Energy-Limited Tolerance to Stress as a Conceptual Framework to Integrate the Effects of Multiple Stressors

Inna M. Sokolova

Department of Biology, University of North Carolina at Charlotte, Charlotte, NC 28223, USA

From the symposium “Physiological Responses to Simultaneous Shifts in Multiple Environmental Stressors: Relevance in a Changing World” presented at the annual meeting of the Society for Integrative and Comparative Biology, January 3–7, 2013 at San Francisco, California.

Synopsis

Integrating the effects of multiple stressors and predicting their consequences for the species’ survival and distribution is an important problem in ecological physiology. This review applies the concept of energy-limited tolerance to stress to develop bioenergetic markers that can assist in integrating the effects of multiple stressors and distinguishing between the moderate stress compatible with long-term survival of populations and bioenergetically unsustainable extreme stress. These markers reflect the progressive decline of the aerobic scope of an organism (defined as the fraction of the energy flux and metabolic power supporting this flux available after the basal maintenance costs of an organism are met) with increasing levels of the environmental stress. During the exposure to moderate stress (i.e., in the pejus range of the environmental conditions), the aerobic scope is positive but reduced compared with the optimum conditions. The reduction of the metabolic scope can be due to the (1) elevated costs of basal metabolism, (2) activation of the mechanisms for protection and damage repair, (3) reduced assimilation of food, and/or (4) stress-induced impacts on the aerobic pathways producing ATP. This leads to suboptimal growth and reproductive rates in the pejus range of environmental conditions and is commonly observed in food-limited and energy-limited wild populations. The tolerance windows of the organisms are delimited by the pessimum range(s) of environmental conditions in which the aerobic scope of the organism disappears (so that all available energy and metabolic capacity are used in support of basal metabolism), energy resources are depleted, and partial anaerobiosis and/or metabolic rate depression set in. The habitats where environmental conditions remain in the pessimum zone long enough to prevent consistent growth and reproduction often coincide with the species’ distributional limits. Thus, focus on the bioenergetic effects of environmental stressors and their immediate consequences for fitness provides a suitable framework for integrating physiology and functional ecology and can assist in understanding the driving forces and limitations of environmental adaptation and improving assessment of ecological risk as well as environmental management in field populations facing multiple stressors.

Introduction

Most organisms (with the notable exception of those from very stable environments such as the deep sea) live in a dynamic, labile environment where several important factors can change rapidly and simultaneously. These natural environmental fluctuations are superimposed with, and exacerbated by, an increasing degree of anthropogenic impacts including pollution, habitat destruction, ocean acidification, and global climatic change. When one or more environmental factors deviate from the species-specific and population-specific optimum, it can lead to physiological disturbance and a decrease in fitness and thus is classified as stress—an environmental factor that causes a potentially injurious change to a biological system with major impacts on evolutionary processes (Hoffmann and Parsons 1994). Studies of tolerance of environmental stress and adaptation to it led to significant advances in our understanding of the molecular, cellular, and physiological mechanisms of responses to a variety of environmental stressors including hypoxia, temperature, salinity,
desiccation, ultraviolet radiation, and pollution (Willmer et al. 2000; Hochachka and Somero 2002). However, the interactive effects of multiple stressors under environmentally realistic scenarios are not yet fully understood. Different stressors that have different physiological and molecular mechanisms can interact in a complex, non-linear way, so that the effects of combined exposures cannot be predicted from the effects of single stressors. Therefore, it is critical to develop an integrative physiological platform from which to assess and predict the effects of multiple stressors in natural populations.

Studies of the interactive effects of multiple environmental stressors pose logistic and conceptual challenges. First, analyses of the effects of multiple stressors require complex factorial experimental designs that can become logistically impractical as the number of analyzed stressors increases. Therefore, the possible stressors need to be screened and ranked according to their potential physiological and ecological impacts. Ranking of stressors can be achieved by multivariate analyses that partition physiological variation among different environmental variables. These include principal component analysis, multivariate regressions, or artificial neural networks (ANNs) analysis; such studies are still relatively rare, but their results are encouraging by revealing that the majority of physiological variation in natural populations is explained by a relatively small suite of environmental factors (James and McCulloch 1990; Lek and Guégan 1999; Chapman et al. 2011). In some cases, ranking of variables can be assisted by a priori knowledge of the stressor’s importance (such as the known effects of individual stressors and the magnitude of their environmental variation). For example, temperature is a key environmental factor that affects the physiology, behavior, and ecology of all organisms, particularly ectotherms, and strongly modifies the effects of other stressors (Schulte 2007; Sokolova and Lannig 2008; Pörtner 2012), making it advisable to consider environmental temperature in all studies of the effects of multiple stressors in natural populations.

It is also important to develop an experimental approach that allows synthesis of the combined effects of multiple stressors on different physiological and molecular mechanisms. Focusing on whole-organism effects (such as survival, growth, and reproductive output) is a common approach that provides the advantage of directly translating the effects of stressors into the fitness consequences for an organism and survival of its populations. However, such “black box” approaches provide no insight into the mechanisms of the effects of multiple stressors and thus limits our ability to extrapolate the results to other organisms or other combinations of the same stressors. To effectively address this challenge, it is important to develop a mechanistic framework that allows integrating the effects of multiple stressors with disparate cellular mechanisms and provides a link between the physiological effects and ecological consequences of exposure to multiple stresses. Global molecular and biochemical approaches (such as genomics, transcriptomics, proteomics, and metabolomics) provide important insights into the molecular mechanisms of the action of multiple stressors, including stressors’ synergy, cross-tolerance, and cross-talk (Chapman et al. 2011; Tomanek 2011; Connor and Gracey 2012). However, these molecular changes are often difficult to translate directly into the changes of the fitness of the whole organism. Recently, integrative approaches focusing on bioenergetics have been proposed to assess the effects and ecological consequences of environmental stress (Pörtner 2010; Sokolova et al. 2012). One of the most comprehensive physiological models that link bioenergetic mechanisms to response to stress is the oxygen-limited and capacity-limited thermal tolerance (OCLTT) concept (Pörtner 2010, 2012). This model serves as a basis for a recently proposed concept of energy-limited tolerance to stress (Sokolova et al. 2012) that merges physiological (i.e., OCLTT; Pörtner 2012) and ecological (i.e., the dynamic energy budget [DEB] model; Kooijman 2010) models of response to stress and provides a framework for linking physiological responses to different stressors and long-term, population-level consequences. In this review, I propose the concept of energy-limited tolerance to stress as a platform for integrating the effects of multiple stressors and I identify bioenergetic markers of the ecologically relevant physiological changes in response to environmental shifts. This review is focused on aquatic ectotherms because many studies of the bioenergetic effects of multiple stressors have been conducted on this group (Heugens et al. 2001; Sokolova and Lannig 2008; Altshuler et al. 2011; Pörtner 2012; Sokolova et al. 2012); however, the general principles of the proposed bioenergetic framework are applicable to other organisms.

**Energy metabolism as an integrator of the effects of multiple stressors**

Bioenergetics plays a central role in the tolerance to environmental stress. For thermodynamically open living systems, a balance between the input and
expenditure of energy is a key requirement for existence; at the organismal level, this requirement translates into the need for sufficient energy supply to cover the costs of basal maintenance (i.e., survival), as well as other fitness-related functions such as activity, growth, and reproduction. The rate of intake and assimilation of energy, as well as the metabolic capacity to convert ingested food to ATP, are inherently limited in any organism and are subject to a metabolic cost (Guderley and Portner 2010). This leads to trade-offs between allocations of energy and metabolic power to different energy-dependent functions and requires optimization of these allocations in ways that maximize Darwinian fitness (Kooijman 2010). The optimal allocation of energy in the absence of environmental stress implies that the aerobic scope of an organism (defined as the fraction of the energy flux and metabolic power available after basal maintenance costs of an organism are met) is at the species-specific and life-stage-specific maximum allowing for maximal investment into development, activity, somatic and gonadal production, and deposition of energy reserves (Fig. 1A).

Environmental stress can affect the optimal allocation of energy by modulating energy demands for survival, as well as capacities for assimilation and conversion of energy, thereby decreasing the aerobic scope (Fig. 1B–F). When an environmental variable
deviates from the optimum, the aerobic scope of an organism diminishes due to increased costs of basal maintenance, limitation of the uptake and delivery systems of oxygen, reduced capacity for acquisition of energy, and aerobic ATP synthesis, or a combination of these factors. Depending on the factor and a degree of its deviation from the population-specific optimum, the mechanisms responsible for a decrease in aerobic scope can differ. For example, a decrease in aerobic scope in response to warming is brought about by the limited capacity of circulation and ventilation systems to meet elevated demand for oxygen by tissues (Pörtner 2010), whereas a reduction of aerobic scope in the cold may be brought about by the low capacity of mitochondria for synthesis of ATP (Sommer and Pörtner 2002; Pörtner et al. 2007), and in extreme cases, by cessation of circulation induced by freezing (Storey and Storey 1988). Moderate concentrations of trace metals such as cadmium reduce the aerobic scope due to a combination of elevated demand of energy for protection from stress and repair of damage, and reduced mitochondrial efficiency (Lannig et al. 2006; Sokolova and Lannig 2008; Kurochkin et al. 2011). In contrast, acutely toxic levels of trace metals can reduce the aerobic scope by limiting oxygen uptake due to damage to the gills and to severe impairment of mitochondrial function (Sokolova and Lannig 2008). Hypoosmotic stress can lead to elevated energetic costs of homeostasis of ions and water, regulation of cell volume, and/or repair of damage (Hawkins and Hilbish 1992; Nelson et al. 1996; Kidder et al. 2006). Hypoxia directly affects the aerobic scope of an organism due to limited availability of oxygen and/or elevated demand of energy for enhanced ventilation (Jackson 2007; Nilsson et al. 2010; Pörtner 2010). Regardless of the exact physiological mechanisms, a decrease in aerobic scope results in a reduction of the energy flux devoted to production and/or to activity, and it can negatively affect the organism’s fitness.

According to the OCLTT concept (Pörtner 2012) and the energy-limited concept of tolerance to stress (Sokolova et al. 2012), the ability to maintain positive aerobic scope is a key determinant in long-term survival of organisms and sustainability of their populations. Under moderate stress (in the pejus range; Fig. 1B and C), aerobic scope is reduced but remains positive so that the long-term survival of the population is possible, albeit at the cost of reduced growth and reproduction. As the environmental factor further deviates from the optimum, the aerobic scope of the organism continues to decline until it reaches zero in the pessimum environmental range (Fig. 1D and E). Under these conditions, all energy flux and metabolic capacity of the organism is devoted to basal maintenance (i.e., survival), and anaerobic metabolism is often engaged to compensate for the insufficient aerobic production of ATP (Pörtner 2012). Some species from extremely variable environments (e.g., intertidal zone, arid, or ephemeral habitats) have evolved a capacity for metabolic rate depression that can be engaged in the pessimum range (Fig. 1E). Metabolic rate depression extends survival time, thereby increasing the chances that an organism stays alive until conditions improve (Guppy and Withers 1999), but it does not increase the aerobic scope so that time-limited survival is achieved at the expense of reproduction, growth, and activity. A further increase in environmental stress eventually results in the aerobic scope becoming negative, and the energy supply from both aerobic and anaerobic pathways is insufficient to cover even the basal metabolic demand (Fig. 1F). Under these conditions (i.e., the lethal range), massive disturbances of homeostasis, as well as cellular and systemic damages, ensue such that cannot be repaired and result in morbidity and mortality.

The proposed energy-limited model of tolerance to stress assumes that basal maintenance (i.e., the energy demand for survival) takes priority over other processes, including growth, reproduction, or storage. This principle has been shown to hold true for many species (Wieser et al. 1988; Guppy and Withers 1999; Rombough 2007; Kooijman 2010); however, under some environmental conditions and/or at some stages of the life cycle, priorities for the investment of energy can change. For example, during breeding semelparous species preferably invest energy into the functions supporting reproduction (Stearns 1992; Farrell et al. 2008; Fisher and Blomberg 2011). Thus, during spawning migrations, Pacific salmon protect their aerobic scope for swimming at the expense of wastage of the muscles that eventually leads to death (Farrell et al. 2008). Similarly, semelparous octopods use their somatic mass to fuel reproduction and die after a short period of intensive parental care (O’Dor and Wells 1978). Short-term shifts in the priorities of investment of energy between reproduction and maintenance also are observed during the spawning season in broadcast-spawning marine bivalves (Cheung 1993; Li et al. 2007; Petes et al. 2008 and references therein). It has also been proposed that similar prioritization of investment of energy into reproduction over maintenance (so-called terminal allocation) can occur in older individuals reaching the limit of their life spans; however, this strategy
appears to be confined to semelparous or semi-semelparous species (Hamel et al. 2010; Weladji et al. 2010; Fisher and Blomberg 2011). Whenever such reversals of the priorities occur, they are by necessity time-limited and can be incorporated into the framework of the energy-limited model of tolerance to stress by integration of the stress-induced changes into the aerobic scope over the organism’s life cycle.

The width and position of the stress-tolerance window (encompassing the optimum and pejus environmental ranges in Fig. 1) differs between different life stages and/or body sizes. Typically, early life stages (such as developing embryos and larvae) have elevated sensitivity to environmental stressors, including temperature, salinity, ocean acidification, and chemical pollutants (review by Sokolova et al. 2012). The mechanisms of high sensitivity to stress in early developmental stages are not yet fully understood and may reflect the lower energy reserves in young organisms and the competing demands of development versus protection from stress (Parsons 2003; Hamdoun and Epel 2007). The position and width of the stress-tolerance window can also be shifted by adaptation, acclimation, or acclimatization that can improve the aerobic scope via adjustments in mechanisms for assimilation of energy, mitochondrial abundance and capacity, enzyme activities, ion and gas transport, and composition of membranes (Willmer et al. 2000; Hochachka and Somero 2002). These adjustments are inevitably constrained by the species’ physiology and environmental conditions (such as availability of food and oxygen).

Bioenergetic markers that assess the impacts of multiple stressors

The energy-limited concept of tolerance to stress ties physiological changes at the cellular and organismic levels to fitness and therefore, to ecological consequences. Although changes in the aerobic scope in response to the environmental stressors are continuous, three important thresholds can be distinguished: (1) a transition between the optimum and pejus range when aerobic scope starts declining but long-term survival, growth, and reproduction are possible, (2) a transition from pejus to pessimum range in which aerobic scope becomes zero and only time-limited survival is possible, and (3) a transition to the lethal range in which the aerobic scope is negative and acute mortality ensues. Bioenergetics markers associated with transitions to the pejus, pessimum, and lethal ranges of environmental conditions can assist in predicting the ecological consequences of environmental stress including exposures to multiple stresses (Table 1). The transition to the pessimum range is especially important in this context, because it marks the bioenergetically unsustainable situation and determines the biogeographic distribution of the species (Pörtner et al. 2001; Pörtner and Knust 2007).

Aerobic scope

Measurement of aerobic scope (i.e., the proportion of the energy and metabolic flux available for supporting fitness-related functions after the cost of the basal maintenance is covered) provides the most direct way of determining the transitions into the pejus and pessimum range of environmental factors and thus assessing the width of the stress-tolerance window in a population. The aerobic scope sets limits to tolerance to temperature, oxygen levels, salinity, pollution, hypercapnia, and combinations of these stressors (Guderley and Pörtner 2010; Pörtner 2012; Sokolova et al. 2012). Recent advances in metabolic physiology and bioenergetic modeling provide several complementary approaches that can be used to measure aerobic scope in a variety of animals. In active species of fish and invertebrates, aerobic scope can be assessed by determining the difference in the basal metabolic rate and maximum aerobic rates during sustainable aerobic exercise (Pörtner 2002c). Such determination requires making certain that animals are fully aerobic during the exercise and that no functional anaerobiosis sets in, a condition that may not be easily met in some sluggish or sedentary species (Pörtner 2002a, 2012). In small organisms lacking ventilatory and circulatory systems (e.g., unicellular organisms or small metazoans such as cnidarians or flatworms), aerobic scope can be determined as the difference between maximal mitochondrial capacity and basal oxygen consumption rates. The aerobic scope of the organism can also be determined using approaches developed in bioenergetics-based ecological models such as the scope for growth (SFG) and DEB family of models (Kooijman 2010; Nisbet et al. 2012). These models assess the proportion of the assimilated energy flux invested into different fitness-related functions (including maintenance, production, storage, excretion, and activity), and thus, aerobic scope can be determined as the proportion of the flux devoted to production and activity. Both the SFG and DEB approaches have been successfully used to model and predict growth and reproduction of field populations of fish and bivalves and to assess the effects of environmental forcing variables (including pollutants, temperature, and salinity) on these fitness-related traits.
Whenever the aerobic scope can be determined, this should be a preferred way to assess the stress-tolerance windows; however, it may not be feasible for all species or habitats. In these cases, it may be possible to use a suite of indirect bioenergetic markers (such as depletion of the energy reserves, induction of the general response to stress, onset of hypoxemia and partial anaerobiosis, disturbances of the cellular energy status, and oxidative stress) to infer transitions into the pejus, pessimum, or lethal range of environmental conditions and thus determine the stress-tolerance window.

### Energy reserves

Deposition of energy reserves (e.g., in the form of carbohydrates and lipids) is a common feature of animals that use storage to save excess energy assimilated from the environment and to buffer against fluctuations in availability of food or demand for energy. Deposition of the energy reserves typically has a lower priority in allocation of energy compared with the energetic costs of survival, reproduction, and growth (Kooijman 2010). Thus, reduction or cessation of the deposition of energy reserves can be viewed as an indicator of transition into the pejus range, while depletion of the energy reserves may be a sign of transition into the bioenergetically unsustainable, pessimum conditions. Depletion of the glycogen and lipid reserves in response to environmental stressors (including temperature and salinity stress, pollutants, and their combination) is commonly found in aquatic ectotherms and may jeopardize their reproduction and growth, thereby indicating cost to fitness (Allen and Downing 1986; Encomio and Chu 2000; Smolders et al. 2004; Li et al. 2007; Ivanina et al. 2011; Dickinson et al. 2012). The amount of glycogen or lipid reserves is usually compared with species-specific and life-stage specific levels found in well-fed, unstressed organisms. The reproductive state of an individual must also be taken into account because energy reserves are used to fuel gametogenesis and thus are affected by the gametogenic cycle (March and Reisman 1995; Ahn et al. 2003; Jackson et al. 2004; Mouneyrac et al. 2008; Benomar et al. 2009; Li et al. 2009).

### Metabolic rate depression

Metabolic rate depression is a strategy that extends survival in the pessimum range of environmental conditions; it evolved in organisms from highly...
variable environments such as the intertidal zone, ephemeral bodies of water, deserts, and other habitats with extreme fluctuations of temperature, oxygen, availability of water, and food. Metabolic rate depression involves a strong coordinated suppression of the demand and supply of energy (and thus a decrease in basal maintenance costs) (Guppy and Withers 1999). This considerably extends survival time by conserving energy reserves when the energy intake or the capacity for synthesis of ATP are limited and can alleviate the necessity of the compensatory onset of anaerobiosis (Guppy and Withers 1999; Sokolova et al. 2011). However, because metabolic rate depression involves suppression production, and consumption of ATP, there is no net increase in aerobic scope, and the organism’s activity decreases while its growth and reproduction cease (Storey and Storey 1990; Sokolova et al. 2000; Sokolova and Pörtner 2001; Marshall et al. 2011). Therefore, an onset of metabolic rate depression can serve as a marker of a transition into the pessimum range that incurs long-term fitness costs and is incompatible with the indefinite survival of the population (Table 1).

Global suppression of cellular protein synthesis is another marker associated with transition into the pessimum environmental range and a hallmark of metabolic rate depression (Hochachka et al. 1996; Kwast and Hand 1996). It can be triggered by exposure to extreme hypoxia, anoxia, freezing, acute exposure to pollutants, and desiccation stress and involves a concerted suppression of mRNA transcription, maturation, and translation processes (Kwast and Hand 1996; Larade and Storey 2007). This down-regulation of protein synthesis is an important energy-saving mechanism but inevitably leads to cessation of growth and reproduction. Notably, despite the overall reduction of the rate of transcription and translation, specific proteins involved in protection from stress (including molecular chaperones, antioxidants, pro-apoptotic proteins, and anti-apoptotic proteins) may be significantly upregulated, thereby supporting essential cellular functions during metabolic depression (Hermes-Lima et al. 1998; Larade and Storey 2002; Teodoro and O’Farrell 2003).

**Partial anaerobiosis**

Onset of hypoxemia and partial anaerobiosis of tissues is considered a hallmark of transition into the bioenergetically unsustainable pessimum range in aquatic ectotherms and was most extensively studied in the context of tolerance to temperature (Pörtner 2012). In aquatic ectotherms, the upper limit of thermotolerance is set by the inability of the ventilatory and circulatory systems to deliver sufficient oxygen to cover the tissues’ demand for energy at high temperatures. As a result, a progressive mismatch between the oxygen consumption of tissues and the delivery of oxygen develops at, and above, the critical temperatures leading to tissue hypoxemia and the onset of partial anaerobiosis, and it heralds transition into the pessimum range. This mechanism was shown in aquatic ectotherms, including fish, crustaceans, polychaetes, and mollusks (Sommer et al. 1997; Pörtner et al. 1999; van Dijk et al. 1999; Frederick and Pörtner 2000; Peck et al. 2002; Sokolova and Pörtner 2003; Melzner et al. 2006; Pörtner and Knust 2007; Schröer et al. 2009) but may be less important in air–breathing ectotherms for which high oxygen content of the air alleviates the onset of hypoxemia (Sokolova and Pörtner 2001; Klok et al. 2004; Stevens et al. 2010; Kutschke et al. 2013). The close relationship between thermotolerance and availability of oxygen in ectotherms is supported by the finding that hypoxia reduces the thermotolerance, whereas elevated concentrations of oxygen alleviate thermal stress (Mark et al. 2002; Pörtner 2002b; Pörtner et al. 2006; Richmond et al. 2006; Le Moullac et al. 2007). Temperature and oxygen concentration are the only two environmental variables that have been systematically studied with regard of stress-induced hypoxemia and anaerobiosis in the pessimum range. Other stressors (such as exposure to the trace metal Cd or to elevated CO2 concentrations) can modulate thermal tolerance by constraining aerobic scope and shifting the upper critical temperatures to lower values (Metzger et al. 2007; Sokolova and Lannig 2008; Lannig et al. 2010); however, whether these and other stressors can also induce hypoxemia and partial anaerobiosis in the absence of thermal stress remains to be investigated.

**Energetic status of cells**

Indices of the energetic status of cells (such as adenylate energy charge [AEC] or ADP:ATP ratios) have been proposed as biomarkers of stress in a variety of organisms although their physiological implications for cellular metabolism are debated (Isani et al. 1997; Thebault et al. 2000; Hardie and Hawley 2001). Studies in the context of the OCLTT and energy-limited tolerance of stress indicate that cellular energy status (including concentrations of adenylates, AEC, ADP/ATP ratios, and Gibbs’ free energy of ATP hydrolysis) is tightly regulated so that significant shifts in these parameters are only observed during exposure to severe stress (typically in the
Oxidative stress

Stress-induced metabolic disturbance can result in oxidative stress due to the increased generation of reactive oxygen species or nitrogen species and/or suppression of the antioxidant defenses. Oxidative stress can be induced by a variety of environmental factors including temperature, hypoxia, and pollutants (Kelly et al. 1998; Livingstone 2001; Abele and Puntarulo 2004). During exposures to moderate stress in the pejus range, antioxidants and other mechanisms of cellular protection are overexpressed, thereby maintaining the redox balance, so that oxidative stress is either not observed or occurs transiently (Abele and Puntarulo 2004; Storey and Wu 2013). The onset of oxidative stress is typically associated with severe stress and with a transition into the pessimum range of environmental conditions (Abele and Puntarulo 2004; Kurochkin et al. 2009; Sokolova et al. 2011; Storey and Wu 2013). It is worth noting that interpretation of the markers of oxidative stress may be complicated due to high species-specific variability and the effects of diet and physiological status (Hörak and Cohen 2010), thereby making them less dependable as markers of the pessimum range.

Other potential markers

Molecular chaperones (such as heat-shock proteins [HSPs]) have been proposed to mark transition into the pejus range (Pörtner 2012). HSPs are constitutively expressed in the cell where they facilitate correct folding of proteins and stabilize proteins and membranes and are induced by a broad variety of stressors including extreme temperatures, exposure to pollutants and toxins, salinity stress, UV light, and hypoxia (Nagao et al. 1990; Sanders 1993). Synthesis and the chaperoning action of HSPs are ATP-demanding, and HSPs account for up to 10% of the total proteins under control conditions, and for an even larger fraction during stress (Kueltz 2003). Thus, elevated expression of HSPs poses a considerable energetic expense and is associated with reduced aerobic scope and fitness costs in the pejus range (Hoffmann and Somero 1995; Anestis et al. 2007; Petes et al. 2008). However, although the onset of HSP response occurs during the transition into the pejus range, elevated expression of HSPs also are often maintained in the pessimum and lethal ranges; this caveat must be taken into account when using HSP expression as a bioenergetic marker for the pejus range of environmental conditions.

Recent studies also indicate that protein kinases involved in sensing of cellular energy and in signaling of stress may serve as potential markers of transitions from the optimum to energy-stressed and energy-deficient (non-sustainable) states. Thus, AMP-activated protein kinase (AMPK) was shown to be a sensitive indicator of transition into the pejus range during heat stress in crustaceans (Frederich et al. 2009). Cyclic AMP-activated protein kinase (protein kinase A [PKA]) is a marker associated with transition to metabolic arrest (and thus in the pessimum range) in many species; however, response to PKA is species-dependent, and PKA is activated during metabolic arrest in some species and suppressed in others (MacDonald and Storey 1999; Ramnanan et al. 2010). Given an important role of reversible protein phosphorylation in coordination of cellular metabolism, protein kinases are likely to play a key role in stress-induced metabolic shifts and future studies should reveal their full potential as markers of ecologically-relevant physiological transitions.

Conclusions and perspectives

The concept of energy-limited tolerance to stress provides a useful tool for assessing the effects of multiple stressors under environmentally realistic scenarios in aquatic ectotherms and potentially in other organisms. This approach focuses on the bioenergetic consequences of exposures to stress that arise from the inevitable trade-offs between allocation of energy to survival, stress-tolerance, and other fitness-related functions. It uses energy balance as a common denominator to determine the combined effects of multiple stressors and their consequences for fitness. This makes this approach applicable to a wide variety of environmental variables that have direct or indirect impacts on energy metabolism. Bioenergetic markers proposed in this review can be used to assess ecologically important transitions into the pejus range (where the long-term survival of the population is possible at the cost of reduced production) and the pessimum range (which coincides with the tolerance limits of the population and defines the distributional range of the species in the face of multiple stressors). These markers will certainly be complemented and refined as more studies on the metabolic effects of environmental stressors become available. A potentially fruitful avenue for such studies that has not been fully explored in
Bioenergetics in stress tolerance

aquatic ectotherms is the study of cellular signaling cascades involved in metabolic regulation and response to stress. Bioenergetic framework may also be useful for understanding the relationships between aging, longevity, and tolerance to stress, which may be directly linked to aerobic scope in energy-limited natural populations (Parsons 2003, 2007). Direct effects of bioenergetic shifts on fitness provide a suitable framework for fusing physiology, functional ecology, and evolutionary biology and to improve our understanding of the driving forces and the constraints of environmental adaptations (including but not limited to, scenarios of exposures to multiple stresses common in nature). Focus on the bioenergetic sustainability of populations also can assist in assessment of ecological risk and of conservation practices by identifying the habitats that are capable of supporting viable populations of ecologically or economically important, or otherwise protected, species.

Acknowledgments
I thank Anne Todgham and Jonathon Stillman for an invitation to present this work at a special symposium (2013 Annual Meeting of the Society for Integrative and Comparative Biology) that provided fruitful discussions for this manuscript. The Society for Integrative and Comparative Biology and the National Science Foundation sponsored this symposium and funded my participation.

Funding
Supported by the National Science Foundation (IOS-0951079 and IBN-0347238).

References


Hofmann GE, Somero GN. 1995. Evidence for protein


