, Matzelle et al. 2014) and can be applied to further analyses of this type. For well-studied mussel species, for example, we need to know the energetic and fitness costs of building tissue and nonliving structures (shells and byssal threads) over a range of physical conditions and when conditions vary (daily, seasonally, or spatially), as they do in many habitats (e.g., Elliott et al. 2008). How does the allocation of energy among multiple compartments affect fitness over a range of environmental conditions (e.g., temperature, pH, wave climate, predation, or food availability)? Areas for future investigation could include, for example, how fitness is affected by increased or decreased allocation to growth and reproduction at different stages in the life cycle and by differential allocation to shell production and byssal thread production.

7. CONCLUSIONS

In this review, we have presented four mechanistic models that describe aspects of mussel biology ranging from biochemistry to organismal traits to population structure. We have highlighted ways in which each model incorporates changing environmental conditions, especially temperature, pH, food supply, and wave action. We have also emphasized how these approaches have advanced in recent years and that their integration can lead to powerful insights that are not possible from correlative models. We have also identified where gaps in our current knowledge exist, which serve as fruitful areas for further study. Although our integrated ecomechanical perspective

was developed for mussels and their byssal attachment in changing environments, this approach could be readily used to describe other aspects of mussel biology and could be extended to other organisms.

SUMMARY POINTS

1. *Mytilus* byssal threads and their constituent proteins show a remarkable reliance on metal ions for self-assembly and solidification. However, this reliance has a cost: Metal-mediated protein-protein interactions are highly sensitive to pH.
2. Reliance on metal ion-mediated load-bearing structures is likely to be extensive among other intertidal invertebrates as well.
3. Seasonal declines in mussel attachment strength are common worldwide and lead to significant mortality in wild and farmed populations.
4. Ocean warming and acidification both weaken the mussel byssus but target different regions of the byssal threads. Whether these are the abiotic factors that trigger weak mussel attachment in nature is unknown.
5. Functional-trait models based on the dynamic energy budget theory are powerful tools to address how changes in the ocean—such as increasing temperature, acidification, contamination, and changes in local food availability—affect the energy allocated to structures such as byssus and shell.
6. A mechanistic approach is useful for predicting how environmental variation affects functional traits and shifts in energetic allocations to life history traits, such as growth and reproduction, that have demographic implications at the population level.
7. Energetics and life history models can be used together to provide fitness estimates for mussels in variable or changing environmental conditions.
8. The trade-off in energy allocation to byssus formation as opposed to growth and reproduction is critical to mussel fitness and population growth.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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Provided the first demonstration of the negative effects of OA on mussel structural mechanics.

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Introduced the use of resonance Raman microscopy with submicron resolution and parts-per-million metal detection in invertebrate sclerotins.

A pivotal article that proposed using the ecological niche as a foundation for mechanistic approaches in ecological research.

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Provides a very useful examination of the fitness concept applied to individuals or trait groups within populations.

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Errata

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