REVIEW

Physiological and behavioural strategies of aquatic animals living in fluctuating environments

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ABSTRACT

Shallow or near-shore environments, such as ponds, estuaries and intertidal zones, are among the most physiologically challenging of all aquatic settings. Animals inhabiting these environments experience conditions that fluctuate markedly over relatively short temporal and spatial scales. Living in these habitats requires the ability to tolerate the physiological disturbances incurred by these environmental fluctuations. This tolerance is achieved through a suite of physiological and behavioural responses that allow animals to maintain homeostasis, including the ability to dynamically modulate their physiology through reversible phenotypic plasticity. However, maintaining the plasticity to adjust to some stresses in a dynamic environment may trade off with the capacity to deal with other stressors. This paper will explore studies on select fishes and invertebrates exposed to fluctuations in dissolved oxygen, salinity and pH. We assess the physiological mechanisms these species employ to achieve homeostasis, with a focus on the plasticity of their responses, and consider the resulting physiological trade-offs in function. Finally, we discuss additional factors that may influence organismal responses to fluctuating environments, such as the presence of multiple stressors, including parasites. We echo recent calls from experimental biologists to consider physiological responses to life in naturally fluctuating environments, not only because they are interesting in their own right but also because they can reveal mechanisms that may be crucial for living with increasing environmental instability as a consequence of climate change.

KEY WORDS: Hypoxia, Hyperoxia, Killifish, Sculpins, Phenotypic plasticity, Salinity

Introduction

Experimental biologists have long sought to understand the responses of animals to changes in their environment. Somewhat paradoxically, we have traditionally kept our experimental laboratory conditions as stable and controlled as possible to avoid 'noise' in the observed responses to experimental treatments. Yet, environmental stability is unnatural. Variability in the environment

can be manifested over multiple temporal scales, ranging from regular tidal or daily changes, through seasonal variation, to occasional stochastic events. The different temporality and magnitude of a given variation will ultimately affect the nature and scale of the physiological response of the organism (Kingsolver et al., 2015). Timing and magnitude are not the sole consequential parameters, as oscillating environmental factors, which result in a temporary change in conditions before a return to the baseline, will induce distinct organismal responses compared with those resulting from gradual directional change (e.g. climate change), or an abrupt environmental disruption (e.g. extreme weather events). There is substantial evidence indicating that extreme events have a more important influence on the performance and survival of ectotherms than the effects of gradually changing climates (Altwegg et al., 2017; Clusella-Trullas et al., 2011; Paaijmans et al., 2013; Román-Palacios and Wiens, 2020; Vasseur et al., 2014). In recognition of all of the above, biologists are increasingly emphasizing the importance of investigating physiological responses to routinely fluctuating environments (e.g. Burggren, 2018, Morash et al., 2018, 2020; Vargas et al., 2017).

In this paper, we address key concepts that facilitate an understanding of the physiological responses of aquatic animals to environmental variation, focusing on fluctuations in oxygen, salinity, pH and environments where multiple stressors might occur. Temperature as an environmental stressor will not be directly addressed as it has been covered extensively in other very recent reviews (Chung and Schulte, 2020; Morash et al., 2020). We consider a fluctuating environment as one where the change is cyclical, consistent and occurs over a relatively short temporal and/or spatial scale (i.e. tidal, daily or seasonal variation). We also address scenarios where climate change is predicted to increase the severity of otherwise naturally occurring fluctuations (seasonal droughts, heat waves, etc.). Environmental changes that are point measurements (i.e. anthropogenic runoff), or those occurring over longer time scales (e.g. glacial cycles) are not considered.

Phenotypic plasticity represents the capacity of an individual to change its phenotype in response to a change in the environment. Phenotypic plasticity provides an organism with the flexibility to keep pace with environmental change, and is generally thought to be a key strategy for coping with fluctuating environmental conditions in nature (Ghalambor et al., 2007; Lande, 2009; Price et al., 2003). However, it is important to note that the magnitude and frequency of the environmental change will alter the phenotypic response to said stressor, and that mechanisms of plasticity will be recruited at different time scales. The extent of phenotypic plasticity shown for a given trait also differs among individuals, populations and species (Borowiec et al., 2020; Jeffries et al., 2019; Norin et al., 2016; Reemeyer and Rees, 2020), suggesting that systems underlying plasticity vary and/or that maintaining plasticity in one or more traits is costly (DeWitt et al., 1998; Fischer et al., 2016; Murren et al.,

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Glossary

Acclimation
Physiological changes within an individual stimulated by exposure to new conditions.

Adaptation
Genetic changes across generations to enhance fitness in an environment.

Aestivation
A state of dormancy, generally associated with reduced metabolic demands, that an organism undergoes during a hot and/or dry period.

Allopatric
Species which occur in geographically distinct areas.

Anadromous
Species that migrate from the ocean to freshwater to spawn.

Anthropocene
A proposed geological epoch encompassing the period during which human activities have substantially impacted the Earth's geology, ecosystems, climate and other environmental features.

Benthic
Ecological region at the lowest level of a body of water.

Brackish water
Water occurring in a natural environment that has a higher salinity than freshwater but lower salinity than seawater.

Brooding
Parental pattern of behaviour exhibited by egg-laying species.

Carbonic anhydrase
An enzyme important for converting CO_2 into carbonic acid and bicarbonate ions for excretion.

Euryhaline
Organisms able to survive in a wide range of salinities.

Eutrophication
The process by which a body of water is enriched with excessive amounts of minerals and nutrients, particularly nitrogen and phosphorus, often leading to excessive growth of algae, areas of low oxygen availability (dead zones) and mass mortality of animals such as fish.

Hyperosmotic
A higher osmotic pressure than that of a surrounding fluid.

Hyperoxia
Oxygenation above normal partial pressures (P_{O_2}>21 kPa).

Hypoxia (aquatic)
A reduction in oxygen that induces a compensatory physiological response in organisms to maintain cellular energy supply–demand balance. From a management perspective, aquatic hypoxia is often defined as dissolved oxygen falling below 2 mg l^{-1}.

Intertidal
The area of a seashore which is covered at high tide and uncovered at low tide.

Ionoregulation
Regulation of ions within an organism.

Osmorespiratory compromise
The conflict between the need for high gill permeability for respiratory gas exchange and low gill permeability to limit ion diffusion.

Oxidative stress
An imbalance between reactive oxygen species (ROS) and antioxidants, where the accumulation of excess ROS leads to molecular damage.

Pelagic
Relating to the open ocean.

Respiratory acidosis
An accumulation of metabolic CO_2 in the body that leads to the accumulation of protons.

Reactive oxygen species (ROS)
A type of unstable molecule that contains oxygen and easily reacts with other molecules in a cell.

Standard metabolic rate
Maintenance rate of oxygen uptake, recorded in a resting and non-digesting ectotherm at a specified temperature.

Subtidal
Area of ocean below the low tide mark.

2015). Although the direct costs of plasticity are debated (Auld et al., 2010; Murren et al., 2015), trade-offs are a prevalent concept in nature and processes that facilitate coping with one environmental stressor often compromise the organismal response to other stressors and the ability to maintain a stable internal environment [homeostasis; e.g. the osmorespiratory compromise (see Glossary) in fish gills; Gonzalez and McDonald, 1992; Randall et al., 1972]. This is particularly relevant for organisms living in shallow and/or near-shore aquatic environments such as ponds, estuaries or intertidal zones (see Glossary). These habitats are subject to large spatial and temporal fluctuations in virtually all physicochemical environmental factors, including the absence/presence of water, temperature, dissolved oxygen, pH, salinity and/or food availability (Hubertz and Cahoon, 1999). They are also frequently contaminated through the direct release of effluents and/or riverine inputs (Matthiessen and Law, 2002), which can threaten homeostasis. Although such environments are typically considered to be stressful, they also harbour high levels of biodiversity. Consequently, animals living in these aquatic environments represent model systems for better understanding the role of reversible, plastic responses in dealing with extreme environmental fluctuations, providing a window into the mechanisms allowing animals to cope with future climatic instability.

We consider responses, including phenotypic plasticity, at several levels of biological organization, comprising genes, cells, tissues and the whole organism. Within each of the environmental concepts, we focus on the following questions: (1) what is the magnitude of the environmental challenge experienced by organisms in temporal and/or spatial terms?; (2) what is the fundamental biology of species experiencing the environmental challenge?; (3) what is the nature of the physiological challenge?; (4) what are the physiological (and/or behavioural) mechanisms employed that facilitate homeostasis, and how successful are they at achieving it?; (5) how are these mechanisms different from those of similar species or congeners living in more stable environments?; and (6) what physiological trade-offs are/may be associated with the mechanisms employed? We also consider the interacting effect of multiple environmental stressors. Overall, our aim is to give examples from a detailed mechanistic approach of phenotypic plasticity using species experiencing fluctuating environmental conditions.

Oxygen

The extent of fluctuations of oxygen levels in aquatic environments can vary profoundly in magnitude, duration and frequency, ranging from relatively high and stable oxygen levels in free-flowing, well-mixed stream environments to extremely variable oxygen conditions in near-shore environments (Booth et al., 2012; Breitburg, 1992; Diaz, 2001; Giomi et al., 2019; McArley et al., 2021; Richards, 2011; Tyler et al., 2009). Relatedly, organisms may experience different patterns of oxygen availability, from near anoxia (no oxygen) to well above air equilibrium (hyperoxia; see Glossary) (McArley et al., 2021; Richards, 2011). Both hypoxia (see Glossary) and hyperoxia, as well as fluctuations in dissolved oxygen levels, may threaten the homeostasis of aquatic organisms.

Aquatic hypoxia is often defined as dissolved oxygen falling below 2 mg l^{-1} (Breitburg et al., 2018). From a physiological perspective, however, aquatic hypoxia is any reduction in dissolved oxygen that induces a compensatory response in an organism to maintain the cellular energy supply–demand balance (e.g. increased anaerobic energy production; Richards, 2009). A level of hypoxia that restricts the ability of animals to support energy demands via

aerobic respiration may eventually result in a lethal disruption of energy homeostasis (Boutilier and St-Pierre, 2000). In contrast, aquatic hyperoxia occurs when dissolved oxygen levels exceed normal atmospheric pressure (i.e. 100% air saturation; McArley et al., 2021). Oxygen levels of 270–400% air saturation have been recorded in high tidepools and shallow tropical ponds during the day, due to high photosynthetic rates from aquatic plants and algae (Domenici et al., 2015; Richards, 2011). Although hyperoxia likely does not appear to significantly disrupt the production of adenosine triphosphate (ATP) by mitochondria, it may potentiate the formation of damaging reactive oxygen species (ROS; see Glossary) (Birben et al., 2012; Dzal, 2018; Welker et al., 2013) and/or induce respiratory acidosis (see Glossary) as a consequence of reduced ventilatory drive and correspondingly increased carbon dioxide (CO_2) accumulation in the body of water-breathing organisms (Gannon and Henry, 2004; McArley et al., 2021; Truchot and Duhamel-Jouve, 1980; Wood and Malvin, 1991). Given that the prevalence and frequency of aquatic hypoxia and hyperoxia are expected to increase as a result of climate change, urbanization and pollution (Diaz and Rosenberg, 2008), studying animals that live in environments naturally experiencing extreme fluctuations in dissolved oxygen levels will enhance our general understanding of mechanisms for surviving oxygen variability.

Phenotypic plasticity and potential genetic adaptation in coping with hypoxic and hyperoxic challenges

Species living in the intertidal zone and estuaries experience fluctuating levels of dissolved oxygen on a daily basis, providing important insight into the mechanisms underlying the ability to cope with fluctuating oxygen concentrations in nature. For example, intertidal mussels and clams that are hypoxia tolerant (e.g. *Mytilus edulis*) exhibited stronger metabolic suppression compared with less hypoxia-tolerant mussels during hypoxia–reoxygenation events, and showed less damage to more oxidation-sensitive amino acids (Haider et al., 2020; Li et al., 2019). More hypoxia-tolerant intertidal clams (*Mercenaria mercenaria*) also showed a decrease in mitochondrial phosphorylation capacity during hypoxia that was quickly recovered after 1 h reoxygenation, relative to less tolerant scallops (*Argopecten irradians*) where mitochondrial phosphorylation capacity remains low after reoxygenation (Ivanina and Sokolova, 2016; Sokolova et al., 2012).

Previous reviews have demonstrated that both phenotypic plasticity and genetic adaptation (see Glossary) are important mechanisms by which aquatic organisms can cope with low oxygen conditions (Bickler and Buck, 2007; Mandic and Regan, 2018). In this respect, the Atlantic killifish (*Fundulus heteroclitus*) and sculpin fishes (family Cottidae) are some of the best-studied examples, and provide valuable insight into how these mechanisms operate within and/or among species.

Intraspecies comparisons of responses to fluctuating oxygen levels

The Atlantic killifish has not only a robust phenotypic response to hypoxia but also a highly specific response that varies according to the exact nature (pattern) of the hypoxia exposure that has not been studied in other aquatic species. Atlantic killifish live in estuaries on the east coast of North America, where they routinely encounter chronic seasonal and intermittent daily fluctuations in dissolved oxygen content (Tyler et al., 2009). Like many fishes, including zebrafish (*Danio rerio*; Rees et al., 2001), goldfish (*Carassius auratus*; Jibb and Richards, 2008; Sollid et al., 2003) and tilapia (*Oreochromis niloticus* or *Oreochromis* hybrid sp.; Li et al., 2018;

Speers-Roesch et al., 2010), *Fundulus* killifishes display a considerable degree of phenotypic plasticity in response to hypoxia, including alterations in oxygen transport capacity and the capacity of anaerobic metabolism, which help maintain ATP supplies in the face of the depressive effect of hypoxia on aerobic metabolism (Abbaraju and Rees, 2012; Borowiec et al., 2015, 2018; Everett et al., 2012; Martínez et al., 2006; Virani and Rees, 2000). Uniquely, the interaction between hypoxia severity and diurnal changes in oxygen availability has been investigated in *F. heteroclitus*. Killifish acclimated to intermittent cycles of diel hypoxia have a physiologically distinct response from those acclimated to constant hypoxia, including rapid and dynamic modulation of blood oxygen-carrying capacity and improved oxidative and gluconeogenetic capacity in the liver (Borowiec and Scott, 2020, 2015, 2018). These alterations may enhance glycolytic capacity and flux and aid in recovering from the heavy use of glycolysis during hypoxic events (Borowiec et al., 2018) to maintain the ATP supply–demand balance during bouts of hypoxia. Conversely, killifish acclimated to constant hypoxia seem to rely on decreasing routine metabolic demands as their main strategy for coping with hypoxia (Borowiec et al., 2018) (Fig. 1A). While similar investigations in other species remain scarce, it is likely that many aquatic organisms display similar fine-tuned responses to not only the severity of hypoxia but also the timing of the hypoxic stressor (both daily and seasonal) and other more subtle modifiers that they routinely encounter in their environment. These findings thus provide insight into how other aquatic species could alter their physiological responses based on the duration of the hypoxia exposure, which is predicted to increase with global warming in combination with increased nutrient discharge to aquatic environments from an increasing global population.

Interspecies comparisons of adaptations to fluctuating oxygen

Various sculpin fishes (family Cottidae) inhabit different regions of the intertidal in the Pacific Northwest, with more hypoxia-tolerant species from the higher intertidal often relying on both genetic adaptation and phenotypic plasticity in coping with habitats with more variable oxygen availability, as compared with less tolerant species in the lower intertidal and subtidal (see Glossary) (Mandic et al., 2009; Richards, 2011). Hypoxia-tolerant intertidal sculpins evolved from less tolerant subtidal ones, and many of the morphological and physiological traits that underlie their differences in hypoxia tolerance appear to have been acquired through genetic adaptations arising through natural selection (Knope, 2013; Knope and Scales, 2013; Mandic et al., 2009). Intertidal sculpins are capable of rapidly responding to hypoxic stress via behavioural responses (i.e. aquatic surface respiration; Sloman et al., 2008) to avoid hypoxic waters. Moreover, they possess adaptive physiological traits that enhance oxygen diffusion through the oxygen transport cascade to improve their inherent ability to extract oxygen at the gills and, finally, oxygen delivery to tissues (Lau et al., 2017; Mandic et al., 2009) (Fig. 1B). Similar to the killifish, more hypoxia-tolerant sculpin species also show higher glycolytic capacity (Mandic et al., 2013). Some evidence points to hypoxia-tolerant sculpins in the higher intertidal employing a more generalist approach, although this was investigated only at the transcriptional level and in a single tissue. In the liver of a more hypoxia-tolerant sculpin species (*Oligocottus maculosus*), changes in gene expression in response to hypoxia occurred after 24–72 h, compared with 3–24 h in a less tolerant species (*Blepsias cirrhosis*) exposed to the same level of hypoxia (~29 Torr, ~3.9 kPa; Mandic
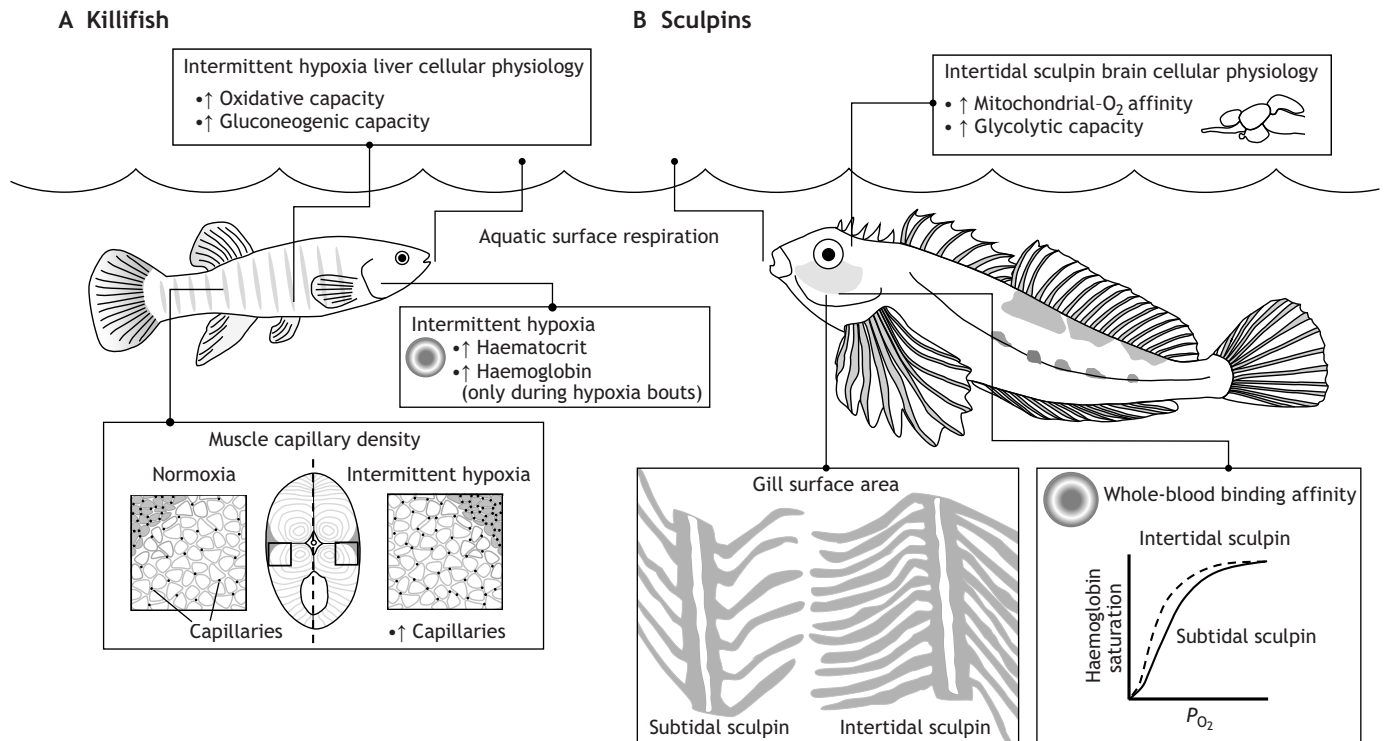
Fig. 1. Examples of phenotypic plasticity in killifish (*Fundulus heteroclitus*) responding to fluctuations in hypoxia and fixed responses of intertidal sculpins. (A) Killifish exposed to intermittent hypoxia exhibit transient increases in haemoglobin and haematocrit and increases in muscle capillary density, enhancing oxygen movement to tissues in hypoxic conditions. (B) Alternatively, intertidal sculpins have increased oxygen uptake through increased gill surface area and increased haemoglobin–oxygen binding compared with subtidal sculpins.

et al., 2014). The difference in the timing of this transcriptional response in the more hypoxia-tolerant species suggests that these transcriptional changes would not occur in the duration of a normal tidal cycle. This onset of transcriptional responses that would then translate into changes in protein expression is probably energetically expensive, and most likely adopted by the more hypoxia-tolerant species in the event of more prolonged changes in environmental oxygen. This pattern of transcriptional changes could be a mechanism other species use to enhance survival in response to more extreme oxygen fluctuations in a more unstable future, but whether or not this is the case warrants further investigation.

Physiological responses to hyperoxia
The physiological responses of organisms to hyperoxia exposure have received less attention than those used to cope with hypoxia; however, many coastal fishes and invertebrates encounter hyperoxic conditions, particularly those living in habitats with high daytime photosynthetic activity (e.g. rock pools, seagrass beds, estuaries, shallow tropical ponds; McArley et al., 2021). Although diel hypoxia–hyperoxia cycles occur naturally in these environments, anthropogenic eutrophication (see Glossary) is causing more extreme fluctuations in environmental oxygen and more severe hyperoxic events (Liu et al., 2012). The formation of ROS is a major challenge associated with exposure to hyperoxia and/or rapid changes in dissolved oxygen (e.g. diel hypoxia–hyperoxia cycling), but plastic changes in antioxidant defence mechanisms can help to combat oxidative stress (see Glossary). A useful model for understanding how species cope with ROS is the burrowing polychaete, *Heteromastus filiformis*, which creates permanent tubes in intertidal sediments that can extend up to 30 cm below the

sediment surface (Cadée, 1979). The head is downward facing so that the worm can feed on organic material buried deep in the anoxic sediment layer (Clough and Lopez, 1993). Gill-like parapods at the caudal end facilitate oxygen uptake near the sediment–water interface where hyperoxic conditions (up to 300% air saturation) can develop during the day owing to algal growth on the sediment surface (Fenchel and Finlay, 1995). This spatial and temporal variation in oxygen along its body length makes *H. filiformis* particularly susceptible to oxidative stress (Abele et al., 1998a,b). *Heteromastus filiformis* appears to rely on both genetic adaptation and phenotypic flexibility to cope with its particular lifestyle. For example, some antioxidant enzymes (e.g. superoxide dismutase) have a constitutively higher activity in *H. filiformis* compared with other benthic (see Glossary) invertebrates (Abele-Oeschger, 1996), whereas the activity of other enzymes (e.g. catalase) rapidly increases in response to acute hyperoxia exposure (Abele et al., 1998a,b).

Many other benthic invertebrates and estuarine fishes have similarly demonstrated an upregulation in ROS scavenging capacity in response to hyperoxia and/or abrupt changes in dissolved oxygen (for reviews, see Canesi, 2015; Martínez-Álvarez et al., 2005). For example, when Ross et al. (2001) compared the activity of important antioxidant enzymes across several estuarine fishes (i.e. *Gillichthys miribilis*, *Leptocottus armatus*, *Leiostomas xanthurus* and *Lagodon rhomboides*), they found the highest activities in species that encountered the most extreme degree of dissolved oxygen cycling and oxygen supersaturation. As antioxidants can be energetically expensive to produce (Pamplona and Costantini, 2011), animals with a high capacity for antioxidant production may face energetic trade-offs in which minimizing oxidative damage results in diminished growth or reproduction.

Exposure to hyperoxia causes immediate hypoventilation in both vertebrates and invertebrates, which can result in CO_2 accumulation in the body (e.g. Berschick et al., 1987; McArley et al., 2021; Sinha and Dejours, 1980; Toulmond and Tchernigovtzeff, 1984; West and Burggren, 1982; Wood, 1991; Zena et al., 2016). Indeed, many fishes, such as rainbow trout (*Oncorhynchus mykiss*), common carp (*Cyprinus carpio*) and turbot (*Scophthalmus maximus*), exhibit elevated CO_2 in the blood within a few hours of hyperoxia exposure, with concomitant decreases in blood pH (respiratory acidosis; McArley et al., 2021; Truchot, 1988). This acidosis is temporary, however, because fish can accumulate bicarbonate ions (HCO_3^-) in the plasma, thereby restoring blood pH to normal levels (Person-Le Ruyet, 2002; Shartau et al., 2017; Wood, 1991). Hyperoxia-driven respiratory acidosis has primarily been investigated in species that would face hyperoxia in an aquaculture setting (e.g. *S. maximus*; Person-Le Ruyet, 2002), but similar mechanisms may be invoked by other species faced with naturally occurring hyperoxia – an interesting avenue for future investigation.

Physiological trade-offs

The physiological strategies used to cope with fluctuations in dissolved oxygen can lead to trade-offs in other performance and fitness-related traits. For instance, in killifish, tolerance to hypoxia increases with acclimation (see Glossary) to either intermittent hypoxia or constant hypoxia (Borowiec et al., 2015). Interestingly, killifish exposed to severe constant hypoxia reduce the total length of their gill filaments (Borowiec et al., 2015), a somewhat counterintuitive response to oxygen limitation that may reflect the necessity to reduce osmoregulatory costs and decrease routine metabolic rates. Killifish acclimated to intermittent hypoxia maintain routine oxygen consumption rates comparable to those of normoxia-exposed controls during hypoxia exposure (Borowiec et al., 2018), but have lower maximum metabolic rates (Borowiec and Scott, 2021), and so presumably have less scope for non-survival functions such as growth and reproduction (Eliason et al., 2011; Holt and Jørgensen, 2015). This phenomenon may be a common feature of intermittent hypoxia as both grass shrimp (*Palaemonetes vulgaris*) and southern flounder (*Paralichthys lethostigma*) showed stunted growth rates under cycling hypoxia conditions (Coiro et al., 2000; Peruzza et al., 2018; Taylor and Miller, 2001).

In terms of hyperoxia, some studies have suggested that the mechanisms used to mitigate oxidative stress can negatively impact various life-history characteristics (e.g. growth, reproduction, immune responses). Cells possess quality control systems to minimize and repair molecular damage (e.g. antioxidant defences), but these systems require significant amounts of energy (Kirkwood, 2005; Monaghan et al., 2009). Consequently, energy allocation trade-offs are hypothesized to exist between the physiological processes that maximize fitness (e.g. growth, reproduction) and processes that repair molecular damage and, thus, increase longevity (Kirkwood, 1977; Lemaître et al., 2015). Indeed, the primary cause of senescence is thought to be the accumulation of molecular damage over time from sources such as ROS (for reviews, see Gems and Partridge, 2013; Kirkwood, 2005; Maynard et al., 2015; Vijg and Campisi, 2008). Although the presence of such energetic trade-offs is still under debate (e.g. Hood et al., 2018), they may be important mediators of fitness in coastal fishes and invertebrates exposed to rapid fluctuations in dissolved oxygen.

Salinity

Aquatic organisms may be challenged with variations in salinity due to migration [e.g. anadromous species (see Glossary)], changing

seasons (e.g. snowmelt), proximity to estuaries or other reasons. Estuaries show substantial spatial and temporal variation in dissolved salt concentration, with salinity increasing with distance from freshwater inputs such as river mouths and deltas (Telesh and Khlebovich, 2010; Whitehead et al., 2011). Seasonal salinity fluctuations in marine environments also occur as a result of changes in terrestrial run-off, such that salinity is relatively low in the spring and high in the autumn (Garvine et al., 1992; Xu et al., 2012). Daily and sometimes hourly alterations in water depth and mixing due to tidal cycles and wind also moderately influence salinity levels in coastal habitats (Garvine et al., 1992; Kuo and Neilson, 1987; Xu et al., 2012). Importantly, shallow coastal marine inhabitants will likely need to cope with greater fluctuations in salinity and/or higher salinity in the near future because of changes in rainfall, streamflow, run-off, upwelling (particularly in the case of stratified environments experiencing more frequent storms) and other effects associated with climate change and urbanization (Gibson and Najjar, 2000; Hong and Shen, 2012).

Salinity fluctuations challenge the ability of aquatic organisms to maintain ion and water homeostasis in body fluids and cells. Many enzymes, transporters and other key subcellular molecules show impaired function outside of normal physiological ion concentrations (Hochachka and Somero, 2002). Moreover, the intricate links between ionoregulation (see Glossary), osmoregulation, acid–base balance and nitrogenous waste excretion in fishes and other aquatic organisms (Evans et al., 2005) mean that osmotic challenges can disrupt multiple physiological systems. Efforts to quantify the energetic cost of ionoregulation and osmoregulation in fishes have achieved mixed results. Some laboratory-based estimates in a wide variety of euryhaline (see Glossary) teleost species suggest that up to ~50% of standard metabolic rate (see Glossary) is needed to osmoregulate when fish are transferred from freshwater to saltwater conditions (Boeuf and Payan, 2001; Christensen et al., 2017; Ern et al., 2014), whereas other studies suggest that osmoregulatory costs are low or negligible at the whole-animal level (Eddy, 1982; Ern et al., 2014; Kirschner, 1995; Morgan and Iwama, 1999; Nordlie and Leffler, 1975; Parker et al., 2020).

Phenotypic plasticity and potential genetic adaptation in coping with hypo-osmotic and hyperosmotic challenges

Many species of fishes live in habitats that experience wide salinity fluctuation over short time scales (Ern et al., 2014; Gonzalez, 2012; McCormick et al., 2013). Among these, Atlantic killifish are a key model for understanding the mechanisms of ionoregulation and osmoregulation in fishes, and how animals respond to extreme fluctuations in salinity. Wild populations, such as those in Chesapeake Bay, inhabit salinities ranging between freshwater and full-strength seawater (Griffith, 1974; Nordlie, 2006; Whitehead, 2010), though the species prefers brackish (see Glossary) or marine waters (~20–30 ppt salinity) (Bucking et al., 2012). In the laboratory, killifish can acclimate to salinities nearly 4 times that of seawater (~114 ppt; Griffith, 1974; Whitehead, 2010).

Rapid transitions between low and high salinities and the associated physiological plastic responses are likely to be more energetically costly than maintaining routine physiological functions in less variable conditions. For example, work investigating the seawater to freshwater transition in the Atlantic killifish suggests that as much as 10% of the animal's total energy budget was dedicated to maintaining homeostasis in the face of salinity fluctuations (Kidder et al., 2006a,b). Killifish maintain osmotic balance (homeostasis) during rapid salinity transitions by decreasing ion permeability across their tissues under osmotic

stress to conserve internal osmotic pressure (Sardella and Brauner, 2016). Under hyperosmotic (see Glossary) conditions *F. heroclitus* uses similar mechanisms of ion and osmotic regulation to those of most other marine fishes (Fig. 2), and readers are directed to the many previous investigations in this area (Evans et al., 2005; Karnaky, 1986; Marshall et al., 1997; Scott et al., 2004a; Whitehead et al., 2012; Wood and Grosell, 2009; Wood and Laurent, 2003; Wood and Marshall, 1994; Wood et al., 2010).

Under hypo-osmotic conditions *F. heteroclitus* responds somewhat differently from other marine teleosts. Like many teleosts, mitochondria-rich cells (MRCs) on the gill epithelium transition from seawater type to freshwater type, but unlike other teleosts, *F. heteroclitus* require a relatively prolonged exposure (e.g. longer than a tidal cycle), and their MRCs are slow to respond, with the shift in cell type only occurring at very low (∼1 ppt) salinity (Copeland, 1950; Evans et al., 2005; Philpott and Copeland, 1963). Such a delayed and insensitive morphological response is unlikely to explain the exceptional salinity tolerance of these organisms. Additionally, killifish are unlike other teleosts in that they effectively lack active chloride uptake at the gills in freshwater (Patrick and Wood, 1999; Patrick et al., 1997; Wood and Laurent, 2003) and have minimal capacity to modulate the rate of active sodium or chloride uptake via the gills in response to internal acid–base disturbances (Patrick and Wood, 1999; Patrick et al., 1997). Instead, *F. heteroclitus* likely rely on modulation of branchial sodium and chloride efflux (Patrick and Wood, 1999; Patrick et al., 1997) and/or other plastic responses to facilitate their rapid and effective acclimation to hypo-osmotic conditions. For example, freshwater transfer leads to alterations in chloride cell morphology (Hossler et al., 1985; Katoh and Kaneko, 2003) in seawater-

acclimated fish, and increases in the gene expression and activity of gill Na^+/K^+-ATPase in killifish acclimated to brackish conditions (Scott et al., 2004b). The activity of the Na^+/K^+-ATPase reaches a new equilibrium within 30 min of salinity transfer (Towle et al., 1977), and within ∼48 h, freshwater-acclimated killifish can modify several aspects of their physiology, from ion uptake to ion secretion (Marshall et al., 1999). Diet also likely plays a key role in maintaining ion balance in freshwater, as chloride and sodium uptake increases in the intestinal tract during freshwater acclimation and may provide an alternative, gill-independent mechanism to modulate chloride uptake (Scott et al., 2006; Wood et al., 2010). Though their ionoregulatory and osmoregulatory responses to low salinity depart considerably from those of other fishes, killifish are nonetheless able to rapidly acclimate to a wide range of salinities, suggesting that potential trade-offs are easily coped with by the animals.

Over longer time scales (e.g. multiple generations sufficient to enable evolution by natural selection), physiological plasticity is complemented in *F. heteroclitus* by interspecific and interpopulation variation in salinity tolerance. For example, freshwater populations typically acclimate more successfully to hypo-osmotic conditions than do brackish or coastal populations, and this is associated with differences in the transcription of genes involved in polyamine regulation, cell volume regulation, signal transduction and other processes (Whitehead et al., 2011). The northern subspecies is more tolerant of freshwater than the southern subspecies, and this appears to largely depend upon their superior capacity to minimize chloride loss, differences in the activity and expression pattern of key ionoregulatory genes and a more complete transition to freshwater MRC morphology (Schulte, 2014; Scott and Schulte, 2005; Scott et al., 2004a; Whitehead et al., 2012).
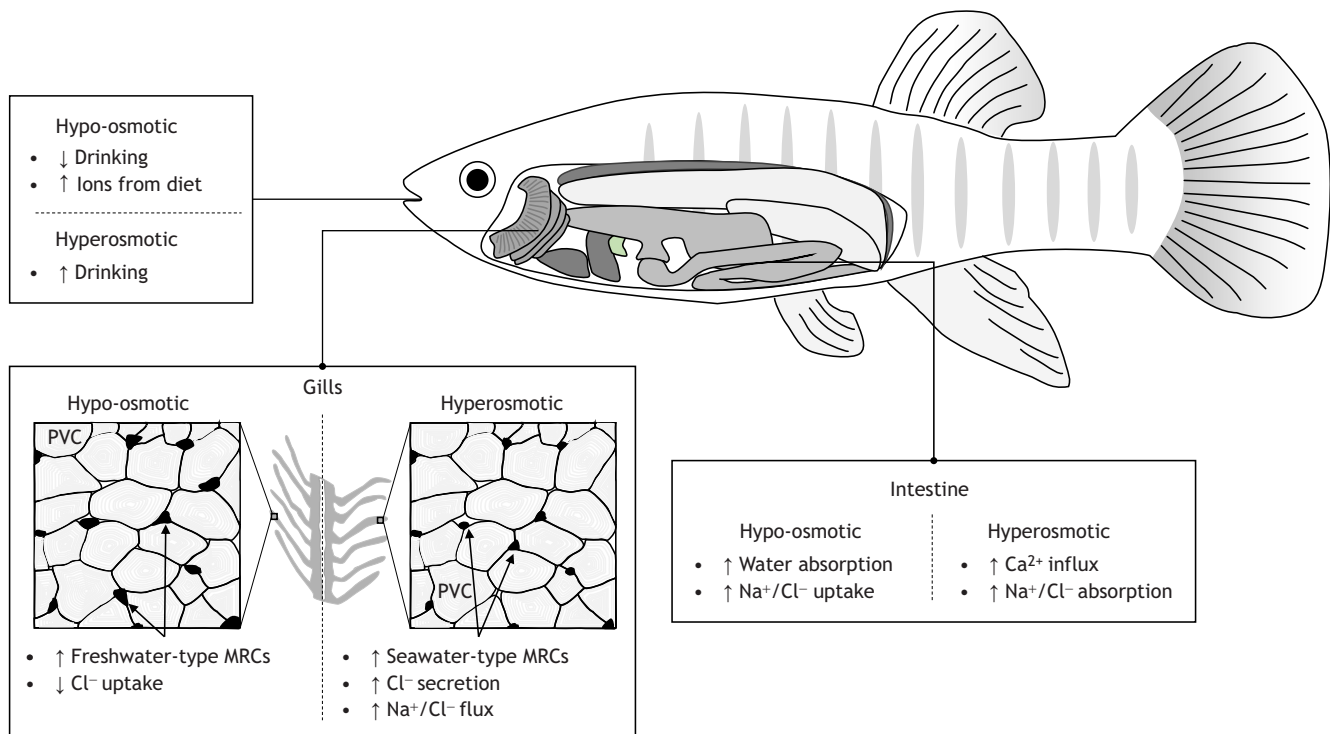

Fig. 2. Examples of phenotypic plasticity that killifish (*F. heteroclitus*) exhibit in hypo-osmotic and hyperosmotic environments. Under hyperosmotic conditions, killifish increase the amount of water they drink, the amount of mitochondria-rich cells (MRCs) with a seawater-type morphology (e.g. deep and narrow apical crypts) and Na^+/Cl^- removal at the gills and intestines, similar to other fish species. Conversely, in hypo-osmotic conditions (<1 ppt), killifish lack Cl^- uptake in the gills and rely on increases in MRCs with a freshwater-type morphology (e.g. wide and shallow apical crypts) and changes in Na^+/Cl^- uptake in the intestine. PVC, pavement cells.

Physiological trade-offs

Coping with salinity challenges can impair gas exchange (osmorespiratory compromise) and impact the ability of killifish to respond to other stressors. For example, freshwater-acclimated killifish are less hypoxia tolerant than those in brackish or marine conditions (Giacomin et al., 2019; B.G.B., unpublished observations). This decrease in tolerance is associated with a reduced respiratory surface area in the gill and corresponding lower oxygen extraction efficiency (Giacomin et al., 2019). Studies incorporating multiple, environmentally relevant stressors remain relatively uncommon (see 'Multiple stressors', below), and we encourage greater integration of salinity challenges with these studies given the interactions between ion balance (and associated counter ions) and several other aspects of homeostasis, such as handling of nitrogenous bases, detoxification and energy balance.

pH

The pH of the open ocean is relatively stable (pH ~8.1); however, there are still many aquatic environments that experience large fluctuations in pH as a result of tidal cycles, upwelling, algal blooms or biological activity. This can be further exacerbated by factors such as diurnal cycle, season and/or biogeochemistry of the environment, which can lead to vast fluctuations in pH on both temporal and spatial scales (Hofmann et al., 2011; Kapsenberg and Hofmann, 2016; Storz et al., 2012). Additionally, specific habitats such as near-shore environments, coral reefs, CO_2 vents and mangrove or kelp forests can also experience great variability in pH across short time and spatial scales (Frieder et al., 2012; Hofmann et al., 2011). Alterations in pH are largely driven by changes in CO_2, as the hydration of CO_2 ultimately results in increasing protons and bicarbonate in solution. Reduced pH can threaten an organism's homeostasis by increasing internal acidity and simultaneously reducing the gradient for CO_2 excretion. In addition to natural variations in aquatic ecosystems, anthropogenic activities are causing increased emissions of CO_2, and approximately one-third of this CO_2 is absorbed into the oceans, leading to acidification (Doney et al., 2011; Gruber, 2011; Reay and Grace, 2007). Owing to rising CO_2 emissions from the Anthropocene (see Glossary), numerous studies have examined the effects of ocean acidification using predicted future scenarios for pCO_2/pH (for reviews, see Clements and Hunt, 2015; Kroeker et al., 2013). These scenarios, however, are sometimes less extreme than the natural variations organisms face in the present day (e.g. Vargas et al., 2017).

Phenotypic plasticity and potential genetic adaptation in coping with pH challenges

pH-driven plasticity can be readily studied in the natural near-shore CO_2 vent ecosystem in the Mediterranean Sea. Here, an underwater volcano releases CO_2 into shallow waters, resulting in a pH ranging from ~6.5 to 8.2, with variation of up to 0.1 units within an hour (Andrea Foo et al., 2018; De Alteriis et al., 2010; Hall-Spencer et al., 2008). Numerous studies have examined this system to predict organismal responses with respect to ocean acidification, as these animals already survive in a pH environment near/beyond future predicted levels (e.g. reduced by 0.3–0.5 pH units by the year 2100; Caldeira and Wickett, 2005) and have done so over a relatively short time scale of ~1850 years, enabling an examination of adaptation versus plasticity (Andrea Foo et al., 2018; Fabricius, 2011; Hall-Spencer et al., 2008). Living in this region are non-calcifying polychaetes that are grouped into low pH/elevated pCO_2-tolerant species (i.e. abundant inside and outside the low pH environment; e.g. *Platynereis dumerilii*, *Amphiglena mediterranea*, *Syllis prolifera*, *Polyophthalmus pictus*; Calosi et al., 2013;

Ricevuto et al., 2014) and low pH/elevated pCO_2-sensitive species (i.e. found away from the vents in a similar habitat; e.g. *Lysidice ninetta*, *Lysidice collaris*, *Sabella spallanzanii*; Calosi et al., 2013). *In situ* transplantation studies are often utilized to investigate differences as a result of plasticity or genetic adaptation.

One tolerant species, *A. mediterranea*, utilizes phenotypic plasticity to inhabit the CO_2 vents. When removed from these high-pCO_2 environments, this species shows a reduction in metabolic rate that becomes consistent with that of individuals living outside the vent habitat (Calosi et al., 2013). Further analysis revealed no distinct differences in genetic make-up between vent-dwelling individuals and those living in more controlled stable environments, supporting the idea that this species uses phenotypic plasticity rather than genetic adaptation to tolerate these drastic changes in pH (Calosi et al., 2013). Contrastingly, *P. dumerilii* living in the vents demonstrate genetic adaptability to their habitat with population-level genetic differences between individuals living inside and outside the vents (Calosi et al., 2013). Metabolic rate is consistently elevated in vent-dwelling animals (Calosi et al., 2013), as is relative mRNA abundance of NADH dehydrogenase, a key enzyme in ATP production (Wäge et al., 2018), demonstrating an elevated energetic cost of living at low pH. Some individuals from the high P_{CO_2} genetic lineage were found outside the vents and vice versa, which may be the result of pelagic (see Glossary) larvae moving between habitats; whether these individuals prosper in the 'opposing' environment remains to be studied (Calosi et al., 2013). Sensitive species not found in the vent system (*S. spallanzanii*, *L. collaris*, *L. ninetta*) were unable to maintain metabolic rate when exposed to high P_{CO_2} and demonstrated species-specific increases or decreases in metabolic rate (Calosi et al., 2013). Furthermore, significant alterations to metabolism were observed in *S. spallanzanii* transplanted from low to high P_{CO_2} (Turner et al., 2015): ATP production increased and arginine dephosphorylation decreased to maintain metabolic rates. However, these plastic responses may not be viable long term owing to significant energetic costs, which could explain why this species is not found inhabiting the vents (Turner et al., 2015).

Physiological trade-offs

Polychaete richness and abundance severely decrease towards the high P_{CO_2} environment, with pH-driven changes in phenotypes observed (Gambi and Danovaro, 2016). Such shifts in species abundance may be driven by the environment, with survival dependent upon the ability to adapt. These adaptations, however, come at a cost. For example, in the P_{CO_2}-sensitive polychaete *S. spallanzanii*, there is a decreased concentration of succinate and lactate (the main products of anaerobiosis) alongside a depressed concentration of carbonic anhydrase (see Glossary) (Turner et al., 2015). Thus, to tolerate elevated P_{CO_2}, these organisms adjust their aerobic capacity while simultaneously experiencing a drop in carbonic anhydrase. This could ultimately have consequences for respiration (e.g. respiratory acidosis; see above) as carbonic anhydrase is necessary for effective CO_2 excretion (Turner et al., 2015), explaining the lack of this species within the vent system. The other species that demonstrate metabolic depression in the face of elevated P_{CO_2} (e.g. *L. collaris* and *L. ninetta*) are likely allocating energy towards managing this environmental challenge, which is unsustainable long term and likewise could explain their absence from the vents (Calosi et al., 2013). In addition to respiratory consequences, organisms living at low pH may also be faced with the stress of maintaining intracellular pH homeostasis, which involves the costly coordinated efforts of ion transporters (e.g.

sodium proton exchanger, NHE). This may explain the aforementioned elevated metabolic rate observed in some vent-dwelling polychaete species (Calosi et al., 2013). A correlated decrease in NHE mRNA abundance in vent-dwelling polychaetes may hint at the energetic expense of ion regulation and suggest those animals living in vents cannot maintain such costly transporters (Wäge et al., 2018) (Fig. 3).

The energy required to effectively handle pH stress could ultimately divert energy away from important processes such as growth and reproduction (Seibel and Walsh, 2003). Indeed, examination of life-history traits within the high-P_{CO_2} vent species showed a shift towards motile, brooding species (see Glossary) as opposed to the sessile species with pelagic larvae that are found in control environments (Calosi et al., 2013; Gambi and Danovaro,
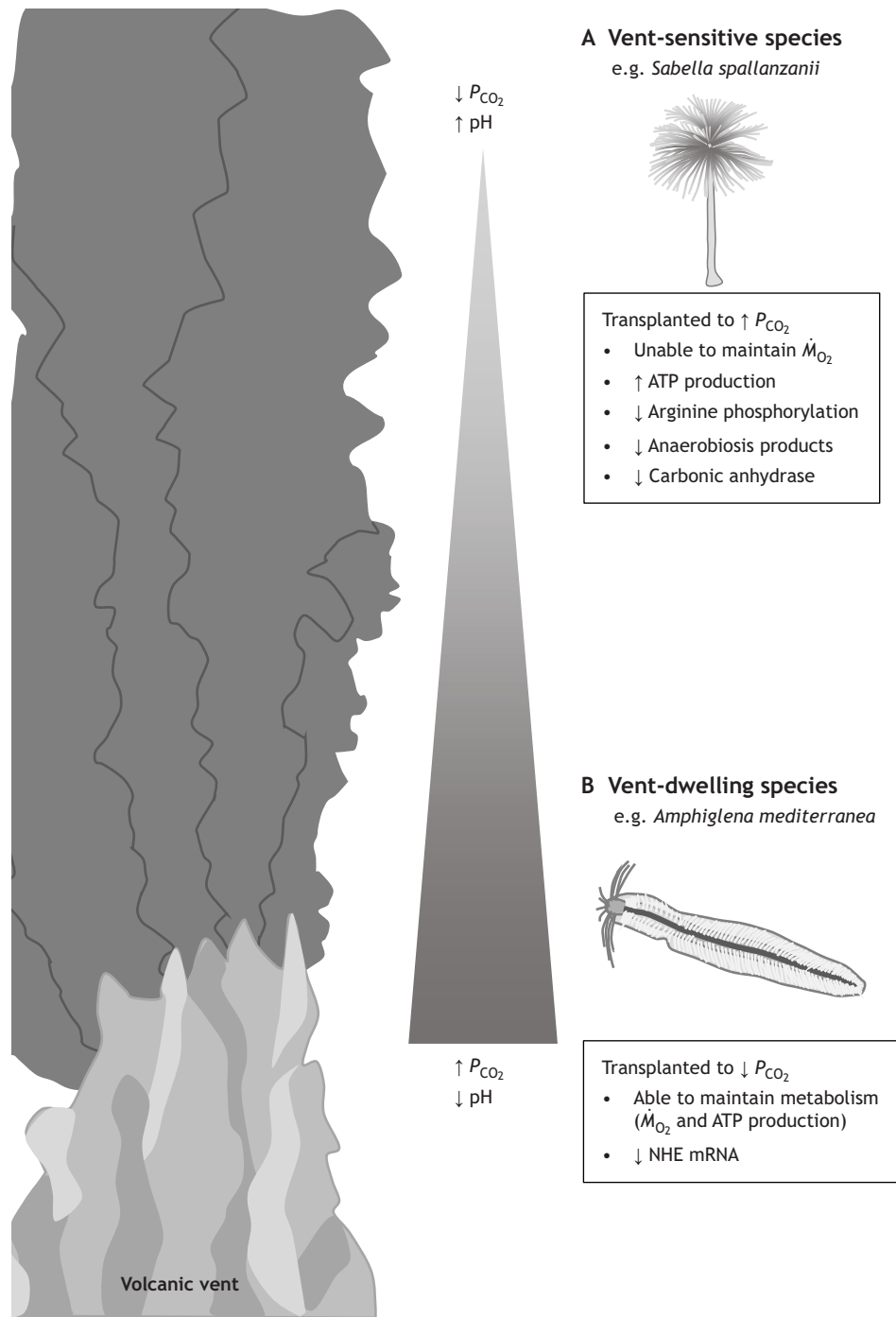

$\downarrow P_{CO_2}$
$\uparrow$ pH

A Vent-sensitive species
e.g. *Sabella spallanzanii*

Transplanted to $\uparrow P_{CO_2}$
- Unable to maintain $\dot{M}_{O_2}$
- $\uparrow$ ATP production
- $\downarrow$ Arginine phosphorylation
- $\downarrow$ Anaerobiosis products
- $\downarrow$ Carbonic anhydrase

B Vent-dwelling species
e.g. *Amphiglena mediterranea*

$\uparrow P_{CO_2}$
$\downarrow$ pH

Transplanted to $\downarrow P_{CO_2}$
- Able to maintain metabolism ($\dot{M}_{O_2}$ and ATP production)
- $\downarrow$ NHE mRNA

Volcanic vent

Fig. 3. Examples of polychaete species living along a pH gradient generated by an underwater volcanic vent. In response to transplantation from low to high P_{CO_2}, species A (e.g. *Sabella spallanzanii* cannot maintain metabolic rate ($\dot{M}_{O_2}$) and ATP production is increased, whereas arginine phosphorylation and anaerobiosis are decreased. This occurs with a reduction in carbonic anhydrase concentration, demonstrating an effect on respiration that indicates these modifications cannot be maintained long term. The vent-dwelling species B (e.g. *Amphiglena mediterranea*), however, can maintain metabolism when transplanted to low P_{CO_2} environments. Further, a decrease in sodium proton exchanger (NHE) mRNA after transplantation may hint at the energetic costs of living in the vents.

2016). Further, *P. dumerilii* collected from within the vents show ~80% reduction in body size when compared with those collected from control conditions (Calosi et al., 2013), thereby demonstrating changes in growth allocation that likely result from this environmental challenge. Species living in these fluctuating pH environments may be better suited for projected scenarios with increasing CO_2 and have genetic adaptations or plastic responses in place that can aid their survivability. However, survival may be dictated by genetic composition and thus result in decreased genetic diversity, causing large-scale, population-level issues during additional environmental stress. This idea extends beyond pH stressors, as any environmental stressor could drive genotype selection that ultimately affects future responses.

Multiple stressors
The examples discussed above largely reflect the responses of aquatic animals to single stressors examined under controlled laboratory conditions. This represents an excellent starting point for elucidating the homeostatic mechanisms employed by an animal to adjust to environmental fluctuations and for establishing the potential trade-offs that may arise from phenotypic plasticity. However, organisms in the natural environment, especially those in the shallow and near-shore environments discussed above, are simultaneously exposed to multiple stressors (Folt et al., 1999; Petitjean et al., 2019). These can include the combined effects of two or more abiotic stressors (e.g. simultaneous fluctuations in dissolved oxygen and temperature) or the addition of a biotic stressor (i.e. parasites or fluctuations in food resources). For example, parasites can potentially render hosts more susceptible to environmental fluctuations by overriding some of the mechanisms

discussed in previous sections: susceptibility to environmental hypoxia is known to increase with parasite infection in rainbow trout (*Salmo gairdneri*, now *Oncorhynchus mykiss*) (Woo and Wehnert, 1986), ninespine stickleback (*Pungitius pungitius*) (Smith and Kramer, 1987) and European eel (*Anguilla anguilla*) (Lefebvre et al., 2007). As our knowledge of the fundamental physiology of aquatic animals grows, additional factors should be considered to enhance our understanding of how these animals exist and thrive in the dynamic environments they inhabit.

When multiple stressors act upon an individual or population, the combined effects can be: (1) additive (the sum of the individual stressors); (2) synergistic (the effect is greater than the sum of the individual stressors); (3) multiplicative (the effect is the product of the individual stressors); or (4) antagonistic (the effect is less than that of the stressors on their own) (Crain et al., 2008; McBryan et al., 2016). The physiological response likely depends upon the mechanism(s) of action for each stressor. For example, synergistic outcomes may be more likely when stressors trigger physiological responses that act through distinct pathways (Fig. 4) (Lange et al., 2018). Similarly, the timing of the stressor exposure can play an important role in the organismal response. For instance, if a second stressor is applied while a response is still being mounted towards an initial stressor, interactive effects are more likely than if more time is permitted between exposures (Gunderson et al., 2016).

Phenotypic plasticity and potential genetic adaptation in coping with multi-stressor challenges
Given the multifaceted responses that can result from exposure to multiple stressors, it is critical to investigate combined effects of stressors at multiple levels of biological organization to better
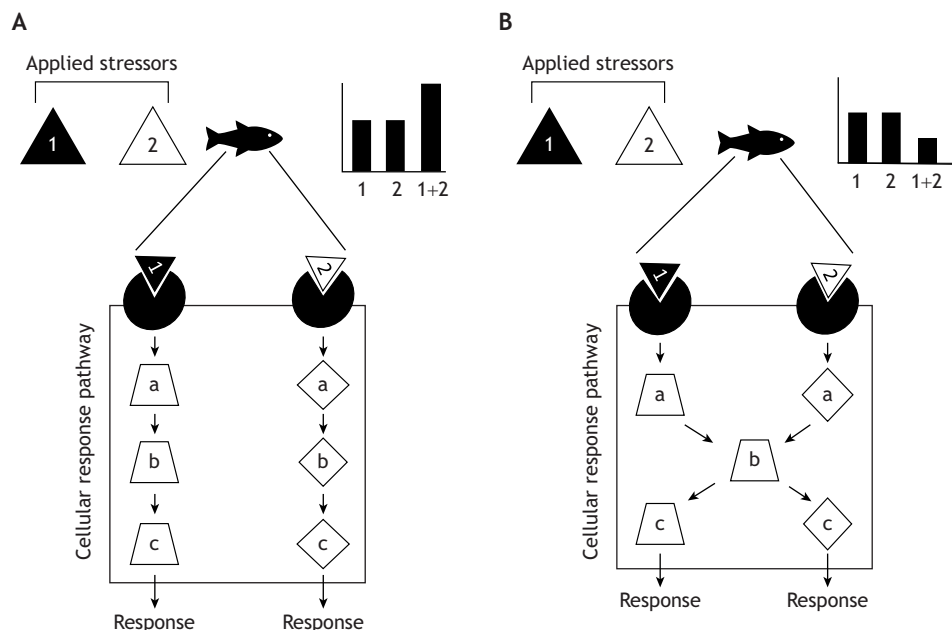

Fig. 4. Schematic representation of multi-stressor activation of cellular response pathways. (A) Each stressor (triangles) triggers a physiological response via two distinct pathways. Tolerance to additional stressors may then decrease as multiple pathways are already triggered. Inset: bar graph illustrating the synergistic effects of combined stressors (1+2) when compared with each stressor on its own. Alternatively, the distinct pathways could provide multiple mechanisms for plastic responses, mitigating the potential for additive effects [i.e. any trade-offs resulting from stressor 1 and 2 are not impacted by each other, as may be the case for the example described in Tietze (2016), where killifish exposed to low pH had increased metabolic rate that was unaffected by altered salinity, suggesting each stressor acted via an independent pathway and had no bearing on the other]. (B) Each stressor (triangles) triggers a separate physiological response acting via overlapping pathways. The overlap could mean that stressor 1 generates a reaction prior to the addition of stressor 2 and mitigates any additional effects, leading to a tolerance that presents as an antagonistic reaction. Inset: bar graph illustrating antagonistic effects of combined stressors (1+2). Alternatively, the prior activation of the shared pathway may result in a multiplicative effect where additional stress is added to an already activated pathway.

understand physiological responses to fluctuating environments. Because these types of multi-stressor experiments can be prohibitively costly and complicated to perform (i.e. each new variable requires exponential increases in the number of replicates), there has been limited experimental investigation on this topic to date and the majority of studies do not focus on fluctuating scenarios. In one multistressor experiment, McBryan et al. (2016) demonstrated how prior acclimation of killifish to elevated temperature increased their tolerance (time to loss of equilibrium) to acute hypoxia, relative to conspecifics acutely exposed to the same elevated temperature and challenged with acute hypoxia. The increased tolerance to hypoxia in thermally acclimated fish was likely due to the observed phenotypic plasticity in gill morphology, whereby respiratory surface area was increased during warm acclimation, which in turn facilitated oxygen extraction in hypoxia (McBryan et al., 2016). Killifish exposed to low pH also showed elevated oxygen consumption rates, with this effect persisting independent of the salinity of the exposure (Tietze, 2016). This last example indicates that the homeostatic responses to these two stressors were likely mediated through distinct pathways, and that the response to salinity did not incur any short-term energetic trade-off that would impact the response to acidity (Fig. 4).

The outcomes of multi-stressor experiments performed to date indicate that effects of multiple stressors are dependent upon the nature of the specific stressors, and their relative strength and duration. For example, in the natural setting, multiple stressors may occur simultaneously, or each individual stressor may have exposures that are continuous, cyclic or recurring, resulting in a myriad of slightly different exposure scenarios which may each yield distinct physiological responses. The cross-tolerance example outlined above, where acclimation to elevated temperature increased tolerance to acute hypoxia (McBryan et al., 2016), is an example of this complexity; it demonstrates that an inability of animals to acclimate to one condition if the environment fluctuates too frequently, as predicted with climate change, can come at the cost of reduced performance to a very different stressor that would be overlooked in single-stressor studies. At an organismal level, factors such as life stage, sex and individual and parental exposure history will also be important factors influencing phenotypic plasticity (Galvez, 2018; Lange et al., 2018). As the complexity of multi-stressor experiments has prevented widespread examination, the number of species examined is limited and reveals a profound need for investigation into multiple taxa to better understand these environmentally relevant combinations.

Physiological trade-offs

The importance of considering the ecological setting also applies to the impact of potential trade-offs associated with phenotypic plasticity. One notable trade-off facilitating homeostasis in fluctuating environments is the modulation of metabolic rate. An example of this is observed in brown mudfish (*Neochanna apoda*), a galaxiid fish inhabiting shallow humus-rich pools in temperate rainforests with peat-rich soils (Eldon, 1968). These pools vary in size and abiotic conditions according to prevailing precipitation patterns, but may become both anoxic and highly acidic (pH <4; White et al., 2015). In drought conditions, these pools can even evaporate to dryness, and the mudfish can persist for months in a state of pseudo-aestivation (see Glossary) within moist soil (Eldon, 1968). Survival in such conditions is achieved partly through phenotypic plasticity of metabolic processes such as reduced carbon dioxide excretion and depressed ion transporter activity, coupled with the physiological trade-off of a very low oxygen consumption

rate (Urbina et al., 2014). However, under heavy precipitation, these isolated pools flood and may become contiguous with more physicochemically benign streams inhabited by another galaxiid fish, the banded kokopu (*Galaxias fasciatus*). This species has a high metabolic rate (White et al., 2017) and, when it infiltrates forest ponds, rapidly extirpates the more sluggish mudfish, principally through outcompeting the mudfish for available resources (White et al., 2015). This effect is so pronounced that even though these species inhabit the same broad geographic ecoregion, their distribution is almost completely allopatric (see Glossary) (White et al., 2015, 2017). The metabolic rate trade-off made by mudfish to withstand the harsh abiotic stressors in their fluctuating environment means that they are highly sensitive to biotic stressors such as competition. This example serves to demonstrate the real-world consequences that physiological trade-offs can have in a fluctuating aquatic setting.

Conclusion

To live and thrive in dynamically fluctuating environments, animals must employ a range of reversible actions that ensure the basal functions that sustain life are maintained. Phenotypic plasticity is one key means by which animals can maintain biological function in the presence of environmental change. However, the importance of processes such as local adaptation (a fixed change in phenotype) and behavioural plasticity, which may be complementary processes that facilitate life in dynamic environments, should not be overlooked. From the studies described here, it seems clear that plasticity comes at a cost. Most commonly, this is an energetic cost, with an enacted change requiring a diversion of energy and resources towards the coping mechanism. Additional costs are then incurred when the environmental conditions and physiology revert to the 'norm'.

As experimental biologists, we are principally interested in controlling exposure scenarios to clearly delineate the effects of individual stressors on organisms. Such analyses are critical for teasing out mechanisms of effect, for making predictions about how stressors may influence organismal function over time. However, as our basal understanding of stressor effects develops, it is imperative that we consider measured laboratory responses in the context of real-world settings and/or develop tools and techniques that may allow us to measure phenotypic plasticity and the resulting physiological trade-offs in natural habitats and/or facing natural biotic stressors. Understanding the mechanistic basis behind survival in fluctuating environments may help us elucidate how many organisms will cope with a rapidly changing climate.

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