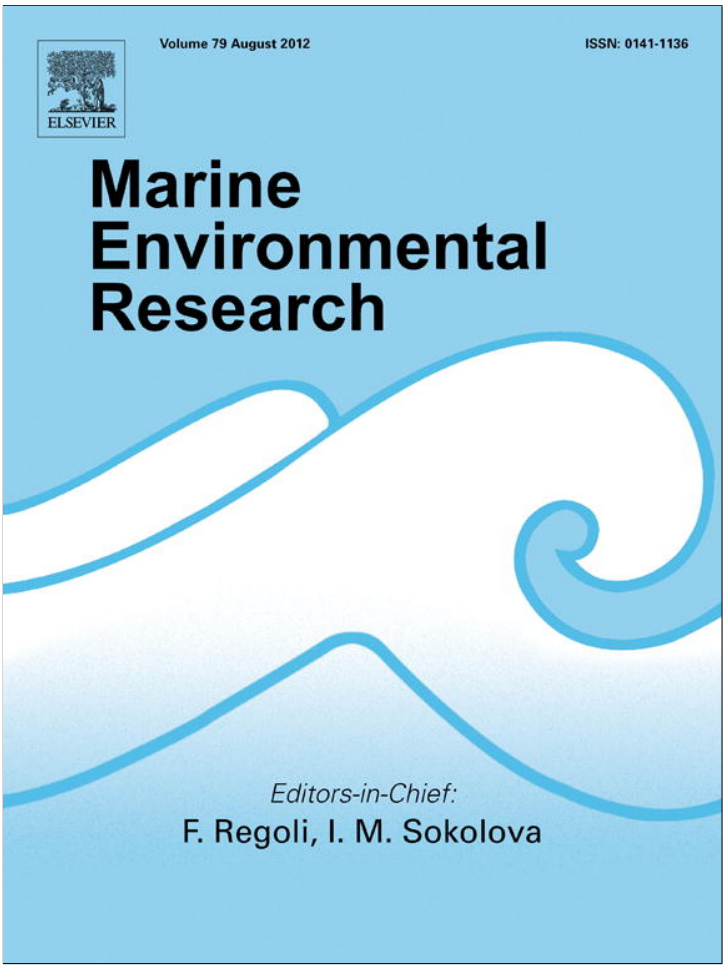

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Energy homeostasis as an integrative tool for assessing limits of environmental stress tolerance in aquatic invertebrates

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ABSTRACT

Energy balance is a fundamental requirement of stress adaptation and tolerance. We explore the links between metabolism, energy balance and stress tolerance using aquatic invertebrates as an example and demonstrate that using key parameters of energy balance (aerobic scope for growth, reproduction and activity; tissue energy status; metabolic rate depression; and compensatory onset of anaerobiosis) can assist in integrating the effects of multiple stressors and their interactions and in predicting the whole-organism and population-level consequences of environmental stress. We argue that limitations of both the amount of available energy and the rates of its acquisition and metabolic conversions result in trade-offs between basal maintenance of a stressed organism and energy costs of fitness-related functions such as reproduction, development and growth and can set limit to the tolerance of a broad range of environmental stressors. The degree of stress-induced disturbance of energy balance delineates transition from moderate stress compatible with population persistence (pejus range) to extreme stress where only time-limited existence is possible (pessimum range). It also determines the predominant adaptive strategy of metabolic responses (energy compensation vs. conservation) that allows an organism to survive the disturbance. We propose that energy-related biomarkers can be used to determine the conditions when these metabolic transitions occur and thus predict ecological consequences of stress exposures. Bioenergetic considerations can also provide common denominator for integrating stress responses and predicting tolerance limits under the environmentally realistic scenarios when multiple and often variable stressors act simultaneously on an organism. Determination of bioenergetic sustainability at the organism's level (or lack thereof) has practical implications. It can help identify the habitats and/or conditions where a population can survive (even if at the cost of reduced reproduction and growth) and those that are incapable of supporting viable populations. Such an approach will assist in explaining and predicting the species' distribution limits in the face of the environmental change and informing the conservation efforts and resource management practices.

1. Introduction

Energy metabolism plays a central role in organism's survival and function, as well as in stress adaptation and tolerance. The amount of available energy, the rate at which it can be gained and metabolically transformed as well as the capacity to store it are inevitably limited in any organism. Therefore, regulation of energy expenditure and its allocation to different functions are fundamental to the organism's fitness. Environmental stress can strongly affect the energy balance of an organism due to the additional energy needed to recover and maintain homeostasis that can put strains on the systems involved in energy acquisition, conversion and conservation. The potential energy cost of stress response and homeostatic regulation against the environmental disturbances have been discussed in several excellent reviews (Calow, 1983, 1989; 1991; Calow and Forbes, 1998; Van Straalen and Hoffmann, 2000). However, up until recently, few experimental studies have explicitly tested the energy cost of stress response and its role in stress tolerance to validate the theoretical framework proposed in these reviews. The past few decades saw a steady growth in the number of studies focusing on metabolic responses to stress. An important common theme emerging from these studies points

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towards energy balance as a key factor that determines stress tolerance limits of an organism and can directly translate into population- and ecosystem-level consequences. Recently, quantitative bioenergetic models [such as the family of the dynamic energy budget (DEB) models proposed by Kooijman (Kooijman, 2010)] have been used to link whole-organism bioenergetics to population growth in a variety of organisms and to incorporate environmental forcing variables such as toxins, food availability and temperature stress (van Haren et al., 1994; Pouvreau et al., 2006; Kooijman et al., 2009; Einarsson et al., 2011). These models are extremely useful in predicting the ecological and population-level consequences of bioenergetic shifts. However, they require extensive parameterization that is time- and effort-consuming and feasible only for a few well-studied species (Van Haren and Kooijman, 1993; van Haren et al., 1994; Bacher and Gangnery, 2006; Kooijman et al., 2009; Sarà et al., 2012). Given enhanced anthropogenic pressure on wild populations, it becomes increasingly important to identify approaches to rapidly assess the degree of stress experienced by the population, to integrate the effects of multiple stressors and predict its likely outcome for the population persistence. In this review, we propose incorporating the physiological models of oxygen- and capacity-limited thermal tolerance (OCLTT) (Pörtner, 2002, 2010) with the fundamental tenets of energy allocation and trade-offs developed in the DEB models (Kooijman, 2010) in a conceptual framework that can be used to integrate the physiological effects of multiple stressors at the organism level and link them to the long-term, population-level consequences. We also argue that the bioenergetic framework provides a common ground for comparison of physiological responses to different stressors regardless of their nature and helps distinguishing between the moderate environmental stress compatible with the long-term population persistence and the extreme stress that can lead to population decline and extinction. Using case studies of aquatic invertebrates, we aim to demonstrate the utility of the proposed bioenergetic framework for determining the limits of stress tolerance and understanding the mechanisms setting these limits, to identify metabolic biomarkers that mark ecologically important physiological transitions during stress exposure and point to the gaps in our knowledge in the hope to stimulate further research on bioenergetic underpinnings of stress physiology and ecology of aquatic ectotherms.

2. Basics of energy balance in animals

Living objects can be defined as non-equilibrium, thermodynamically open systems relying on the external energy sources and constant energy flow. In its simplest form, the net energy exchange in an organism can be described by a balance Eq. (1) (Winberg, 1960):

$$C = P + R + U + F \quad (1)$$

where C is consumption (energy acquired through food ingestion); P (production) is the energy incorporated into e.g. somatic and gonad tissue growth, storage tissues, gamete production, and exoskeleton and mucus deposition; R (respiration) is the basal maintenance cost including metabolic costs of growth, development and reproduction and is equivalent to total metabolic heat losses; U is energy excreted with the products of protein metabolism (ammonium, urea, etc.); F is part of the energy of ingested food which is not assimilated and excreted with feces. Importantly, the net energy exchange and energy balance of an organism depends not only on the amount of the available food, but also on the rates of its incorporation and metabolic conversion that channel the energy flux to different processes. Thus, all terms in the Eq. (1) include a time component (i.e. are calculated per unit time). The DEB models (Kooijman, 2010) represent further development of these ideas and can be broadly applied to assess the bioenergetic consequences of homeostasis and stress response [e.g. (Hall et al., 2007; Jager et al., 2010) and references therein]. The standard DEB model assumes that energy and matter assimilated from food is first directed into the reserve pool and distributed throughout the organism (Kooijman, 2010). The utilization of this energy occurs through its allocation into two main sinks - building and maintenance of somatic structures (including somatic maintenance and growth, as well as activity) and reproduction (metabolic needs for development, maturation and production of gametes). The fixed fraction of energy/matter (κ) is allocated in somatic needs, while the rest of the flux is directed to reproduction (so-called κ-rule).

In order to survive and to maximize Darwinian fitness, the organism must be able to balance its energy gains from the environment against its metabolic losses and to ensure an optimal allocation of surplus energy to somatic growth and to reproduction (Kozłowski, 1992; Perrin and Sibly, 1993). Importantly for our subsequent discussion, the components of an organism's energy budget are functionally linked together, so that changes in any of the processes have consequences for one or more of the others. This functional linkage is provided by two major constraints that require allocation of available energy (in terms of calories) and metabolic power: 1) the limitation in energy input including the overall amount of available food and the rate of its assimilation (Barnes, 1974; Barnes and de Villiers, 2000; Beukema and Cadeé, 2001); 2) limitation of the maximum metabolic capacity (e.g. in oxygen supply capacities or mitochondrial efficiency) for conversion of the food and channeling it toward different physiological processes (Guderley and Pörtner, 2010).

3. Metabolic principles of stress response and the role of energy balance in setting stress tolerance limits

Energy balance plays a critical role in environmental stress tolerance and in setting limits for the survival of organisms and their populations under stressful conditions. Metabolic adaptations to environmental stress can involve flexible allocation of energy resources and/or metabolic power as well as a switch between different metabolic processes responsible for energy acquisition and conversion. In the DEB model (Kooijman, 2010), the energy assimilated by an organism is incorporated in a common pool from which it is used for maintenance, activity, growth/development and reproduction (Fig. 1). A certain amount of assimilated energy is incorporated into the energy reserve in the form of lipids, carbohydrates, and/or proteins thus providing for the storage of surplus energy. Although deposition of energy reserves falls under the overall umbrella of growth, the distinction is important in the context of the metabolic responses to environmental stress, because energy reserves play an important role in the rapid provision for elevated energy demands during stress exposure. This involvement of energy reserves in metabolic stress responses may compete with other functions such as provision for high energy flux during reproduction (gametogenesis and spawning) or buffering of the fluctuations in food availability to ensure continuous supply of metabolic energy.

The maintenance costs encompass the energy demand for basal cellular and organismal maintenance to fuel key cellular processes (e.g. ion and acid–base regulation, protein turnover, anabolism) and essential systemic activities such as ventilation, circulation and excretion (Figs. 1 and 3). Somatic maintenance is the major component of the energy budget and unlike activity, growth and reproduction cannot be reduced below a certain limit. These maintenance costs can be measured as standard or basal metabolic

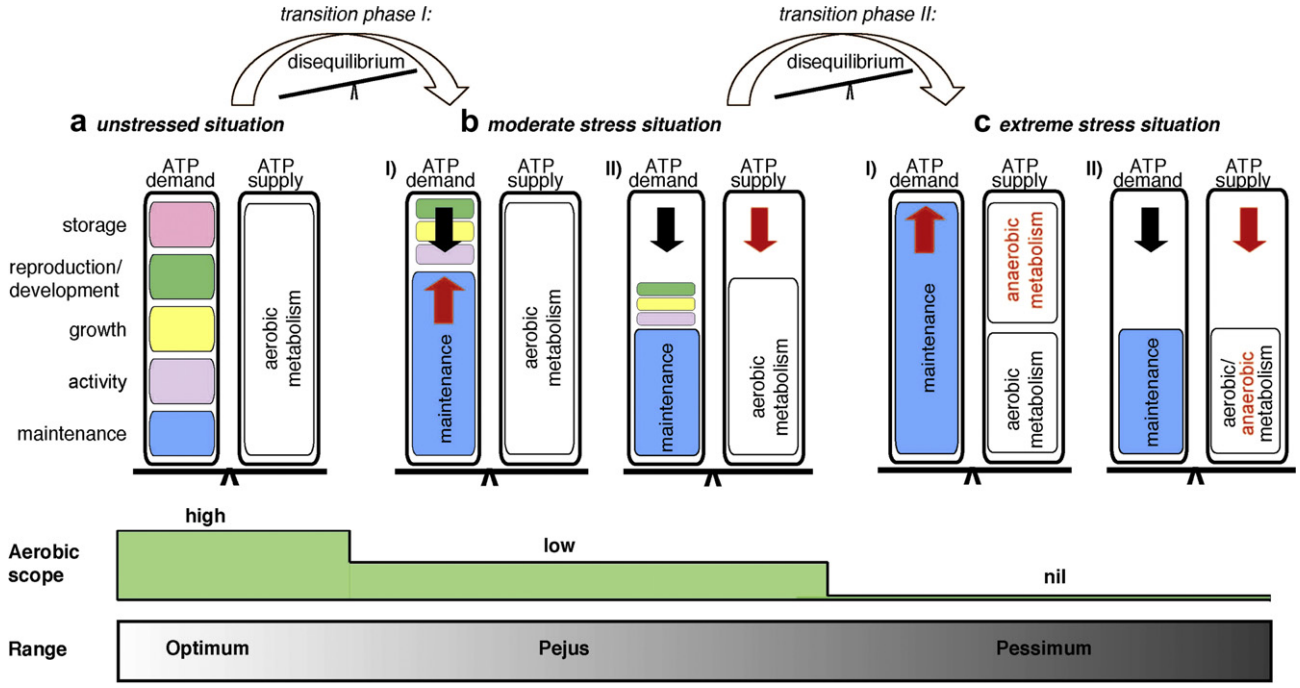

Fig. 1. Bioenergetic framework for assessing the stress impacts on organism's physiology based on the integration of the dynamic energy budget (DEB) and oxygen- and capacity-limited thermal tolerance concept (OCLTT). Red arrows indicate the impact on and the direction of stress-induced changes in ATP demand or ATP supply. Black arrows indicate the direction of the resulting trade-offs. a) Under the normal conditions (optimum range), ATP supply via aerobic metabolism is sufficiently high to cover the maintenance costs as well as activity, growth, reproduction/development and their respective energy costs. It is worth noting that the size of the boxes corresponding to different energy-demanding processes are made equal for the sake of clarity and do not reflect the actual energy allocation. The excess of energy (if emerges) can be deposited in storage compounds/tissues. These reserves are used for energy provision during times of elevated energy demand (e.g. reproduction) or temporary reduction in food availability. Aerobic scope is high and the metabolic strategy involves normal trade-offs in energy allocation between these processes (whereby maintenance costs take priority) and ensures maximum fitness of the organism. b) During moderate stress (pejus range), either the maintenance costs increase in order to cover additional energy demands for stress protection and damage repair (bI), or metabolism and/or food assimilation is impaired by the stressor (bII). As a result, the aerobic scope declines. There is a mismatch energy demand and supply during the onset of moderate stress (transition phase I), and later during acclimation/acclimatization the energy balance is reinstated via energy trade-offs to cover maintenance costs and ensure survival at the expense of other processes such as growth and reproduction. Energy accumulated in storage tissues is used up to fuel these essential processes. c) During extreme stress situations (pessimum range), the progressive rise in ATP demand for maintenance (cI) or the progressive impairment of aerobic metabolism (cII) overrides ATP supply via aerobic metabolism (transition phase II). The aerobic scope disappears and metabolism switches to partial anaerobiosis to compensate for the insufficient aerobic energy supply to fuel the essential maintenance costs and to allow for short-term survival of the organism. Eventually, metabolic rate depression sets in to ensure energy balance at reduced energy turnover rates; however, this balance is maintained at the expense of shutting down of many ATP-demanding functions to ensure immediate survival. This represents a time-limited situation and does not permit for a long-term population survival because of the lack of energy to invest in growth, reproduction and activity. However, metabolic depression can extend survival time of an organism from several days up to several months and even years. In the lethal range of environmental stressors (not shown) the balance of ATP supply and demand is disrupted resulting in the ultimate death of an organism. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

rate (SMR or BMR) (Guderley and Pörtner, 2010; Kooijman, 2010). Typically, allocation of energy and metabolic power to maintenance has priority over growth, storage or reproduction, so that these processes cease if the available energy flux is only sufficient to support the basal maintenance costs (Wieser et al., 1988; Rombough, 1994; Kooijman, 2010).

The aerobic scope – the proportion of the energy flux (and the corresponding amount of metabolic power used to support this flux) that is left after the basal maintenance costs of an organism are met – provides a useful measure of the energy balance (Fry, 1971). It reflects the surplus energy flux that can be invested into somatic and gametes production and other fitness-related functions of an organism (Fig. 1). According to the OCLTT concept (Pörtner, 2002, 2010) the ability (or lack thereof) to sustain aerobic scope emerges as a major criterion that distinguishes between moderate and extreme stress. In an energy-based stress classification, the overall scope of the values of an environmental factor experienced by an organism is divided into several ecologically and physiologically relevant ranges (Pörtner and Farrell, 2008): 1) optimal range where the energy balance is positive and the maximum aerobic scope is available for activity, growth, development, reproduction and storage; 2) pejus range (from Latin "worse") where the aerobic scope is still positive but diminished compared to the optimal range due to the elevated basal metabolic costs and/or impaired aerobic capacity; 3) pessimum range (from Latin "the worst") where aerobic scope disappears and anaerobic metabolism is engaged to partially cover the energy costs of basal maintenance; 4) lethal range where the energy homeostasis is disrupted and a short-term survival depends on "emergency" stress protection such as molecular chaperones and antioxidants (Figs. 1 and 2). Transition from the pejus into the pessimum range can be determined by a partial onset of anaerobic metabolism at the critical threshold value for a given stressor, and transition into the lethal range is typically marked by a negative energy balance indicated by a disturbance of the cellular energy status (Fig. 2). This energy-based classification of environmental stressors allows comparing the effects of various unrelated stressors and focuses on those physiological effects that are linked to fitness and thus can directly translate into the population-level consequences.

The position and breadth of the stress tolerance windows (encompassing the optimum and pejus ranges on Fig. 2) are flexible within the limits and can be shifted by adaptation, acclimation or acclimatization. Metabolic reorganisation induced by acclimatization, acclimation or adaptation involves adjustments in

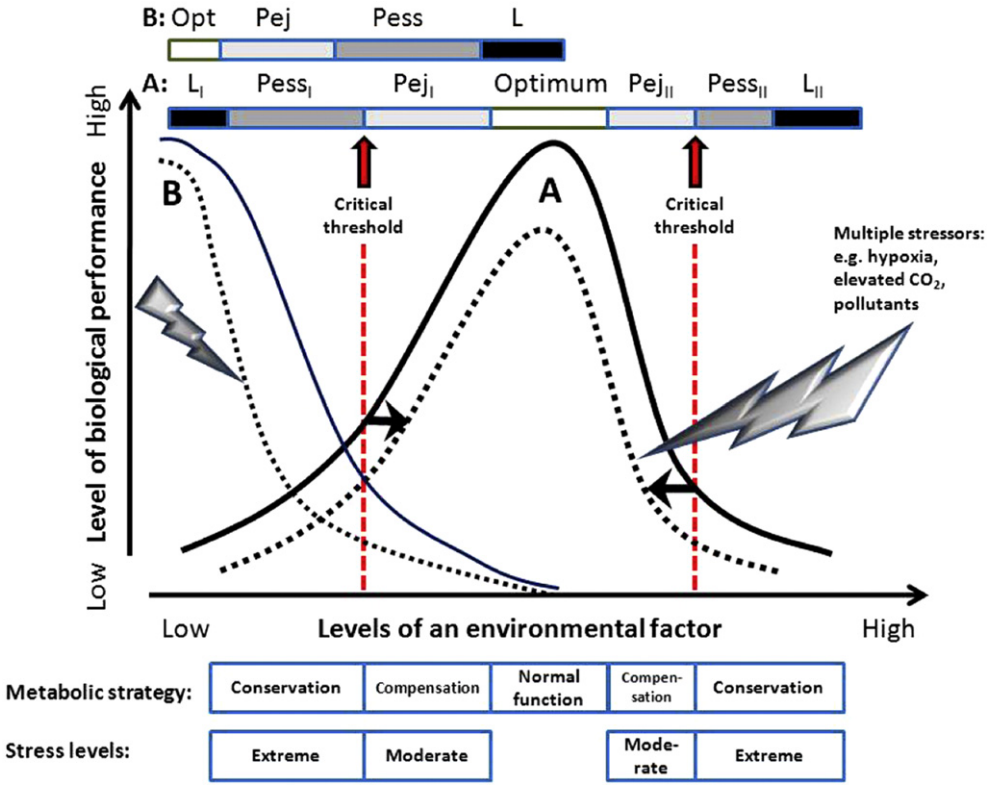

Fig. 2. The concept of energy-limiting stress tolerance limits and classification of environmental stressors based on their effects of energy balance. Modified after Pörtner and Farrell (2008). Solid lines refer to a single environmental factor/stressor situation, and dotted lines refer to a combined exposure to multiple stressors (e.g. hypoxia, hypercapnia or pollution) that can negatively affect the aerobic scope and thus narrow the tolerance window for another environmental factor/stressor (e.g. temperature). The level of the biological performance of an organism is proportional to the available aerobic scope and diminishes as an organism transits from the optimum to pejus, pessimum and then lethal range. The depicted curves show schematic representation of a generalized stressor, and the shape and the symmetry of the actual curves will depend on the nature of the stressor. Thus, for some stressors (such as temperature, salinity or levels of essential metals) the relationship between the level of the stressor and the organism's performance follows a bell-shaped curve (similar to curve A on Fig. 2), which may be symmetrical or skewed. As a result, there are lower and upper pejus (Pej_I and Pej_{II}), pessimum ($Pess_I$ and $Pess_{II}$) and lethal (L_I and L_{II}) ranges for these factors (A: bar shown above the curves). For other stressors (such as pollutants, toxins or UV irradiation) the optimum lays near zero level of the stressor such as shown by curve B. For these stressors, there are only the upper pejus, pessumum and lethal ranges (Pej, Pess and L, respectively) (B: bar shown above the curves). The critical thresholds indicating the transition from moderate to extreme stress range correspond to the transition from the pejus to pessimum ranges; for the sake of clarity these thresholds are shown for curve A only. The shift in the critical thresholds of tolerance induced by multiple stressors is indicated by black horizontal arrows. Ranges depicting different metabolic strategies (conservation vs. compensation) and stress levels (moderate vs. extreme) refer to the single-stressor situation shown by the solid line (curve A). This conceptual framework is theoretically applicable to any stressor that negatively affects the aerobic scope of an organism and has been experimentally tested for temperature, oxygen levels, pollution and their combinations; additional studies on other stressors are needed to further test the generality of this conceptual framework.

mitochondria, enzymes, ion and gas transport capacities as well as membrane composition (Hazel, 1995; Willmer et al., 2000; Hochachka and Somero, 2002). Thus, seasonal acclimatization or laboratory acclimation to different temperatures shifts the thermal tolerance windows in invertebrates and fish due to the readjustment of energy metabolism (Sommer et al., 1997; van Dijk et al., 1999; Pörtner, 2002; Sommer and Pörtner, 2002; Sokolova and Pörtner, 2003; Wittmann et al., 2008; Schröer et al., 2009). Similarly, long-term acclimation to changing salinity in aquatic invertebrates and fish often involves metabolic readjustments to reduce SMR and/or preserve the aerobic scope except when salinity change is extreme (Shumway and Koehn, 1982; Nelson et al., 1996; Sangiao-Alvarellos et al., 2005; Kidder et al., 2006). Evolutionary adaptation to different thermal or salinity regimes can also shift the respective tolerance windows (review in: Kinne, 1967, 1971b; Berger and Kharazova, 1997; Pörtner, 2002, 2010). As a result of these adjustments, the position of optimum, pejus, and pessimum ranges (Fig. 2) can shift (within the constraints of species' physiology) to improve the aerobic scope and the net energy available for fitness-related functions under the prevailing environmental conditions (Beiras et al., 1995; Pörtner, 2002, 2010). In some cases (such as shown in an intertidal crustacean), adaptation can enhance the thermal tolerance by expanding the optimum thermal range and shrinking the pejus range so that an organism transitions directly from optimum to pessimum (Jost et al., 2012). Notably, evolutionary adaptations to elevated pollutant levels have been reported to shift the lethal tolerance limits in invertebrates and plants (Klerks and Weis, 1987; Klerks and Bartholomew, 1991; Shaw, 1994; Levinton et al., 2003; Xie and Klerks, 2004); however, the effects of these adaptations on the optimum, pejus and pessimum ranges (as defined on Fig. 2) have not been tested and require further investigation.

The position and breadth of the tolerance windows can also vary with the life stage and/or size of an organism. Thus, developing larvae commonly have elevated sensitivity to environmental stressors such as temperature, salinity, ocean acidification and chemical pollutants (Woltering, 1984; Giesy and Graney, 1989; Hamdoun and Epel, 2007; Kurihara, 2008; Kurihara et al., 2008; Dupont and Thorndyke, 2009; Ross et al., 2011). The temperature range compatible with the normal growth and development can also be narrower for embryos than juveniles and adults (O'Connor and Heasman, 1998; Drent, 2002; O'Connor and Lawler, 2004). The reasons of high stress sensitivity of early developmental stages are currently debated and likely involve the competing demands of

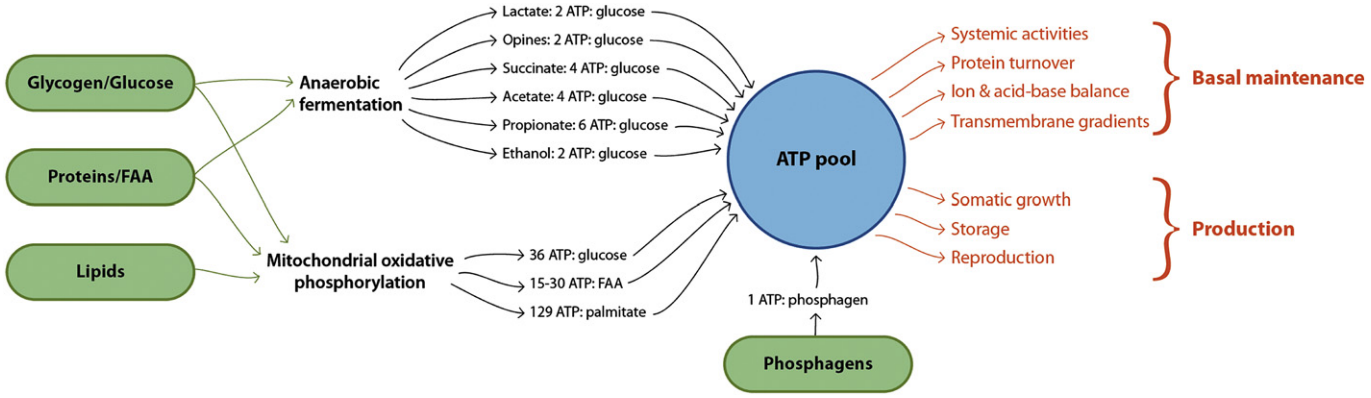

Fig. 3. A summary of the major ATP-producing and ATP-consuming pathways in aquatic invertebrates. Carbohydrates (glycogen and glucose), and free amino acids (FAA) from proteins and/or intracellular FAA pools can be oxidized aerobically or anaerobically, while lipids are predominantly catabolized in aerobic pathways. Aerobic catabolism of glucose, FAA or lipids yields CO_2 and water as end products while anaerobic end products can vary depending on the species and/or environmental conditions. ATP yields for anaerobic fermentation depend on the pathway and vary from 2 to 6 moles ATP $mole^{-1}$ glucose. ATP yields (mole ATP $mole^{-1}$ glucose) are given for each anaerobic end product. Note that succinate and propionate can also be generated by anaerobic fermentation of amino acids (aspartate or glutamate) yielding 1 or 2 moles ATP $mole^{-1}$ FAA (for succinate and propionate, respectively). Aerobic ATP yields are significantly higher than even the most efficient anaerobic pathway ranging from 15 to 129 moles ATP $mole^{-1}$ substrate. Note that under the normal circumstances, aerobic metabolism is mostly fueled by lipids and carbohydrates, while proteins and FAA are used only during extreme energy deficiency (e.g. starvation). ATP pool can also be buffered by phosphagens (such as phospho-L-arginine, phosphocreatine or phospholumbricine) that generate ATP in a one-step substrate-level phosphorylation reaction catalyzed by phosphagen kinases; this may occur under aerobic or anaerobic conditions. ATP generated aerobically or anaerobically is used to support basal maintenance functions including systemic activities (e.g. ventilation, circulation) and cellular protein turnover, ion and acid–base balance and maintenance of electrochemical ion gradients across the cell membrane and intracellular membranes (e.g. mitochondrial, lysosomal or endoplasmic reticulum), and can also be invested in production including reproduction, growth and deposition of energy stores.

developmental, proliferative and stress protection processes for the limited energy supply (Hamdoun and Epel, 2007). Elevated energy demands for reproduction and spawning can also reduce tolerance of sexually mature adults to temperature stress, pollution, parasites and pathogens compared with juveniles or non-reproducing adults (Li et al., 2007, 2009a,b,c; Song et al., 2007; Petes et al., 2008).

In the following chapters, we demonstrate that changes of energy balance are a common denominator of response to a variety of environmental stressors, and show how bioenergetic framework can be used to effectively integrate the effects of multiple stressors in environmentally realistic contexts using examples of marine ectotherms. Marine ectotherms have proven excellent models for studies of stress-induced bioenergetic shifts under the premises of both DEB and OCLTT models. They are especially prone to energy limitations during stress exposures due to the lower O_2 content and slower diffusion of O_2 in water than in air. We also review the metabolic strategies and corresponding bioenergetic markers associated with two important physiological transitions that are critical from the ecological standpoint: 1) a transition from the optimum into the pejus range that is indicative of the stress-induced fitness costs but is compatible with the long-term survival of individuals and potential persistence of their populations; 2) a transition from the pejus into the pessimum range where the long-term survival of an individual is impossible (unless the conditions change to the optimum or at least pejus range) and thus the populations become bioenergetically non-sustainable. This can provide the framework for future integrative studies of the physiological mechanisms setting limits of stress tolerance in marine organisms and identify the suite of metabolic markers that can serve as a rapid assessment tool for environmental stress and/or ecological sustainability of the wild and aquaculture populations of marine organisms.

4. The role of the aerobic scope and energy balance in stress tolerance of marine ectotherms

The role of the aerobic scope and energy balance in stress tolerance has been experimentally tested for several important environmental factors including temperature, oxygen levels, pollution and their combinations. Below we provide a brief overview of these studies as well as studies that demonstrate metabolic shifts induced by other stressors (that such as salinity and ocean acidification) and suggest that these stressors can also reduce the general stress tolerance of marine organisms via the negative effects on the aerobic scope.

4.1. Temperature stress

The role of diminishing aerobic scope in setting the thermal tolerance limits is well documented in aquatic ectotherms and has been a subject of several excellent reviews (Pörtner, 2001, 2002; Pörtner and Farrell, 2008). In water-breathing ectotherms such as fish, crustaceans, annelids and mollusks, a strong deviation of the environmental temperature from the optimum results in a disappearance of the aerobic scope and onset of partial anaerobiosis in the pessimum temperature range (Fig. 2) as indicated by accumulation of anaerobic end products [review in: (Pörtner, 2002, 2010)]. This transition to partial anaerobiosis occurs well before the onset of lethal temperatures but heralds a time-limited situation, where only a temporary survival of a few days to few weeks is possible. Notably, the so called critical temperatures (T_c) at which the transition to partial anaerobiosis occurs (Fig. 2), correlate with the environmental temperatures in the marginal populations of many aquatic ectotherms and can explain the current biogeographic distribution of these organisms as well as its shifts due to the global climate change (Pörtner and Knust, 2007; Pörtner and Farrell, 2008). Transition to partial anaerobiosis at critical temperatures in marine ectotherms is attributed to the limited capacity of oxygen uptake and transport mechanisms rather than to neural and/or mitochondrial dysfunction (Frederich and Pörtner, 2000; Sokolova and Pörtner, 2003; Eliason et al., 2011). However, other factors such as temperature-induced changes in the oxygen-carrying capacities of respiratory pigments (Melzner et al., 2007; Wittmann et al., 2008) or season-dependent differences in mitochondrial properties and density (Hawkins, 1995; Keller et al., 2004; Sommer and Pörtner, 2004) may also contribute to this transition. Notably,

elevated environmental oxygen concentrations (via water hyperoxia and/or air breathing) can alleviate the temperature-induced decrease in the aerobic scope supporting the notion that impaired oxygen delivery via ventilation and circulation are at the root of the temperature-induced transition to the partial anaerobiosis in aquatic invertebrates (Sokolova and Pörtner, 2001; Pörtner et al., 2006).

4.2. Oxygen deficiency

Oxygen levels are directly related to the aerobic scope of an organism because oxygen is required for ATP production via oxidative phosphorylation (Fig. 3). Most aquatic invertebrates respond to declining oxygen concentration either as oxygen conformers (i.e. display a direct positive correlation between ambient P_{O_2} and oxygen consumption rates) or partial oxygen regulators (i.e. maintain constant oxygen consumption rates more or less independently of ambient P_{O_2} until a critical P_{O_2} threshold is reached after which they become oxygen conformers) (Newell, 1979; Herreid and Clyde, 1980; Le Moullac et al., 2007). It has been postulated that oxygen levels can directly affect stress tolerance of aquatic ectotherms via the changes in the aerobic scope, with lower aerobic scope (and stress tolerance) in hypoxic compared to normoxic and hyperoxic waters (Pörtner, 2010). Thus, an experimentally induced increase in the aerobic scope due to the elevated oxygen concentrations led to increased thermal tolerance of an Antarctic bivalve *Laternula elliptica* (Pörtner et al., 2006). Elevated aerobic scope due to a seasonal depression of basal metabolism also resulted in a concomitant increase in both hypoxia and temperature tolerance in *L. elliptica* (Morley et al., 2007; Pörtner et al., 2007). Hypoxia/anoxia can also indirectly affect energy metabolism due to an increase in oxidative stress during hypoxia and recovery (Hermes-Lima and Zenteno-Savín, 2002; Abele et al., 2007; Clanton, 2007; Diaz and Rosenberg, 2008) and/or elevated SMR (so called "oxygen debt") during post-hypoxic/anoxic recovery (Herreid and Clyde, 1980; Ellington, 1983; Vismann and Hagerman, 1996, 2008; Lewis et al., 2007; Kurochkin et al., 2009). However, more studies are needed to determine whether these indirect effects of hypoxia incur large enough energy costs to sensitize aquatic organisms to other stressors in intertidal and coastal habitats that commonly experience intermittent hypoxia.

4.3. Pollution

Estuarine and coastal areas are often the most polluted areas in the ocean due to the urban development and human industrial and agricultural activities. The increase in the toxic stresses in aquatic habitats has a direct effect on fitness of aquatic organisms and survival of their populations as indicated by a decrease in biodiversity in polluted areas (Clark, 1997; Grant and Briggs, 2002; Dolbeth et al., 2007). The cellular mechanisms of toxicity and detoxification have been extensively studied for a broad variety of pollutants and reviewed previously (Malins and Ostrander, 1994; Smital and Kurelec, 1998; Walker et al., 2001; Zalups and Koropatnick, 2010). The bioenergetic aspect of toxicity has not been well explored for most aquatic pollutants with the notable exception of trace metals. Recent studies indicate that exposure to toxic metals can diminish the aerobic scope of aquatic invertebrates and disrupt metabolic functions and energy balance in these organisms (Sokolova and Lannig, 2008). Metal exposures can result in elevated basal metabolic demand due to the costs of upregulation of cellular protective mechanisms such as metallothioneins, glutathione, molecular chaperones, antioxidants and/or cellular repair pathways (Calow, 1989, 1991; Cherkasov et al., 2006; Ivanina et al., 2008; Ivanina and Sokolova, 2008; Sokolova and Lannig, 2008). This increase in basal metabolism can in turn lead to the reduced scope for activity, reproduction and growth due to the energy trade-offs between basal maintenance and other energy-requiring functions. Moreover, metal exposures have a direct effect on the cellular bioenergetics interfering with the ATP-producing pathways. Thus, toxic metals such as cadmium, copper, zinc and mercury have negative effects on mitochondrial function and capacity for aerobic ATP production resulting in reduced mitochondrial efficiency and coupling, elevated proton leak and in severe cases, depolarization (Stohs and Bagchi, 1995; Li et al., 2003; Sokolova, 2004; Sokolova et al., 2005; Kurochkin et al., 2011). Recent studies showed that some metals such as cadmium can also negatively affect anaerobic metabolism in a marine bivalve *Crassostrea virginica* (Eastern oyster) (Ivanina et al., 2010) suggesting that this metal can be a potent metabolic toxicant with global effects on all aspects of cellular bioenergetics. Reduction in the aerobic scope induced by exposure to toxic metals can narrow the thermal tolerance limit in aquatic ectotherms shifting the upper critical temperature to lower values (Sokolova and Lannig, 2008). Interestingly, the mechanisms of this metal-induced reduction in thermal tolerance appear to differ depending on the metal concentrations. During exposure to low sub-lethal metal concentrations, the reduction of the aerobic scope is mostly due to the metal-induced elevation of the basal metabolic costs (presumably for detoxification and damage repair) whereas during acute exposures to high metal levels toxic effects on mitochondria and cellular aerobic capacity dominate (Sokolova and Lannig, 2008). Regardless of the exact physiological and molecular mechanisms, the metal-induced reduction of the aerobic scope has negative effects on the temperature stress tolerance in aquatic ectotherms (Sokolova and Lannig, 2008), and future studies are needed to demonstrate whether this can also be extended to the tolerance of other environmental stressors such as oxygen deficiency, salinity or hypercapnia.

4.4. Salinity

Effects of salinity on energy metabolism of aquatic invertebrates have been extensively studied during the second part of the 20th century (Kinne, 1971a; Lange et al., 1972; Shumway and Koehn, 1982; Prosser, 1991; Berger and Kharazova, 1997). Salinity typically has mild effects on the oxygen demand and bioenergetics of aquatic invertebrates within the environmentally relevant range of salinities suggesting low energy costs of osmoregulation in fully acclimated organisms. An important exception occurs in some extremely variable environments such estuaries and intertidal zones that experience large and rapid salinity fluctuations. A common adaptive strategy in invertebrates from these environments includes breakdown of cellular proteins to increase the concentration of free amino acids in the cytosol during the periods of high salinity and excretion of the excess amino acids when salinity drops down in order to prevent cell shrinking and swelling, respectively (Kinne, 1971a; Deaton et al., 1984; Berger and Kharazova, 1997). In variable environments, these rapid cycles of the protein breakdown and amino acid release may deplete the intracellular protein pool, incur considerable energy costs and result in the reduced fitness (Koehn, 1978, 1983; Hilbish et al., 1982; Deaton et al., 1984).

4.5. Ocean acidification

Ocean acidification (OA) driven by the increasing atmospheric CO_2 levels is an emerging concern in marine environments. The CO_2-driven change in seawater pH and carbonate chemistry can potentially lead to complex biological effects in exposed organisms

affecting biomineralization, acid–base balance and energy metabolism (Council, 2010). Current studies indicate that OA may affect energy balance indirectly, due to the elevated energy expenditures for biomineralization and acid–base homeostasis, and directly via negative effects of the reduced extra- and intracellular pH on energy metabolism.

Biomineralization in marine calcifying organisms is a highly biologically regulated and potentially energy-demanding process. Palmer estimated the total cost for protein synthesis at approximately 29 J mg^{-1} in molluscan shells, while the estimated total cost for inorganic shell material was much lower at 1–2 J mg^{-1} $CaCO_3$ (Palmer, 1981, 1983, 1992). However, the latter is a conservative estimate because it does not take into account such energy-dependent aspects of calcification as production of enzymes involved in calcium carbonate deposition, transport of $CaCO_3$ crystals by hemocytes and acid–base regulation at the site of $CaCO_3$ deposition (Digby, 1968; Wheeler, 1992; Mount et al., 2004). Even with this conservative estimate, energetic costs for calcification could account for 75% of the total energy needed for somatic growth and may be up to four times higher than the amount of energy invested in reproduction, as shown in a rocky shore gastropod *Tegula funebralis* (Palmer, 1992). After the shell is deposited, mollusks spend additional energy on its maintenance in order to counteract dissolution and erosion; in some intertidal limpets of the genus *Patella* the annual costs of shell erosion accounted for 8–20% of the total energy invested in production (somatic and shell growth and gonadal output) (Day et al., 2000). Such high-energy expenditure for shell deposition and maintenance makes it likely that energetic trade-offs can also occur between shell, somatic growth and reproduction. Such trade-offs are yet to be directly measured, although there is indirect evidence supporting this notion.

Exposure to elevated CO_2 and reduced seawater pH was shown to inhibit biomineralization rates and increase shell dissolution in marine calcifiers (Gazeau et al., 2007; Hall-Spencer et al., 2008; Kurihara, 2008; Kurihara et al., 2008; McClintock et al., 2009; Ries et al., 2009; Nienhuis et al., 2010). This can result in elevated metabolic costs of shell deposition and maintenance. In juvenile oysters *C. virginica*, elevated P_{CO_2} (~0.36 kPa) induced an increase in oxygen consumption indicating elevated energy costs for basal maintenance (Beniash et al., 2010). In the brittle star *Amphiura filiformis* the OA-induced increase in calcification rates coincided with an increase in respiration rates and was accompanied by a partial resorption of the arm muscles indicating high-energy demand in OA-exposed organisms (Wood et al., 2008). In contrast, OA-exposed cephalopods *Sepia officinalis* that showed significantly higher calcification rates in OA-exposed than in control animals gained soft body mass at a similar rate to the controls with no evidence of tissue resorption (Gutowska et al., 2008). However, incorporation of organic matrix in the calcified structure was reduced in OA-exposed cephalopods (Gutowska et al., 2010) possibly indicating an energetic trade-off due to the high-energy cost of shell protein production. Interestingly, reduced calcification rates were observed during the periods of high energy demand associated with reproduction in mollusks (Fairbridge, 1953; Vahl, 1981a,b; Bricelj et al., 1987, 1992) supporting the notion that shell deposition is an energetically costly function. The relationships between biomineralization rates and energy balance in response to OA are likely to be complex, species-specific and depend on the degree of acidification. Indeed, calcification rates in different species of mollusks, crustaceans and echinoderms were found to increase, decrease with increasing P_{CO_2} or show an optimum curve with the highest calcification rates at intermediate P_{CO_2} levels (Wood et al., 2008; Miller et al., 2009; Ries et al., 2009; Gutowska et al., 2010). The observed variation in response of marine calcifiers to OA may be partially due to the differences in shell mineralogy (specifically, the content of the two isoforms of $CaCO_3$, aragonite and calcite, of which the latter is significantly less soluble) and may also reflect species-specific physiological differences in biomineralization which currently are poorly understood (Fabry, 2008; Doney et al., 2009). The bioenergetic consequences of this variable biomineralization response are also likely to be variable and require further investigation.

Disturbance of ion and acid–base homeostasis in response to OA can also lead to a reduction of aerobic scope in aquatic organisms. The data about the effects of hypercapnia on the energy cost of ion regulation of marine ectotherms are sparse. Recent studies suggest that Na^+/K^+ ATPase, a major cellular ATP consumer (Wieser and Krumschnabel, 2001), may be susceptible to the effects of hypercapnia in aquatic organisms. In the peanut worm, *Sipunculus nudus*, extracellular pH was shown to modulate the energy costs for acid–base regulation inducing a shift to more ATP-efficient ion transporters during hypercapnia that resulted in a reduction of Na^+/K^+ ATPase activity (Reipschläger and Pörtner, 1996; Pörtner and Bock, 2000; Pörtner et al., 2000). A similar shift to more ATP-efficient ion transporters was suggested in OA-exposed oysters due to the observed lowering of extracellular $[Na^+]$ and elevation of $[K^+]$ compared to controls (Lannig et al., 2010). In fish, elevated Na^+/K^+ ATPase activity was found in some species after acclimation to long-term hypercapnia (1–5 kPa P_{CO_2})(Ishimatsu et al., 2005; Deigweiher et al., 2008), while in others Na^+/K^+ ATPase did not change (2 kPa P_{CO_2}) (Seidelin et al., 2001). In cod, Na^+/K^+ ATPase activity in gill cells was not affected at 0.3 kPa P_{CO_2}, but increased at 0.6 kPa P_{CO_2} (Melzner et al., 2009). Further studies are urgently needed to determine the effects of elevated P_{CO_2} on acid–base and ion homeostasis and their consequences for bioenergetics and general stress tolerance in fish and aquatic invertebrates.

5. Metabolic strategies of stress tolerance in pejus vs. pessimum range: compensation vs. conservation

Strategies of metabolic adaptation to environmental stress in aquatic organisms depend both on the nature and the degree of stress (Hochachka and Guppy, 1987; Hochachka and Somero, 2002; Gracey et al., 2008). The concept of the energy-limited stress tolerance allows delineating between moderate and extreme stressors that have principally different consequences for long-term survival of the populations reflecting the degree of disturbance and the long-term sustainability of the energy balance. As a rule, exposure to low and moderate stress (corresponding to the pejus range in Fig. 2) causes metabolic and ATP turnover acceleration that allows to compensate for additional energy expenses for increased physiological activity, cellular maintenance and damage repair. In contrast, extreme stress (indicated by the transition to the pessimum range on Fig. 2) often results in a general slow-down of activity and suppression of metabolic rate. This permits conservation of metabolizable resources and decelerates the unfavorable change in intracellular milieu due to accumulation of metabolic waste. A distinctive characteristic of an extreme stressor from the bioenergetic viewpoint is the lack of the long-term sustainability of the energy balance: an organism temporarily suspends metabolic activity biding its time until the return of more favorable environmental conditions but the long-term survival is impossible. This general rule broadly applies to a wide range of environmental stressors including low-oxygen availability, fluctuations in temperature, pH or salinity, exposure to pollutants and others. Below, we briefly outline the major metabolic events and bioenergetic consequences associated with transitions into the pejus and pessimum range (corresponding to moderate stress compatible with the long-term survival of the individual and extreme stress

where only time-limited survival is possible, respectively) that can be used for determining stress tolerance limits and/or for risk assessment of marine populations in the face of environmental stress.

5.1. Metabolic response to moderate stressors during transition to the pejus range: compensation

Organisms subjected to low levels of environmental stress when the conditions moderately deviate from the optima and fall within the pejus range (Fig. 2) employ numerous cellular, physiological and behavioral mechanisms that reduce negative impact of stress. At the cellular level, moderate stress induces a set of common responses that include the repair of DNA and protein damage, cell cycle arrest or apoptosis, the removal of cellular and molecular debris generated by stress, and an overall transition from a state of cellular growth to one of cellular repair (Kültz, 2005; Kassahn et al., 2009). At the whole-organism level, the compensatory mechanisms may involve escape behavior, acceleration of ventilation and oxygen uptake and stimulation of feeding (Willmer et al., 2000). Overall, the cellular and whole-organism compensatory processes while essential in surviving stress, are energetically costly and may divert energy flux and metabolic power from fitness-related functions such as reproduction and growth towards maintenance and repair (Fig. 1b).

Metabolic compensation during the moderate stress exposure in the pejus range (Fig. 2) often involves elevated protein turnover (and associated increase in the basal maintenance costs) to cover the energy cost of expression of stress proteins and/or activation of the protein degradation pathways. Protein synthesis and turnover are among the most important ATP sinks in the cell (Hochachka and Somero, 2002). One of the common mechanisms of maintaining protein homeostasis during stress exposure is activation and expression of heat shock proteins (HSPs). These proteins possess a chaperoning function folding newly synthesized polypeptide chains and repairing molecular structure or removing damaged protein molecules. Many types of stress, such as exposure to heat and cold, organic pollutants, heavy metals, oxidants, UV light and hypoxia induce HSPs (Sanders, 1993; Clegg et al., 1998; Soti and Csermely, 2000; Buckley et al., 2001; Dahlhoff, 2004; Piano et al., 2004; Anestis et al., 2007, 2008; Gonzalez-Riopedre et al., 2007; Ivanina et al., 2009). Some HSPs can also stabilize lysosomes protecting against spilling of their contents into the cytoplasm and the cell death (Kirkegaard et al., 2010). Severely damaged proteins that cannot be repaired by HSPs are ubiquitin-conjugated and targeted for proteolytic degradation and elimination.

Antioxidants represent another group of functional molecules that are constitutively expressed as well as induced by stressors incurring energy cost for their synthesis and turnover. They include highly conserved enzymatic (e.g., superoxide dismutase, catalase, and enzymes that use glutathione as a reactive oxygen species (ROS) quencher) and non-enzymatic components (e.g., carotenoids, flavonoids, vitamins C, A and E, and glutathione) that scavenge, transform and detoxify ROS thereby mitigating oxidative stress (Halliwell and Gutteridge, 1999). A variety of environmental stressors such as heat stress, UV radiation, metals and xenobiotics induce elevated ROS generation above the normal background levels and thus require energy-costly upregulation of antioxidant defenses (Winston and Giulio, 1991; Valko et al., 2005; Bertin and Averbeck, 2006; Abele et al., 2007; Kakkar and Singh, 2007). Exposure to pollutants also upregulates pollutant-specific biotransformation and detoxification pathways in aquatic invertebrates including multixenobiotic resistance proteins (MXR) involved in ATP-dependent elimination of organic pollutants and some trace metals as Cd and Hg (Broeks et al., 1996; Smital and Kurelec, 1998; Bard, 2000; Callaghan, 2002; Ivanina and Sokolova, 2008; Bošnjak et al., 2009); Phase I and Phase II biotransformation enzymes (Livingstone, 1993; Stegeman and Hahn, 1994); and metal-binding proteins such as metallothioneins (MTs) that are responsible for metal homeostasis and maintenance of the cellular redox status (Stillman, 1995; Palmiter, 1998; Haq et al., 2003).

Chaperoning action of HSPs as well as ubiquitination are ATP-dependent processes which together with the energy cost of stress protein synthesis contribute to the elevated cellular and the whole-organism energy demand during stress exposures and require the support of high levels of aerobic metabolism (Calow, 1991; Feder and Hofmann, 1999). Production and functioning of such stress proteins can strongly increase cellular and whole-organism energy expenditure in response to mild stressors. Indeed, energy costs of protein synthesis increase from 5 to 10% of the total cellular ATP demand under control conditions to up to 30–40% during heavy metal stress in mollusks (Cherkasov et al., 2006; Ivanina et al., 2008). These additional energy costs of homeostasis can reduce aerobic scope of the organism and lead to the trade-offs in terms of the reduced scope for growth, activity and reproductive output (Calow, 1989, 1991; Calow and Forbes, 1998).

Salinity, pH fluctuations and other changes in the seawater chemistry may also lead to metabolic costs and/or reduced aerobic scope due to the disturbances in cell volume, ion and acid–base regulation as well as ventilation and circulation (Caldeira and Wickett, 2003, 2005; Ishimatsu et al., 2004; Pörtner et al., 2004; Ishimatsu et al., 2005; Shirayama and Thornton, 2005; Metzger et al., 2007; Walther et al., 2009). Osmoregulators such as all freshwater and many marine organisms spend a significant part of their energy budget (5–30% of resting metabolic rate) to maintain water and ion balance by actively excreting water and countergradient ion transport. Changes in water composition may cause additional energy costs on osmoregulation. In osmoconformers blood and intracellular milieu is isoosmotic to the environment and thus the basal costs of osmoregulation are low. However, fluctuating salinity and ion composition may incur indirect energy costs due to the need to rapidly regulate osmolarity and body volume immediately after the salinity change. During the decline in environmental salinity, inorganic ions (especially Na^+, K^+ and Cl^-) and organic osmolytes such as free amino acids and methylamines are excreted from the cells and need to be replenished by *de novo* synthesis, transamination and breakdown of the proteins, which in turn need to be re-synthesized (Kinne, 1971a; Hochachka and Somero, 2002) resulting in elevated ATP demand (Hawkins and Hilbish, 1992).

Elevated cellular energy demands for basal maintenance often leads to an increased rate of food consumption and/or changes in energy allocation in order to cover maintenance needs at the expense of growth and reproduction (Fig. 1). For example, temperature increase in the environmentally relevant range is reflected by a corresponding acceleration of most cellular and physiological processes ensuring metabolic coupling of the energy-consuming and energy-conserving pathways (Hochachka and Somero, 2002). At the cellular level, this coupling is mainly achieved by balancing the rates of ATP consumption governed by membrane processes and ATP synthesis (Hochachka, 1988; Hulbert and Else, 1999; Hochachka and Somero, 2002). At the whole-organism level, the balance is achieved by temperature-dependent changes in the feeding and respiration rates ensuring a high and relatively constant scope for growth (SFG) in the optimum range of temperatures (Winberg, 1983). Elevated temperatures can shift this balance inflating energy demands which first reduces the SFG and eventually results in negative growth or mortality events at extreme temperatures when the capacity of feeding processes to compensate is exceeded (De Wilde,

1975; Navarro and Thompson, 1996; Delaporte et al., 2006; Soletchnik and Goulletquer, 2006; Samain and McCombie, 2008; Anestis et al., 2010). A recent modeling effort that used DEB models to integrate the effects of temperature stress on energy budgets also showed that high thermal tolerance and high temperature optima for growth were associated with lower maintenance costs in marine fish and crustaceans (Freitas et al., 2010).

Moderate levels of pollution can also significantly change the energy allocation in aquatic animals (Calow, 1991). Typically, energy intake is suppressed due to decline in either feeding activity or assimilation efficiency (Toro et al., 2003; Wang et al., 2005), while the metabolic maintenance costs either remain constant (Widdows and Johnson, 1988) or often increase due to the compensatory upregulation of detoxification and cellular protection mechanisms (Cherkasov et al., 2006; Lannig et al., 2006a,b; Jeong and Cho, 2007; Sokolova and Lannig, 2008). Elevated metabolic costs of somatic maintenance and reproduction as a response to sub-lethal pollution stress have been predicted by DEB and recorded in *Daphnia magna* (Jager et al., 2010). High energy demand during moderate stress exposure is often fueled by breaking down tissue energy reserves (such as glycogen and lipid stores), and associated with hyperglycemia and increased amounts of energy excreted with nitrogen-containing substances (Wijsman et al., 1988; Ansaldo et al., 2006; Jeong and Cho, 2007; Moolman et al., 2007; Sokolova and Lannig, 2008).

5.2. Metabolic response to extreme stressors during transition to the pessimum range: conservation

Upon transition to the pessimum range, the aerobic scope disappears and all available energy and metabolic capacity is devoted to the somatic maintenance supporting time-limited survival of an organism but preventing growth and reproduction (Fig. 1c). Initially, this transition is heralded by the onset of partial anaerobiosis to partially compensate for energy deficiency (Sokolova and Pörtner, 2001; Pörtner et al., 2006). If this compensation is insufficient, an organism can enter metabolically arrested state – a temporary reversible suppression of metabolic rates below SMR. Metabolic arrest allows extending survival and stretching out available metabolizable resources until the environmental conditions improve and the organism can resume normal functioning. It is especially common in animals from extreme environments such as intertidal zones of the oceans, ephemeral freshwater bodies, low-oxygen zones or highly polluted biotopes (Sokolova et al., 2000a; Sokolova and Pörtner, 2002; Marshall et al., 2011), and involves coordinated suppression of anaerobic and aerobic ATP production with the commensurate reduction of the energy demand. During metabolic arrest, an organism usually seeks out a shelter and becomes inactive, and all available energy is devoted to the maintenance of homeostasis with no investment into somatic or gonad growth, reproduction or locomotion. However, the metabolically arrested state is by no means passive; during this state, organisms actively upregulate protection mechanisms that allow preserving intracellular structures against damage due to the changes in intracellular milieu such as shifts in pH, redox and energy status and prepare the organism for resuming of the normal function upon return to more favorable environmental conditions. The detailed analysis of the mechanisms of metabolic arrest has been a subject of several excellent reviews (e.g. (Hochachka and Guppy, 1987; Storey, 1998; Hochachka and Lutz, 2001; Storey and Storey, 2004)) and is beyond the scope of this work. Here, we briefly outline the aspects of metabolic rate depression that have a potential as biomarkers of transition to metabolic pessimum and thus can herald a bioenergetically non-sustainable state in aquatic organisms.

5.2.1. ATP production

Stress-induced metabolic arrest in aquatic organisms is usually associated with the strong suppression of ATP-producing pathways and often also with the oxygen deficiency either due to insufficient oxygen in the environment, limitations of gas exchange or disrupted tissue perfusion. When the oxygen concentration falls below the mitochondrial K_m for oxygen, oxidative phosphorylation is completely shut down and the organism must rely on the substrate-level phosphorylation to synthesize ATP (Fig. 3). Given that anaerobic metabolism is much less efficient than oxidative phosphorylation in terms of the ATP output and results in accumulation of the potentially toxic waste products and acidification of intracellular milieu, anaerobic animals face two interrelated problems: the necessity to conserve fermentable substrates and to avoid self-poisoning by metabolic wastes. At the same time, ATP-consuming pathways must be down-regulated to allow survival under conditions of the reduced ATP output. As a result, the so-called reverse Pasteur effect is observed during metabolic arrest - a strong reduction in the catabolism rates of carbohydrates and amino acids. Increased energy stores (especially glycogen levels that are typically 3–4 times higher in facultative anaerobes than in other species) coupled with the reverse Pasteur effect represent a major metabolic adaptation to extreme environmental stressors (Hochachka and Guppy, 1987). Successful facultative anaerobes such as marine intertidal mollusks have also evolved alternative pathways of substrate-level phosphorylation that produce more ATP per unit metabolized substrate, and/or result in less toxic or more volatile and thus easily excretable end products compared to lactate (Fig. 3). Thus, during early stages of environmental anaerobiosis, substrate-level fermentation predominantly occurs in cytosol with formation of lactate or alanine (Kluytmans and Zandee, 1983; Hochachka and Somero, 2002). At the later stage, mitochondrial pathways producing succinate, propionate and/or acetate become engaged that yield 2–3 times more ATP per unit metabolized glucose and allow more ATP to be turned over per mole accumulated H^+ than lactate (Hochachka and Mommsen, 1983; Hochachka and Somero, 2002).

Accumulation of acidic end products such as lactate, succinate, propionate or acetate can lead to acidification of the intracellular milieu. While moderate metabolic acidosis may be beneficial by supporting the metabolic rate depression (Langenbuch et al., 2006; Pörtner, 2008), extreme intracellular acidification is toxic. Successful facultative anaerobes such as marine intertidal mollusks are capable of counteracting this potentially toxic acidification and delay or slow the decrease in intracellular pH. Due to a strong inhibition of ATP production during metabolic arrest the active ATP-dependent mechanisms play a minor role in keeping the acid–base homeostasis which is maintained using the passive tissue buffering systems (such as imidazol, phosphate and calcium carbonate)(Sokolova et al., 2000b). Some earlier reports also suggest that the shell $CaCO_3$ material may also be used to regulate pH during anaerobiosis in mollusks (Dugal, 1939; Akberali et al., 1977). These buffering mechanisms have limited efficiency, and long-term anaerobiosis is typically associated with a moderate intracellular acidification (Sokolova et al., 2000b).

5.2.2. ATP consumption

A certain degree of reduction in ATP use during metabolic arrest is achieved by curtailing locomotory activity and all functions that are non-essential for survival (such as reproduction and growth). However, a significant suppression of the metabolic rate below SMR requires a decrease in the amount of energy spent on homeostasis. This is achieved by slowing down ventilation and/or circulation as

well as by decreasing the rates of the two most energy-consuming functions of the cell: ion pumping across the cellular membranes and macromolecular (especially protein) synthesis.

Both ventilation and circulation are greatly suppressed during the exposure to extreme stressors that induce metabolic arrest. Ventilation is an energetically costly activity due to the need of constantly propelling water (and often also mucus) across the ventilatory surfaces using ciliary or muscular action (Wells, 1990; Kennedy et al., 1996). A decrease in ventilation (and the concomitant decrease in oxygen consumption) is among the first responses to oxygen-deficient situations. Due to this behavior, animals can maintain energy balance via aerobic mechanisms until a critical P_{O_2} for anaerobiosis (P_{O_2CII}) is reached. Beyond the P_{O_2CII} anaerobic metabolism sets in to compensate for the decline in aerobic energy production and to maintain the vital processes (such as ion regulation) that cannot be shut down. When the ambient P_{O_2} reaches complete anoxia, ventilation usually ceases. Cessation of ventilation is also observed during air exposure of intertidal mollusks (except periodical brief bouts of gaping for atmospheric oxygen), as well as during exposure to other stressors that trigger shell closure such as extreme salinities, desiccation, and acute exposure to pollutants or pathogens.

Metabolic arrest is also associated with decreased heart rate (bradycardia) and reduced amplitude of heart beats. A pronounced bradycardia was observed in aquatic invertebrates as a response to salinity shock (Marshall and McQuaid, 1993; Bakhmet et al., 2005), hypoxia and air exposure (Marshall and McQuaid, 1993; Nicholson, 2002), and high levels of a contaminant (copper) (Marshall et al., 2004). Interestingly, in marine mollusks the ability to suppress heart rate correlated with the overall ability of metabolic rate depression and the survival of prolonged periods of extreme stress, such as desiccation during air exposure (Marshall and McQuaid, 1991). Such controlled suppression of circulation and the cardiac activity (which often coincides with the reduction of the oxygen uptake) may play an important role in reducing the ATP turnover (Fig. 3).

The cellular plasma membrane is a major ATP consumer accounting for 25–30% of ATP use during resting conditions (Hulbert and Else, 2005). This energy is mostly spent on the maintenance of ion gradients (particularly those of Na^+ and K^+) across the cell membrane. Additional 20–40% of ATP turnover is spent on maintaining the proton gradient across the mitochondrial membrane and counteracting proton leak (Brand et al., 1991, 1994; Cherkasov et al., 2006). Thus, it is hardly surprising that down-regulation of the membrane ATPases is a major event during metabolic arrest in organisms capable of metabolic rate depression. During metabolic rate depression, activity of Na^+/K^+ ATPase is strongly suppressed (Ramnanan and Storey, 2006). Despite this suppression, hypometabolic cells maintain essentially the same electrochemical potential across the cell membrane as active cells due to the so-called "channel arrest" – a strong decline in the membrane permeability (Hochachka et al., 1996; Hochachka and Lutz, 2001). In hypoxia-tolerant organisms, such channel arrest is promoted by an inherently low permeability of the membrane reflecting low channel densities and/or activities. In some cell types the membrane permeability may be further suppressed during the hypometabolic state (Hochachka et al., 1996). Unlike the cell membrane, permeability of mitochondrial membrane remains unchanged during the metabolic arrest. Instead, mitochondrial membrane potential is decreased during the metabolic arrest reducing the drive for ATP turnover and proton leak (St-Pierre et al., 2000; Bishop et al., 2002). However, the mitochondrial membrane potential cannot be fully dissipated even during the deepest metabolic arrest because it is required for the protein transport and mitochondrial maintenance which is crucial for stress survival and subsequent recovery (Wagner et al., 2009). Despite the high efficiency of the channel arrest and inhibition of the mitochondrial ATPase in reducing the cellular ATP demand, the maintenance of ion gradients across the cellular and mitochondrial membranes claims the lion's share of the ATP demand during metabolic arrest (up to 70–85%, of which around 60–75% is used by Na^+/K^+ ATPase and around 10% by mitochondrial ATPase) (Hochachka et al., 1996; St-Pierre et al., 2000).

Protein synthesis is a close second to the ion homeostasis in terms of the energy costs accounting for 10–25% of the total ATP consumption (Hand and Hardewig, 1996; Cherkasov et al., 2006). Thus, down-regulation of protein synthesis is an important energy saving mechanism during stress-induced metabolic arrest during extreme hypoxia, anoxia, freezing, acute exposure to pollutants and desiccation stress (Steinert and Pickwell, 1988; Tomanek and Somero, 2000; Larade and Storey, 2002, 2007; Storey and Storey, 2004). Unlike the activity of ion pumps which is only suppressed by about 75%, synthesis of cytosolic and mitochondrial proteins is usually inhibited by 93–94% in metabolically arrested states (Hochachka et al., 1996; Kwast and Hand, 1996). This tremendous down-regulation can be achieved by two complementary mechanisms: 1) suppression of gene transcription and mRNA synthesis (Larade and Storey, 2002; Teodoro and O'Farrell, 2003); and 2) a global inhibition of mRNA translation due to disassembling of the polyribosome complexes (polysomes), selective inactivation of translation initiation factors and low intracellular pH (Kwast and Hand, 1996; Koumenis et al., 2002; Koritzinsky and Wouters, 2007; Larade and Storey, 2007). Interestingly, despite the overall reduction of the rate of transcription and translation, specific mRNAs and proteins that are involved in stress tolerance may be significantly upregulated (Hochachka and Lutz, 2001; Larade and Storey, 2002) allowing fine-tuning of the cellular metabolism, supporting the essential cellular functions during metabolic depression and preparing the organism for the subsequent recovery.

The potentially important players in metabolic rate depression that regulate both ATP-consuming and ATP-producing processes are protein kinases such as AMP-activated protein kinase (AMPK) and cyclic AMP-activated protein kinase (protein kinase A, or PKA). AMPK is highly conserved during evolution and well described in mammalian systems. By phosphorylating key enzymes of energy metabolism pathways AMPK maintains a constant cellular ATP concentration during stress-induced energy shortage [for review see (Karagounis and Hawley, 2009)]. Little is known about AMPK in invertebrates; however recent studies in the rock crab, *Cancer irroratus*, the green crab *Carcinus maenas*, and the lobster *Homarus americanus* showed that AMPK can be a cellular indicator of the transition into the pejus range during heat stress (Frederich et al., 2009). AMPK is also activated during the metabolic rate depression of aestivating land snails *Otala lactea* where it appears to facilitate the suppression of anabolism, without activating ATP-producing pathways (Ramnanan et al., 2010). PKA is involved in metabolic rate depression as well as in transitions from metabolically depressed to active metabolic states (Brooks and Storey, 1994, 1995; MacDonald and Storey, 1999). It can activate or suppress enzyme activities, depending on the species and the enzyme (Michaelidis and Storey, 1990, 1991; Fernández et al., 1997; Fernández et al., 1998; Evans and Somero, 2010). Thus, metabolic rate depression during anoxia or freezing was associated with a decrease in PKA activity in marine snails *Littorina littorea* (MacDonald and Storey, 1999) while in mussels *Mytilus galloprovincialis* anoxia-induced metabolic rate depression led to an increase in PKA-dependent protein phosphorylation (Michaelidis and Storey, 1990, 1991). Given an important role of the reversible protein phosphorylation in the cellular response to environmental

shifts, further studies are needed to better characterize the involvement of protein kinases in the invertebrate bioenergetics under stressful conditions and determine their role in metabolic transitions from the optimum to the pejus and pessimum ranges.

As a corollary, metabolic adaptations and survival strategies to extreme stress require coordinated decrease in energy-conserving and -consuming mechanisms in order to maintain tissue energy balance as indicated by the minimal depletion of tissue ATP and phosphagens even during the prolonged metabolic arrest (Isani et al., 1989; Storey and Churchill, 1995; Churchill and Storey, 1996; Sokolova et al., 2000a; Kurochkin et al., 2009). Physiological and molecular changes that accompany this (including a cessation of feeding, assimilation and locomotion, reduction in SMR, partial transition to anaerobiosis, down-regulation of membrane transport and global protein synthesis and/or activation of AMPK) can serve as biomarkers of transition to the bioenergetically non-sustainable situation and therefore transition into the pessimum range.

6. Perspectives and significance

Studies in aquatic ectotherms demonstrate that energetic considerations can be used as a common yardstick by which to measure, compare and integrate the effects of different stressors on organisms. Most importantly, focus on energy balance provide means to directly link physiological stress effects to the organism's fitness and thus to the population-level consequences. Determining bioenergetic dimensions of stress response can be invaluable in predicting stress responses and tolerance limits under the environmentally realistic scenarios when multiple and often variable stressors are acting simultaneously on an organism. Thus, bioenergetic markers of transition into the environmental pejus range (including elevated SMR, high protein synthesis costs associated with the over-expression of stress proteins and/or stimulation of the protein degradation pathways, and reduced aerobic scope) indicate situations and/or environments where an organism is likely to suffer fitness consequences due to the stress exposure, but the long-term persistence of the population is possible at a cost of the reduced reproductive output and growth rates. In contrast, metabolic indicators of the pessimum range (such as transition to partial anaerobiosis, metabolic rate depression, reduced ventilation and circulation rates, suppression of the global protein synthesis, inhibition of ion pumps or channel arrest) are characteristic of the environment and/or conditions when individuals and thus populations cannot survive indefinitely unless changing environmental conditions provide a window of opportunity for growth and reproduction. Bioenergetic markers are likely to be more tightly correlated with the population performance than common stress biomarkers such as oxidative stress markers or expression of stress proteins; the latter are useful in indicating the stress response but are difficult to link to the organism's and population's survival. Bioenergetic sustainability at the organism's level (or lack thereof) can help identify the habitats and/or conditions that are or are not capable of supporting viable populations, thus assisting in explaining and predicting the species' distribution limits in the face of environmental change and informing the conservation efforts and resource management practices. Analysis of energy status can also provide important insights into the mechanisms of the interactive effects (e.g. synergy or antagonism) between different stressors and help in predicting the effects of new stressor combinations if the effects of individual stressors on energy balance are well understood. Overall, bioenergetic aspects of responses to environmental stressors (especially under the environmentally realistic conditions of multiple stress exposures) deserve most serious consideration in future studies seeking to determine the ecological consequences of physiological stress in the present-day dramatically and rapidly changing environment affected by pollution, global climate change and ocean acidification – to name but a few examples.

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