Oceanography and Marine Biology: An Annual Review, 2022, **60**, 625-680

Taylor & Francis

ADAPTATIONS AND PLASTIC PHENOTYPIC RESPONSES OF MARINE ANIMALS TO THE ENVIRONMENTAL CHALLENGES OF THE HIGH INTERTIDAL ZONE

ROBINE H.J. LEEUWIS & A. KURT GAMPERL

Department of Ocean Sciences, Memorial University of Newfoundland, St. John's, Canada

Abstract The high intertidal zone is home to an incredible variety of marine animals, as it offers an escape from low intertidal/subtidal predation and competition, among other advantages. However, this area of the shore also comes with many tide-driven and emersion-associated environmental stressors, such as desiccation, high temperatures and freezing stress, hypoxia, salinity fluctuations, nitrogenous waste accumulation, ultraviolet (UV) radiation, wave and ice disturbance and hydrogen sulphide (H_2S) toxicity. This review explores the diversity of evolutionary adaptations and plastic phenotypic responses that high intertidal animals use to cope with these challenges. Examples are provided of behavioural, morphological, physiological and biochemical adaptations/responses, along with some of the underlying molecular mechanisms that have been elucidated to date. Adaptations of many different worms, anemones, molluscs, crustaceans and fishes are highlighted. Many adaptations and mechanisms of plasticity are universal among animal phyla, and some are multifunctional (serve more than one function) or provide tolerance to multiple stressors (i.e. 'cross-tolerance'). High intertidal animals have received considerable attention by scientists, given their accessibility and that they can provide valuable insights into the transition from a marine to a terrestrial lifestyle. Nevertheless, further research is needed to understand the adaptations/responses of these animals more thoroughly, and the future holds great promise for accomplishing this with recent advances in epigenetics, transcriptomics, protein biochemistry and other molecular tools.

Keywords: Intertidal zone, marine animals, evolutionary adaptations, phenotypic plasticity, cross-tolerance.

Introduction

Scope of review

The intertidal zone is the interface between the marine and terrestrial realms and can be described as the area on the shore that is 'in between tides', i.e. the habitat between the lowest low tide and the highest high tide (Figure 1A) (Nybakken 1993). The intertidal can be divided into low, middle and high zones, although there is no standard/universal classification in the literature to distinguish them. For the purpose of this review, the high intertidal zone (also known as the upper eulittoral zone; Raffaelli & Hawkins 1999) is defined as the area most distantly removed from the ocean (the subtidal) and is generally characterized by exposure to air (i.e. emersion) during the majority of the day. Exceptions are high-shore tidepools and the upper intertidal region of estuaries where animals may be continuously submerged in water, and which can be better distinguished by the shore height relative to the tidal amplitude (e.g. at 1.4 m above the lowest low tide level with the highest high tide at 2.1 m).

DOI: 10.1201/9781003288602-13

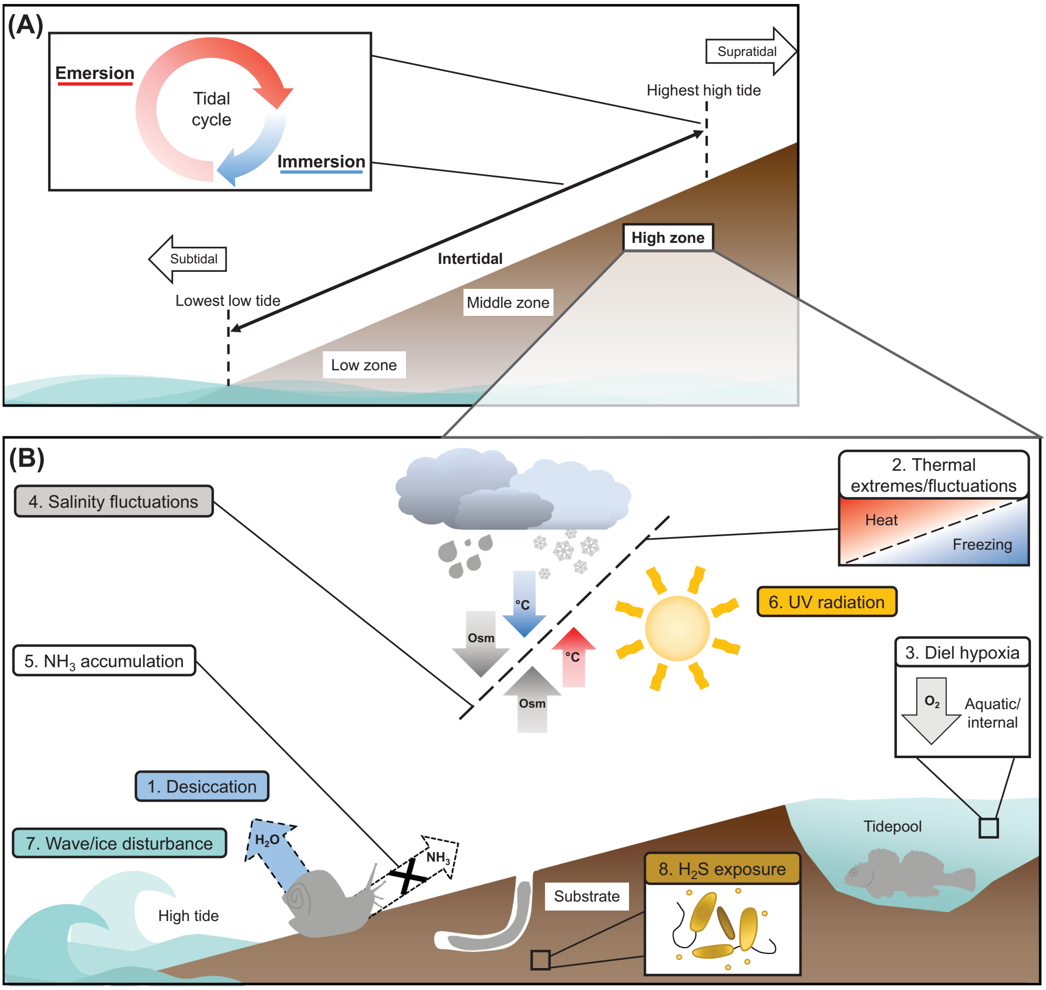

Figure 1 The location and definition of the high intertidal zone, and overview of the main abiotic challenges that marine animals are exposed to in this environment. (A) The intertidal (eulittoral) zone is the area on the shore that covers the distance between the lowest low tide and the highest high tide (Nybakken 1993), and it can usually be divided into a low, middle and high zone. The high intertidal zone is defined here as the furthest removed from the ocean/located at the highest relative shore height (e.g. at 1.4 m above the lowest low tide level with a 2.1 m tidal amplitude), and is often characterized by exposure to air (emersion) during the majority of the day (e.g. 16 hours). The intertidal zone is bordered at the lower end by the subtidal zone, which is never air-exposed during the tidal cycle, and at the upper end by the supratidal zone, which may only be wetted with seawater by wave splash. (B) Inhabitants of the high intertidal zone encounter various abiotic stressors in their environment that are often the result of the daily shift between immersion and emersion, and that are typically less severe in lower areas on the shore (Raffaelli & Hawkins 1999). Many of these stressors may occur simultaneously or at different times of the day (see Figure 2). The following abiotic challenges faced by these organisms are described in this review. (1) Emersion of considerable duration (hours) at low tide puts animals at risk of losing too much water through evaporation (i.e. desiccation stress). (2) High intertidal animals are exposed to a more thermally variable environment and greater heat stress and/or risk of freezing compared with low intertidal animals. This is largely due to the lower specific heat capacity of air as compared to that of seawater (see Table 1). (3) Aquatic hypoxia (e.g. in tidepools) can occur daily during low tide because of crowding, warming (during the day) or insufficient photosynthesis/excess respiration (at night) (Schulte 2011). Internal hypoxia may also be experienced by animals as a result of the collapse or retraction of respiratory structures during emersion, which are no longer able to take up oxygen (O_2), or due to

(Continued)

Figure 1 (*Continued*) stagnated haemolymph flow during freezing, which limits the O_2 supply to tissues. (4) Tidal movements, evaporation and freshwater input from precipitation and/or rivers can cause dramatic fluctuations in salinity (osmolarity) on the upper shore, which challenges animals to maintain their water and ionic balance. (5) Emerged animals are unable to excrete ammonium (NH_3) into the water, leading to a potential build-up of this toxic waste product in the body. (6) Because ultraviolet (UV) radiation is not filtered out by shallow seawater or during emersion, high intertidal animals may be at a greater risk of suffering UV damage (direct or indirect through the generation of reactive oxygen species) to their macromolecules (Denny & Gaines 2007). (7) Intertidal animals are subjected to wave action and other physical disturbances (e.g. from ice) at high tide, and this disturbance is more pronounced than in the subtidal zone given the ocean-to-land transition in the intertidal. (8) In intertidal habitats with a muddy or sandy substrate, hydrogen sulphide (H_2S) is produced by sulphate-reducing bacteria in the anoxic region of the sediment, and burrowing animals are exposed to high concentrations of this toxic compound.

There are many environmental challenges associated with the high intertidal zone (Figures 1B and 2), and these are often more pronounced/severe than elsewhere in the intertidal. For instance, the shift between immersion and emersion is problematic for marine animals, as seawater and air have very distinct, often opposite, characteristics (Dejours 1989) (Table 1). Compared to seawater, air is highly desiccating, has a very limited capacity to buffer temperature fluctuations, a low solubility for nitrogenous waste, and it does not filter out ultraviolet (UV) radiation (Nybakken 1993, Moyle & Cech 1996, Raffaelli & Hawkins 1999, Wright & Turko 2016). As a result, due to long emersion times, animals in the high intertidal are exposed to desiccation stress, temperature extremes and UV radiation, and face difficulties with nitrogenous waste excretion. High intertidal animals also frequently experience diel hypoxia (Richards 2011, Schulte 2011). Furthermore, large fluctuations in salinity can occur in estuaries and tidepools (Marshall 2012), and rocky intertidal zones are exposed to physical stress from waves and ice (Raffaelli & Hawkins 1999). Finally, in many intertidal environments, the sediment contains high concentrations of hydrogen sulphide (H_2S) which is toxic to most animals (Schulte 2011).

The aim of this review is to describe, and provide mechanistic bases for, the variety of adaptations and plastic (phenotypic) responses that high intertidal marine animals use to survive these various environmental challenges and flourish on this part of the shore. This topic has not been revisited since Raffaelli & Hawkins (1999), and to our knowledge, a synthesis entirely dedicated to this subject does not exist. In each of the following sections, adaptations are discussed and organized according to the environmental challenges that they protect against – although some are ‘multifunctional’ (serve multiple functions, e.g. see Hui et al. 2019) and/or allow for ‘cross-tolerance’ (tolerance against multiple stresses, e.g. see Sinclair et al. 2013); with the latter summarized at the end of this review. The high intertidal zone is an extremely diverse environment: it exists in all climates (from polar to tropical), is associated with substrates of different particle sizes (e.g. rock, gravel, sand, mud), can contain a large amount of decomposing seaweeds and debris (strandlines), terrestrial vascular plants can be present/absent (mangroves and salt marshes versus mudflats), and it can be associated with significant freshwater input (estuaries) (Nybakken 1993, Raffaelli & Hawkins 1999, Schulte 2011). Therefore, the exact nature of the local environment can greatly determine the type and severity of abiotic stressors that are encountered (Figure 3), and in turn, which adaptations and responses are required from its animal inhabitants; this will be discussed in the sections below.

In this review, the adaptations/physiological responses of a wide range of marine intertidal animals is illustrated with examples from fishes, crustaceans, molluscs, anemones and worms. This comprehensive exploration allows for the identification of adaptations that are universal among animal groups, and these are discussed in the concluding section of this article. Adaptations can be categorized as general strategies (e.g. avoidance versus tolerance) and can occur at various levels of biological organization – genetic, biochemical, physiological, morphological or complex

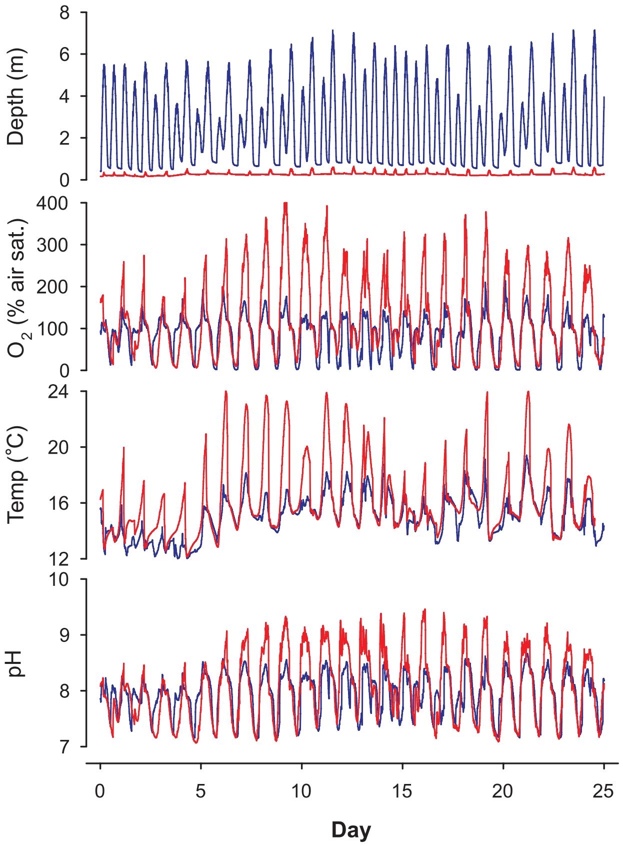

Figure 2 On-site measurements of several environmental variables (i.e. water depth, O_2 level, temperature, pH) within tidepools in the marine rocky intertidal zone at Wizard Island near the Bamfield Marine Sciences Centre, Bamfield, British Columbia, Canada. The data were collected over the course of 25 days, illustrating the type and scope of abiotic challenges that animals can experience in lower and upper parts of the shore. High tidepools (red lines) are shallower and have a very variable environment, with large fluctuations and greater extremes in O_2 level, temperature and pH. Environmental parameters in low tidepools (blue lines) are much more stable. Figure is reproduced/adapted from Richards (2011) with permission from the Journal of Experimental Biology (Company of Biologists, Cambridge, the UK).

behaviours. In some cases, it is possible to distinguish between responses that are short term and reversible within an animal's lifetime (i.e. phenotypically plastic), and those that are long-term and involve heritable genetic changes (i.e. evolutionary adaptations). We define phenotypic plasticity herein as the flexibility of animals with the same genetic background to develop different phenotypes depending on their environment (West-Eberhard 1989). The review concludes with brief perspectives on: (1) what makes the high intertidal zone an attractive habitat for marine animals, despite its harsh environmental conditions; (2) the valuable insights that can be obtained from research on high intertidal animals, especially in the current era of accelerated global climate change; and (3) important knowledge gaps and recommendations for future research on intertidal animals.

Table 1 Some important properties of seawater versus air, and the physiological consequences of emersion

Property	Seawater (35‰)	Air	Consequences of emersion
Water availability	High	Low	Desiccation[a]
Heat capacity[b]	5	1	Rapid heating/cooling
O_2 solubility	1	33	Higher O_2 availability if able to breathe air
NH_3 solubility	939[c]	1	Accumulation of NH_3
UV radiation absorption	Yes[d]	No	Higher UV radiation exposure
Density	821	1	Effect of gravity is more noticeable

Source: After Dejours (1989), Nybakken (1993), Moyle & Cech (1996) and Wright & Turko (2016).

[a] Seawater is desiccating for hypoosmotic animals due to osmotic water loss; however, air is more desiccating.

[b] Amount of heat (joules or calories) required to raise the temperature of 1 g of a substance by 1°C.

[c] In fresh water. There is no information available on the NH_3 solubility in seawater.

[d] The rate of UV absorption by seawater depends primarily on the concentration of dissolved organic matter (Tedetti & Sempéré 2006).

Values are ratios. If properties are dependent on temperature, the average is taken of the values at 0°C and 20°C.

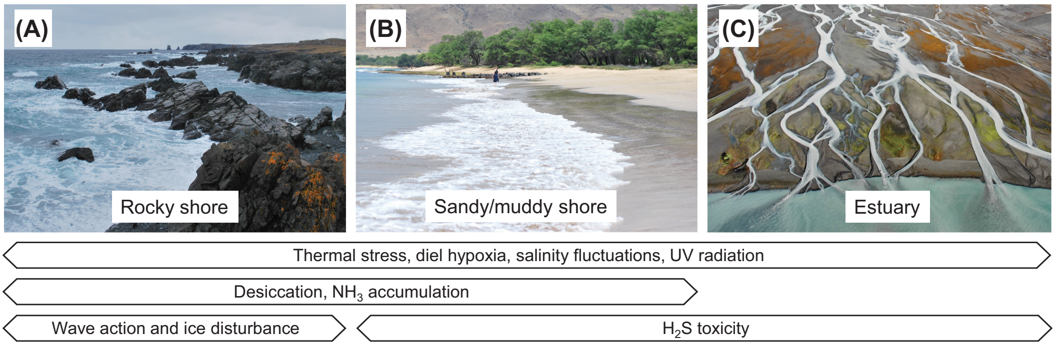

Figure 3 The abiotic stressors encountered by animals depends on the type of high intertidal habitat. For instance, the type of thermal stress (i.e. heat or freezing stress) depends on the climate of the intertidal habitat. Here, three main shore types are shown: rocky shores, sandy/muddy shores and estuaries. The occurrence of a stressor in these particular types of intertidal habitat is indicated by the horizontal bars. (A) Example of a rocky shore in Bonavista, Newfoundland, Canada. The rocky intertidal usually encounters more severe wave action than sandy/muddy shores, but generally lacks an anoxic sediment layer in which sulphate-reducing bacteria produce high levels of H_2S (Raffaelli & Hawkins 1999). (B) Example of a sandy beach in Kihei, Maui, Hawaii. Intertidal habitats with sandy/muddy shores include sand and mud flats, salt marshes and mangroves (Peterson 1991). (C) Example of an estuary in Kachemak Bay, Alaska, the USA. Because estuaries receive freshwater input from a river, estuarine animals are typically not exposed to many of the environmental challenges that are a consequence of emersion (Table 1). Estuaries are also often sheltered shores with minimal exposure to wave action. Photographs by the authors (panel A–B) and Alaska ShoreZone (panel C).

Topics not addressed

There are certainly more stressors associated with the high intertidal zone than those listed above. Large pH variations occur in tidepools and mudflats (due to fluctuations in the production and consumption of carbon dioxide, CO_2) (Figure 2) (Ganning 1971, Morris & Taylor 1983, Richards 2011). Due to frequent emersion on the upper shore, filter-feeders have limited time for feeding which can reduce the amount of energy available for growth and reproduction (Bayne et al. 1988), animals experience reduced buoyancy (Table 1), and may be unable to release sufficient quantities of metabolically produced CO_2 (Schulte 2011, Martin 2014, Wright & Turko 2016). High intertidal animals are also exposed to terrestrial pathogens and parasites, with characteristics that may differ from those in the marine environment (You et al. 2014, 2018). However, these stressors/challenges are not the focus of this review, because information on these topics is limited or only available/relevant for a specific animal group (e.g. suspension-feeders). Adaptations of high intertidal animals that allow them to avoid terrestrial predation (e.g. the use of camouflage in rockpool fish; see Stevens et al. 2014) or that are important in competitive/territorial behaviour (e.g. the role of the decapod chelipeds in agonistic interactions; see Lee 1995) are also not discussed, because this review concentrates on abiotic stressors, and similar adaptations against biotic stressors are found among animals in other marine environments. Thus, the emphasis of this work is on adaptations and phenotypic responses that are unique to animals inhabiting the high intertidal zone or driven/magnified by the tidal cycle.

This review focuses on studies that specifically compare high intertidal animals with low intertidal species of similar taxonomic classification (e.g. the high shore sculpin *Oligocottus maculosus* versus the low shore sculpin *Blepsias cirrhosus*), or with individuals of the same species that also inhabit the low intertidal zone (e.g. high shore versus low shore *Mytilus edulis* mussels), because this approach minimizes confounding phylogenetic effects. Studies on species that are abundant in the eulittoral fringe (the extreme upper end of the shore that is only wetted by waves during spring tides) are also highlighted whenever possible (e.g. the snail *Echinolittorina malaccana*, Marshall & Ng 2013). Sometimes, there are few studies of high intertidal animals related to a particular topic, and in such cases, adaptations of high intertidal animals that have not (yet) been recorded/observed among subtidal animals are also briefly discussed. Marine animals that are tidal migrants [i.e. move along with the wave splash, like the common sand crab (*Emerita analoga*); Lastra et al. 2004], or that exclusively inhabit the supratidal/splash zone (the area above the eulittoral zone/highest high tide level), are not covered by this review. The same applies to the few terrestrial animals found in the high intertidal zone that can tolerate exposure to the marine environment to some degree, such as insects, centipedes and pseudoscorpions (Raffaelli & Hawkins 1999). Shores with gravel or very coarse sand as a substrate are not discussed in this review, because they appear inhospitable to marine animals and are regarded as ‘intertidal deserts’ (Raffaelli & Hawkins 1999).

Almost all phylogenetic groups are represented in the high intertidal environment and covered in this review, with the exception of echinoderms which are typically restricted to the low intertidal zone. The high intertidal zone has tidal and seasonal visitors, but this review is focused on residents that inhabit this area during the majority of the year and/or their life cycle (Moyle & Cech 1996, Lam et al. 2006). Therefore, the adaptations of subtidal animals that temporarily remain in the high intertidal to build nests, deposit their eggs and/or provide parental care (e.g. plainfin midshipman, *Porichthys notatus*; and sharpnose sculpin, *Clinocottus acuticeps*) (Martin et al. 2004), are also not discussed here.

Adaptations to the environmental challenges of the high intertidal zone

Desiccation tolerance and resistance

Animals in the high intertidal zone are typically air-exposed for the majority of the time (e.g. 20 hours/day in the high intertidal snail *Littorina scutulata*, Stickle et al. 2017; and 98% of the time in some eulittoral fringe snail species as an extreme case, Monaco et al. 2017). With the exception of animals on exposed rocky shores that receive a large amount of wave splash, the most important coping mechanisms during emersion are probably those against the desiccating effects of air exposure (Table 1) (McMahon 1990, Raffaelli & Hawkins 1999). Some intertidal invertebrates (e.g. sipunculid worms and the high intertidal limpet *Lottia digitalis*) are highly tolerant of desiccation and can survive large short-term water losses (up to ~45% of their total body water content) (Oglesby 1969, Roland & Ring 1977). Likewise, there are examples of intertidal fishes (e.g. clingfish species *Tomicodon humeralis* and *Pherallodiscus funebris* from the Gulf of California) that are able to sustain extreme (as great as 60% of their total body water content) water losses (Horn & Gibson 1988). Differences in desiccation tolerance have also been reported between high versus low intertidal gastropods. In Brunei Darussalam, the high intertidal tropical snail species *Echinolittorina malaccana* only experiences low levels of mortality (<20%) when exposed to warm air for several days despite a ~13% water loss, while two local lower intertidal species *Planaxis sulcatus* and *Echinolittorina vidua* experience 100% mortality after only a ~7% water loss, and cannot withstand more than 1–2 days under these conditions (Miranda et al. 2019). Although differences in upper thermal tolerance between these tropical species is also a contributing factor, it is clear that the greater survival time during emersion (LT_{50}) and the amount of water loss until mortality (LD_{50}) both facilitate the upward extension of the intertidal distribution of *Echinolittorina malaccana*. In the South African limpet *Siphonaria oculus* from the uppermost intertidal zone, both LT_{50} and LD_{50} are also higher than reported for the local lower shore limpet *Patella granularis* (Marshall & McQuaid 1992a). Among snail species of the *Nucella* and *Littorina* genera from the northern Washington coast, LT_{50} increases similarly with shore height (Stickle et al. 2017).

However, it appears that an enhanced desiccation tolerance is not the most common strategy used by high intertidal animals when emersed. Instead, they appear to be primarily adapted to avoid, or reduce the rate of, water loss (e.g. see McMahon 1990 with regard to the decline in evaporative rates in eulittoral fringe gastropods). Further, several high shore ecotypes of the rough periwinkle *Littorina saxatilis* (from the White and North Seas) survive longer in air because they avoid water loss better than their low intertidal relatives; although the mechanisms underlying the reduced evaporation rates/improved water conservation were not identified (Sokolova et al. 2000, Sokolova & Pörtner 2001a). Ecotypes are morphological variants within a species that have undergone incomplete speciation, and they are often well studied, and can be useful models for understanding adaptations across a vertical shore gradient. *Littorina saxatilis* ecotypes and populations (also described as a ‘species aggregation’) have received particular attention as they display substantial phenotypic variation in response to environmental factors, while genetic divergence is limited (Panova et al. 2014). In the avoidance strategy, the first line of defence against desiccation upon emersion is a behavioural response (Table 2). High intertidal animals minimize exposed surfaces by, for instance, retracting feeding appendages (tentacles in anemones and cirri in barnacles) or tightly closing their shell (bivalves) (Shick 1991, Wolcott & Wolcott 2001, Denny & Gaines 2007). The mussel *Mytilus californianus* frequently uses this strategy, and individuals in the high intertidal zone close their shell for ~81% of the time compared to ~39% in low intertidal individuals (Gleason et al. 2017). In addition to contracting their tentacles, anemones commonly attach gravel or shells to themselves. In *Anthopleura elegantissima*, this is particularly important at high temperatures (>20°C) and wind speeds (2.2 m/s), as under these conditions such actions reduce the rate of water loss by ~64% and increase survival 2-fold (Hart & Crowe 1977). High intertidal individuals also cover themselves with more gravel than low intertidal individuals when exposed to sunlight (Dykens & Shick 1984). Finally, high intertidal *Littorina* species in Panama substantially reduce water loss during emersion by retracting inside their shell and sealing off the shell’s opening with an opercula (Garrity 1984).

Mobile animals are also able to cope behaviourally by finding shelter/a favourable microhabitat. Many high intertidal animals have been reported to seek refuge in tidepools, crevices/moist places underneath rocks (e.g. snails, crabs, stichaeid fishes, clingfish) (Garrity 1984, Denny & Gaines 2007, Martin 2014, Wright & Turko 2016), in beach wrack (e.g. isopods, amphipods) (Wolcott & Wolcott 2001), inside galleried mangrove logs (e.g. the mangrove killifish) (Taylor et al. 2008), or in burrows

Table 2 Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
Desiccation	↑ Avoidance	Behavioural	Attaching gravel (anemones); contracting/clamming up (anemones, molluscs, barnacles); seeking shelter (snails, crustaceans, fishes); retreating to low tidal area/tidepool; rolling in mud (fishes)
		Morphological	↑ Shell volume:circumference and height; ↓ aperture size (snails); ↑ body size (crabs); ↓ surface area:weight (fishes)
		Physiological/biochemical	↑ Mucus (snails, fishes); ↑ embryo/total body water content (crabs, fishes); ↓ water permeability of gills/skin/eggs/embryos; staying in water triggered by vaso- and isotocin release; ↑ water intake by activation of forebrain thirst centre (fishes)
	↑ Tolerance	Physiological/biochemical	↑ Ability to survive large water losses (worms, snails, fishes)
Heat stress	↑ Avoidance	Behavioural	Aggregate, strategic shell orientation/standing (snails); use thermal refuge in crevice/tunnelled wood (snails, fishes); sponging/emergence to ↑ evaporative cooling (crabs, fishes); cooling by wave splash (fishes)
	↑ Tolerance	Physiological/biochemical	↑ Heat stability of metabolic enzymes (molluscs); metabolic depression (snails); ↑ heat resilience of the heart; ↑ aerobic/air-breathing capacity (snails, crabs); ↑ constitutive and/or heat-induced heat shock protein levels (molluscs, fishes)
Freezing stress	↑ Avoidance	Physiological/biochemical	↓ Ice crystal growth by antifreeze proteins (worms) or viscous mucus (snails)
	↑ Tolerance	Morphological	↑ Body size to ↓ freezing rate (snails)
		Physiological/biochemical	Extracellular ice-nucleating agents; ↑ intracellular compatible organic osmolytes; ↑ anaerobiosis (molluscs); ↓ protein synthesis by RNA interference (snails)
Thermal fluctuations	↑ Avoidance	Behavioural	Thermoregulatory shuttling (crabs)
	↑ Tolerance	Physiological/biochemical	Ability to tolerate extreme temperature changes (snails, fishes); ↓ thermal sensitivity of metabolism (Q_{10}) or aerobic scope (crabs, fishes)

(*Continued*)

Table 2 (*Continued*) Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
Diel hypoxia	↑ Aerobic capacity	Behavioural	Gaping (bivalves); aquatic surface respiration (shrimps, fishes); emergence; tidepool hopping; ferrying air into burrows (fishes)
		Morphological	Respiratory leg structures, water recirculation over gills (crabs); ↑ epithelial vascularization (crabs, fishes); ↑ gill surface area and structural support; ↓ thickness of gill secondary lamellae (fishes)
		Physiological/biochemical	↑ O_2 affinity of haemoglobin/haemocyanin or stability thereof (decapods, fishes); ↑ haemoglobin level; ↑ efficiency of oxidative phosphorylation; ↑ maximum O_2 consumption/O_2 affinity by cytochrome *c* oxidase (fishes)
	↑ Anaerobic capacity	Physiological/biochemical	↑ Fermentable energy reserves (snails, crabs, fishes); ↑ lactate dehydrogenase activity (fishes)
	↓ Metabolism	Physiological/biochemical	↓ Heart rate (mussels, fishes); ↓ metabolic enzyme activities (constitutively/temporarily) (molluscs); ↓ expression of genes involved in energy-consuming and O_2-dependent processes (fishes)
Salinity stress	↑ Avoidance	Behavioural	Contracting/clamming up (bivalves, barnacles), seeking wetter microhabitats (amphipods, crabs)
		Physiological/biochemical	↓ Respiration and heart rate (worms, snails, fishes); ↓ ion loss by ionocyte retraction; ↓ gill/skin/intestinal water permeability (fishes)
	↑ Osmoregulatory capacity	Physiological/biochemical	Adjustments in free amino acid levels by changing metabolic/biosynthetic gene expression (anemones, worms, molluscs); regulating urine tonicity (crustaceans, fishes); NaCl uptake/secretion by gill/operculum ionocytes (crabs, fishes)
NH_3 accumulation	↓ NH_3 production	Physiological/biochemical	↓ Protein and (partial) amino acid catabolism (fishes)
	↑ NH_3 excretion	Morphological	Water trapping in the gills (amphipods, fishes)
		Physiological/biochemical	↑ Nitrogenous secretion rates (snails); NH_3 volatilization (isopods, fishes); active secretion and ↑ NH_3 transporter effectivity in the gills (fishes)
	↑ NH_3 detoxification	Physiological/biochemical	Recycling through endosymbiotic algae (anemones); ↑ conversion into glutamine and/or urea (snails, crustaceans, fishes)
	↑ NH_3 tolerance	Physiological/biochemical	↑ NH_3 tolerance of brain (fishes)

(*Continued*)

Table 2 (*Continued*) Overview of the adaptations and plastic phenotypic responses of marine animals to the main environmental challenges of the high intertidal zone

Challenge	Strategy	Type of adaptation	Examples of adaptations across animal groups
UV radiation	↑ Avoidance	Behavioural	↓ Exposed external surface (anemones, molluscs); seeking shelter (fishes)
	↑ Protection	Physiological/biochemical	↑ Sunscreens (mycosporine-like amino acids) (fishes); ↑ antioxidant levels in column (anemones) and gametes (bivalves); ↑ gene expression of antioxidants and DNA repair enzymes (snails)
Wave action/ice disturbance[*]	↑ Avoidance	Behavioural	Restricting activity to low tide (snails)
	↑ Physical resilience	Morphological	Adhesion with byssus threads (mussels), cement (barnacles), suction (snails, fishes); ↓ wave drag by ↓ spiral shell height/weight; ↑ foot/aperture size (snails)
		Physiological/biochemical	↑ Shell strength by ↑ constitutive gene expression of shell matrix proteins (snails)
H_2S toxicity	↑ Avoidance	Behavioural	Staying at surface of sediment/water; emergence (fishes)
	↑ H_2S detoxification	Physiological/biochemical	Use of H_2S as electron donor for ATP production (chemolithoheterotrophy); symbiosis with H_2S-oxidizing bacteria (worms, bivalves); ↑ enzymatic detoxification by oxidation into thiosulphate (worms, bivalves, fishes)

[*] The intertidal zone is exposed to a greater amount of wave action and ice disturbance than the subtidal zone. However, this stressor is more severe in the lower than in the higher intertidal zone (see text). Adaptations that are found among intertidal animals, in general, are included in this table, whereas adaptations specific to low intertidal animals are excluded.

in the mud or sand (e.g. mudskippers) (Marshall 2012). Shelter seeking is particularly important for intertidal individuals of the mud crab *Eurypanopeus depressus*, as their desiccation tolerance is not different from the subtidal population (Grant & McDonald 1979). Finally, in addition to withdrawing to their burrows, mudskippers also retreat to other sources of water (e.g. tidepools or the low tidal zone) to drink/rehydrate (Sakamoto et al. 2015), and regularly roll onto their sides on wet substrates (e.g. the mud) to keep their skin moist (Gordon et al. 1968, Fenwick & Lam 1988).

Among intertidal snails, it has been well-documented that a specific shell morphology is often associated with greater desiccation avoidance (e.g. see Branch 1985 and references below). In limpets, for instance, water loss is reduced by having a higher volume to circumference ratio (e.g. in intertidal versus subtidal *Nacella concinna*; Weihe & Abele 2008) and a higher shell height (e.g. in high versus low shore *Siphonaria lessonii*; Nuñez et al. 2018). The latter general trend towards taller shells with increasing shore height has also been suggested to be a morphological adaptation to avoid/reduce heat stress, as it facilitates convective cooling by the wind, and the relatively smaller contact area with the substratum reduces conductive heat transfer (Harley et al. 2009). In periwinkles, a smaller aperture (i.e. the main shell opening) has also been shown to minimize desiccation (e.g. in high versus low shore *Littorina saxatilis* from Spain) (Martínez-Fernández et al. 2008). In eel-like stichaeid fishes, both desiccation tolerance and resistance are greatest in species that live high in the intertidal zone. This has been linked to a higher whole-body water content (thus, larger water losses can be sustained) and a reduced surface area to weight ratio (i.e. a more cylindrical body with a wider cross section, which allows for lower weight-specific water evaporation rates) (Horn & Riegle 1981). Total body water content is the same among sympatric hermit crab species (i.e. species that evolved in the same environment from a common ancestor) that have different vertical distributions within the intertidal. However, high intertidal species (*Clibanarius vittatus* and *C. sclopetarius*) have the largest body size, and this was associated with a slower rate of water loss upon emersion and a longer survival time (Turra & Denadai 2001).

There are also examples of anti-desiccation adaptations associated with physiology in high intertidal animals. High intertidal limpet species from the genus *Lottia* (also known as the genus *Acmaea*) have desiccation rates 1/10th of their low intertidal relatives, which is attributed to the production of a mucous barrier between the shell and the substrate (Wolcott 1973). Mudskippers and rockskippers (blennies) also secrete considerable amounts of mucus onto their gills and skin to reduce evaporation when exposed to air (Whitear & Mittal 1984, Yokoya & Tamura 1992, Lam et al. 2006). In various intertidal and estuarine fishes (e.g. Australian, Atlantic and mangrove killifish, *Austrofundulus limnaeus*, *Fundulus heteroclitus*, *Kryptolebias marmoratus*; and the giant mudskipper, *Periophthalmodon schlosseri*), water permeability of the gills, skin and eggs/embryos is very low, or can be reduced via remodelling/plasticity, and this minimizes water loss on land (Marshall 2012). *Kryptolebias marmoratus* can maintain whole-body water levels while emersed for several days, and is also able to increase cutaneous water influx during this time, which may benefit water absorption through the skin (e.g. while remaining in a moist refuge during the dry season) (LeBlanc et al. 2010, Heffell et al. 2018). These changes in water permeability could be, in part, related to a rapid (i.e. within hours) increase in the expression of aquaporin genes (e.g. aquaporin 3) (Dong et al. 2021). Finally, females of some upper intertidal porcelain crab species (e.g. *Petrolisthes laevigatus* and *P. violaceus*) provide ~20% more water to their embryos than subtidal species (such as *Allopetrolisthes punctatus*) from the same local environment, which likely improves their survival in air (Viña et al. 2018). The higher embryo water content is either accomplished during oogenesis, or during embryonic development as brooding females may behaviourally select more environmentally stable/favourable microhabitats.

Among amphibious fishes – which are able to spend time out of the water as a normal part of their life history – the mudskippers (Periophthalminae) are probably the most 'terrestrial' and active on land, so they face the greatest risk of desiccation (Sayer & Davenport 1991, Martin 1995). A detailed overview of the behavioural, morphological and physiological adaptations in mudskippers (and other fishes) to the tropical intertidal environment, including adaptations to lower the risk of desiccation, can be found in Lam et al. (2006) and Jaafar & Murdy (2017). The mudskipper's behaviour of seeking refuge in burrows is associated with an increase in water intake and an enhancement of water reabsorption in the gut and kidney (see review by Katayama et al. 2018). All of these responses appear to be regulated/stimulated by the release of various hormones, such as prolactin and the neurohypophysial hormones vasotocin and isotocin. During terrestrial exposure, prolactin mRNA levels in the pituitary, and prolactin levels in the blood, increase in the mudskippers *Periophthalmus modestus* (Sakamoto et al. 2005) and *P. chrysospilos* (Lee & Ip 1987), respectively. Further, when the high intertidal *Periophthalmus modestus* is kept out of water, the expression of vaso- and isotocin precursors in the brain is upregulated, and after an intracerebroventricular injection of vaso- and isotocin, there is an increased tendency to remain in the water (Sakamoto et al. 2015). Interestingly, these hormones also regulate water balance in aquatic fishes. However, mudskippers are unique in that they need to experience the sensation of thirst, and actively move to water, to rehydrate (Katayama et al. 2018). In the shuttles mudskipper (*Periophthalmus modestus*), a putative thirst centre similar to that of mammals has been identified in the forebrain (lamina terminalis), which is neuronally activated by dehydration, and that has vascular permeability (i.e. it is permeable to circulating proteins, in contrast to the blood-brain barrier), and thus, may be involved in sensing blood osmolarity (Hamasaki et al. 2016).

Tolerance to temperature-related stress

Temperature is another dominant environmental factor impacting the lives of intertidal animals. Mechanisms responsible for the heat tolerance of intertidal animals have also received a considerable amount of attention among scientists, because of their relevance in the context of global warming. Animals in the high intertidal zone, which are isolated from the ocean (i.e. in tidepools) for prolonged periods, experience greater temperature extremes and more rapid thermal fluctuations than those in the lower intertidal zone (Figure 2) (Raffaelli & Hawkins 1999, Richards 2011). Further, as seawater has a 5-fold greater heat capacity as compared to air (Table 1), these thermal challenges are magnified in those species that are emerged for long durations. Adaptations that help them to cope with heat, freezing temperatures and high temperature variability, are discussed below.

Heat tolerance

Numerous field measurements in tidepools, rocky shores and mussel beds in temperate and tropical climates have shown that animals in the high intertidal zone typically experience much higher temperatures (on a diurnal and seasonal basis) than those in the low intertidal zone (Figure 2) (Morris & Taylor 1983, Huggett & Griffiths 1986, Roberts et al. 1997, Nakano & Iwama 2002, Pulgar et al. 2005, Petes et al. 2008, Fangue et al. 2011, Richards 2011, Gleason et al. 2017, Monaco et al. 2017, Stickle et al. 2017). For example, during the summer months, the high edge of mussel beds (*Mytilus californianus*) (at ~2 m above the lowest tide level) on the north-western Pacific coast can experience average high temperatures of ~30°C, in contrast to ~15°C for the low edge beds (at ~1 m above the lowest tide level) (Petes et al. 2008). In tropical regions, high intertidal animals can be exposed to extreme heat, such as in Hong Kong, where the two high-shore snail species *Echinolittorina malaccana* and *E. radiata* are found on rock surfaces that reach temperatures of 52°C–55°C (Seuront & Ng 2016). Although heat stress can occur in all intertidal habitat types (Figure 3), extreme high temperatures in the tropics may be more common on rocky shores as compared to sandy shores or mudflats. For example, in Singapore, temperatures on the upper rocky shore often exceed 50°C, while they rarely reach 40°C on sandy/muddy substrates (Nguyen et al. 2011). This may be related to differences in physical substrate characteristics such as colour and wettability, or because of the higher occurrence of shade-providing vegetation on soft substrate shores. For instance, the maximum estimated body temperatures of tropical snails in sheltered/shaded mangroves across various geographical locations are ~20°C lower than in sun-exposed rocky shores (Marshall et al. 2015).

Some animals, such as the clingfish *Sicyases sanguineus*, are able to behaviourally avoid high temperature extremes as they remain close to the waves and are cooled by the spray (Ebeling et al. 1970). The tropical high intertidal gastropod *Nerita scabricosta* also successfully lowers its body temperature (by ~6°C) by avoiding open rock surfaces and seeking refuge in crevices, and can substantially reduce warming by aggregating with conspecifics (Garrity 1984). In contrast, the periwinkle *Echinolittorina peruviana* appears to use a thermoregulatory orientation behaviour. On warm days it faces the sun frontally, so that the small lateral side of the shell is exposed, and this reduces heat gain by as much as 7°C (Muñoz et al. 2005). These examples of thermoregulatory behaviour represent two types of responses that high intertidal ectotherms can exhibit to cope with high temperatures: the 'flight' response (to escape the most extreme heat by seeking a cooler environment, i.e. the clingfish using wave spray, and *Nerita scabricosta* hiding in crevices) and the 'fight' response (where individuals modify their own environment to minimize extreme temperatures, i.e. *N. scabricosta* aggregations, and the periwinkles' shell orientation strategy) (Ng et al. 2017). The importance of thermoregulatory behaviours was highlighted by Marshall et al. (2015) in a study of 40 tropical eulittoral snail species. This research showed that animals in rocky shore habitats can experience extreme temperatures that on average exceed their heat coma temperature (HCT) and upper lethal limit temperatures (ULT), and thus, finding sheltered microhabitats is essential to survival.

High intertidal Littorinid snails have been reported to display various flight and fight thermoregulatory behaviours (reviewed by Ng et al. 2017), and 'shell standing' is an interesting illustration of a multifunctional adaptation in this taxa. Shell standing is when aestivating snails (withdrawn into their shells during the hottest time of the day) attach their shell to the substratum with mucus in such a way that the aperture faces perpendicularly to the surface, as opposed to downwards. This posturing behaviour allows for reduced heat gain/improved cooling, but has also been suggested to play a role in mating (e.g. in the rocky-shore, eulittoral fringe species *Echinolittorina malaccana*) (Marshall & Ng 2013, Seuront & Ng 2016). Likewise, the multifunctional 'sponging' behaviour (lowering of the body onto the sediment to take up pore water by setal capillary action) of the high tropical shore crab *Scopimera intermedia* helps with cooling (reduces average body temperature by ~1.3°C), while also benefitting feeding (supplies water for flotation feeding where lighter food particles are separated from heavier sediment grains) (Hui et al. 2019). In the latter, cooling is likely facilitated through evaporation and increasing the animal's specific heat capacity. Another example of a multifunctional adaptation is seen in dartfish (*Parioglossus interruptus*) that seek refuge during low tide in tunnelled mangrove wood (Hendy et al. 2013). This behaviour's primary purpose is most likely to avoid high water temperatures, as within the wood daytime thermal maxima are 6.5°C lower as compared to adjacent tidepools. However, these cryptic niches may also allow for protection against predation and desiccation.

Although behavioural thermoregulation is an important strategy that allows high intertidal animals to stay cool during warm days, it is not always possible. The 'gaping' behaviour of mussels (intermittently opening the shell to allow for aerial O_2 uptake, see 'Tolerance to diel hypoxia' section), for instance, could theoretically also allow for evaporative cooling. Nevertheless, it was shown in *Mytilus californianus* that gaping has no significant effect on body temperature (Fitzhenry et al. 2004), probably as the shell is only occasionally opened (<20% of the time) to minimize water loss (Gleason et al. 2017). Littorinid snails restricted to the eulittoral fringe appear to rely little on evaporative cooling (or are limited in the use thereof) compared to species in lower parts of the eulittoral zone, because of the greater need to conserve water (McMahon 1990). Furthermore, slow-moving animals (e.g. limpets) are not always able to effectively utilize behavioural thermoregulation, because they may not be able to get to a favourable microhabitat in a relevant timescale (i.e. within a few hours) (Harley et al. 2009). For fish in tropical tidepools, thermal refuge options can also be limited, and they may need to stay at high temperatures until they decrease naturally (Lam et al. 2006). Even when escaping stressful thermal conditions in the water is possible, there may be important benefits to remaining in this habitat. For instance, when mangrove rivulus (*Kryptolebias marmoratus*) are exposed to hot water temperatures, they can jump out of the water to take advantage of evaporative cooling on the land (Gibson et al. 2015). However, remaining in the water allows the fish to continue to forage for aquatic prey and avoid terrestrial predators.

Because of the high temperatures encountered, high intertidal animals also typically acquire an intrinsically higher heat tolerance. For instance, a survey of 60 intertidal gastropod species representing seven superfamilies showed that thermal tolerance (measured as HCT) increases with shore height (McMahon 2001). This trend was particularly well-supported given that species included in the survey occupied various habitat types (rocky shores, mangroves and salt marshes), and were from ten geographically separate sites with different climates (temperate, subtropical and tropical). Littorinid snails have also been reported to have a 2.7°C greater mean HCT than non-littorinid snails, which may explain the worldwide dominance of this superfamily on high shores. In another study, involving 34 tropical invertebrate species, acute thermal tolerance was also found to be positively correlated with tidal height (Nguyen et al. 2011). Sometimes, this relationship is visible even among individuals of the same species. For example, tidepool sculpins (*Oligocottus maculosus*) from the high intertidal, have a slightly (0.5°C) higher critical thermal maximum (CT_{max}; the temperature at which loss of equilibrium occurs) than those from the low intertidal (Fangue et al. 2011).

There are several physiological/biochemical mechanisms that have been linked to the higher heat tolerance of high intertidal animals. In high intertidal crustaceans and molluscs, the heat resilience of the heart and the maintenance of O_2 supply/consumption play an important role. *Petrolisthes* porcelain crabs exhibit a clear correlation between thermal tolerance (measured as the LT_{50}; the temperature at which 50% mortality occurs) and intertidal vertical position (Stillman & Somero 2000). In the higher intertidal porcelain crab *Petrolisthes cinctipes*, which can be emerged for more than half of the day, the temperature of cardiac failure (Arrhenius breakpoint temperature; ABT) was 5°C higher than in its lower intertidal relative *Petrolisthes eriomerus*, which is emerged only during low spring tides (Stillman & Somero 1996). During emersion, *P. cinctipes* could also maintain a higher rate of O_2 consumption ($\dot{M}O_2$) and better avoid lactate accumulation than *P. eriomerus*. This was attributed, in part, to *P. cinctipes* having a specialized membranous structure on each walking leg that has an aerial respiratory function. Similar to porcelain crabs, there is a strong relationship between vertical distribution, thermal tolerance and ABT in several intertidal snail species from both temperate and tropical climates (Tomanek & Somero 1999, Stenseng et al. 2005, Dong & Williams 2011, Monaco et al. 2017). For instance, the ABT of the limpet *Cellana grata* which is abundant in the high zone of tropical rocky shores (at ~1.5–2 m above the lowest tide level) is ~5.6°C higher than in the local low intertidal congener *Cellana toreuma* (at ~1.25–1.5 m above the lowest tide level) (Dong & Williams 2011). Further, $\dot{M}O_2$, cardiac performance and thermal limits are linked when the upper intertidal limpet *Lottia digitalis* from California is exposed to increasing temperatures, and surprisingly, these parameters are higher under emersion than immersion (Bjelde & Todgham 2013, Bjelde et al. 2015). The fact that cardiac and metabolic performance are optimal in *L. digitalis* when aerially exposed, highlights how well adapted it is to the high intertidal environment. Interestingly, the high intertidal tropical snail *Echinolittorina malaccana* reduces crawling speed and depresses metabolic rate during heat stress, as $\dot{M}O_2$ is completely thermally insensitive between 35°C and 46°C (Marshall & McQuaid 2011, Marshall et al. 2011). Cardiac activity is also depressed in this species within this thermal range, and enzymatic and metabolomic analyses suggest that there is a switch to anaerobic metabolism up to this species' ULT (Chen et al. 2021). Thus, under heat stress, *Echinolittorina malaccana* reduces O_2 demand rather than enhancing O_2 uptake and supply. This ability to enter a state of protective metabolic depression may contribute to the extremely high thermal tolerance of this littorinid snail (i.e. its LT_{50} or ULT is ~50°C) (Lee & Lim 2009, Nguyen et al. 2011).

High intertidal molluscs also often have metabolic enzymes with a higher heat stability than their low intertidal relatives, and these differences can be found both between and among species, so they are driven by both evolution and phenotypic plasticity. In the genera *Lottia* and *Echinolittorina*, for instance, upper intertidal snail species that are more thermally tolerant than their lower intertidal congeners (Wolcott 1973, Li 2012) have cytosolic enzyme malate dehydrogenase (cMDH) isoforms/orthologues that are more resistant to heat inactivation (Dong & Somero 2009, Liao et al. 2017). Interestingly, these differences in enzyme thermal stability result from only minor amino acid substitutions (i.e. at one or two positions) that each arise from a single nucleotide polymorphism. cMDH is involved in many metabolic pathways, such as the tricarboxylic acid cycle, amino acid synthesis and gluconeogenesis (Goward & Nicholls 1994), so it is feasible that this enzyme is under strong selective pressure for thermal adaptation. Aminotransferases, which catalyse the conversion between amino acids and keto acids and are important in amino acid metabolism, also appear to have undergone selection for heat resistance; this conclusion is based on comparisons among several species of littorinids that occupy different tidal heights. For instance, in Britain, high intertidal species had higher activity levels of aspartate aminotransferase than low intertidal species (Hull et al. 1999), and this enzyme was more heat stable than alanine aminotransferase which was more active in low intertidal species. Furthermore, within one periwinkle species (*Littorina saxatilis*) that inhabits different intertidal heights, both aspartate and alanine aminotransferase were more heat tolerant in high intertidal compared to low intertidal individuals. In Singapore, the heat stability of alanine aminotransferase (also known as glutamate oxaloacetate transaminase) was the highest in the snail species living the furthest up the shore (*Echinolittorina malaccana*) (Lee & Lim 2009).

The heat shock protein (Hsp) response has been particularly well studied in intertidal animals (Hofmann 1999), and Hsp expression levels clearly mark the onset of cellular heat stress (e.g. in tidepool sculpins at 2°C below their CT_{max}) (Fangue et al. 2011). Hsps are molecular chaperones, whose expression is upregulated by heat shock transcription factors (HSF) during thermal stress (or another form of stress), and they limit the denaturation (unfolding) of proteins, aid with their repair and inhibit the induction of apoptosis (Morimoto 1998, Tomanek & Somero 2002, Beere 2004). In four *Chlorostoma* snail species (formerly the genus *Tegula*), the temperatures at which the synthesis of Hsps began, peaked and was heat-inactivated (ceased), were positively correlated with their intertidal vertical distribution and upper thermal tolerance limits (Tomanek & Somero 1999). In the rocky shore limpet *Patella vulgata*, Hsp70 levels were also higher in warmer sun-exposed microhabitats compared to cooler shaded sites (Lima et al. 2016). These data strongly suggest that these chaperone proteins play a key ecological role. In numerous intertidal molluscs found in temperate and tropical climates [e.g. in *Lottia* limpets (Sanders et al. 1991, Dong et al. 2008), *Cellana* limpets (Dong & Williams 2011) and *Mytilus californianus* mussels (Roberts et al. 1997, Halpin et al. 2004, Gracey et al. 2008)], increasing shore height, heat exposure and heat tolerance have also been associated with greater constitutive and/or heat-induced Hsp levels. Very similar trends have also been observed among sculpin species (Nakano & Iwama 2002). This indicates that in high intertidal molluscs and fishes, Hsps are thermoprotective during heat exposure, but can also be used as a 'preparative defence' against future heat stress. This latter strategy involves the maintenance of high baseline (constitutive) Hsp levels that may protect against protein damage during extreme and unpredictable high temperature events (Nakano & Iwama 2002, Dong et al. 2008). Because maintaining higher constitutive Hsp levels likely requires the allocation of energy (given that protein synthesis can be a major energy expenditure in ectotherms; Hawkins 1985, Houlihan 1991), this may not be advantageous for animals in the low intertidal, less thermally stressed, environment.

The production of Hsps in intertidal animals is highly plastic and affected by thermal history, which further helps intertidal animals to adjust to the highly variable thermal environment in which they live. This plasticity is illustrated in *Mytilus* mussels, for example, where: the induction temperature for Hsp70 production is 5°C higher in the summer than in the winter (a form of seasonal acclimatization), and similar Hsp70 induction temperatures are measured in high bed mussels as compared to low bed mussels transplanted to the former habitat (a form of acclimatization to microhabitat temperature) (Buckley et al. 2001, Halpin et al. 2004). It has also been suggested that intertidal animals induce the Hsp response more frequently than animals in thermally stable marine environments (e.g. the subtidal Antarctic, coral reefs), whereby the associated metabolic cost is likely outweighed by the benefits (Tomanek 2010). In the tidepool sculpin *Oligocottus maculosus*, the transcription and production of Hsps is not directed by an endogenous rhythm, but directly by tidepool temperature fluctuations (Todgham et al. 2006). Interestingly, in the same species, Hsp70 production following heat shock increases the tolerance to hyperosmotic and hypoxic stress exposure 8–48 hours later, which is a timeframe similar to the interval between low tides (Todgham et al. 2005). This finding suggests that the production of Hsps may allow for cross-tolerance, by helping the animal deal with multiple, and different, environmental stressors.

Freezing resistance

High intertidal animals can sometimes be exposed to extreme cold, which can occur in any environment (e.g. see Firth et al. 2011), but especially in boreal and polar climates (Raffaelli & Hawkins 1999). For example, in the Antarctic high intertidal, mean winter temperatures of –15°C are often recorded, whereas in the low intertidal/subtidal, –5°C is typically not surpassed (Waller et al. 2006, Clark et al. 2008). The duration of exposure to freezing temperatures (hours/days of temperatures below 0°C) also increases with intertidal height, as shown in several gastropod species on the Alaska and Washington coasts (Stickle et al. 2015, 2017). Temperatures close to zero can slow down the metabolism of animals to the extent that cardiac activity ceases, as seen in *Mytilus trossulus* mussels (Braby & Somero 2006). However, the freezing of body fluids is more problematic, because ice crystals cause physical damage to delicate cell structures, and ice crystal growth leads to desiccation by removing intracellular water (Denny & Gaines 2007). This section focuses on freezing, because it is the most severe form of cold stress, and resistance to its effects also involves avoidance and tolerance (Ansart & Vernon 2003, Yancey 2005, Duman 2015, Peck 2018). The body fluids of ‘freezing avoiders’ do not freeze, whereas ‘tolerators’ allow their extracellular body fluids to freeze.

Intertidal molluscs and barnacles are generally considered to be freeze tolerant, and survive with up to 65%–80% of their tissue water frozen (Roland & Ring 1977, Murphy 1983, Storey & Storey 1996, Ansart & Vernon 2003). There are a few bivalves and gastropods that also display an evolutionary trend or plasticity towards higher freezing tolerance with increasing shore height, based on comparisons among congeners or conspecifics, respectively. For instance, *Nacella concinna* sampled from the Antarctic intertidal zone in the summer have higher rates of survival following (almost) complete freezing of their body fluids as compared to individuals from the subtidal (95% versus 67% survival, respectively) (Waller et al. 2006). In the winter, high intertidal *Mytilus trossulus* from the Vancouver coast (at 3.5 m above the lowest tide level) had a 1°C–1.5°C lower freezing lethal temperature (LLT_{50}, temperature which causes 50% mortality) than low intertidal individuals (at 1 m above the lowest tide level) (Kennedy et al. 2020). A similar relationship between freezing LLT_{50} and intertidal distribution has been reported among *Nucella* and *Littorina* snail species from the Pacific Northwest (Stickle et al. 2015, 2017).

Information regarding the drivers of patterns of freezing tolerance across shore heights is still limited, compared to what is known about heat resilience-related adaptations in intertidal animals. In the periwinkle *Littorina littorea*, a larger body size provides increased freeze tolerance (i.e. a lower freezing mortality) by reducing the freezing rate, thus minimizing the length of time being frozen (Murphy & Johnson 1980). This might explain why larger individuals of this species are more common in the high intertidal zone (Gendron 1977). An important physiological adaptation allowing for the high freeze tolerance in intertidal invertebrates is the secretion of ice-nucleating proteins. The mussel *Mytilus edulis* (Aunaas 1982, Lundheim 1997, Denny & Gaines 2007) and snail *Melampus bidentatus* (Loomis 1985), for instance, deposit ice-nucleating proteins in the extracellular space that induce ice formation at a relatively high temperature, before intracellular fluids begin to freeze. As the extracellular compartment freezes, water is drawn out of the cell due to osmotic pressure, and this reduces the probability of intracellular ice formation. In the ribbed mussel *Geukensia demissa*, the gill-associated bacterium *Pseudomonas fulva* serves as an ice-nucleating agent (Loomis & Zinser 2001). Compatible organic osmolytes such as free amino acids (see the section ‘Resistance to salinity fluctuations’ below) may also improve freeze tolerance (i.e. serve as cryoprotectants), as they prevent excessive cellular water loss to growing extracellular ice crystals by retaining water inside the cell (Storey 1997). In the saltmarsh snail *Melampus bidentatus*, the amount of such osmolytes (glycerol and proline) was slightly elevated in the winter (Loomis 1985). Recently, using a metabolomics approach, it was shown that the concentration of several osmolytes [taurine, betaine, trimethylamine N-oxide (TMAO), glycine and alanine] was also elevated in *Mytilus trossulus* in the winter, and that this was correlated with an increase in freezing tolerance (i.e. reduced LLT_{50}) during this season in both low and high intertidal individuals (Kennedy et al. 2020). However, osmolyte concentrations did not explain the increase in freezing tolerance with shore height, so this must be facilitated by other mechanisms.

Freezing avoiders typically produce antifreeze proteins or glycoproteins, which inhibit the growth of ice crystals, or they accumulate high levels of compatible osmolytes such as glycerol to lower their freezing temperature. Although these mechanisms are used by various fishes (e.g. rainbow smelt, winter flounder, Antarctic notothenioids, Arctic gadids) and terrestrial arthropods (Yancey 2005, Duman 2015, Peck 2018), they are quite uncommon among intertidal animals (Storey & Storey 1996, Ansart & Vernon 2003). In a survey of 11 Antarctic intertidal invertebrates, only the nemertean worm *Antarctonemertes validum* was found to use antifreeze proteins, which results in 1.4°C of thermal hysteresis in its haemolymph (Waller et al. 2006). Thermal hysteresis is the delay in the growth of ice crystals in the blood/haemolymph when cooled below 0°C (i.e. the separation of the melting and freezing point of a liquid), and it is characteristic of the presence of antifreeze proteins (whereas osmolytes do not produce this phenomenon) (Peck 2018). Waller et al. (2006) also showed that the Antarctic intertidal snails *Margarella antarctica* and *Nacella concinna* have a lower freezing point than subtidal individuals of the same species, and that all intertidal species included in their study had freezing points well below that of the surrounding seawater (i.e. below −5°C). The mechanism(s) underlying freezing point depression in these animals still remain to be elucidated – although in both subtidal and intertidal *Nacella concinna*, the mucus secreted by the foot has been reported to contribute to its freezing avoidance, as the physical viscosity of this mucal film delays the proliferation of ice crystals (Hawes et al. 2010).

In molluscs, the ability to switch to anaerobiosis or to suppress metabolism, is important during hypoxia survival (see the section ‘Tolerance to diel hypoxia’ below), and freeze tolerance may rely on a very similar metabolic adaptation. Freezing has been shown to induce anaerobiosis in the periwinkle *Littorina littorea* (Churchill & Storey 1996) and mussel *Geukensia demissa* (Storey & Churchill 1995). During freezing, haemolymph flow is halted, which might limit O_2 supply and generate internal hypoxia, and this would require the generation of ATP though O_2-independent means to maintain energy balance. In *Littorina littorea*, freezing was also associated with the upregulation of many microRNAs and protein levels of Dicer (an enzyme that processes pre-microRNAs into mature ones) (Biggar et al. 2012). This suggests a role for ‘RNA interference’ in the suppression of protein synthesis (an energetically expensive process) during freezing survival in *L. littorea*, possibly as part of a metabolic depression response. As Biggar et al. (2012) argue, this would explain the lack of a decrease in overall mRNA levels. In RNA interference, mRNA transcription is unaffected, but mRNA translation into protein is suppressed through the binding of microRNAs and mRNA is subsequently degraded.

Hypoosmotic marine teleosts are particularly vulnerable to freezing compared to isosmotic invertebrates. However, research on freezing resistance in intertidal fishes is lacking. Perhaps, this is because intertidal fishes in boreal climates (e.g. the rock gunnel *Pholis gunnellus*) leave the intertidal zone during the winter months to avoid exposure to freezing temperatures (Sawyer 1967, Moring 1990). Antarctic intertidal areas are usually covered by ice in the winter (Aghmich et al. 2016), resulting in harsh conditions that may not allow for the survival of fish. Even in the summer, the Antarctic high intertidal often experiences temperatures below zero (Waller et al. 2006). Indeed, summer surveys of faunal assemblages rarely report the presence of fish (Aghmich et al. 2016), and indicate that the few species found are confined to the middle and low intertidal zones (with exception of the Antarctic spiny plunderfish *Harpagifer antarcticus* which can be found in high tidepools) (Sakurai et al. 1996). This pattern is likely driven by exposure to freezing temperatures, although it is conceivable that summer high temperatures, desiccation and other factors also make the upper tidal area in the Antarctic uninhabitable to fish.

Tolerance to high thermal variability

From polar to tropical climates, *in situ* temperature recordings show that the high intertidal zone typically experiences more rapid and larger thermal fluctuations than lower areas, on both a daily and a seasonal basis (Morris & Taylor 1983, Richards 2011, Schulte 2011, Kuklinski & Balazy 2014, Monaco et al. 2017) (Figure 2). For instance, on a single day in the tropics, the temperature can fluctuate on an emerged high shore by ~20°C versus ~15°C on an emerged low shore (Monaco et al. 2017). Further, during the summer in the Antarctic, the average temperature variability was 10°C higher at upper tidal sites as compared to lower tidal sites (Kuklinski & Balazy 2014). Temperature variability can differ substantially across intertidal environments, as it may depend on factors such as substratum type (Nguyen et al. 2011), whether the shore side receives sun exposure (Seabra et al. 2011, Amstutz et al. 2021), and the presence of vegetation that provides shading (Marshall et al. 2015). On the Atlantic coast of the Iberian Peninsula, for example, data loggers mimicking limpets ('robolimpets', see Lima & Wethey 2009) recorded far greater temperature variability on south-facing sun-exposed shores than on north-facing shaded shores (Seabra et al. 2011); and subtleties like these are important to consider in the study of high intertidal animals. Adaptations to cope with high thermal variability also have some overlap with those covered by the two preceding sections on heat and freezing stress, hence, the discussion of this topic is limited.

Rapid temperature fluctuations in the high intertidal zone have a considerable impact on animals given that a change in only 1°C can alter the rate of biological reactions by 10% (Lam et al. 2006). Therefore, mobile animals will often avoid exposure to the largest temperature changes using behavioural thermoregulation, i.e. by shuttling between cooler and warmer microenvironments, or between the air and water in which they may cool down/warm up more quickly. This is seen, for instance, in the middle to high intertidal crab *Hemigrapsus nudus*, which in the laboratory manages to maintain its body temperature within much narrower limits (~8°C to ~20°C) than that of the environment (~5°C to 35°C) (McGaw 2003). These results have been confirmed in the field. Intertidal animals are also typically eurythermal, and capable of tolerating large changes in temperature over short periods of time. For instance, limpets (*Lottia gigantea*) can tolerate an increase in body temperature of >20°C within a day (Denny & Harley 2006). The killifish *Fundulus heteroclitus* can live at near freezing temperatures, but also tolerates heating from 5°C to 37°C over as little as an hour (Moyle & Cech 1996, Schulte 2011).

A physiological adaptation that might improve tolerance to thermal fluctuations, is having a less thermally sensitive metabolism. Among hermit crabs, the temperature sensitivity of aerobic metabolism ($\dot{M}O_2$) in intertidal species (*Pagurus granusimanus* and *P. hirsutiusculus*) is lower than in subtidal species (*Paguristes turgidus* and *Elassochirus tenuimanus*), as indicated by lower values for the temperature coefficient Q_{10} (1.4–1.6 versus 2.1–2.4) (Burggren & McMahon 1981). An organism's Q_{10} is the degree of change for a physiological process (in this case $\dot{M}O_2$) resulting from a 10°C change in temperature. The intertidal hermit crab species also had a smaller $\dot{M}O_2$ 'overshoot' (i.e. quick increase in O_2 consumption) in response to an abrupt 10°C increase above acclimation temperatures, and a more rapid acclimation (i.e. return of $\dot{M}O_2$ to normal resting levels), than the subtidal species. This could be advantageous by reducing the use of energy resources during the summer, as well as allowing the animal to remain active during winter. In killifish (*Fundulus heteroclitus*), the large thermal tolerance range is associated with an extremely wide thermal breath (at least 5°C–33°C) over which it can maintain a positive aerobic scope (i.e. the difference between maximum and basal/resting $\dot{M}O_2$) (Healy & Schulte 2012). Furthermore, thermal acclimation is not required to establish the majority of this breadth; and therefore, it is an inherent (fixed) characteristic of this species that its aerobic capacity suffers few negative effects from acute temperature changes.

Tolerance of diel hypoxia

Aquatic hypoxia occurs in many marine ecosystems (e.g. oxygen minimum zones and estuaries) (Breitburg et al. 2018). In the intertidal zone, this environmental challenge is characterized by short-term (acute), and often severe, decreases in available oxygen (O_2) that frequently become more extreme higher up the shore (Figure 2) (Morris & Taylor 1983, Raffaelli & Hawkins 1999, Richards 2011, Schulte 2011, Mandic & Regan 2018, McArley et al. 2019). During high tide, conditions are usually normoxic, whereas during low tide, water O_2 levels can drop to near zero in high tidepools on rocky shores and in the high tidal zone of estuaries, mangroves, salt marshes and mudflats (Figures 2 and 3) (Richards 2011, Schulte 2011). Aquatic hypoxia may occur during the day because of increased organismal respiration due to issues such as crowding (e.g. many animals are aggregated in a small tidepool) and/or overheating (which increases the metabolic demands of animals), and during the night because O_2 is not being replenished by primary production (Ganning 1971, Morris & Taylor 1983, Huggett & Griffiths 1986). Although, intertidal rockpools can also become hyperoxic (supersaturated) at low tide during the day due to photosynthetic processes (Figure 2) (Ganning 1971, Morris & Taylor 1983, Huggett & Griffiths 1986, Richards 2011, Schulte 2011, McArley et al. 2018, 2019). In addition to aquatic hypoxia, high intertidal animals can also experience 'internal hypoxia' (hypoxemia) when emerged, as a result of the retraction or collapse of respiratory structures which renders them non-functional (Raffaelli & Hawkins 1999, Wolcott & Wolcott 2001, Turko et al. 2014). Emersion-induced hypoxemia and aquatic hypoxia affect animals in a similar way (both involve constrained O_2 uptake), although there can be subtle differences in the adaptations/plastic responses against these environmental cues (e.g. see Turko et al. 2014, 2018). This section is more detailed/comprehensive than many other sections in this review because of the abundance of information on the topic, which perhaps reflects the multitude of adaptations that intertidal animals have to deal with the stress of limited O_2. These various adaptations and responses are divided into three main strategies (Table 2).

Enhanced aerobic capacity

To avoid depending on anaerobiosis during hypoxia, many high intertidal animals enhance their aerobic capacity by improving O_2 uptake from water/air, O_2 transport and/or mitochondrial O_2 utilization. For more elaborate discussions of the adaptations of intertidal fishes to hypoxia, see the recent reviews by Martin (2014), Wright & Turko (2016) and Mandic & Regan (2018). Intertidal fishes generally use two types of behaviour to enhance O_2 uptake: aquatic surface respiration (ASR), whereby the O_2-rich water is skimmed from the surface, and emergence for aerial respiration. The mangrove rivulus, *Kryptolebias marmoratus*, even has physical characteristics compatible with ASR, including an upward-facing mouth and a flattened head (Taylor 2012). Fishes such as sculpins, killifish, clingfish and mudskippers, actively leave hypoxic waters to exchange gases using their skin, gills or other epithelial surfaces, which are more vascularized to allow for better aerial O_2 uptake (Ebeling et al. 1970, Wright & Turko 2016, Mandic & Regan 2018, Livingston et al. 2018). For instance, intertidal sculpins perform ASR and emergence behaviours at a much higher frequency than subtidal relatives to escape aquatic hypoxia (Mandic et al. 2009a), and in *Oligocottus maculosus* and *O. snyderi*, O_2 uptake from the air can be as high as from the water (Yoshiyama & Cech 1994, Sloman et al. 2008). Amphibious fishes (e.g. the high intertidal mudskipper *Periophthalmodon schlosseri*) commonly have modified gills, for instance, with improved support structures, which do not collapse during emersion and can be used for effective aerial respiration (Ebeling et al. 1970, Low et al. 1988, Sayer 2005, Lam et al. 2006). However, emergence behaviour in fish might not always be for the purpose of air breathing. Emergence of the rockpool blenny *Praealticus tanegasimae* during low tide is often followed by migration to nearby tidepools ('tidepool hopping'), likely to find more favourable conditions (Kimura & Sakai 2016). Several species of intertidal burrow-inhabiting mudskippers, such as *Periophthalmodon schlosseri* (Ishimatsu et al. 1998), *Periophthalmus modestus* (Ishimatsu et al. 2007) and *Scartelaos histophorus* (Lee et al. 2005), repeatedly emerge at the surface of their burrows to take in mouthfuls of air. These are then deposited into the burrow's air chamber to avoid hypoxic exposure of the inhabitant and any eggs or embryos that it is guarding.

When aerial predation is present, high intertidal fishes may remain submerged in hypoxic water, and instead rely on physiological/biochemical strategies to maintain aerobic metabolism while exposed to limited O_2 levels (Mandic & Regan 2018). Such strategies have been described in fishes such as sculpins (Cottidae family), triplefins (Tripterygiidae family) and the mangrove rivulus (*Kryptolebias marmoratus*), and include increases in mass-specific gill surface area, haematocrit (Hct), haemoglobin levels and/or haemoglobin O_2-binding affinity (as indicated by a lower P_{50}, the O_2 level at which haemoglobin is 50% saturated with O_2) (Mandic et al. 2009b, Turko et al. 2014, 2018, Mandic & Regan 2018, McArley et al. 2019). Intertidal triplefin species also have thinner gill secondary lamellae, which results in a shorter diffusion distance for O_2 to reach the blood during ventilation (McArley et al. 2019). In addition to these adaptations at the level of O_2 uptake and transport, changes in mitochondrial respiration/the electron transport chain have been identified. In the intertidal triplefin *Bellapiscis medius*, oxidative phosphorylation in the heart is more efficient (coupled), and maximum O_2 consumption by cytochrome *c* oxidase (COX) is higher, as compared to the two subtidal species *Forsterygion varium* and *F. malcolmi* (Hilton et al. 2010). This may explain why *Bellapiscis medius* is more hypoxia tolerant (i.e. has a lower critical O_2 tension or P_{crit}; the O_2 level at which the O_2 consumption rate becomes dependent on the O_2 level) than the other triplefin species. Recently, it was also shown that the upper intertidal sculpin *Oligocottus maculosus* has a higher COX and mitochondrial O_2-binding affinity in the brain compared to lower intertidal species, which is associated with a higher hypoxia tolerance (Lau et al. 2017). *In silico* analysis of the COX3 subunit, which is the putative entry point for O_2 into the COX protein catalytic core, showed that the higher COX O_2 affinity of *Oligocottus maculosus* could be attributed to higher COX3 protein stability. This increased stability was further linked to two amino acid substitutions in the COX3 v-cleft structure (i.e. at residue positions 55 and 224, phenylalanine is replaced by leucine, and leucine by phenylalanine, respectively), which likely strengthen the high-affinity interaction with cardiolipin from the mitochondrial membrane.

Similar to fishes, intertidal crustaceans such as crabs and shrimps use ASR when exposed to hypoxia, or are capable of switching to air breathing during emersion. The temperate rockpool shrimp (*Palaemon elegans*) moves to shallow water at low O_2 levels, exploiting the higher O_2 level at the air/water interface, and this allows it to maintain a higher haemolymph O_2 content and resist increases in lactate levels (Taylor & Spicer 1988). This partial-emergence, hypoxia-induced behaviour, has also been described in the tropical burrowing ghost shrimp *Upogebia africana* (Hill 1981) and other intertidal shrimp species (Hagerman 1998). In air-exposed *Pagurus* hermit crabs, intertidal species are relatively well-adapted to aerial respiration, as they are better able to defend aerobic metabolic rate (O_2 consumption; $\dot{M}O_2$) as compared to their subtidal relatives (Burggren & McMahon 1981). The estuarine crab *Chasmagnathus granulatus* uses highly vascularized branchial chambers for air breathing (Halperin et al. 2000). Finally, some intertidal porcelain crabs (*Petrolisthes*) have an aerial gas exchange organ on each of their walking legs, which appears to be especially important in larger bodied species (Stillman 2000), and a similar structure (a 'gas window') can be found in small burrowing intertidal crabs of the genera *Scopimera* and *Dotilla* (Maitland 1986).

Some intertidal crabs have evolved other mechanisms to sustain/optimize gas exchange while in or out of the water. The high intertidal mudflat crab *Helice crassa* is able to recirculate water in the branchial chambers for re-oxygenation, whereas the mid-intertidal crab *Macrophtalmus hirtipes* is unable to do this; which might be why it is restricted to a lower area of the intertidal zone (Hawkins & Jones 1982). Intertidal crabs can also improve aerial or aquatic O_2 uptake by having haemocyanin with a higher O_2 affinity (a lower P_{50}) (DeFur 1988). Among gammaridean, hyalid and talitrid amphipods, other adaptations of respiratory pigments have been described in high intertidal species that optimize O_2 supply concordant with their semi-terrestrial/amphibious lifestyle. For instance, in *Talitrus saltator*, which inhabits sandy shores in Europe around the high tide mark, haemocyanin-O_2 affinity is relatively insensitive to organic (lactate) and inorganic ionic (Ca^{2+}, Cl^-) fluctuations in the haemolymph (Spicer et al. 1990), which would help to maintain O_2 uptake during the desiccation/acidosis that is associated with emersion. Spicer (1993) and Spicer & Taylor (1994a) confirmed this in other amphipod species, and further described a general decline in the Bohr effect in upper tidal amphipod genera as compared to aquatic amphipods. This avoids a loss of haemocyanin-O_2 affinity due to high haemolymph CO_2 levels during emersion.

In bivalves, gaping behaviour allows for aerial O_2 uptake, and is used by several intertidal species in response to emersion. This is a trade-off between shell closure to avoid desiccation, and shell opening to avoid hypoxia (Nicastro et al. 2010). Bivalves that engage in gaping activity have higher aerial O_2 uptake rates (~75% of the aquatic $\dot{M}O_2$ in *Mytilus californianus* and *Cardium edule*) than non-gaping species (~17% of the aquatic $\dot{M}O_2$ in *Mytilus edulis*) (Bayne et al. 1976, Widdows & Shick 1985, Zippay & Helmuth 2012). In addition, differences in air-breathing abilities are seen among mollusc species that inhabit both the intertidal and subtidal zone [e.g. in the mussels *Mytilus edulis* and *M. galloprovincialis* (Altieri 2006, Tagliarolo et al. 2012) and the limpet *Nacella concinna* (Weihe & Abele 2008)] and this underscores the role of phenotypic plasticity in allowing intertidal colonization. Interestingly, in *Mytilus edulis*, the difference in survival during emersion and aquatic hypoxia among inter- and subtidal individuals was almost completely reversed 7 weeks after reciprocal transplantation (Altieri 2006). Reciprocal transplantations are a useful tool to elucidate processes such as local adaptation in a species, the level of phenotypic plasticity and its reversibility, and epigenetic effects; and this specific finding highlights the flexibility of *M. edulis* to acclimate to different shore heights. Although this study does not explore the mechanisms behind this ability, it is very likely that changes in gene transcription are a key driver.

Enhanced anaerobic capacity

Bivalves from the high intertidal zone – including those that gape – also rely greatly on anaerobiosis to survive emersion, given that their air-tight shells are closed for most of the time and the water retained within the valves is depleted of O_2 within minutes (Bayne et al. 1976, Zippay & Helmuth 2012). This enhanced anaerobiosis may be reflected by a greater accumulation of anaerobic end products such as alanine, as seen in *Mytilus californianus,* when individuals from the high versus low intertidal zone are compared (Gleason et al. 2017). Estuarine oysters, such as *Ostrea chilensis*, also use anaerobiosis when 'clammed up' in response to reduced salinities (see the 'Tolerance to salinity fluctuations' section below), including embryos that are brooded by females inside the mantle cavity (Segura et al. 2015). The disadvantage of this anaerobic strategy is that an 'O_2 debt' must be repaid during re-immersion to allow for the aerobic processing of anaerobic end products, as seen in several bivalve species (Bayne et al. 1976, Widdows & Shick 1985, Zippay & Helmuth 2012). *Mytilus californianus* has a strong tide-driven metabolic cycle of aerobic metabolism during submergence and anaerobiosis during emersion, and interestingly, this intrinsic property is still visible under continuous experimental submergence (Connor & Gracey 2012, Gracey & Connor 2016). These data emphasize the importance of this metabolic cycle for *M. californianus*, which apparently does not rely on external signals from the environment that are associated with emersion.

Some high intertidal invertebrates appear to increase the amount of anaerobic fuel sources compared to their low intertidal counterparts, to help maintain energy levels under O_2 limited conditions. This is reported, for instance, in high versus low intertidal periwinkles of the species *Littorina saxatilis*, although anaerobiosis accounts only for a relatively small fraction of the ATP turnover compared to aerobic metabolism (Sokolova & Pörtner 2001a). Females of high intertidal crab species have also been reported to produce embryos with higher levels of organic matter (total protein, carbohydrates and lipids, etc.), and thus, with more available anaerobic substrates, as compared to subtidal species (Viña et al. 2018).

Although high intertidal sculpins have many adaptations to enhance aerobic capacity, they still use anaerobiosis when access to aquatic surface respiration and emergence is denied (Sloman et al. 2008), and have an enhanced capacity to do so. In several species of sculpin with differing vertical distributions on the Pacific coast, a high maximal lactate dehydrogenase (LDH) enzyme activity in the brain is linked to high hypoxia tolerance (Mandic et al. 2012). For instance, hypoxia tolerance and LDH activity were highest in the upper tidal species *Oligocottus maculosus*, and lowest in the subtidal species *Myoxocephalus scorpius* and *Blepsias cirrhosus* (i.e. P_{crit} of ~30 versus ~50 mm Hg, and enzymatic substrate conversion rate of ~170 versus ~100 µmol/min/g wet tissue, respectively). Optimizing the brain's LDH activity allows for maximum glycolytic ATP synthesis capacity, which might be particularly important in sculpins given that they elicit complex behaviours during low tide. The glycogen content in brain, liver and muscle of *Oligocottus maculosus* is also generally greater under normoxia and after hypoxic exposure, as compared to that of *Blepsias cirrhosus* (Speers-Roesch et al. 2013). Thus, *Oligocottus maculosus* appears to have larger anaerobic fuel stores in its tissues, and to deplete these stores at a slower rate. Similarly, New Zealand intertidal triplefin species have higher brain and muscle glycogen levels compared to subtidal, and less hypoxia-tolerant, congeners (McArley et al. 2019).

Metabolic depression

For the purposes of this review, metabolic depression is defined as the temporary (and reversible) reduction of metabolism below what would be considered normal at a particular temperature. Similar to anaerobiosis, metabolic depression as a means to survive hypoxia/hypoxemia during emersion is common among intertidal molluscs, and high intertidal animals appear to use this strategy to the greatest extent. For instance, in the mussel *Mytilus edulis* and cockle *Cardium edule*, both aerial O_2 uptake and heat dissipation rates were lower in intertidal compared to subtidal individuals (i.e. this reduced aerobic respiration rate was not compensated for by anaerobiosis) (Widdows & Shick 1985). Likewise, aerial O_2 consumption rates in the upper shore limpet *Siphonaria oculus* were below that of the lower shore limpet *Patella granularis*, and no 'O_2 debt' repayment was observed upon re-immersion (Marshall & McQuaid 1992a). Finally, high intertidal periwinkles (*Littorina saxatilis*) have been reported to use metabolic depression, as they accumulate less anaerobic end products during emersion while maintaining the same $\dot{M}O_2$ as individuals from the low intertidal (Sokolova & Pörtner 2001a).

In some high intertidal animals, metabolic depression as an adaptive response during emersion or aquatic hypoxia might be reflected by, or linked with, a decline in cardiac activity (though keeping in mind that heart rate and O_2 consumption are not always correlated, e.g. see Marshall & McQuaid 1992b). In the mussels *Mytilus californianus* (Connor & Gracey 2012) and *M. edulis* (Curtis et al. 2000), a greatly reduced heart rate (bradycardia), to near zero beats per min (acardia), is observed during emersion/valve closure, which would reduce O_2 requirements of the heart and of the whole organism. This bradycardic response does not change even in the absence of a tidal cycle (akin to the switch to anaerobiosis described above), and this suggests that this is an innate and crucial mechanism for survival (Gracey & Connor 2016). Similarly, exposure to hypoxia (~50 mm Hg or ~30% air saturation) induces bradycardia in the limpets *Siphonaria capensis* and *Patella granularis*, whereby *Siphonaria capensis* which occupies high shore tidepools appears to depress metabolic rate more effectively, and does not undergo the heart rate overshoot (tachycardia) upon return to normoxia as is typical for the lower intertidal species *Patella granularis* (Marshall & McQuaid 1993). Bradycardia has also been observed in the clingfish *Sicyases sanguineus* when out of the water, concordant with a reduction in $\dot{M}O_2$, a loss of respiratory movements and minimal lactate accumulation – all of which likely reflect metabolic depression (Gordon et al. 1970). However, it appears that metabolic depression in response to short-term hypoxia/emersion-related hypoxemia (i.e. hours to days) is not that common among intertidal fishes. This is possibly because it increases predation risk (i.e. the capacity for quick escape movements is likely diminished greatly). Although, it can play an important role during prolonged exposure (i.e. weeks to months) (Livingston et al. 2018, Mandic & Regan 2018, Turko et al. 2019).

Constitutively lower enzyme activities may also facilitate metabolic depression in high intertidal animals, and reduce overall energy demands. In *Littorina saxatilis* from Sweden, there are two allele variants of the metabolic enzyme alanine aminotransferase (AAT), whereby AAT^{100} and AAT^{120} homozygotes represent >70% and >80% of the population on the low and high shore, respectively (Panova & Johannesson 2004). AAT^{120} has a lower activity than AAT^{100}, which might help high shore periwinkles conserve energy during low tide. There are several more aerobic and anaerobic metabolic enzymes that have been reported to have a lower activity in high intertidal *Littorina saxatilis* from the White and North Sea coast (Sokolova & Pörtner 2001b). The mechanism(s) underlying these differences in allozyme activity (e.g. non-synonymous amino acid substitutions) have not yet been elucidated, but they appear to be under strong selection given that their vertical gradients within the intertidal zone occur in various geologically separated areas (Johannesson 2016). A temporary reduction in enzyme activity and energy-consuming processes to downregulate metabolism during hypoxia, is also seen among intertidal molluscs (Storey & Storey 1990). In *Littorina littorea* exposed to anoxia, for instance, there is a considerable decrease in the activity of two important glycolytic enzymes, phosphofructokinase (PFK) and pyruvate kinase (PK) (Greenway & Storey 2001). In the case of PK, the change in activity is the result of reversible phosphorylation, a type of covalent modification that affects the enzyme's kinetic properties, and that can switch it from an active (dephosphorylated) to an inactive (phosphorylated) form. This anoxia-induced regulatory mechanism of PK activity has also been described in the whelk *Busycotypus canaliculatus* and mussel *Mytilus edulis* (Storey & Storey 1990). Recently, it was shown that metabolic suppression during hypoxia in the hypoxia-tolerant intertidal clam *Mercenaria mercenaria* is facilitated by a drastic downregulation of protein synthesis and ion transport, as indicated by the inactivation of translation initiation factor EIF-1a, and a reduction of 26S proteasome and Na^+/K^+ ATPase activity (Ivanina et al. 2016). In contrast, this response was not observed in the hypoxia-sensitive subtidal scallop *Argopecten irradians*.

Despite the fact that intertidal fishes do not appear to use metabolic depression to the same extent as in molluscs, there is some evidence that they reduce the expression of genes involved in energetically expensive processes. In the intertidal mudskippers *Periophthalmus magnuspinnatus* and *Boleophthalmus pectinirostris*, genes involved in cell growth and proliferation were downregulated during 6 hours of air exposure, which suggests that they employ this as an energy-saving strategy (You et al. 2014). However, no comparison with subtidal mudskipper species was made in that study, leaving it uncertain as whether this strategy is a specific adaptation to the intertidal zone. In the upper tidal sculpin *Oligocottus maculosus*, on the other hand, it was reported that genes involved in O_2-dependent (e.g. fatty acid oxidation, oxidative phosphorylation) and energy-consuming (e.g. cell proliferation, protein synthesis) processes were downregulated during aquatic hypoxia, whereas this was not the case in the less hypoxia-tolerant, low intertidal sculpin *Blepsias cirrhosus* (Mandic et al. 2014). However, these transcriptional changes did not occur until after 24 hours of hypoxia exposure, which is beyond the typical duration of diel hypoxia (6–8 hours) in the intertidal zone. These authors suggested that rapid changes in gene transcription may not be used for daily hypoxia survival in *Oligocottus maculosus*, and that it instead uses translational or post-translational regulation to modulate gene expression (i.e. changes in protein synthesis or folding). Alternatively, it may maintain a 'fixed generalist phenotype' because of the metabolic costs associated with the continuous alteration of mRNA transcription during the tidal cycle. Indeed, many physiological/biochemical adaptations to diel hypoxia have already evolved in this sculpin species, and with its innate hypoxia resilience, the benefits of additional phenotypic plasticity may be limited. This theory would be somewhat consistent with the 'plastic floors and concrete ceilings' concept that was recently put forward by Sandblom et al. (2016). This concept suggests that limitations in physiological plasticity constrain the ability of fish to cope with high temperatures.

Tolerance to salinity fluctuations

All intertidal habitat types can experience tremendous fluctuations in salinity (Figure 3), which can be more extreme higher up the shore. River input or heavy precipitation (e.g. tropical monsoon rains) can reduce salinity down to that of fresh water, whereas evaporation or droughts can increase it above that of seawater (hypersaline conditions) (Ganning 1971, Morritt et al. 2007, Firth & Williams 2009, Schulte 2011, Marshall 2012). For instance, high shore rockpools may fill with seawater at high tide, but with fresh water at low tide (Marshall 2012), resulting in more pronounced salinity fluctuations than in low rockpools (Ganning 1971, Morris & Taylor 1983). In estuaries, salinity can vary between ~5‰ and ~30‰–35‰ (parts per thousand, ppt) throughout the year (e.g. as recorded on the Vancouver and Hong Kong coasts by Chan et al. 2001, Chan & Williams 2004 and Covernton & Harley 2020), and in mangroves and lagoons, evaporation can increase salinity to over 50‰–60‰ (Raffaelli & Hawkins 1999, Lam et al. 2006). Therefore, intertidal animals are typically euryhaline (able to cope with a range of salinities), and both osmoconformers [animals that allow their internal osmolarity to fluctuate along with the environmental osmolarity (predominantly invertebrates)] and osmoregulators [animals that maintain a constant internal osmotic pressure (most vertebrates and some crustaceans)] are able to withstand these fluctuations (Yancey 2005, Rivera-Ingraham & Lignot 2017). For example, among osmoconformers, the intertidal and estuarine Pacific oyster (*Crassostrea gigas*) tolerates salinities between 5‰ and 40‰ (Meng et al. 2013), and the boreo-Arctic high intertidal barnacle *Semibalanus balanoides* can tolerate/remain active at salinities from ~15‰ up to ~50‰ (Foster 1970, 1971). Among osmoregulators, the mangrove rivulus (*Kryptolebias marmoratus*), estuarine Nile tilapia (*Oreochromis niloticus*) and common killifish (*Fundulus heteroclitus*) can be acclimated to salinities ranging between 0‰ and 60‰–80‰ (King et al. 1989, Genz & Grosell 2011, Marshall 2012, Taylor 2012).

The first coping mechanisms with regard to salinity challenges are behavioural. These are particularly important for osmoconformers in the high intertidal zone (although osmoregulators use them as well), and this is probably because they are economical/do not require large physiological adjustments. Behavioural responses revolve around seeking refuge in a more favourable microhabitat, or reducing the amount of exposed surface area; that is, they are much like the behavioural defences against desiccation (Table 2). For instance, intertidal worms may move vertically in the sediment/their burrow in response to a salinity gradient (Oglesby 1969). The oyster *Ostrea chilensis* that inhabits estuaries isolates its tissues from the environment by 'clamming up' (closing the shell) when exposed to low salinity stress (Segura et al. 2015) and this is a common behavioural response among intertidal bivalves and barnacles when exposed to hypo- or hypersaline conditions (Foster 1970, Wolcott & Wolcott 2001, Chan et al. 2001). For example, the barnacle *Tetraclita japonica,* which is common on the high shore in Hong Kong (at 1.5–2 m above the lowest tide level), closes its opercular plates during acute hyposalinity stress (immersion in 10‰ seawater for up to 36 hours) and stops cirral activity/beating to prevent the influx of water into the mantle cavity (Chan et al. 2001). Although this prevents the barnacles from feeding (or reproducing, which has negative consequences in the long term), in the short term it allows them to protect their haemolymph and mantle water osmolality (by maintaining this at >500 mOsmol/kg above that of the surrounding seawater). Limpets may also use this type of 'behavioural osmoregulation' by tightly clamping to the substrate and isolating soft tissues from the environment. Such a behaviour may play a role in ionoregulation in the high intertidal *Cellana grata*, which has a limited ability to hyper-osmoregulate during monsoonal rainfall (Morritt et al. 2007, Williams et al. 2011).

An important physiological mechanism that allows intertidal osmoconformers to regulate cellular volume under salinity stress (i.e. to avoid excessive cell swelling/shrinkage), is the accumulation or catabolism of intracellular organic osmolytes, in particular free amino acids and their derivatives (Yancey 2005). In contrast to inorganic osmolytes, which at high concentrations can destabilize proteins and nucleotides, these organic osmolytes are 'compatible' as they do not interact with or damage macromolecules (Yancey et al. 1982, Yancey 2005, Schulte 2011). Therefore, their concentrations can safely fluctuate. In intertidal anemones, the amount of free amino acids (predominantly glycine, alanine and glutamate) increases along with salinity, which is not the result of the passive diffusion of water, but from their active production (Shick 1991). The high intertidal rockpool copepod *Tigriopus californicus* also appears to modulate its free fatty acid pool in response to hypo- or hyperosmotic stress (Burton & Feldman 1982). The Pacific oyster (*Crassostrea gigas*) likely uses free amino acids in a very similar way, as their concentration in the gills is positively correlated with acclimation salinity, with taurine being the most abundant free amino acid (up to 80% of total amino acids) (Hosoi et al. 2003, Meng et al. 2013, Zhang et al. 2016). Underlying the control of free amino acid levels in the gills, is the differential mRNA expression of key enzymes involved in free amino acid metabolism, such as cysteine sulphinate decarboxylase (CSAD), the main biosynthetic enzyme for taurine (Meng et al. 2013, Zhao et al. 2017). Taurine, together with glycine, betaine, alanine and trimethylamine N-oxide (TMAO), are also the most abundant organic osmolytes in intertidal mussels (Kennedy et al. 2020), polychaete worms, snails and clams (Yancey 2005). However, not all intertidal invertebrates are reported to actively modulate their intracellular osmolyte pool during salinity stress. For instance, the snail *Littorina littorea* appears to, instead, rely on a passive tolerance to large changes in cell volume (Taylor & Andrews 1988). Interestingly, in both high and low intertidal *Mytilus trossulus*, the accumulation of osmolytes in response to hypersalinity exposure is associated with a higher freezing tolerance, thus providing an example of cross-tolerance (Kennedy et al. 2020).

Osmoregulators use regulatory organs (e.g. gills, kidneys, antennal glands) to keep internal fluids at the same osmolarity (as much as possible) during salinity stress, and organic osmolytes do not play a major role (Morritt & Spicer 1998, Yancey 2005). Intertidal crustaceans vary in their capacity for osmoregulation and reliance on it as a coping strategy. For instance, the saltmarsh and estuarine gammarid amphipod *Gammarus duebeni* produces urine hypotonic to its haemolymph to conserve ions in the body during acute low salinity exposure (Lockwood 1961). The high intertidal hyalid amphipod *Hyale nilssoni* is also able to hyper-osmoregulate when exposed to dilute seawater, although it is unable to hypo-osmoregulate (Morritt 1988). Instead, this species likely adapts behaviourally rather than physiologically to hyperosmotic stress, by actively moving down the shore into wetter microhabitats (e.g. into seaweed or under gastropod shells). This appears to generally be the case for talitrid amphipods, as well as other crustaceans such as anomuran crabs, in intertidal habitats (for details, see reviews by Morritt & Spicer 1998 and Greenaway 2003). Although, the talitrid amphipod (beach flea) *Traskorchestia traskiana* that occurs at and above the high water mark, has the ability to hyper- and hypo-osmoregulate in diluted and full-strength seawater, respectively, and probably uses its gills to exchange ions accordingly (Spicer 2013). Other high intertidal crustaceans, e.g. isopods *Ligia occidentalis* and *L. pallasii* (Wilson 1970) and several crab species (e.g. *Pachygrapsus crassipes* and *Chasmagnathus granulatus*) (Gross 1964, Luquet et al. 2002), are also capable of a high degree of osmoregulation (relative to subtidal species). In the estuarine *Chasmagnathus granulatus*, which encounters salinities from <1‰ to hypersaline conditions in tidepools, the gill epithelium contains characteristics typical of a salt-transporting tissue, e.g. with numerous mitochondria and a high Na^+/K^+ ATPase activity that is involved in both NaCl uptake and secretion (Luquet et al. 2002, Genovese et al. 2004).

The osmoregulatory mechanisms/adaptations in (tropical) intertidal and estuarine fishes are reviewed by Lam et al. (2006) and Marshall (2012), and thus, will only be described here very briefly, with a focus on responses to short-term/cycling salinity stress (as this is most reflective of changes encountered in the intertidal environment). Intertidal and estuarine fish are typically able to quickly (and reversibly) adjust their gill functionality to external salinity (Schulte 2011, Marshall 2012). For instance, when the mummichog (*Fundulus heteroclitus*) is transferred from fresh- to seawater, the Na^+/K^+ ATPase in the gills is upregulated within 3 hours, allowing for a rapid increase in NaCl secretion by the ionocytes (Mancera & McCormick 2000). The chloride cell-rich opercular membrane of the mummichog also rapidly enhances NaCl secretion when exposed to a hypertonic shock (Zadunaisky et al. 1995). Hypotonic exposure of mudskippers (*Periophthalmus modestus*; Sakamoto et al. 2000) and killifish (*Fundulus heteroclitus*; Daborn et al. 2001), on the other hand, results in the retraction of gill ionocytes from the surface (i.e. the chloride cells close/contract the opening to their apical crypt). This response occurs within an hour, which provides almost immediate protection against ion loss. The contraction is facilitated by a ring of actin in the apex of chloride cells, although the signalling/triggering mechanism(s) has/have not been described. In addition to these dynamic physiological responses, intertidal fishes have a low gill, skin and intestinal permeability to water, which reduces the overall costs of osmoregulation (Schulte 2011, Marshall 2012).

High intertidal animals (both osmoconformers and -regulators) may also use a hypometabolic response (i.e. a limited form of metabolic depression, or behavioural quiescence) to help tolerate hyposalinity exposure. In seawater-acclimated upper tidal black prickleback (*Xiphister atropurpureus*), O_2 consumption ($\dot{M}O_2$) dropped by twofold and opercular beating stopped almost entirely, after transfer to a salinity of 6‰, whereas these parameters changed little in the low intertidal/subtidal penpoint gunnel (*Apodichthys flavidus*) from the same local environment and with a similar gross morphology (Haynes et al. 2009). By reducing breathing rate and water flow over the gills, the black prickleback is potentially minimizing ion loss and water gain. A decline in heart rate (bradycardia), and sometimes episodes of no cardiac activity (acardia), have also been observed in the high intertidal limpets *Cellana grata* and *Siphonaria capensis* when submerged in fresh water (Chelazzi et al. 2001), diluted seawater (20‰) (Marshall & McQuaid 1993), or exposed to simulated monsoonal rainfall (Morritt et al. 2007). This strongly suggests a degree of metabolic depression; although the cardiac response in *Cellana grata* was not seen in a later study (Williams et al. 2011) and the local low intertidal species *Cellana toreuma* and *Patella granularis* displayed bradycardia and acardia as well (Marshall & McQuaid 1993, Chelazzi et al. 2001). In the upper intertidal flatworm *Macrostomum lignano*, respiration rate decreases by ~50% during low salinity exposure (transfer from 35‰ to 5‰), and this is associated with a ~75% reduction in activity levels (Rivera-Ingraham et al. 2016). Hypersalinity exposure (transfer from 35‰ to 55‰), on the other hand, was associated with an increase in respiration and mitochondrial density, and the upregulation of antioxidant genes such as superoxide dismutase. Similarly, the mangrove rivulus (*Kryptolebias marmoratus*) increases its metabolic rate during acute hypersalinity exposure, as opposed to its hypometabolic response to hyposalinity stress (Sutton et al. 2018). These findings highlight that, in contrast to tolerance to low salinity, tolerance to high salinity comes with an energetic cost, and that it potentially induces oxidative stress which the animal may need to invoke protective measures against. The concept of salinity-induced oxidative stress and the corresponding antioxidant defences may also apply to other intertidal/estuarine animals, given that they are all frequently subject to salinity stress (Rivera-Ingraham & Lignot 2017).

Coping with nitrogenous waste accumulation

The catabolism of amino acids releases ammonia (NH_3), which has deleterious effects on many cellular processes including ion regulation (Ip & Chew 2018). Typically, marine animals are ammoniotelic and excrete NH_3 directly into the seawater due to its high solubility in this medium (Table 1). Animals with gills usually excrete NH_3 at this interface, because of its large surface area and the large volume of seawater that runs over it. However, during emersion, this mode of NH_3 excretion is interrupted (e.g. as seen in the mangrove rivulus, Livingston et al. 2018), potentially resulting in a build-up of NH_3 in the body. Nevertheless, high intertidal animals typically do not accumulate high systemic levels of NH_3, and the clingfish *Sicyases sanguineus* can even avoid NH_3 build-up while being air-exposed for over a day (Gordon et al. 1970). Because the risk of nitrogenous waste accumulation is related to air exposure, it may not be a major stressor for animals in estuaries or tidepools that remain immersed throughout the tidal cycle. Thus, this section focuses on animals in high intertidal habitats (i.e. rocky and sandy/muddy shores) that are typically emerged during low tide (Figure 3).

The adaptations that allow high intertidal (amphibious) fishes to cope with NH_3 accumulation and excretion during emersion have recently been reviewed (Chew & Ip 2014, Wright & Turko 2016, Ip & Chew 2018), and detailed information specifically for mudskippers is also available in Clayton (1993) and Jaafar & Murdy (2017); therefore, these adaptations are only briefly summarized here. Three major strategies can be identified that avoid NH_3 accumulation: (1) a reduction in NH_3 production by reducing proteolysis and amino acid catabolism; (2) active excretion of NH_3 or ammonium (NH_4^+) by the gills, skin and gut; and (3) NH_3 detoxification by conversion into glutamine and, to a lesser extent, urea (Table 2). When excreting NH_3 across a non-branchial surface, some species [e.g. the mangrove rivulus *Kryptolebias marmoratus* (Frick & Wright 2002) and common blenny *Blennius pholis* (Davenport & Sayer 1986)] are able to release NH_3 as a gas (volatilization). In *Kryptolebias marmoratus*, this can account for over 40% of total ammonia secretion, which is likely promoted/facilitated by the increase in NH_4^+ concentration and the pH (alkalinization) on its cutaneous surfaces upon emersion, and by ammonia-transporting Rhesus glycoproteins (Rhcg1 and Rhcg2) detected in the skin that are normally absent in fully aquatic fish (Litwiller et al. 2006, Cooper et al. 2013, Livingston et al. 2018). It should be noted that this mechanism of NH_3 volatilization is not limited to intertidal fish; as it has also been described in freshwater amphibious fishes (Livingston et al. 2018). Urea synthesis (ureotely) is not a major detoxifying mechanism among intertidal fishes (as compared to lungfishes), and few species possess a complete and functional ornithine-urea cycle (i.e. the enzymes needed to convert NH_3 into urea in the liver, and at sufficient levels of activity) (Chew & Ip 2014, Wright & Turko 2016, Ip & Chew 2018, Livingston et al. 2018). This is likely because it is an energetically expensive process, as a total of 5 mol of ATP are hydrolysed for each mol of urea synthesized. Indeed, tidepool sculpins (*Oligocottus maculosus*) only secrete 8%–17% of their nitrogenous waste as urea (Wright & Wood 1995). In addition to these preventative strategies against NH_3 build-up, intertidal fishes can have a high tolerance to elevated NH_3 levels in the brain, an organ that is particularly sensitive to NH_3 toxicity (Chew & Ip 2014, Ip & Chew 2018).

The high intertidal mudskipper *Periophthalmodon schlosseri* has adaptations that illustrate many of the above strategies, that are often not shared by mudskipper species inhabiting lower shore heights. During emersion, it reduces protein and amino acid catabolism (or it only partially catabolizes amino acids, forming alanine) and mitochondria-rich branchial cells actively excrete NH_3 against the concentration gradient (Wilson et al. 2000, Lim et al. 2001, Randall et al. 2004).

The modified gills of *Periophthalmodon schlosseri* have branched filaments and fused lamellae, which trap water during emersion allowing NH_3 excretion to continue, whereas the gills of the lower tidal relative *Boleophthalmus boddaerti* are comparable to those of aquatic fishes and are not able to hold water (Ip & Chew 2018). This trapped water could also reduce the risk of desiccation of the gills during air exposure, and hence, can be regarded as a multifunctional adaptation (Lam et al. 2006). Furthermore, *Periophthalmodon schlosseri* excretes more NH_3 than *Boleophthalmus boddaerti* once re-immersed (Chew et al. 2003). This may be explained, on a molecular level, by the fact that an important gill NH_3 transporter (Rhcg1) in two species of high intertidal mudskippers (*Periophthalmodon schlosseri* and *P. magnuspinnatus*) contains three more hydrophobic amino acid residues at the central pore, than in their low intertidal relative *Boleophthalmus pectinirostris* (You et al. 2014, 2018). Such an adaptation might allow for more effective NH_3 excretion. Finally, *Periophthalmodon schlosseri* can excrete 26% of its nitrogenous waste as urea (Ip et al. 2004b), and both high and low intertidal species of mudskippers (*Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*) can tolerate high levels of NH_3 in the brain (>14 µmol/g whereas mammalian brains rarely tolerate >1 µmol/g) and are able to detoxify NH_3 into glutamate in this tissue (Ip et al. 2005, Lam et al. 2006).

Intertidal invertebrates vary in which mechanism(s) they utilize to avoid emersion-related nitrogenous waste accumulation, although, some mechanisms are similar to those observed in fishes. High intertidal *Anthopleura elegantissima* anemones that are exposed to air for up to 18 hours/day, do not excrete NH_3 at a higher rate during immersion than low intertidal individuals (Zamer 1986, Zamer & Shick 1987). However, they appear to use metabolic depression during emersion, which would not only reduce energy expenditure, but also NH_3 production. It is also possible that anemones recycle NH_3 by passing it to their endosymbiotic algae, reducing the requirement for exogenous nitrogen uptake by these endosymbionts (Shick 1991). The high intertidal snail *Littorina saxatilis* has a higher nitrogenous excretion rate in seawater, and excretes proportionally more nitrogen as urea as compared to its lower intertidal relative *Littorina obtusata* (Aldridge et al. 1995). Finally, the littoral fringe beachflea *Orchestia gammarellus* retains exosomatic water during emersion, which allows NH_4^+ excretion from the gills to continue unimpeded (Spicer & Taylor 1994b).

Among other intertidal crustaceans, some isopod species (*Alloniscus perconvexus*, *Tylos punctatus* and *Ligia occidentalis*) accumulate glutamine as a non-toxic intermediary nitrogen store, whereas *A. perconvexus* and *T. punctatus* can also release considerable amounts of NH_3 into the air by volatilization (Nakamura & Wright 2013). Although all three isopod species still excrete NH_3 directly into the seawater during high tide, similar to their subtidal relatives, these adaptations are key innovations that are also seen in terrestrial isopods (Wright & Pena-Peralta 2005). Glutaminase catalyses the release of NH_3 from glutamine, which is needed to create a high concentration of NH_3 for volatilization. In intertidal isopods, glutaminase activity is half that of terrestrial species, which reflects their dual mode of nitrogen excretion (Nakamura & Wright 2013). The intertidal crab *Carcinus maenas* can also detoxify NH_3 by accumulating glutamine in the muscle during air exposure (Durand & Regnault 1998, Durand et al. 1999). This allows *Carcinus maenas* to avoid high NH_3 levels in the haemolymph (below or equal to 0.1 mmol/L) for at least 72 hours of emersion. This is in contrast to the subtidal crab *Necora puber* from the same local environment, which continuously accumulated NH_3 (up to 10.4 mmol/L) during 18 hours of emersion because it is unable to detoxify NH_3 into glutamine.

Protection against UV radiation

All marine animals that inhabit shallow waters are exposed to ultraviolet (UV) radiation, so this environmental stressor is not unique to the high intertidal zone (Shick & Dunlap 2002). However, compared to the subtidal zone, the intertidal zone experiences more UV radiation – with the high intertidal zone at the extreme end (Peterson 1991). This is because the lower water depth and longer duration of air exposure result in less UV radiation being filtered out by seawater (i.e. there is less attenuation of short wavelength radiation) (Table 1 and Figure 2) (Raffaelli & Hawkins 1999, Rawlings 1999, Denny & Gaines 2007, Richards 2011); although, field measurements of the UV radiation intensity (in mW/m^2) across the vertical intertidal gradient are still lacking. Nonetheless, it is apparent from on-site data that intertidal animals need to cope with large variations in UV radiation intensity throughout the day, on a seasonal basis, and with changes in cloud cover (Carrasco-Malio et al. 2014, Pulgar et al. 2015, 2017). UV radiation is harmful because UV-B rays (~280–320 nm) are absorbed by pyrimidine bases and cause DNA mutations. Further, the photoreactivity of UV-A (~320–400 nm) leads to the formation of reactive oxygen species (ROS), which in turn damage macromolecules (Cockell & Knowland 1999, Shick & Dunlap 2002, Carrasco-Malio et al. 2014). In a few intertidal animals, the harmful effects of UV have been illustrated, in particular in vulnerable early life-stages (e.g. in mollusc embryos/larvae/juveniles when the UV-protective shell is still absent or translucent/thin). For instance, in the intertidal gastropods *Nucella emarginata* (Rawlings 1996), *Siphonaria denticulata* and *Bembicium nanum* (Przeslawski et al. 2005), UV exposure of embryos was reported to cause substantial mortality, especially in combination with high temperature and salinity conditions (in the case of the latter two species).

There are various DNA repair mechanisms, and damaged proteins/lipids can be synthesized anew (Cockell & Knowland 1999). However, this requires energy, which is observed, for example, in *Girella laevifrons*. This is a fish species that occupies tidepools in the high intertidal zone during its 2-year juvenile stage (after which it migrates to the subtidal zone for reproduction) (Pulgar et al. 2005). Exposure to UV radiation at an intensity and time period similar to that experienced in its natural habitat, was shown to increase metabolic rate ($\dot{M}O_2$) and reduce growth (weight gain) (García-Huidobro et al. 2017, Pulgar et al. 2017). Therefore, a more energetically efficient strategy is to avoid or protect against UV radiation (Table 2) (Cockell & Knowland 1999, Shick & Dunlap 2002). Intertidal animals minimize UV radiation exposure by using several behaviours. These include reducing the amount of exposed external surfaces and seeking shelter; which are the same behavioural responses used to avoid desiccation, and heat and salinity stress (discussed above). For instance, peak levels of UV irradiation cause contraction of the anemone *Anthopleura elegantissima* (with and without endosymbiotic algae) even when it is continuously immersed, and it also attaches debris to its column (Shick & Dykens 1984). The high tidepool fish *Girella laevifrons* actively seeks shelter underneath rocks when experimentally exposed to UV radiation (Pulgar et al. 2017). Embryos of the snail *Nucella emarginata* are encased in a thick egg capsule. This capsule functions as a form of shield or shelter, and blocks out a substantial amount of UV radiation (>95% of UV-B and >45% of UV-A) and this improves survival during UV exposure; although the properties of the capsule that block UV radiation remain unclear (Rawlings 1996).

Adaptations that are particularly/more unique to this type of stressor include the use of: (1) 'sunscreens' (UV-absorptive pigments) such as melanin and mycosporine-like amino acids (MAAs) in the skin and mucus; and (2) antioxidants such as carotenoids, ascorbic acid, superoxide dismutase (SOD) and catalase that quench and deactivate ROS (Cockell & Knowland 1999, Shick & Dunlap 2002). These adaptations are very widespread across phylogenetic groups and climates. For example, MAAs are found in temperate Pacific intertidal *Anthopleura* anemones (Shick et al. 2002, Stochaj et al. 1994) and numerous Antarctic intertidal species of annelids, molluscs and crustaceans (Karentz et al. 1991). Though, these UV-absorptive compounds are not exclusive to intertidal marine animals and are also synthesized by subtidal animals such as corals. In the intertidal *Anthopleura elegantissima*, SOD and catalase activity are disproportionally high in the external column tissue, which is exposed to the most sunlight during contraction (Dykens & Shick 1984). The endosymbiotic algae in *Anthopleura elegantissima* could also induce oxidative stress. However, there are only a few algae in the column, which suggests that the higher antioxidant activities are an adaptation to protect against oxidative stress from UV exposure rather than from photosynthesis.

The upper tidepool fish *Girella laevifrons* also actively increases catalase and SOD enzyme activities upon UV exposure (Carrasco-Malio et al. 2014).

In a few cases, high intertidal animals have been specifically shown to exhibit greater defences against UV radiation than their low intertidal relatives. Upper intertidal sculpin species have higher mucus MAA concentrations than lower intertidal species (Zamzow 2003). In *Mytilus californianus*, individuals on the high edge of the mussel bed accumulate more carotenoids in their gonadal tissues than those on the low edge of beds (Petes et al. 2008), possibly to protect their gametes or vulnerable early developmental stages against UV radiation once they are released into the water column and no longer sheltered by the parent shell. SOD activity levels in the digestive gland and gills were also higher in middle/high versus low intertidal *Mytilus edulis* (Letendre et al. 2008); although in this case, it is doubtful that this trend in oxidative stress defence is explicitly associated with UV exposure. In adult mussels, UV rays are unlikely to reach these internal tissues (thus, a different abiotic stressor may be driving this response).

Intertidal animals, in general, also appear to enhance their protection against UV radiation compared to subtidal animals. For instance, in the Antarctic limpet *Nacella concinna*, transcriptome analysis (i.e. contrasting gene expression in inter- versus subtidal individuals) showed that the intertidal ecotype upregulated genes for DNA repair enzymes (such as DNA ligase 1), antioxidants (including SOD and others) and the pentose shunt enzyme 6-phosphogluconate dehydrogenase (PGD) (Clark et al. 2018). PGD generates NADH, which can be used as an energy donor by antioxidant enzymes to combat ROS formation. Interestingly, after transplantation to the subtidal zone for 1 month, and even after 9 months of submergence in the laboratory, the intertidal limpets maintained a higher expression of most antioxidant genes. This underlines the importance of this protective mechanism in allowing limpets to inhabit the intertidal zone. DNA methylation, a form of epigenetic regulation which is often linked to reduced gene expression (Nelson & Cox 2000, Bossdorf et al. 2008), is at least partially responsible for creating the distinct, and generally permanent, gene expression profile of intertidal limpets (Clark et al. 2018). The methylation of cytosine residues (5-methylcytosine) was strongly associated with ecotype shore height, and while this pattern was largely unchanged by reciprocal transplantation, it disappeared after laboratory submergence. This phenotype reversal indicates that laboratory conditions can be very different from the environment experienced by limpets in the field.

Protection against wave action or ice disturbance

Animals in the intertidal zone experience considerably more wave action than those in the subtidal zone, because of the breaking waves that are formed at the sea-to-land transition. The severity of wave action varies greatly among intertidal habitats (Figure 3). For instance, the shores of isolated oceanic islands are usually exposed to heavy waves, as winds can build up over large distances, whereas in sheltered areas such as estuaries and fjords, wave exposure can be minimal (Raffaelli & Hawkins 1999). For the inhabitants of the high intertidal zone, wave action is not necessarily stressful and can be beneficial, as it helps with the supply of oxygen and nutrients, and in warmer climates the wave spray offers some cooling. On wave-exposed rocky shores certain predators (e.g. green crab, *Carcinus maenas*) may also be less abundant, so particular species (e.g. dog whelk, *Nucella lapillus*) may experience a lower predation risk (e.g. see Kitching et al. 1966, Hughes & Elner 1979, Gibbs 1993). However, for many sessile and slow-moving animals, being swept away from the substrate by waves has severe consequences; for example, by increasing vulnerability to subtidal predation or causing physical damage (Denny & Gaines 2007). In the rocky intertidal, wave exposure is typically the most substantial, especially when smaller rocks are ‘thrown’ against the shore by waves, and act as projectiles that can lead to mortality of barnacles and limpets (Shanks & Wright 1986). In colder climates, brash ice also mechanically disturbs the animals in the intertidal zone.

To avoid being swept away by waves, intertidal animals use several strategies to strongly adhere to the substrate: for example, by secreting cement (barnacles) or byssus threads (mussels) (Denny & Gaines 2007) or using suction (snails, clingfishes) (Table 2) (Ebeling et al. 1970, Branch 1985). The clingfish *Sicyases sanguineus*, which inhabits heavily wave-exposed high intertidal areas, attaches itself to vertical rock surfaces with a ventral sucking disc ('sucker') formed by modified pelvic fins (Ebeling et al. 1970). Among two ecotypes of the snail *Trophon geversianus* found on rocky shores in Patagonia, the intertidal ecotype has an expanded aperture and lower-spired shell compared to the subtidal ecotype. This morphology allows for better attachment to the substrate because it accommodates a larger foot and reduces wave drag (Márquez et al. 2015). Similar plastic changes in shell morphology (i.e. larger aperture and lighter shell construction) have been described in the Atlantic dogwinkle *Nucella lapillus* in response to wave action based on comparisons of individuals from wave-exposed and sheltered shores (see Kitching et al. 1966, Hughes & Elner 1979, Gibbs 1993 and references below). However, as wave exposure and predation pressure, which is also known to directly or indirectly affect shell shape and thickness in *Nucella lapillus*, can interact, it can be challenging to tease apart their individual effects (e.g. Bourdeau 2010, Pascoal et al. 2012). In Antarctica, the intertidal ecotype of the limpet *Nacella concinna* has a stronger/thicker shell compared to the subtidal ecotype, and this provides better protection against mechanical stress from ice (Clark et al. 2018). This adaptation is associated with higher constitutive expression levels of genes putatively involved in the formation of the shell matrix (thrombospondin- and proline-rich proteins) (Clark et al. 2018, Marin 2020). Interestingly, some limpet species have evolved rhythms of activity as a behavioural adaptation that works in concert with strong physical adhesion. This ensures that they are not off their 'scars' (i.e. homing site on the rock where the shell fits tightly into the substrate) when wave action can dislodge them (Branch 1985). This behaviour is seen, for instance, in the high intertidal *Siphonaria capensis* from South Africa which only feeds at low tide (Branch & Cherry 1985).

It is important to note that among intertidal animals, those in the upper zone are on average exposed to a lower frequency and force of wave action/ice disturbance than in the lower zone (e.g. see McQuaid 1981). This is simply because of the shorter contact time with the sea and the larger distance from the strongest breaking waves. Thus, this environmental stressor can result in the opposite vertical intertidal gradient in morphological and physiological adaptations as compared to the previously discussed stressors. For instance, small-sized chiton species (e.g. *Acanthopleura echinata*) have been found to be more abundant in the low intertidal than larger-sized species (e.g. *Chiton barnesi*) (Otaíza & Santelices 1985), and this pattern might be explained by the lower wave drag associated with a small size. Similarly, the wave-exposed low intertidal ecotype of the periwinkle *Littorina saxatilis* – also called the 'wave type' – has a smaller shell than the high intertidal ecotype (Johannesson 2016). Finally, the *L. saxatilis* wave type has a larger aperture along with a 1.4-fold larger foot muscle, and this helps it to avoid being dislodged by the waves (Carvajal-Rodríguez et al. 2005, Martínez-Fernández et al. 2008). Proteomic analysis of whole-animal tissue homogenates has also revealed that the wave type expresses ~3-fold higher levels of the glycolytic enzyme fructose-bisphosphate aldolase, and ~73-fold higher levels of arginine kinase which catalyses the transfer of phosphate to ADP from phosphoarginine (Martínez-Fernández et al. 2008). This allows the low intertidal ecotype to enhance ATP supply to support the energy demands of withstanding wave action.

Adaptations to H_2S toxicity

In sandy/muddy intertidal habitats such as mangroves, mudflats and salt marches, animals are exposed to the toxic gas hydrogen sulphide (H_2S), which is produced by sulphate-reducing bacteria in anoxic regions of the sediment (Figure 3) (Schulte 2011, Dufour 2018). This anoxic region is the result of oxygen consumption by bacterial decomposers in the upper sediment layer, and the subsequent use of sulphate as an alternative chemical substrate for O_2 leads to the accumulation of H_2S (Dufour 2018). Animals in muddy intertidal environments are, in particular, exposed to H_2S as O_2 diffusion into fine sediment is more limited than in coarse substrate (i.e. the anaerobic layer starts at less than 1 cm below the surface whereas in sandy shores it begins at a considerably deeper depth). H_2S is toxic to animals because it binds to the ferric haem site of the cytochrome *c* oxidase 3 subunit (COX3). This prevents O_2 from binding to COX3, inhibiting aerobic respiration and blocking the production of ATP through the electron transport chain (Ip et al. 2004a, Tobler et al. 2016, Cochrane et al. 2019). The binding is reversible, but more potent than that of cyanide, as 50% inhibition occurs at H_2S concentrations as low as 1 µmol/L. Hydrogen sulphide also binds to the haem groups of haemoglobin to form sulphhaemoglobin (SHb), which inhibits the binding and transport of O_2. Muddy/sandy intertidal zones are certainly not the only H_2S-rich marine environments (e.g. cold seeps and hydrothermal vents also exhibit high H_2S levels) (Dubilier et al. 2008, Tobler et al. 2016). However, H_2S concentrations in intertidal environments can show diel fluctuations (i.e. low during the day, high at night; Hagerman 1998, Lam et al. 2006), and the exposure of intertidal animals to H_2S can be linked to tidal movements. For instance, the intertidal mudskipper *Boleophthalmus boddaerti* is actively emerged on mudflats at low tide, but during high tide it seeks shelter in burrows in the sediment (to avoid aquatic/terrestrial predation), where it can be exposed to up to 0.3 mmol/L of H_2S (Ip et al. 2004a). Other intertidal animals, such as the mudflat worm *Urechis unicinctus*, permanently reside in burrows and are constantly exposed to high levels of H_2S (Liu et al. 2015).

Among fish, intertidal species generally exhibit higher H_2S tolerance than those which inhabit the open ocean (Lam et al. 2006) and some persist in environments with extremely high H_2S levels (>1.1 mmol/L) (Rossi et al. 2019). Because H_2S is concentrated in the sediment, fishes that live on mudflats and among mangroves avoid exposure to high H_2S levels by remaining at the top layer of the sediment or at the water surface, or by exiting the water altogether (e.g. a behaviour displayed by the mangrove rivulus *Kryptolebias marmoratus* and mangrove molly *Poecilia orri*) (Table 2) (Schulte 2011, Taylor 2012, Cochrane et al. 2019, Rossi et al. 2019). In the mangrove rivulus, the outer epithelial skin layer may also act as a barrier that limits the entry of H_2S into the body (Martin & Currie 2020). When behavioural avoidance or emersion is not possible, and harmful amounts of H_2S diffuse into internal tissues, an alternative coping strategy for intertidal animals is to switch to anaerobic respiration, which is independent of COX3 function. However, this does not appear to be a major strategy among animals that inhabit H_2S-rich environments, possibly because of the low sustainability of anaerobiosis. For instance, the mudskipper *Boleophthalmus boddaerti* does not accumulate lactate during H_2S exposure (Ip et al. 2004a), and the expression of glycolytic genes is low in the intertidal mudflat worm *Urechis unicinctus* which also inhabits H_2S-rich burrows (Liu et al. 2015). A more common adaptation is to enzymatically detoxify H_2S by oxidation. In *Urechis unicinctus* (Liu et al. 2015) and *Boleophthalmus boddaerti* (Ip et al. 2004a), the mRNA expression and activity of detoxifying mitochondrial enzymes increases upon H_2S exposure. These enzymes include sulfur transferase (ST) and sulphide-quinone oxidoreductase (SQR), which convert H_2S into thiosulphate ($S_2O_3^{2-}$) and other harmless oxidized compounds. The sand clam (*Donax serra*) primarily detoxifies H_2S into $S_2O_3^{2-}$, and this mechanism appears to be very effective during acute H_2S exposure as H_2S itself was not detected in the tissues (Laudien et al. 2002). In the saltmarsh resident *Fundulus parvipinnis*, erythrocyte mitochondrial H_2S oxidation also plays an important role, and results in lower SHb formation (Lam et al. 2006). Although, it is important to note that H_2S detoxification by oxidation can only occur in the presence of sufficient O_2. This is why the H_2S tolerance of animals can be reduced when combined with anoxia/hypoxia (e.g. in the green crab *Carcinus maenas*, see Hagerman 1998), which is relevant given that these two environmental stressors often co-occur.

In contrast to avoiding or detoxifying H_2S, many intertidal invertebrates are able to take advantage of H_2S exposure. For example, *Urechis unicinctus* is thought to use a super-complex composed of SQR and electron transport chain complexes III and IV (which are also upregulated during H_2S exposure) to catalyse the production of ATP by using H_2S as an electron donor instead of NADH and $FADH_2$ (Liu et al. 2015). The oxidation of H_2S releases electrons that can be transferred into the oxidative phosphorylation pathway (Tobler et al. 2016). A few sulphide-tolerant intertidal animals such as the ribbed mussel *Geukensia demissa* (Doeller et al. 2001) and the lugworm *Arenicola marina* (Völkel & Grieshaber 1997) indeed appear to use sulphide to produce ATP, which is a remarkable form of chemolithoheterotrophy. Unlike most animals that solely produce ATP from organic molecules such as glucose, these particular invertebrates are able to obtain energy from the oxidation of inorganic compounds (although they still require organic molecules as a carbon source). However, far more common are intertidal animals (nematodes, annelids and bivalves) with chemosymbiotic relationships, where H_2S is detoxified by ecto- or endosymbiotic H_2S-oxidizing chemosynthetic/chemolithoautotrophic bacteria. These bacterial symbionts can occupy the gut lumen (in nematodes), or are associated with the gills (in clams), for instance, and are able to convert carbon dioxide (CO_2) into organic carbon-molecules using the energy from H_2S oxidation. This type of symbiosis does not only allow for H_2S detoxification, but the bacteria are also an essential food source for the intertidal animals. See reviews by Taylor & Glover (2000), Dubilier et al. (2008), Taylor et al. (2011) and Dufour (2018) for detailed information on H_2S-oxidizing chemosymbiotic invertebrates (such as lucinid bivalves) in the intertidal zone, as well as in other marine ecosystems (e.g. the deep sea).

Concluding remarks

The high intertidal zone is a unique marine environment, because many of its animal inhabitants need to be highly adapted to living in two worlds: the marine and the terrestrial environment. As a result of the typical daily transition between immersion in seawater and emersion in air (which have very different properties, see Table 1), and given that the duration of emersion is often greater than that of immersion, these animals are exposed to severe/extreme physicochemical conditions (Figures 1 and 2). Which specific abiotic stressors are encountered depends on the type of intertidal habitat (e.g. the climate, substrate, extent of freshwater input), although high intertidal habitats have many challenges in common (Figure 3). This review has provided a comprehensive overview of the various adaptations and responses of marine animals that help them deal with the many stressors characteristic of the high intertidal zone: desiccation, thermal and osmotic stress, hypoxia, nitrogenous waste accumulation, UV exposure, wave/ice disturbance and H_2S toxicity (Table 2). For each specific stressor, these adaptations can be further categorized according to the strategy utilized to deal with them. Often, these strategies first involve behaviours used to avoid the stressor, followed by adaptations or responses that improve the physiological tolerance of the animal to the stressor. Adaptations and plastic phenotypic responses can be related to changes in animal behaviour, morphology, physiology or biochemistry, but frequently several levels of biological organization are integrated into the animal's coping mechanism(s). For instance, the release of vaso- and isotocin from the neurohypophysis in intertidal mudskippers triggers their retreat into burrows to avoid desiccation (i.e. this adaptation is behavioural with a physiological driver) (Katayama et al. 2018), and the higher constitutive gene expression for shell matrix proteins in intertidal limpets strengthens the shell as a defence against wave and ice disturbance (i.e. it is a biochemical change that underlies this morphological trait) (Clark et al. 2018). Interestingly, an adaptation/response to a particular environmental stressor can have various effects (synergistic, additive, antagonistic or potentiating) with respect to the animal's ability to respond to or tolerate another stressor, and when the stressors co-occur, animals need to find a balance between adaptive strategies. An example of a trade-off (antagonistic effect) is gaping in intertidal bivalves. This behavioural strategy allows for evaporative cooling during heat stress and aerial O_2 uptake to avoid hypoxemia during emersion; however, this comes with the risk of desiccation, and this is likely why this behaviour is only sparsely used by some high intertidal mussels (Gleason et al. 2017). The opposite situation is when adaptations allow for cross-tolerance, which appears to be more common (see the 'Adaptations allowing for cross-tolerance' section below).

Another conclusion that can be drawn from this review is that many adaptations are shared among diverse animal groups. For example, high intertidal sculpins (Speers-Roesch et al. 2013), crabs (Viña et al. 2018) and periwinkles (Sokolova & Pörtner 2001a) improve their survival during diel hypoxia (i.e. aquatic hypoxia or internal hypoxia due to air exposure) by enhancing their anaerobic capacity and by increasing fermentable energy reserves. In addition, increased (gene expression/activity) levels of antioxidants to protect against reactive oxygen species (ROS), which are generated by UV radiation, are used by high intertidal mussels (Letendre et al. 2008, Petes et al. 2008) as well as intertidal limpets (Clark et al. 2018). The examples provided in Table 2 illustrate the types of adaptations that occur in multiple taxa. It is striking that these adaptations are often universal among animals that are very different in their mobility (sessile or mobile) or phylogeny (invertebrate or vertebrate), and perhaps they can be considered as examples of convergent evolution/phenotypic convergence. Finally, this review shows that several adaptations are phenotypically plastic (i.e. gained during the animal's lifetime through exposure to the high intertidal environment), and sometimes can be reversible. Examples of phenotypic plasticity are common among higher and lower intertidal mollusc ecotypes. Plastic responses may be energetically expensive for an animal (Wright & Turko 2016), but the advantage to a species as a whole is that they provide flexibility with regard to where an individual settles across the vertical range of the intertidal zone. On the other hand, many high intertidal animals have evolved adaptations that are permanent and embedded in their genome (e.g. non-synonymous amino acid substitutions that alter the function of a protein), and these 'fixed' changes may allow them to be more optimally adapted to cope with the specific challenges of the upper tidal environment.

Adaptations allowing for cross-tolerance

In this review we have identified several adaptations that appear to confer cross-tolerance to other abiotic stressors (Sinclair et al. 2013). The following is a list of exemplar adaptations. (1) In intertidal snails (*Lottia* and *Nucella* limpets) and crabs (*Petrolisthes* species), adaptations that improve air breathing ability (i.e. capacity for O_2 uptake from the air and the maintenance of aerobic metabolism) allow for a greater tolerance to emersion-induced hypoxemia as well as high temperatures. These data strongly suggest that heat tolerance is O_2 limited in these animals. This hypothesis is consistent with the widely known, but also controversial, Oxygen and Capacity Limited Thermal Tolerance concept (see Pörtner et al. 2017, Jutfelt et al. 2018) which provides a theoretical framework that relates O_2 consumption and aerobic scope to the thermal tolerance of aquatic animals. (2) In fishes (tidepool sculpins), a heat-induced heat shock protein (Hsp) response can improve tolerance against subsequent osmotic or hypoxic stress. (3) The trend towards high-spired shells in high-shore limpets has the benefit of reducing water loss in air. However, this morphological adaptation also potentially avoids heat stress by reducing conductive heat gain and increasing convective heat loss. (4) Organic compatible osmolytes are essential for maintaining osmotic balance and avoiding excess cellular water loss/gain in intertidal osmoconforming invertebrates (anemones, worms, molluscs) during salinity fluctuations, but also appear to have an important role in freeze tolerance. (5) In some high intertidal fish (mudskippers) and crustaceans (amphipods and crabs), water trapping in the gills allows both gas exchange and NH_4^+ secretion to continue while emerged. (6) The behaviour of seeking refuge underneath some form of cover and reducing exposed surfaces during emersion is truly multifunctional, as it helps a diversity of intertidal animals to avoid exposure to various environmental stressors including desiccation, heat exposure, salinity fluctuations and UV radiation. (7) Anaerobiosis, and/or the use of varying degrees of metabolic depression, are strategies that improve hypoxia survival in all major intertidal animal groups, but have also been shown to contribute to the heat and freeze tolerance of intertidal molluscs, and to the tolerance of worms, snails and fishes to low salinity. (8) Finally, in intertidal and estuarine fishes (e.g. killifish, mudskippers), the water permeability of the skin, gills and intestines is lower, which helps protect against desiccation during air exposure, and minimizes the costs of osmoregulation during salinity stress.

More adaptations allowing for cross-tolerance are likely to exist in high intertidal animals, given that this review only covers a selection of challenges that animals need to respond and/or adapt to in their environment, and further examples of cross-tolerance may be uncovered by future research. The existence of 'multi-stressor'-related, multifunctional, adaptations is not at all surprising, as it reflects the wide range of environmental stressors that high intertidal animals are exposed to, often simultaneously (Figure 2). For instance, on rocky shores in warmer climates, desiccation stress is typically encountered along with heat stress, whereas in soft substrate environments, hypoxia is frequently associated with high H_2S levels. Although it is important for our understanding of adaptions and phenotypic plasticity that we isolate individual stressors in experimental studies, these stressors inevitably co-occur in nature. This is why it is key to also perform 'multi-stressor' studies in a laboratory setting (e.g. see Nancollas 2020 and below).

Perspectives

Benefits of life in the high intertidal zone

The inevitable question that comes up after discussing the multitude of challenges in the high intertidal zone, is why this ecological region is inhabited by such a great diversity of marine animals (why are these animals going through all the trouble?). However, the high intertidal zone has some important biotic and abiotic advantages over lower coastal areas. For some animals, the upper tidal zone allows for an escape from low intertidal/subtidal predators that are unable to survive in this environment. Although these animals may then be exposed to terrestrial predators (e.g. birds), it seems that the 'risk of being eaten' is less than in lower intertidal areas. For instance, the common killifish *Fundulus heteroclitus* has higher survival rates in the intertidal compared to the subtidal zone (Banikas & Thompson 2012), and the subtidal ecotype of the snail *Trophon geversianus* suffers higher predation pressure from crabs than the intertidal ecotype (Márquez et al. 2015). Periods of avian predation on intertidal sandy/muddy shores are also usually brief, coinciding with migration, so this may only temporarily affect intertidal animals (Peterson 1991). The high intertidal zone, specifically, offers protection to *Mytilus* mussels and acorn barnacles from low intertidal predatory *Pisaster* seastars that are unable to extend beyond the mid-intertidal (Denny & Gaines 2007, Harley 2011). In the case of the snail *Littorina sitkana* from rocky shore habitats in British Columbia, Canada, predation by the pile perch *Rhacochilus vacca* is also virtually absent at high tidal levels, but considerable at low tidal levels (McCormack 1982). In many species, juveniles find a refuge in the high intertidal, as adult counterparts dominate the low areas with more favourable conditions. For example, recruits of the sculpin *Oligocottus snyderi* use suboptimal shallow tidepools that are more common in the high intertidal zone to avoid the aggression and cannibalism by adults that dominate the more favourable deeper tidepools in the low intertidal zone (Ritter 2017).

In addition, intertidal fishes may take advantage of the relatively high O_2 level in air (Table 1) which benefits the development of their eggs (Sayer 2005, Martin 2014). In air, eggs may also be incubated at a higher temperature (which speeds up embryo development), and they are protected from aquatic predators (Martin et al. 2004). For example, the common killifish (*Fundulus heteroclitus*) purposefully lays its eggs at the upper limit of the seawater during spring tide (i.e. the highest high tide of the tidal cycle), which leaves them on the beach exposed to the air for the rest of the tidal cycle (Martin et al. 2004, Schulte 2011). There are also several subtidal fishes (e.g. plainfin midshipman, *Porichthys notatus*; sharpnose sculpin, *Clinocottus acuticeps*; white stickleback, *Gasterosteus* sp.) that enter the high intertidal zone solely to construct nests, spawn and, in some cases, provide parental care (e.g. guarder males), in order to take advantage of its several benefits (Martin et al. 2004, Bose et al. 2019).

Mudskippers that spend the majority of their life emerged, and are capable of air breathing, take full advantage of the O_2-rich air. Although there are many adjustments required to survive/thrive as this more terrestrial phenotype, it appears to have several advantages as compared to dealing with poor water conditions in their environment (such as hypoxia, high temperatures and toxic H_2S levels) (Giomi et al. 2014, Martin 2014, Wright & Turko 2016). Finally, there may be a lower energetic cost to ventilating air for O_2 uptake as compared to seawater, because of its much lower density and higher O_2 content (Table 1). These benefits have even been suggested to underlie the greater physiological performance and thermal tolerance of some intertidal invertebrates during emersion (Giomi et al. 2014, Bjelde et al. 2015).

High intertidal animals as model organisms

High intertidal marine animals have received considerable attention from biologists, which is partially due to their accessibility compared to animals found in other marine ecosystems (e.g. the deep sea) (Nybakken 1993). However, there are other reasons why high intertidal animals have garnered so much attention. These marine animals have adjusted to life that often involves little immersion in water, and this may provide a unique opportunity for gaining insights into the evolution of early terrestrial animals and the adaptations needed to make the water-to-land transition (Sayer 2005, You et al. 2014, Wright & Turko 2016, Katayama et al. 2018). For instance, paleontological studies imply that the intertidal zone is the place from where the tetrapods entered the terrestrial realm (Graham & Lee 2004, Lam et al. 2006). High intertidal animals may also reflect the selective pressures that animals experienced when moving towards a terrestrial lifestyle. For instance, aquatic hypoxia is often a trigger for modern intertidal fishes (such as sculpins and mudskippers) to actively emerge (Table 2), and it is generally thought that hypoxia was an initial driver of the development of air-breathing in vertebrates that allowed for terrestrial colonization early in the evolutionary record (Sayer & Davenport 1991, Martin 1995, Bayley et al. 2019). On the other hand, it has been proposed that heat stress in the intertidal environment triggered the development of air-breathing abilities in some marine animals (Giomi et al. 2014), as aerial respiratory structures appear to be a key adaptation with regard to enhancing the thermal tolerance of present-day intertidal crabs in the tropics (Table 2). What extant intertidal animals can reveal about the evolutionary transition to the land, and specifically whether mudskippers provide a good model for early tetrapods, is an ongoing topic of discussion (Graham & Lee 2004).

In the light of global warming and climate change, a case can be also made for the importance of research on high intertidal animals. Because they are exposed to environmental challenges from both terrestrial and marine climate regimes, these animals could serve as early warning systems for the impacts of climate change (Helmuth et al. 2006). For instance, upper shore *Mytilus californianus* are projected to undergo larger changes in body temperature compared to lower shore conspecifics with global warming, due to the additive effect of warming water and air temperatures (Gilman et al. 2006). Furthermore, compared to low intertidal animals that inhabit less thermally variable environments, high intertidal species experience more severe heat stress, have optimal performance temperatures and upper thermal limits that are generally closer to their environmental temperatures (i.e. they have a smaller 'thermal safety margin', TSM), and they appear to have a reduced capacity to increase their heat tolerance through phenotypic plasticity/acclimation (Stillman 2002, 2003, Berger & Emlet 2007, Tomanek 2010, Somero 2010, 2012, Nguyen et al. 2011, Bjelde & Todgham 2013). The latter finding may be counterintuitive, but is based on experimental results collected from a large variety of animals groups, such as *Balanus* barnacles, *Mytilus* mussels, *Lottia* limpets and *Petrolisthes* crabs. In other words, despite their relatively high thermal tolerance, high intertidal animals might be especially vulnerable in this era of accelerated climate change, given that the susceptibility of all animals to climate change appears to depend to a large extent on the level of exposure to environmental temperature extremes, and their physiological tolerance and acclimation potential (Williams et al. 2008, Huey et al. 2012). Although, some of the impacts of global warming may be ameliorated ('buffered') by behavioural thermoregulation, as suggested by Marshall et al. (2015), given that snail species on the tropical rocky shore already have negative TSMs (i.e. the maximum environmental temperatures exceed their lethal temperatures). On the other hand, indirect effects from ecological biotic interactions can add a layer of complexity with regards to how high intertidal animals are affected by climate change, and they can further exacerbate the direct physiological impacts of warming. For instance, *Mytilus* mussels and acorn barnacles that rely on the higher intertidal zone as a refuge from lower intertidal/subtidal predators (e.g. the *Pisaster ochraceus* seastar) are losing a large amount of this predator-free habitat due to rising shore temperatures that have gradually limited their upper range (Harley 2011).

Future research directions

Although intertidal animals have been extensively studied, research comparing high versus low intertidal relatives or conspecifics are in relatively short supply. This is especially true for adaptations related to freezing and salinity stress, and UV radiation exposure, where the effect of shore height is little explored. Such comparative studies will be instrumental to understanding the specific evolutionary adaptations or plastic phenotypic responses of high intertidal animals while minimizing phylogenetic effects. In particular, studies using species comparisons that follow the 'comparative phylogenetic method', whereby phylogeny is carefully incorporated in the statistical contrasts (Garland et al. 2005), would contribute greatly to our understanding of evolutionary trends among high intertidal animals, i.e. by ensuring that the effect of interest (shore height) is not confounded by phylogenetic affinities. A small amount of research included in this review did apply this phylogenetically independent approach (e.g. Mandic et al. 2009a,b, 2012), but it is expected that this will become a more frequently followed methodology. The use of reciprocal transplantations is also a helpful experimental tool that allows for investigations of the specific role of phenotypic plasticity in sessile or sluggish species that colonize the high intertidal – provided that appropriate controls are included (i.e. transplantations to the same vertical level on the shore) to account for the effect of disorientation that an animal may experience after transplantation (see Chapman 2000, Underwood 2000). This is now a standard practice (e.g. Halpin et al. 2004, Altieri 2006), but was less common in the older literature (e.g. McQuaid 1981, McCormack 1982).

To a large extent, research efforts so far have concentrated on the behavioural, morphological, physiological and biochemical adaptations of high intertidal animals. While this research has revealed numerous and intriguing responses/adaptations (Table 2), recent studies on the genetics, epigenetics, transcriptomics, proteomics, metabolomics and protein biochemistry of high intertidal animals have provided additional insights into the underlying mechanisms that allow them to be successful in this environment (e.g. Mandic et al. 2014, You et al. 2014, Kennedy et al. 2020, Chen et al. 2021, Dong et al. 2021). However, there are only a small number of these molecular studies, and thus, they are a promising venue for further research that may further advance this field. In comparative physiology, 'omics' approaches can, for instance, allow for a better understanding of factors involved in cross-tolerance, by looking for common transcriptional responses to different environmental stressors (Torson et al. 2020). Rapid changes (i.e. ranging from minutes to hours) in gene expression or in the proteome (e.g. post-transcriptional and post-translational modifications, respectively) that play a role in the response of high intertidal animals to the tidal cycle can also be elucidated with these methods and/or described in more detail.

There is also a need for research that attempts to better reflect the complex physicochemical conditions encountered by high intertidal animals in the wild – including experiments that specifically account for the high variability, unpredictability (stochasticity) and the combined and repeated occurrence of stressors that are characteristic of this environment (Figure 2) (Raffaelli & Hawkins

1999, Gunderson et al. 2016). Such work (e.g. multi-stressor studies) has been done with several high intertidal animals, for instance *Lottia* sp. and *Cellana grata* limpets (Williams et al. 2011, Denny & Dowd 2012, Pasparakis et al. 2016, Drake et al. 2017), porcelain and green crabs (*Petrolisthes cinctipes* and *Carcinus maenas*; Paganini et al. 2014, Nancollas 2020, Nancollas & McGaw 2021) and the tidepool sculpin (*Oligocottus maculosus*; Somo et al. 2020), but overall, this area is still little explored (e.g. the majority of multi-stressor studies appears to be focused on subtidal animals; see review by Gunderson et al. 2016). Multi-stressor experiments can evaluate the interactive effects of various abiotic factors, many of which may be exacerbated with anthropogenic climate change. For instance, along with rising temperatures, high intertidal animals may encounter greater increases in environmental salinity through evaporation, or sudden exposure to hyposalinity stress due to more frequent and extreme rainfall events (Williams et al. 2011, Rivera-Ingraham & Lignot 2017). Multi-stressor experimental regimes are also helpful when trying to determine whether there is cross-tolerance to environmental stressors (i.e. if response X to stressor A increases tolerance to subsequent exposure to stressor B) (Sinclair et al. 2013).

In the area of freezing tolerance and avoidance, there is a lack of understanding of whether, and how, high intertidal animals cope with freezing stress as compared to those in the low intertidal (even though freezing exposure is evidently more severe on the high shore) – so this area is ripe for discovery. The potential link between oxidative stress and defences, and abiotic stressors on the high shore (e.g. UV radiation and salinity fluctuations), could also be further investigated (Rivera-Ingraham & Lignot 2017). Even in areas where there is abundant literature, for example on adaptations associated with heat stress in high intertidal animals, there are knowledge gaps. While studies where high intertidal animals are acclimated to warmer conditions for a few weeks or months are relatively common (see Stillman 2002, Berger & Emlet 2007, Tomanek 2010, Somero 2010), their informative value is somewhat unclear. Transgenerational experiments that involve the acclimation of multiple generations to a particular stressor may more profoundly improve our assessment of their capacity to adapt to global warming and other climate change-related environmental challenges. This is because they also consider potential transgenerational plasticity and evolutionary shifts (through epigenetics and genetic changes). This type of research can be time-consuming, but has already been done with animals from other aquatic environments (e.g. tropical reef and freshwater fish, see Donelson et al. 2011 and Morgan et al. 2020, respectively). Further, it would be a novel approach in the field of intertidal animal biology and may be pivotal in better defining their adaptive abilities and strategies.

BOX 1. GLOSSARY

Amphibious fishes
Fishes that spend periods of time out of the water as a natural part of their life history.

Antifreeze proteins or glycoproteins
Proteins that inhibit the growth of ice crystals, which is observed as thermal hysteresis – the delayed growth of an ice crystal during cooling below 0°C.

Antioxidants
Enzymes and other bioactive compounds that protect against oxidative stress by quenching and deactivating reactive oxygen species.

Aquatic surface respiration
A behaviour by aquatic breathers where the O_2-rich layer is skimmed from the water's surface.

Bradycardia
A significant decline in heart rate, for instance in response to aquatic hypoxia.

Compatible organic osmolytes
Organic solutes, such as free amino acids, that do not interact with or damage macromolecules when their concentration fluctuates.

Cross-tolerance
When an adaptation that enhances tolerance to a specific environmental stressor also allows for greater tolerance to another, or other, stressor(s).

Ecotype
Morphological variant within a species that occupies a particular habitat and that has undergone incomplete speciation.

Gaping
Opening of the shell by bivalves during emersion to allow for aerial O_2 uptake and/or evaporative cooling.

Heat shock proteins
Molecular chaperones whose expression is upregulated during heat stress (or another form of stress), and that improve the stability and repair of other proteins in the cell.

Ice-nucleating agents
Compounds that trigger ice crystal formation at a relatively high temperature.

LT_{50}
The emersion time or temperature at which 50% mortality occurs in a group of animals, which can be used as a measure for their desiccation tolerance or upper thermal tolerance, respectively.

Metabolic depression
A temporary, and reversible, reduction in metabolism below the standard (minimal) metabolic rate.

$\dot{M}O_2$
Mass-specific O_2 consumption rate.

Multifunctional adaptation
An adaptation that serves multiple functions.

NH_3 volatilization
Release of NH_3 as a gas, instead of excreting it into the water.

Phenotypic plasticity
The flexibility that allows animals with the same genetic make-up to express multiple phenotypes depending on the environment.

Q_{10}
The fold change for a physiological process (e.g. metabolic rate) following a 10°C increase or decrease in temperature.

Reciprocal transplantations
The transplantation of animals (typically sessile or sluggish species) from one site to another, and vice versa.

RNA interference
The suppression of mRNA translation into protein through the binding of microRNAs followed by degradation of the mRNA.

Acknowledgements

We thank Drs. Javier Santander, Ian Fleming and Andy J. Turko for input into earlier versions of this chapter; Dr. Amanda Bates for inviting RHJL to submit it for consideration as a review in OMBAR and for providing guidance during the revision process; and two anonymous reviewers for their valuable feedback. This work was supported by research funding awarded to AKG through the Natural Sciences and Engineering Research Council of Canada's Discovery Grant Program (249926-2011) and by the Ocean Frontier Institute (OFI) through an award from the Canada First Research Excellence Fund. During the writing of this paper, RHJL was supported by the OFI and a MUN School of Graduate Studies fellowship.

References

Aghmich, A., Taboada, S., Toll, L. & Ballesteros, M. 2016. First assessment of the rocky intertidal communities of Fildes Bay, King George Island (South Shetland Islands, Antarctica). *Polar Biology* **39**, 189–198.

Aldridge, D.W., Russell-Hunter, W.D. & McMahon, R.F. 1995. Effects of ambient temperature and of temperature acclimation on nitrogen excretion and differential catabolism of protein and nonprotein resources in the intertidal snails, *Littorina saxatilis* (Olivi) and *L. obtusata* (L.) *Hydrobiologia* **309**, 101–109.

Altieri, A.H. 2006. Inducible variation in hypoxia tolerance across the intertidal–subtidal distribution of the blue mussel *Mytilus edulis*. *Marine Ecology Progress Series* **325**, 295–300.

Amstutz, A., Firth, L.B., Spicer, J.I. & Hanley, M.E. 2021. Facing up to climate change: Community composition varies with aspect and surface temperature in the rocky intertidal. *Marine Environmental Research* **172**, 105482.

Ansart, A. & Vernon, P. 2003. Cold hardiness in molluscs. *Acta Oecologica* **24**, 95–102.

Aunaas, T. 1982. Nucleating agents in the haemolymph of an intertidal mollusc tolerant to freezing. *Experientia* **38**, 1456–1457.

Banikas, E.M. & Thompson, J.S. 2012. Predation risk experienced by mummichog, *Fundulus heteroclitus*, in intertidal and subtidal salt marsh habitats. *Estuaries and Coasts* **35**, 1346–1352.

Bayley, M., Damsgaard, C., Thomsen, M., Malte, H. & Wang, T. 2019. Learning to air-breathe: The first steps. *Physiology* **34**, 14–29.

Bayne, B.L., Bayne, C.J., Carefoot, T.C. & Thompson, R.J. 1976. The physiological ecology of *Mytilus californianus* Conrad. *Oecologia* **22**, 229–250.

Bayne, B.L., Hawkins, A.J.S. & Navarro, E. 1988. Feeding and digestion in suspension-feeding bivalve molluscs: The relevance of physiological compensations. *American Zoologist* **28**, 147–159.

Beere, H.M. 2004. 'The stress of dying': The role of heat shock proteins in the regulation of apoptosis. *Journal of Cell Science* **117**, 2641–2651.

Berger, M.S. & Emlet, R.B. 2007. Heat-shock response of the upper intertidal barnacle *Balanus glandula*: Thermal stress and acclimation. *The Biological Bulletin* **212**, 232–241.

Biggar, K.K., Kornfeld, S.F., Maistrovski, Y. & Storey, K.B. 2012. MicroRNA regulation in extreme environments: differential expression of microRNAs in the intertidal snail *Littorina littorea* during extended periods of freezing and anoxia. *Genomics, Proteomics & Bioinformatics* **10**, 302–309.

Bjelde, B.E. & Todgham, A.E. 2013. Thermal physiology of the fingered limpet *Lottia digitalis* under emersion and immersion. *Journal of Experimental Biology* **216**, 2858–2869.

Bjelde, B.E., Miller, N.A., Stillman, J.H. & Todgham, A.E. 2015. The Role of Oxygen in Determining Upper Thermal Limits in *Lottia digitalis* under Air Exposure and Submersion. *Physiological and Biochemical Zoology* **88**, 483–493.

Bose, A.P.H., Borowiec, B.G., Scott, G.R. & Balshine, S. 2019. Nesting on high: Reproductive and physiological consequences of breeding across an intertidal gradient. *Evolutionary Ecology* **33**, 21–36.

Bossdorf, O., Richards, C.L. & Pigliucci, M. 2008. Epigenetics for ecologists. *Ecology Letters* **11**, 106–115.

Bourdeau, P.E. 2010. An inducible morphological defence is a passive by-product of behaviour in a marine snail. *Proceedings of the Royal Society B: Biological Sciences* **277**, 455–462.

Braby, C.E. & Somero, G.N. 2006. Following the heart: Temperature and salinity effects on heart rate in native and invasive species of blue mussels (genus *Mytilus*). *Journal of Experimental Biology* **209**, 2554–2566.

Branch, G.M. 1985. Limpets: Evolution and adaptation. In: *The Mollusca, Volume 10, Evolution*, E.R. Trueman et al. (eds.) Academic Press, 187–220.

Branch, G.M. & Cherry, M.I. 1985. Activity rhythms of the pulmonate limpet *Siphonaria capensis* Q. & G. as an adaptation to osmotic stress, predation and wave action. *Journal of Experimental Marine Biology and Ecology* **81**, 153–168.

Breitburg, D., Levin, L.A., Oschlies, A., Grégoire, M., Chavez, F.P., Conley, D.J., Garçon, V., Gilbert, D., Gutiérrez, D., Isensee, K., Jacinto, G.S., Limburg, K.E., Montes, I., Naqvi, S.W.A., Pitcher, G.C., Rabalais, N.N., Roman, M.R., Rose, K.A., Seibel, B.A., Telszewski, M., Yasuhara, M. & Zhang, J. 2018. Declining oxygen in the global ocean and coastal waters. *Science* **359**, doi:10.1126/science.aam7240.

Buckley, B.A., Owen, M.E. & Hofmann, G.E. 2001. Adjusting the thermostat: The threshold induction temperature for the heat-shock response in intertidal mussels (genus *Mytilus*) changes as a function of thermal history. *Journal of Experimental Biology* **204**, 3571–3579.

Burggren, W.W. & McMahon, B.R. 1981. Oxygen uptake during environmental temperature change in hermit crabs: Adaptation to subtidal, intertidal, and supratidal habitats. *Physiological Zoology* **54**, 325–333.

Burton, R.S. & Feldman, M.W. 1982. Changes in free amino acid concentrations during osmotic response in the intertidal copepod *Tigriopus californicus*. *Comparative Biochemistry and Physiology Part A: Physiology* **73**, 441–445.

Carrasco-Malio, A., Díaz, M., Mella, M., Montoya, M.J., Miranda, A., Landaeta, M.F., Sánchez, G. & Hidalgo, M.E. 2014. Are the intertidal fish highly resistant to UV-B radiation? A study based on oxidative stress in *Girella laevifrons* (Kyphosidae). *Ecotoxicology and Environmental Safety* **100**, 93–98.

Carvajal-Rodríguez, A., Conde-Padín, P. & Rolán-Alvarez, E. 2005. Decomposing shell form into size and shape by geometric morphometric methods in two sympatric ecotypes of *Littorina saxatilis*. *Journal of Molluscan Studies* **71**, 313–318.

Chan, B.K.K., Morritt, D. & Williams, G.A. 2001. The effect of salinity and recruitment on the distribution of *Tetraclita squamosa* and *Tetraclita japonica* (Cirripedia; Balanomorpha) in Hong Kong. *Marine Biology* **138**, 999–1009.

Chan, B.K.K. & Williams, G.A. 2004. Population dynamics of the acorn barnacles, *Tetraclita squamosa* and *Tetraclita japonica* (Cirripedia: Balanomorpha), in Hong Kong. *Marine Biology* **146**, 149–160.

Chapman, M.G. 2000. Poor design of behavioural experiments gets poor results: examples from intertidal habitats. *Journal of Experimental Marine Biology and Ecology* **250**, 77–95.

Chen, Y.-q., Wang, J., Liao, M.-i., Li, X.-x. & Dong, Y.-w. 2021. Temperature adaptations of the thermophilic snail *Echinolittorina malaccana*: Insights from metabolomic analysis. *Journal of Experimental Biology* **224**, doi:10.1242/jeb.238659.

Chew, S.F., Hong, L.N., Wilson, J.M., Randall, D.J. & Ip, Y.K. 2003. Alkaline environmental pH has no effect on ammonia excretion in the mudskipper *Periophthalmodon schlosseri* but inhibits ammonia excretion in the related species *Boleophthalmus boddaerti*. *Physiological and Biochemical Zoology* **76**, 204–214.

Chew, S.F. & Ip, Y.K. 2014. Excretory nitrogen metabolism and defence against ammonia toxicity in air-breathing fishes. *Journal of Fish Biology* **84**, 603–638.

Churchill, T.A. & Storey, K.B. 1996. Metabolic responses to freezing and anoxia by the periwinkle *Littorina littorea*. *Journal of Thermal Biology* **1**, 57–63.

Clark, M.S., Geissler, P., Waller, C., Fraser, K.P., Barnes, D.K. & Peck, L.S. 2008. Low heat shock thresholds in wild Antarctic inter-tidal limpets (*Nacella concinna*). *Cell Stress and Chaperones* **13**, 51–58.

Clark, M.S., Thorne, M.A., King, M., Hipperson, H., Hoffman, J.I. & Peck, L.S. 2018. Life in the intertidal: Cellular responses, methylation and epigenetics. *Functional Ecology* **32**, 1982–1994.

Clayton, D.A. 1993. Mudskippers. In: *Oceanography and Marine Biology: An Annual Review*, A.D. Ansell et al. (eds.) UCL Press, **31**, 507–577.

Cochrane, P.V., Rossi, G.S., Tunnah, L., Jonz, M.G. & Wright, P.A. 2019. Hydrogen sulphide toxicity and the importance of amphibious behaviour in a mangrove fish inhabiting sulphide-rich habitats. *Journal of Comparative Physiology B* **189**, 223–235.

Cockell, C.S. & Knowland, J. 1999. Ultraviolet radiation screening compounds. *Biological Reviews* **74**, 311–345.

Connor, K. & Gracey, A.Y. 2012. High-resolution analysis of metabolic cycles in the intertidal mussel *Mytilus californianus*. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **302**, R103–R111.

Cooper, C.A., Wilson, J.M. & Wright, P.A. 2013. Marine, freshwater and aerially acclimated mangrove rivulus (*Kryptolebias marmoratus*) use different strategies for cutaneous ammonia excretion. *American Journal of Physiology-Regulatory, Integrative and Comparative Physiology* **304**, R599–R612.

Coverton, G.A. & Harley, C.D.G. 2020. Multi-scale variation in salinity: A driver of population size and structure in the muricid gastropod *Nucella lamellosa*. *Marine Ecology Progress Series* **643**, 1–19.

Curtis, T.M., Williamson, R. & Depledge, M.H. 2000. Simultaneous, long-term monitoring of valve and cardiac activity in the blue mussel *Mytilus edulis* exposed to copper. *Marine Biology* **136**, 837–846.

Daborn, K., Cozzi, R.R.F. & Marshall, W.S. 2001. Dynamics of pavement cell–chloride cell interactions during abrupt salinity change in *Fundulus heteroclitus*. *Journal of Experimental Biology* **204**, 1889–1899.

Davenport, J. & Sayer, M.D.J. 1986. Ammonia and urea excretion in the amphibious teleost *Blennius pholis* (L.) in sea-water and in air. *Comparative Biochemistry and Physiology Part A: Physiology* **84**, 189–194.

DeFur, P.L. 1988. Systemic respiratory adaptations to air exposure in intertidal decapod crustaceans. *American Zoologist* **28**, 115–124.

Dejours, P. 1989. From comparative physiology of respiration to several problems of environmental adaptations and to evolution. *Journal of Physiology* **410**, 1–19.

Denny, M.W. & Dowd, W.W. 2012. Biophysics, environmental stochasticity, and the evolution of thermal safety margins in intertidal limpets. *Journal of Experimental Biology* **215**, 934–947.

Denny, M.W. & Gaines, S.D. (eds.) 2007. *Encyclopedia of Tidepools and Rocky Shores*. University of California Press.

Denny, M.W. & Harley, C.D. 2006. Hot limpets: Predicting body temperature in a conductance-mediated thermal system. *Journal of Experimental Biology* **209**, 2409–2419.

Doeller, J.E., Grieshaber, M.K. & Kraus, D.W. 2001. Chemolithoheterotrophy in a metazoan tissue: Thiosulfate production matches ATP demand in ciliated mussel gills. *Journal of Experimental Biology* **204**, 3755–3764.

Donelson, J.M., Munday, P.L., McCormick, M.I. & Pitcher, C.R. 2011. Rapid transgenerational acclimation of a tropical reef fish to climate change. *Nature Climate Change* **2**, doi:10.1038/nclimate1323.

Dong, Y., Miller, L.P., Sanders, J.G. & Somero, G.N. 2008. Heat-shock protein 70 (Hsp70) expression in four limpets of the genus *Lottia*: Interspecific variation in constitutive and inducible synthesis correlates with *in situ* exposure to heat stress. *The Biological Bulletin* **215**, 173–181.

Dong, Y. & Somero, G.N. 2009. Temperature adaptation of cytosolic malate dehydrogenases of limpets (genus *Lottia*): Differences in stability and function due to minor changes in sequence correlate with biogeographic and vertical distributions. *Journal of Experimental Biology* **212**, 169–177.

Dong, Y.-w., Blanchard, T.S., Noll, A., Vasquez, P., Schmitz, J., Kelly, S.P., Wright, P.A. & Whitehead, A. 2021. Genomic and physiological mechanisms underlying skin plasticity during water to air transition in an amphibious fish. *Journal of Experimental Biology* **224**, doi:10.1242/jeb.235515.

Dong, Y.-w. & Williams, G.A. 2011. Variations in cardiac performance and heat shock protein expression to thermal stress in two differently zoned limpets on a tropical rocky shore. *Marine Biology* **158**, 1223–1231.

Drake, M.J., Miller, N.A. & Todgham, A.E. 2017. The role of stochastic thermal environments in modulating the thermal physiology of an intertidal limpet, *Lottia digitalis*. *Journal of Experimental Biology* **220**, 3072– 3083.

Dubilier, N., Bergin, C. & Lott, C. 2008. Symbiotic diversity in marine animals: The art of harnessing chemosynthesis. *Nature Reviews Microbiology* **6**, 725–740.

Dufour, S.C. 2018. Bivalve chemosymbioses on mudflats. In: *Mudflat Ecology, Aquatic Ecology Series 7*, P.G. Beninger (ed.). Springer, 169–184.

Duman, J.G. 2015. Animal ice-binding (antifreeze) proteins and glycolipids: An overview with emphasis on physiological function. *Journal of Experimental Biology* **218**, 1846–1855.

Durand, F.A., Chausson, F.A. & Regnault, M.I. 1999. Increases in tissue free amino acid levels in response to prolonged emersion in marine crabs: An ammonia-detoxifying process efficient in the intertidal *Carcinus maenas* but not in the subtidal *Necora puber*. *Journal of Experimental Biology* **202**, 2191–2202.

Durand, F.A. & Regnault, M.I. 1998. Nitrogen metabolism of two portunid crabs, *Carcinus maenas* and *Necora puber*, during prolonged air exposure and subsequent recovery: A comparative study. *Journal of Experimental Biology* **201**, 2515–2528.

Dykens, J.A. & Shick, J.M. 1984. Photobiology of the symbiotic sea anemone, *Anthopleura elegantissima*: Defenses against photodynamic effects, and seasonal photoacclimatization. *The Biological Bulletin* **167**, 683–697.

Ebeling, A.W., Bernal, P. & Zuleta, A. 1970. Emersion of the amphibious Chilean clingfish, *Sicyases sanguineus*. *The Biological Bulletin* **139**, 115–137.

Fangue, N.A., Osborne, E.J., Todgham, A.E. & Schulte, P.M. 2011. The onset temperature of the heat-shock response and whole-organism thermal tolerance are tightly correlated in both laboratory-acclimated and field-acclimatized tidepool sculpins (*Oligocottus maculosus*). *Physiological and Biochemical Zoology* **84**, 341–352.

Fenwick, J.C. & Lam, T.J. 1988. Calcium Fluxes in the Teleost Fish Tilapia (*Oreochromis*) in Water and in Both Water and Air in the Marble Goby (*Oxyeleotris*) and the Mudskipper (*Periophthalmodon*). *Physiological Zoology* **61**, 119–125.

Firth, L.B. & Williams, G.A. 2009. The influence of multiple environmental stressors on the limpet *Cellana toreuma* during the summer monsoon season in Hong Kong. *Journal of Experimental Marine Biology and Ecology* **375**, 70–75.

Firth, L.B., Knights, A.M. & Bell, S.S. 2011. Air temperature and winter mortality: Implications for the persistence of the invasive mussel, *Perna viridis* in the intertidal zone of the south-eastern United States. *Journal of Experimental Marine Biology and Ecology* **400**, 250–256.

Fitzhenry, T., Halpin, P.M. & Helmuth, B. 2004. Testing the effects of wave exposure, site, and behavior on intertidal mussel body temperatures: Applications and limits of temperature logger design. *Marine Biology* **145**, 339–349.

Foster, B.A. 1970. Responses and acclimation to salinity in the adults of some balanomorph barnacles. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **256**, 377–400.

Foster, B.A. 1971. On the determinants of the upper limit of intertidal distribution of barnacles (Crustacea: Cirripedia). *Journal of Animal Ecology* **40**, 33–48.

Frick, N.T. & Wright, P.A. 2002. Nitrogen metabolism and excretion in the mangrove killifish *Rivulus marmoratus* II. Significant ammonia volatilization in a teleost during air-exposure. *Journal of Experimental Biology* **205**, 91–100.

Ganning, B. 1971. Studies on chemical, physical and biological conditions in Swedish rockpool ecosystems. *Ophelia* **9**, 51–105.

García-Huidobro, M.R., Aldana, M., Duarte, C., Galbán-Malagón, C. & Pulgar, J. 2017. Seawater-temperature and UV-radiation interaction modifies oxygen consumption, digestive process and growth of an intertidal fish. *Marine Environmental Research* **129**, 408–412.

Garland, T., Bennett, A.F. & Rezende, E.L. 2005. Phylogenetic approaches in comparative physiology. *Journal of Experimental Biology* **208**, 3015–3035.

Garrity, S.D. 1984. Some adaptations of gastropods to physical stress on a tropical rocky shore. *Ecology* **65**, 559–574.

Gendron, R.P. 1977. Habitat selection and migratory behaviour of the intertidal gastropod *Littorina littorea* (L.). *Journal of Animal Ecology* **46**, 79–92.

Genovese, G., Luchetti, C.G. & Luquet, C.M. 2004. Na^+/K^+–ATPase activity and gill ultrastructure in the hyper- hypo-regulating crab *Chasmagnathus granulatus* acclimated to dilute, normal, and concentrated seawater. *Marine Biology* **144**, 111–118.

Genz, J. & Grosell, M. 2011. *Fundulus heteroclitus* acutely transferred from seawater to high salinity require few adjustments to intestinal transport associated with osmoregulation. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **160**, 156–165.

Gibbs, P.E. 1993. Phenotypic changes in the progeny of *Nucella lapillus* (Gastropoda) transplanted from an exposed shore to sheltered inlets. *Journal of Molluscan Studies* **59**, 187–194.

Gibson, D.J., Sylvester, E.V.A., Turko, A.J., Tattersall, G.J. & Wright, P.A. 2015. Out of the frying pan into the air—emersion behaviour and evaporative heat loss in an amphibious mangrove fish (*Kryptolebias marmoratus*). *Biology Letters* **11**, doi:10.1098/rsbl.2015.0689.

Gilman, S.E., Wethey, D.S. & Helmuth, B. 2006. Variation in the sensitivity of organismal body temperature to climate change over local and geographic scales. *Proceedings of the National Academy of Sciences* **103**, 9560–9565.

Giomi, F., Fusi, M., Barausse, A., Mostert, B., Pörtner, H.-O. & Cannicci, S. 2014. Improved heat tolerance in air drives the recurrent evolution of air-breathing. *Proceedings of the Royal Society B: Biological Sciences* **281**, doi:10.1098/rspb.2013.2927.

Gleason, L.U., Miller, L.P., Winnikoff, J.R., Somero, G.N., Yancey, P.H., Bratz, D. & Dowd, W.W. 2017. Thermal history and gape of individual *Mytilus californianus* correlate with oxidative damage and thermoprotective osmolytes. *Journal of Experimental Biology* **220**, 4292–4304.

Gordon, M.S., Boëtius, J., Evans, D.H. & Oglesby, L.C. 1968. Additional Observations on the Natural History of the Mudskipper, *Periophthalmus sobrinus*. *Copeia* **1968**, 853–857.

Gordon, M.S., Fischer, S. & Tarifeño, E. 1970. Aspects of the Physiology of Terrestrial Life in Amphibious Fishes: II. The Chilean Clingfish, *Sicyases Sanguineus*. *Journal of Experimental Biology* **53**, 559–572.

Goward, C.R. & Nicholls, D.J. 1994. Malate dehydrogenase: A model for structure, evolution, and catalysis. *Protein Science* **3**, 1883–1888.

Gracey, A.Y., Chaney, M.L., Boomhower, J.P., Tyburczy, W.R., Connor, K. & Somero, G.N. 2008. Rhythms of gene expression in a fluctuating intertidal environment. *Current Biology* **18**, 1501–1507.

Gracey, A.Y. & Connor, K. 2016. Transcriptional and metabolomic characterization of spontaneous metabolic cycles in *Mytilus Californianus* under subtidal conditions. *Marine Genomics* **30**, 35–41.

Graham, J.B. & Lee, H.J. 2004. Breathing air in air: In what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? *Physiological and Biochemical Zoology* **77**, 720–731.

Grant, J. & McDonald, J. 1979. Desiccation Tolerance of *Eurypanopeus depressus* (Smith) (Decapoda: Xanthidae) and the Exploitation of Microhabitat. *Estuaries* **2**, 172–177.

Greenaway, P. 2003. Terrestrial adaptations in the Anomura (Crustacea: Decapoda). *Memoirs of Museum Victoria* **60**, 13–26.

Greenway, S.C. & Storey, K.B. 2001. Effects of seasonal change and prolonged anoxia on metabolic enzymes of *Littorina littorea*. *Canadian Journal of Zoology* **79**, 907–915.

Gross, W.J. 1964. Trends in water and salt regulation among aquatic and amphibious crabs. *The Biological Bulletin* **127**, 447–466.

Gunderson, A.R., Armstrong, E.J. & Stillman, J.H. 2016. Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. *Annual Review of Marine Science* **8**, 357–378.

Hagerman, L. 1998. Physiological flexibility; a necessity for life in anoxic and sulphidic habitats. *Hydrobiologia* **375/376**, 241–254.

Halperin, J., Ansaldo, M., Pellerano, G.N. & Luquet, C.M. 2000. Bimodal breathing in the estuarine crab *Chasmagnathus granulatus* Dana 1851—physiological and morphological studies. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **126**, 341–349.

Halpin, P.M., Menge, B.A. & Hofmann, G.E. 2004. Experimental demonstration of plasticity in the heat shock response of the intertidal mussel *Mytilus californianus*. *Marine Ecology Progress Series* **276**, 137–145.

Hamasaki, S., Mukuda, T., Kaidoh, T., Yoshida, M. & Uematsu, K. 2016. Impact of dehydration on the forebrain preoptic recess walls in the mudskipper, *Periophthalmus modestus*: A possible locus for the center of thirst. *Journal of Comparative Physiology B* **186**, 891–905.

Harley, C.D. 2011. Climate change, keystone predation, and biodiversity loss. *Science* **334**, 1124–1127.

Hart, C.E. & Crowe, J.H. 1977. The effect of attached gravel on survival of intertidal anemones. *Transactions of the American Microscopical Society* **96**, 28–41.

Hawes, T.C., Worland, M.R. & Bale, J.S. 2010. Freezing in the Antarctic limpet, *Nacella concinna*. *Cryobiology* **61**, 128–132.

Hawkins, A.J.S. 1985. Relationships between the synthesis and breakdown of protein, dietary absorption and turnovers of nitrogen and carbon in the blue mussel, *Mytilus edulis* L. *Oecologia* **66**, 42–49.

Hawkins, A.J.S. & Jones, M.B. 1982. Gill area and ventilation in two mud crabs, *Helice crassa* Dana (Grapsidae) and *Macrophthalmus hirtipes* (Jacquinot) (Ocypodidae), in relation to habitat. *Journal of Experimental Marine Biology and Ecology* **60**, 103–118.

Haynes, T.B., Phillips-Mentzos, E. & Facey, D.E. 2009. A Comparison of the Hyposaline Tolerances of Black Prickleback (*Xiphister atropurpureus*) and Penpoint Gunnel (*Apodichthys flavidus*). *Northwest Science* **83**, 361–366.

Healy, T.M. & Schulte, P.M. 2012. Thermal Acclimation Is Not Necessary to Maintain a Wide Thermal Breadth of Aerobic Scope in the Common Killifish (*Fundulus heteroclitus*). *Physiological and Biochemical Zoology* **85**, 107–119.

Heffell, Q., Turko, A.J. & Wright, P.A. 2018. Plasticity of skin water permeability and skin thickness in the amphibious mangrove rivulus *Kryptolebias marmoratus*. *Journal of Comparative Physiology B* **188**, 305–314.

Helmuth, B., Mieszkowska, N., Moore, P. & Hawkins, S.J. 2006. Living on the edge of two changing worlds: Forecasting the responses of rocky intertidal ecosystems to climate change. *Annual Review of Ecology, Evolution, and Systematics* **37**, 373–404.

Hendy, I.W., Eme, J., Dabruzzi, T.F., Nembhard, R.V., Cragg, S.M. & Bennett, W.A. 2013. Dartfish use teredinid tunnels in fallen mangrove wood as a low-tide refuge. *Marine Ecology Progress Series* **486**, 237–245.

Hill, B. 1981. Respiratory adaptations of three species of *Upogebia* (Thalassinidea, Crustacea) with special reference to low tide periods. *The Biological Bulletin* **160**, 272–279.

Hofmann, G.E. 1999. Ecologically relevant variation in induction and function of heat shock proteins in marine organisms. *American Zoologist* **39**, 889–900.

Horn, M.H. & Gibson, R.N. 1988. Intertidal fishes. *Scientific American* **258**, 64–71.

Horn, M.H. & Riegle, K.C. 1981. Evaporative water loss and intertidal vertical distribution in relation to body size and morphology of stichaeoid fishes from California. *Journal of Experimental Marine Biology and Ecology* **50**, 273–288.

Hosoi, M., Kubota, S., Toyohara, M., Toyohara, H. & Hayashi, I. 2003. Effect of salinity change on free amino acid content in Pacific oyster. *Fisheries Science* **69**, 395–400.

Houlihan, D.F. 1991. Protein turnover in ectotherms and its relationships to energetics. In *Advances in Comparative and Environmental Physiology, Volume 7*, R. Gilles et al. (eds.) Springer, 1–43.

Huggett, J. & Griffiths, C.L. 1986. Some relationships between elevation, physicochemical variables and biota of intertidal rock pools. *Marine Ecology Progress Series* **29**, 189–197.

Hughes, R.N. & Elner, R.W. 1979. Tactics of a predator, *Carcinus maenas*, and morphological responses of the prey, *Nucella lapillus*. *Journal of Animal Ecology* **48**, 65–78.

Hull, S.L., Grahame, J. & Mill, P.J. 1999. Heat stability and activity levels of aspartate aminotransferase and alanine aminotransferase in British Littorinidae. *Journal of Experimental Marine Biology and Ecology* **237**, 255–270.

Hui, T.Y., Yuan, F.L., Bonebrake, T.C. & Williams, G.A. 2019. Multifunctional behaviour in a sandy shore crab enhances performance in extreme intertidal environments. *Oecologia* **189**, 79–89.

Huey, R.B., Kearney, M.R., Krockenberger, A., Holtum, J.A.M., Jess, M. & Williams, S.E. 2012. Predicting organismal vulnerability to climate warming: Roles of behaviour, physiology and adaptation. *Philosophical Transactions of the Royal Society of London B: Biological Sciences* **367**, 1665–1679.

Ip, Y.K. & Chew, S.F. 2018. Air-breathing and excretory nitrogen metabolism in fishes. *Acta Histochemica* **120**, 680–690.

Ip, Y.K., Kuah, S.S. & Chew, S.F. 2004a. Strategies adopted by the mudskipper *Boleophthalmus boddaerti* to survive sulfide exposure in normoxia or hypoxia. *Physiological and Biochemical Zoology* **77**, 824–837.

Ip, Y.K., Leong, M.W., Sim, M.Y., Goh, G.S., Wong, W.P. & Chew, S.F. 2005. Chronic and acute ammonia toxicity in mudskippers, *Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*: Brain ammonia and glutamine contents, and effects of methionine sulfoximine and MK801. *Journal of Experimental Biology* **208**, 1993–2004.

Ip, Y.K., Lim, C.K., Lee, S.L., Wong, W.P. & Chew, S.F. 2004b. Postprandial increases in nitrogenous excretion and urea synthesis in the giant mudskipper *Periophthalmodon schlosseri*. *Journal of Experimental Biology* **207**, 3015–3023.

Ishimatsu, A., Hishida, Y., Takita, T., Kanda, T., Oikawa, S., Takeda, T. & Huat, K.K. 1998. Mudskippers store air in their burrows. *Nature* **391**, 237–238.

Ishimatsu, A., Yoshida, Y., Itoki, N., Takeda, T., Lee, H.J. & Graham, J.B. 2007. Mudskippers brood their eggs in air but submerge them for hatching. *Journal of Experimental Biology* **210**, 3946–3954.

Ivanina, A.V., Nesmelova, I., Leamy, L, Sokolov, E.P. & Sokolova, I.M. 2016. Intermittent hypoxia leads to functional reorganization of mitochondria and affects cellular bioenergetics in marine molluscs. *Journal of Experimental Biology* **219**, 1659–1674.

Jaafar, Z. & Murdy, E.O. (eds.) 2017. *Fishes Out of Water: Biology and Ecology of Mudskippers*. CRC Marine Science Series, Taylor & Francis.

Johannesson, K. 2016. What can be learnt from a snail? *Evolutionary Applications* **9**, 153–165.

Jutfelt, F., Norin, T., Ern, R., Overgaard, J., Wang, T., McKenzie, D.J., Lefevre, S., Nilsson, G.E., Metcalfe, N.B., Hickey, A.J.R., Brijs, J., Speers-Roesch, B., Roche, D.G., Gamperl, A.K., Raby, G.D., Morgan, R., Esbaugh, A.J., Gräns, A., Axelsson, M., Ekström, A., Sandblom, E., Binning, S.A., Hicks, J.W., Seebacher, F., Jørgensen, C., Killen, S.S., Schulte, P.M. & Clark, T.D. 2018. Oxygen- and capacity-limited thermal tolerance: Blurring ecology and physiology. *Journal of Experimental Biology* **221**, doi:10.1242/jeb.169615.

Karentz, D., McEuen, F.S., Land, M.C. & Dunlap, W.C. 1991. Survey of mycosporine-like amino acid compounds in Antarctic marine organisms: Potential protection from ultraviolet exposure. *Marine Biology* **108**, 157– 166.

Katayama, Y., Sakamoto, T., Takanami, K. & Takei, Y. 2018. The amphibious mudskipper: A unique model bridging the gap of central actions of osmoregulatory hormones between terrestrial and aquatic vertebrates. *Frontiers in Physiology* **9**, doi:10.3389/fphys.2018.01112.

Kennedy, J.R., Harley, C.D.G. & Marshall, K.E. 2020. Drivers of plasticity in freeze tolerance in the intertidal mussel, *Mytilus trossulus*. *Journal of Experimental Biology* **223**, doi:10.1242/jeb.233478.

Kimura, Y. & Sakai, Y. 2016. Emergence behavior of a tide pool fish *Praealticus tanegasimae* (Teleostei; Blenniidae) on subtropical reefs. *Journal of Ethology* **34**, 175–181.

King, J.A.C., Abel, D.C. & DiBona, D.R. 1989. Effects of salinity on chloride cells in the euryhaline cyprinodontid fish *Rivulus marmoratus*. *Cell and Tissue Research* **257**, 367–377.

Kitching, J.A., Muntz, L. & Ebling, F.J. 1966. The ecology of Lough Ine. XV. The ecological significance of shell and body forms in Nucella. *Journal of Animal Ecology* **35**, 113–126.

Kuklinski, P. & Balazy, P. 2014. Scale of temperature variability in the maritime Antarctic intertidal zone. *Journal of Sea Research* **85**, 542–546.

Lam, K., Tsui, T., Nakano, K. & Randall, D.J. 2006. Physiological adaptations of fishes to tropical intertidal environments. In *Fish Physiology, Volume 21, The Physiology of Tropical Fishes*, A.L. Val et al. (eds.) Elsevier, 501–581.

Lastra, M., Jaramillo, E., López, J., Contreras, H., Duarte, C. & Rodríguez, G. 2004. Population abundances, tidal movement, burrowing ability and oxygen uptake of *Emerita analoga* (Stimpson) (Crustacea, Anomura) on a sandy beach of south-central Chile. *Marine Ecology* **25**, 71–89.

Lau, G.Y., Mandic, M. & Richards, J.G. 2017. Evolution of cytochrome *c* oxidase in hypoxia tolerant sculpins (Cottidae, Actinopterygii). *Molecular Biology and Evolution* **34**, 2153–2162.

Laudien, J., Schiedek, D., Brey, T., Pörtner, H.-O. & Arntz, W.E. 2002. Survivorship of juvenile surf clams *Donax serra* (Bivalvia, Donacidae) exposed to severe hypoxia and hydrogen sulphide. *Journal of Experimental Marine Biology and Ecology* **271**, 9–23.

LeBlanc, D.M., Wood, C.W., Fudge, D.S. & Wright, P.A. 2010. A fish out of water: Gill and skin remodeling promotes osmo- and ionoregulation in the mangrove killifish *Kryptolebias marmoratus*. *Physiological and Biochemical Zoology* **83**, 932–949.

Lee, S.Y. 1995. Cheliped size and structure: The evolution of a multi-functional decapod organ. *Journal of Experimental Marine Biology and Ecology* **193**, 161–176.

Lee, C.G.L. & Ip, Y.K. 1987. Environmental effect on plasma thyroxine (t_4), 3,5,3'-triido-$_L$-thyronine (t_3), prolactin and cyclic adenosine 3', 5'-monophosphate (camp) content in the mudskippers *Periophthalmus chrysospilos* and *Boleophthalmus boddaerti*. *Comparative Biochemistry and Physiology Part A: Physiology* **87**, 1009–1014.

Lee, H.J., Martinez, C.A., Hertzberg, K.J., Hamilton, A.L. & Graham, J.B. 2005. Burrow air phase maintenance and respiration by the mudskipper *Scartelaos histophorus* (Gobiidae: Oxudercinae). *Journal of Experimental Biology* **208**, 169–177.

Lee, S.-L. & Lim, S.S.L. 2009. Vertical zonation and heat tolerance of three littorinid gastropods on a rocky shore at Tanjung Chek Jawa, Singapore. *The Raffles Bulletin of Zoology* **57**, 551–560.

Letendre, J., Chouquet, B., Rocher, B., Manduzio, H., Leboulenger, F. & Durand, F. 2008. Differential pattern of Cu/Zn superoxide dismutase isoforms in relation to tidal spatio-temporal changes in the blue mussel *Mytilus edulis*. *Comparative Biochemistry and Physiology Part C: Toxicology & Pharmacology* **148**, 211–216.

Livingston, M.D., Bhargav, V.V., Turko, A.J., Wilson, J.M. & Wright, P.A. 2018. Widespread use of emersion and cutaneous ammonia excretion in Aplocheiloid killifishes. *Proceedings of the Royal Society B: Biological Sciences* **285**, doi:10.1098/rspb.2018.1496.

Li, H.T. 2012. *Thermal Tolerance of Echinolittorina species in Hong Kong: Implications for Their Vertical Distributions*. MPhil thesis, University of Hong Kong, China.

Liao, M.-L., Zhang, S., Zhang, G.-Y., Chu, Y.-M., Somero, G.N. & Dong, Y.-W. 2017. Heat-resistant cytosolic malate dehydrogenases (cMDHs) of thermophilic intertidal snails (genus *Echinolittorina*): Protein underpinnings of tolerance to body temperatures reaching 55°C. *Journal of Experimental Biology* **220**, 2066–2075.

Lim, C.B., Chew, S.F., Anderson, P.M. & Ip, Y.K. 2001. Reduction in the rates of protein and amino acid catabolism to slow down the accumulation of endogenous ammonia: A strategy potentially adopted by mudskippers (*Periophthalmodon schlosseri* and *Boleophthalmus boddaerti*) during aerial exposure in constant darkness. *Journal of Experimental Biology* **204**, 1605–1614.

Lima, F.P., Gomes, F., Seabra, R., Wethey, D.S., Seabra, M.I., Cruz, T., Santos, A.M. & Hilbish, T.J. 2016. Loss of thermal refugia near equatorial range limits. *Global Change Biology* **22**, 254–263.

Lima, F.P. & Wethey, D.S. 2009. Robolimpets: Measuring intertidal body temperatures using biomimetic loggers. *Limnology and Oceanography: Methods* **7**, 347–353.

Litwiller, S.L., O'Donnell, M.J. & Wright, P.A. 2006. Rapid increase in the partial pressure of NH_3 on the cutaneous surface of air-exposed mangrove killifish, *Rivulus marmoratus*. *Journal of Experimental Biology* **209**, 1737–1745.

Liu, X., Zhang, L., Zhang, Z., Ma, X. & Liu, J. 2015. Transcriptional response to sulfide in the Echiuran Worm *Urechis unicinctus* by digital gene expression analysis. *BMC Genomics* **16**, doi:10.1186/s12864-015-2094-z.

Lockwood, A.P.M. 1961. The urine of *Gammarus duebeni* and *G. pulex*. *Journal of Experimental Biology* **38**, 647–658.

Loomis, S.H. 1985. Seasonal changes in the freezing tolerance of the intertidal pulmonated gastropod *Melampus bidentatus* Say. *Canadian Journal of Zoology* **63**, 2021–2025.

Loomis, S.H. & Zinser, M. 2001. Isolation and identification of an ice-nucleating bacterium from the gills of the intertidal bivalve mollusc *Geukensia demissa*. *Journal of Experimental Marine Biology and Ecology* **261**, 225–235.

Low, W.P., Lane, D.J.W. & Ip, Y.K. 1988. A Comparative Study of Terrestrial Adaptations of the Gills in Three Mudskippers—*Periophthalmus chrysospilos*, *Boleophthalmus boddaerti*, and *Periophthalmodon schlosseri*. *Biological Bulletin* **175**, 434–438.

Lundheim, R. 1997. Ice nucleation in the blue mussel (*Mytilus edulis*). *Marine Biology* **128**, 267–271.

Luquet, C.M., Genovese, G., Rosa, G.A. & Pellerano, G.N. 2002. Ultrastructural changes in the gill epithelium of the crab *Chasmagnathus granulatus* (Decapoda: Grapsidae) in diluted and concentrated seawater. *Marine Biology* **141**, 753–760.

Maitland, D.P. 1986. Crabs that breathe air with their legs–*Scopimera* and *Dotilla*. *Nature* **319**, 493–495.

Mancera, J.M. & McCormick, S.D. 2000. Rapid Activation of Gill Na^+, K^+-ATPase in the Euryhaline Teleost *Fundulus heteroclitus*. *Journal of Experimental Zoology* **287**, 263–274.

Mandic, M., Ramon, M.L., Gracey, A.Y. & Richards, J.G. 2014. Divergent transcriptional patterns are related to differences in hypoxia tolerance between the intertidal and the subtidal sculpins. *Molecular Ecology* **23**, 6091–6103.

Mandic, M. & Regan, M.D. 2018. Can variation among hypoxic environments explain why different fish species use different hypoxic survival strategies? *Journal of Experimental Biology* **221**, doi:10.1242/jeb.161349.

Mandic, M., Sloman, K.A. & Richards, J.G. 2009a. Escaping to the surface: A phylogenetically independent analysis of hypoxia-induced respiratory behaviors in sculpins. *Physiological and Biochemical Zoology* **82**, 730–738.

Mandic, M., Speers-Roesch, B. & Richards, J.G. 2012. Hypoxia tolerance in sculpins is associated with high anaerobic enzyme activity in brain but not in liver or muscle. *Physiological and Biochemical Zoology* **86**, 92–105.

Mandic, M., Todgham, A.E. & Richards, J.G. 2009b. Mechanisms and evolution of hypoxia tolerance in fish. *Proceedings of the Royal Society B: Biological Sciences* **276**, 735–744.

Marin, F. 2020. Mollusc shellomes: Past, present and future. *Journal of Structural Biology* **212**, doi:10.1016/j.jsb.2020.107583.

Marshall, D.J., Dong, Y.W., McQuaid, C.D. & Williams, G.A. 2011. Thermal adaptation in the intertidal snail *Echinolittorina malaccana* contradicts current theory by revealing the crucial roles of resting metabolism. *Journal of Experimental Biology* **214**, 3649–3657.

Marshall, D.J. & McQuaid, C.D. 1992a. Comparative aerial metabolism and water relations of the intertidal limpets *Patella granularis* L. (Mollusca: Prosobranchia) and *Siphonaria oculus* Kr. (Mollusca: Pulmonata). *Physiological Zoology* **65**, 1040–1056.

Marshall, D.J. & McQuaid, C.D. 1992b. Relationship between heart rate and oxygen consumption in the intertidal limpets *Patella granularis* and *Siphonaria oculus*. *Comparative Biochemistry and Physiology Part A: Physiology* **103**, 297–300.

Marshall, D.J. & McQuaid, C.D. 1993. Effects of hypoxia and hyposalinity on the heart beat of the intertidal limpets *Patella granularis* (Prosobranchia) and *Siphonaria capensis* (Pulmonata). *Comparative Biochemistry and Physiology Part A: Physiology* **106**, 65–68.

Marshall, D.J. & McQuaid, C.D. 2011. Warming reduces metabolic rate in marine snails: Adaptation to fluctuating high temperatures challenges the metabolic theory of ecology. *Proceedings of the Royal Society B: Biological Sciences* **278**, 281–288.

Marshall, D.J. & Ng, T.P.T. 2013. Shell standing in Littorinid snails: A multifunctional behaviour associated with mating? *Journal of Molluscan Studies* **79**, 74–75.

Marshall, D.M., Rezende, E.L., Baharuddin, N., Choi, F. & Helmuth, B. 2015. Thermal tolerance and climate warming sensitivity in tropical snails. *Ecology and Evolution* **5**, 5905–5919.

Marshall, W.S. 2012. Osmoregulation in estuarine and intertidal fishes. In: *Fish Physiology, Volume 32, Euryhaline Fishes*, S.D. McCormick et al. (eds.) Elsevier, 395–434.

Martin, K.E. & Currie, S. 2020. Hydrogen sulphide sensitivity and tolerance in genetically distinct lineages of a selfing mangrove fish (*Kryptolebias marmoratus*). *Journal of Comparative Physiology B* **190**, 761–770.

Martin, K.L. 1995. Time and tide wait for no fish: Intertidal fishes out of water. *Environmental Biology of Fishes* **44**, 165–181.

Martin, K.L. 2014. Theme and variations: Amphibious air-breathing intertidal fishes. *Journal of Fish Biology* **84**, 577–602.

Martin, K.L.M, van Winkle, R.C., Drais, J.E. & Lakisic, H. 2004. Beach-spawning fishes, terrestrial eggs, and air breathing. *Physiological and Biochemical Zoology* **77**, 750–759.

Martínez-Fernández, M., Rodríguez-Piñeiro, A.M., Oliveira, E., Páez de la Cadena, M. & Rolán-Alvarez, E. 2008. Proteomic comparison between two marine snail ecotypes reveals details about the biochemistry of adaptation. *Journal of Proteome Research* **7**, 4926–4934.

Márquez, F., Vilela, R.A.N., Lozada, M. & Bigatti, G. 2015. Morphological and behavioral differences in the gastropod *Trophon geversianus* associated to distinct environmental conditions, as revealed by a multidisciplinary approach. *Journal of Sea Research* **95**, 239–247.

McArley, T.J., Hickey, A.J.R. & Herbert, N.A. 2018. Hyperoxia increases maximum oxygen consumption and aerobic scope of intertidal fish facing acutely high temperatures. *Journal of Experimental Biology* **221**, doi:10.1242/jeb.189993.

McArley, T.J., Hickey, A.J.R., Wallace, L., Kunzmann, A. & Herbert, N.A. 2019. Intertidal triplefin fishes have a lower critical oxygen tension (P_{crit}), higher maximal aerobic capacity, and higher tissue glycogen stores than their subtidal counterparts. *Journal of Comparative Physiology B* **189**, 399–411.

McCormack, S.M.D. 1982. The maintenance of shore-level size gradients in an intertidal snail (*Littorina sitkana*). *Oecologia* **54**, 177–183.

McGaw, I.J. 2003. Behavioral thermoregulation in *Hemigrapsus nudus*, the amphibious purple shore crab. *The Biological Bulletin* **204**, 38–49.

McMahon, R.F. 1990. Thermal tolerance, evaporative water loss, air-water oxygen consumption and zonation of intertidal prosobranchs: A new synthesis. *Hydrobiologia* **193**, 241–260.

McMahon, R.F. 2001. Acute thermal tolerance in intertidal gastropods relative to latitude, superfamily, zonation and habitat with special emphasis on the Littorinoidea. *Journal of Shellfish Research* **20**, 459–1167.

McQuaid, C.D. 1981. The establishment and maintenance of vertical size gradients in populations of *Littorina africana knysnaensis* (Philippi) on an exposed rocky shore. *Journal of Experimental Marine Biology and Ecology* **54**, 77–90.

Meng, J., Zhu, Q., Zhang, L., Li, C., Li, L., She, Z., Huang, B. & Zhang, G. 2013. Genome and transcriptome analyses provide insight into the euryhaline adaptation mechanism of *Crassostrea gigas*. *PLoS One* **8**, doi:10.1371/journal.pone.0058563.

Miranda, N.A.F., Peer, N., Ishak, M.Z.B. & Marshall, D.J. 2019. Heat-wave tolerance in tropical intertidal animals: Accounting for thermal and desiccation tolerances. *Ecological Indicators* **107**, doi:10.1016/j.ecolind.2019.105561.

Monaco, C.J., McQuaid, C.D. & Marshall, D.J. 2017. Decoupling of behavioural and physiological thermal performance curves in ectothermic animals: A critical adaptive trait. *Oecologia* **185**, 583–593.

Morgan, R., Finnøen, M.H., Jensen, H., Pélabon, C. & Jutfelt, F. 2020. Low potential for evolutionary rescue from climate change in a tropical fish. *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.2011419117.

Morimoto, R.I. 1998. Regulation of the heat shock transcriptional response: Cross talk between a family of heat shock factors, molecular chaperones, and negative regulators. *Genes & Development* **12**, 3788–3796.

Moring, J.R. 1990. Seasonal absence of fishes in tidepools of a boreal environment (Maine, USA). *Hydrobiologia* **194**, 163–168.

Morris, S. & Taylor, A.C. 1983. Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science* **17**, 339–355.

Morritt, D. 1988. Osmoregulation in littoral and terrestrial talitroidean amphipods (Crustacea) from Britain. *Journal of Experimental Marine Biology and Ecology* **123**, 77–94.

Morritt, D., Leung, K.M., De Pirro, M., Yau, C., Wai, T.-C. & Williams, G.A. 2007. Responses of the limpet, *Cellana grata* (Gould 1859), to hypo-osmotic stress during simulated tropical, monsoon rains. *Journal of Experimental Marine Biology and Ecology* **352**, 78–88.

Morritt, D. & Spicer, J.I. 1998. The physiological ecology of talitrid amphipods: An update. *Canadian Journal of Zoology* **76**, 1965–1982.

Moyle, P.B. & Cech, J.J., Jr. 1996. *Fishes: An Introduction to Ichthyology*, third ed. S.L. Snavely (ed.). Prentice-Hall Inc.

Muñoz, J.L., Finke, G.R., Camus, P.A. & Bozinovic, F. 2005. Thermoregulatory behavior, heat gain and thermal tolerance in the periwinkle *Echinolittorina peruviana* in central Chile. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **142**, 92–98.

Murphy, D.J. 1983. Freezing resistance in intertidal invertebrates. *Annual Review of Physiology* **45**, 289–299.

Murphy, D.J. & Johnson, L.C. 1980. Physical and temporal factors influencing the freezing tolerance of the marine snail *Littorina littorea* (L.). *The Biological Bulletin* **158**, 220–232.

Nakamura, M. & Wright, J.C. 2013. Discontinuous ammonia excretion and glutamine storage in littoral Oniscidea (Crustacea: Isopoda): Testing tidal and circadian models. *Journal of Comparative Physiology B* **183**, 51–59.

Nakano, K. & Iwama, G.K. 2002. The 70-kDa heat shock protein response in two intertidal sculpins, *Oligocottus maculosus* and *O. snyderi*: Relationship of hsp70 and thermal tolerance. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **133**, 79–94.

Nancollas, S.J. 2020. *The Importance of Tidal Acclimation When Assessing the Physiological Responses of Carcinus maenas to Environmental Stressors*. MSc thesis, Memorial University of Newfoundland, Canada.

Nancollas, S.J. & McGaw, I.J. 2021. Acclimation to tidal conditions alters the physiological responses of the green shore crab, *Carcinus maenas*, to subsequent emersion. *Journal of Experimental Biology* **224**, doi:10.1242/jeb.242220.

Nelson, D.L. & Cox, M.M. (eds.) 2000. Regulation of gene expression. In: *Lehninger Principles of Biochemistry*, sixth ed. New York: W. H. Freeman and Company.

Ng, T.P., Lau, S.L., Seuront, L., Davies, M.S., Stafford, R., Marshall, D.J., & Williams, G.A. 2017. Linking behaviour and climate change in intertidal ectotherms: Insights from littorinid snails. *Journal of Experimental Marine Biology and Ecology* **492**, 121–131.

Nguyen, K.D.T., Morley, S.A., Lai, C.-H., Clark, M.S., Tan, K.S., Bates, A.E. & Peck, L.S. 2011. Upper temperature limits of tropical marine ectotherms: Global warming implications. *PLoS One* **6**, doi:10.1371/journal.pone.0029340.

Nicastro, K.R., Zardi, G.I., McQuaid, C.D., Stephens, L., Radloff, S. & Blatch, G.L. 2010. The role of gaping behaviour in habitat partitioning between coexisting intertidal mussels. *BMC Ecology* **10**, doi:10.1186/1472-6785-10-17.

Nuñez, J.D., Iriarte, P.F., Ocampo, E.H., Madrid, E. & Cledón, M. 2018. Genetic and morpho-physiological differentiation in a limpet population across an intertidal gradient. *Helgoland Marine Research* **72**, doi:10.1186/s10152-018-0519-1.

Nybakken, J.W. 1993. *Marine Biology: An Ecological Approach*, third volume. G. Davies (ed.). HarperCollins College Publishers.

Oglesby, L.C. 1969. Salinity-stress and desiccation in intertidal worms. *American Zoologist* **9**, 319–331.

Otaíza, R.D. & Santelices, B. 1985. Vertical distribution of chitons (Mollusca: Polyplacophora) in the rocky intertidal zone of central Chile. *Journal of Experimental Marine Biology and Ecology* **86**, 229–240.

Paganini, A.W., Miller, N.A. & Stillman, J.H. 2014. Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*. *Journal of Experimental Biology* **217**, 3974–3980.

Panova, M. & Johannesson, K. 2004. Microscale variation in *Aat* (aspartate aminotransferase) is supported by activity differences between upper and lower shore allozymes of *Littorina saxatilis*. *Marine Biology* **144**, 1157–1164.

Panova, M., Johansson, T., Canbäck, B., Bentzer, J., Rosenblad, M.A., Johannesson, K., Tunlid, A. & André, C. 2014. Species and gene divergence in *Littorina* snails detected by array comparative genomic hybridization. *BMC Genomics* **15**, doi:10.1186/1471-2164-15-687.

Pascoal, S., Carvalho, G., Creer, S., Mendo, S. & Hughes, R. 2012. Plastic and heritable variation in shell thickness of the intertidal gastropod *Nucella lapillus* associated with risks of crab predation and wave action, and sexual maturation. *PLoS One* **7**, doi:10.1371/journal.pone.0052134.

Pasparakis, C., Davis, B.E. & Todgham, A.E. 2016. Role of sequential low-tide-period conditions on the thermal physiology of summer and winter laboratory-acclimated fingered limpets, *Lottia digitalis*. *Marine Biology* **163**, doi:10.1007/s00227-015-2779-5.

Peck, L.S. 2018. Antarctic marine biodiversity: Adaptations, environments and responses to change. In *Oceanography and Marine Biology: An Annual Review*. S.J. Hawkins et al. (eds.) Taylor & Francis, **56**, 2–133.

Peterson, C.H. 1991. Intertidal zonation of marine invertebrates in sand and mud. *American Scientist* **79**, 236–249.

Petes, L.E., Menge, B.A. & Harris, A.L. 2008. Intertidal mussels exhibit energetic trade-offs between reproduction and stress resistance. *Ecological Monographs* **78**, 387–402.

Pörtner, H.O., Bock, C. & Mark, F.C. 2017. Oxygen-and capacity-limited thermal tolerance: Bridging ecology and physiology. *Journal of Experimental Biology* **220**, 2685–2696.

Przeslawski, R., Davis, A.R. & Benkendorff, K. 2005. Synergistic effects associated with climate change and the development of rocky shore molluscs. *Global Change Biology* **11**, 515–522.

Pulgar, J.M., Bozinovic F. & Ojeda, F.P. 2005. Local distribution and thermal ecology of two intertidal fishes. *Oecologia* **142**, 511–520.

Pulgar, J., Lagos, P., Maturana, D., Valdés, M., Aldana, M. & Pulgar, V.M. 2015. Effect of UV radiation on habitat selection by *Girella laevifrons* and *Graus nigra* (Kyphosidae). *Journal of Fish Biology* **86**, 812–821.

Pulgar, J., Waldisperg, M., Galbán-Malagón, C., Maturana, D., Pulgar, V.M. & Aldana, M. 2017. UV radiation impacts body weight, oxygen consumption, and shelter selection in the intertidal vertebrate *Girella laevifrons*. *Science of the Total Environment* **578**, 317–322.

Raffaelli, D. & Hawkins, S. 1999 (eds.) *Intertidal Ecology*, second ed. Dordrecht: Kluwer Academic Publishers.

Randall, D.J., Ip, Y.K., Chew, S.F. & Wilson, J.M. 2004. Air breathing and ammonia excretion in the giant mudskipper, *Periophthalmodon schlosseri*. *Physiological and Biochemical Zoology* **77**, 783–788.

Rawlings, T.A. 1996. Shields against ultraviolet radiation: An additional protective role for the egg capsules of benthic marine gastropods. *Marine Ecology Progress Series* **136**, 81–95.

Rawlings, T.A. 1999. Adaptations to physical stresses in the intertidal zone: The egg capsules of *Neogastropod molluscs*. *American Zoologist* **39**, 230–243.

Richards, J.G. 2011. Physiological, behavioral and biochemical adaptations of intertidal fishes to hypoxia. *Journal of Experimental Biology* **214**, 191–199, doi:10.1242/jeb.047951.

Ritter, A.F. 2017. Adult avoidance behavior leads to ontogenetic shifts in habitat use of an intertidal fish. *Marine Ecology Progress Series* **585**, 143–154.

Rivera-Ingraham, G.A. & Lignot, J.-H. 2017. Osmoregulation, bioenergetics and oxidative stress in coastal marine invertebrates: Raising the questions for future research. *Journal of Experimental Biology* 220, 1749–1760.

Rivera-Ingraham, G.A., Nommick, A., Blondeau-Bidet, E., Ladurner, P. & Lignot, J.-H. 2016. Salinity stress from the perspective of the energy-redox axis: Lessons from a marine intertidal flatworm. *Redox Biology* **10**, 53– 64.

Roberts, D.A., Hofmann, G.E. & Somero, G.N. 1997. Heat-shock protein expression in *Mytilus californianus*: Acclimatization (seasonal and tidal-height comparisons) and acclimation effects. *The Biological Bulletin* **192**, 309–320.

Roland, W. & Ring, R.A. 1977. Cold, freezing, and desiccation tolerance of the limpet *Acmaea digitalis* (Eschscholtz). *Cryobiology* **14**, 228–235.

Rossi, G.S., Tunnah, L., Martin, K.E., Turko, A.J., Taylor, D.S., Currie, S. & Wright, P.A. 2019. Mangrove fishes rely on emersion behavior and physiological tolerance to persist in sulfidic environments. *Physiological and Biochemical Zoology* **92**, 316–325.

Sakamoto, T., Amano, M., Hyodo, S., Moriyama, S., Takahashi, A., Kawauchi, H. & Ando, M. 2005. Expression of prolactin-releasing peptide and prolactin in the euryhaline mudskippers (*Periophthalmus modestus*): Prolactin-releasing peptide as a primary regulator of prolactin. *Journal of Molecular Endocrinology* **34**, 825–834.

Sakamoto, T., Nishiyama, Y., Ikeda, A., Takahashi, H., Hyodo, S., Kagawa, N. & Sakamoto, H. 2015. Neurohypophysial hormones regulate amphibious behaviour in the mudskipper goby. *PLoS One* **10**, doi:10.1371/journal.pone.0134605.

Sakamoto, T., Yokota, S. & Ando, M. 2000. Rapid morphological oscillation of mitochondrion-rich cell in estuarine mudskipper following salinity changes. *Journal of Experimental Zoology* **286**, 666–669.

Sakurai, H., Sato, T., Arai, H., Takasaki, A. Tada, S., Hori, H., Kimpara, I., Matsuyama, T. & Kodama, M. 1996. Habitats of fish and epibenthic invertebrates in Fildes Bay, King George Island, Antarctica. *Proceedings of the NIPR Symposium on Polar Biology* **9**, 231–242.

Sandblom, E., Clark, T.D., Gräns, A., Ekström, A., Brijs, J., Sundström, L.F., Odelström, A., Adill, A., Aho, T. & Jutfelt, F. 2016. Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nature Communications* **7**, doi:10.1038/ncomms11447.

Sanders, B.M., Hope, C., Pascoe, V.M. & Martin, L.S. 1991. Characterization of the stress protein response in two species of *Collisella* limpets with different temperature tolerances. *Physiological Zoology* **64**, 1471–1489.

Sawyer, P.J. 1967. Intertidal life-history of the rock gunnel, *Pholis gunnellus*, in the Western Atlantic. *Copeia* **1967**, 55–61.

Sayer, M.D.J. 2005. Adaptations of amphibious fish for surviving life out of water. *Fish and Fisheries* **6**, 186–211.

Sayer, M.D.J. & Davenport, J. 1991. Amphibious fish: Why do they leave water? *Reviews in Fish Biology and Fisheries* **1**, 159–181.

Schulte, P.M. 2011. Physiological specializations of different fish groups: Intertidal fishes. In: *Encyclopedia of Fish Physiology: From Genome to Environment.* A.P. Farrell (ed.). Elsevier.

Seabra, R., Wethey, D.S., Santos, A.M. & Lima, F.P. 2011. Side matters: Microhabitat influence on intertidal heat stress over a large geographical scale. *Journal of Experimental Marine Biology and Ecology* **400**, 200–208.

Segura, C.J., Montory, J.A., Cubillos, V.M., Diederich, C.M., Pechenik, J.A. & Chaparro, O.R. 2015. Brooding strategy in fluctuating salinity environments: Oxygen availability in the pallial cavity and metabolic stress in females and offspring in the Chilean oyster *Ostrea chilensis*. *Journal of Comparative Physiology B* **185**, 659–668.

Seuront, L. & Ng, T.P.T. 2016. Standing in the sun: Infrared thermography reveals distinct thermal regulatory behaviours in two tropical high-shore littorinid snails. *Journal of Molluscan Studies* **82**, 336–340.

Shanks, A.L. & Wright, W.G. 1986. Adding teeth to wave action: The destructive effects of wave-borne rocks on intertidal organisms. *Oecologia* **69**, 420–428.

Shick, J.M. 1991. *A Functional Biology of Sea Anemones*. P. Calow (ed.) Chapman & Hall.

Shick, J.M. & Dunlap, W.C. 2002. Mycosporine-like amino acids and related gadusols: Biosynthesis, accumulation, and UV-protective functions in aquatic organisms. *Annual review of Physiology* **64**, 223–262.

Shick, J.M., Dunlap, W.C., Pearse, J.S. & Pearse, V.B. 2002. Mycosporine-like amino acid content in four species of sea anemones in the genus *Anthopleura* reflects phylogenetic but not environmental or symbiotic relationships. *The Biological Bulletin* **203**, 315–330.

Shick, J.M. & Dykens, J.A. 1984. Photobiology of the symbiotic sea anemone *Anthopleura elegantissima*: Photosynthesis, respiration, and behavior under intertidal conditions. *The Biological Bulletin* **166**, 608–619.

Sinclair, B.J., Ferguson, L.V., Salehipour-shirazi, G. & MacMillan, H.A. 2013. Cross-tolerance and cross-talk in the cold: Relating low temperatures to desiccation and immune stress in insects. *Integrative and Comparative Biology* **53**, 545–556.

Sloman, K.A., Mandic, M., Todgham, A.E., Fangue, N.A., Subrt, P. & Richards, J.G. 2008. The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **149**, 284–292.

Sokolova, I.M., Granovitch, A.I., Berger, V.J. & Johannesson, K. 2000. Intraspecific physiological variability of the gastropod *Littorina saxatilis* related to the vertical shore gradient in the white and north seas. *Marine Biology* **137**, 297–308.

Sokolova, I.M. & Pörtner, H.O. 2001a. Physiological adaptations to high intertidal life involve improved water conservation abilities and metabolic rate depression in *Littorina saxatilis*. *Marine Ecology Progress Series* **224**, 171–186.

Sokolova, I.M. & Pörtner, H.O. 2001b. Temperature effects on key metabolic enzymes in *Littorina saxatilis* and *L. obtusata* from different latitudes and shore levels. *Marine Biology* **139**, 113–126.

Somero, G.N. 2010. The physiology of climate change: How potentials for acclimatization and genetic adaptation will determine 'winners' and 'losers'. *Journal of Experimental Biology* **213**, 912–920.

Somero, G.N. 2012. The physiology of global change: Linking patterns to mechanisms. *Annual Review of Marine Science* **4**, 39–61.

Somo, D.A., Onukwufor, J.O., Wood, C.W. & Richards, J.G. 2020. Interactive effects of temperature and hypoxia on diffusive water flux and oxygen uptake rate in the tidepool sculpin, *Oligocottus maculosus*. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **250**, doi:10.1016/j.cbpa.2020.110781.

Speers-Roesch, B., Mandic, M., Groom, D.J.E. & Richards, J.G. 2013. Critical oxygen tensions as predictors of hypoxia tolerance and tissue metabolic responses during hypoxia exposure in fishes. *Journal of Experimental Marine Biology and Ecology* **449**, 239–249.

Spicer, J.I. 1993. Oxygen binding by amphipod (Crustacea) haemocyanins. *Marine Behaviour and Physiology* **24**, 123–136.

Spicer, J.I. 2013. Physiological changes accompanying the presence of black gill syndrome in the high shore amphipod *Traskorchestia traskiana*. *Journal of Experimental Marine Biology and Ecology* **446**, 131–138.

Spicer, J.I. & Taylor, A.C. 1994a. Oxygen-binding by haemocyanins from an ecological series of amphipod crustaceans. *Marine Biology* **120**, 231–237.

Spicer, J.I. & Taylor, A.C. 1994b. The origin and metabolic significance of exosomatic water in the semi-terrestrial beachflea, *Orchestia gammarellus* (Crustacea: Amphipoda). *Journal of Zoology* **232**, 617–632.

Spicer, J.I., Taylor, A.C. & McMahon, B.R. 1990. O_2-binding properties of haemocyanin from the sandhopper *Talitrus saltator* (Montagu, 1808) (Crustacea: Amphipoda). *Journal of Experimental Marine Biology and Ecology* **135**, 213–228.

Stenseng, E., Braby, C.E. & Somero, G.N. 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (genus *Tegula*): Implications for vertical zonation. *The Biological Bulletin* **208**, 138–144.

Stevens, M., Lown, A.E. & Denton, A.M. 2014. Rockpool gobies change colour for camouflage. *PLoS One* **9**, doi:10.1371/journal.pone.0110325.

Stickle, W.B., Carrington, E. & Hayford, H. 2017. Seasonal changes in the thermal regime and gastropod tolerance to temperature and desiccation stress in the rocky intertidal zone. *Journal of Experimental Marine Biology and Ecology* **488**, 83–91.

Stickle, W.B., Lindeberg, M. & Rice, S.D. 2015. Comparative freeze tolerance and physiological adaptations of three species of vertically distributed rocky intertidal gastropods from southeast Alaska. *Journal of Experimental Marine Biology and Ecology* **463**, 17–21.

Stillman, J.H. 2000. Evolutionary history and adaptive significance of respiratory structures on the legs of intertidal porcelain crabs, Genus *Petrolisthes*. *Physiological and Biochemical Zoology* **73**, 86–96.

Stillman, J.H. 2002. Causes and consequences of thermal tolerance limits in rocky intertidal porcelain crabs, Genus *Petrolisthes*. *Integrative and Comparative Biology* **42**, 790–796.

Stillman, J.H. 2003. Acclimation capacity underlies susceptibility to climate change. *Science* **301**, 65.

Stillman, J.H. & Somero, G.N. 1996. Adaptation to temperature stress and aerial exposure in congeneric species of intertidal porcelain crabs (genus *Petrolisthes*): Correlation of physiology, biochemistry and morphology with vertical distribution. *Journal of Experimental Biology* **199**, 1845–1855.

Stillman, J.H. & Somero, G.N. 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: Influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiological and Biochemical Zoology* **73**, 200–208.

Stochaj, W.R., Dunlap, W.C. & Shick, J.M. 1994. Two new UV-absorbing mycosporine-like amino acids from the sea anemone *Anthopleura elegantissima* and the effects of zooxanthellae and spectral irradiance on chemical composition and content. *Marine Biology* **118**, 149–156.

Storey, K.B. 1997. Organic solutes in freezing tolerance. *Comparative Biochemistry and Physiology Part A: Physiology* **117**, 319–326.

Storey, K.B. & Churchill, T.A. 1995. Metabolic responses to anoxia and freezing by the freeze tolerant marine mussel *Geukensia demissus*. *Journal of Experimental Marine Biology and Ecology* **188**, 99–114.

Storey, K.B. & Storey, J.M. 1990. Metabolic rate depression and biochemical adaptation in anaerobiosis, hibernation and estivation. *Quarterly Review of Biology* **65**, 145–174.

Storey, K.B. & Storey, J.M. 1996. Natural freezing survival in animals. *Annual Review of Ecology and Systematics* **27**, 365–386.

Sutton, A.O., Turko, A.J., McLaughlin, R.L. & Wright, P.A. 2018. Behavioral and physiological responses of an amphibious, euryhaline mangrove fish to acute salinity exposure. *Copeia* **106**, 305–311.

Tagliarolo, M., Clavier, J., Chauvaud, L., Koken, M. & Grall, J. 2012. Metabolism in blue mussel: Intertidal and subtidal beds compared. *Aquatic Biology* **17**, 167–180.

Taylor, A.C. & Spicer, J.I. 1988. Functional significance of a partial-emersion response in the intertidal prawn *Palaemon elegans* (Crustacea: Palaemonidae) during environmental hypoxia. *Marine Ecology Progress Series* **44**, 141–147.

Taylor, D.S. 2012. Twenty-four years in the mud: What have we learned about the natural history and ecology of the mangrove rivulus, *Kryptolebias marmoratus*? *Integrative and Comparative Biology* **52**, 724–736.

Taylor, D.S., Turner, B.J., Davis, W.P. & Chapman, B.B. 2008. A novel terrestrial fish habitat inside emergent logs. *The American Naturalist* **171**, 263–266.

Taylor, P.M. & Andrews, E.B. 1988. Osmoregulation in the intertidal gastropod *Littorina littorea*. *Journal of Experimental Marine Biology and Ecology* **122**, 35–46.

Taylor, J.D. & Glover, E.A. 2000. Functional anatomy, chemosymbiosis and evolution of the Lucinidae. In: *The Evolutionary Biology of the Bivalvia*, E.M. Harper et al. (eds.) London: Geological Society, Special Publications 177, 207–225.

Taylor, T.D., Glover, E.A., Smith, L., Dyal, P. & Williams, S.T. 2011. Molecular phylogeny and classification of the chemosymbiotic bivalve family Lucinidae (Mollusca: Bivalvia). *Zoological Journal of the Linnean Society* **163**, 15–49.

Tedetti, M. & Sempéré, R. 2006. Penetration of ultraviolet radiation in the marine environment. A review. *Photochemistry and Photobiology* **82**, 389–397.

Tobler, M., Passow, C.N., Greenway, R., Kelley, J.L. & Shaw, J.H. 2016. The evolutionary ecology of animals inhabiting hydrogen sulfide-rich environments. *Annual Review of Ecology, Evolution, and Systematics* **47**, 239–262.

Todgham, A.E., Iwama, G.K. & Schulte, P.M. 2006. Effects of the natural tidal cycle and artificial temperature cycling on Hsp levels in the tidepool sculpin *Oligocottus maculosus*. *Physiological and Biochemical Zoology* **79**, 1033–1045.

Todgham, A.E., Schulte, P.M. & Iwama, G.K. 2005. Cross-tolerance in the tidepool sculpin: The role of heat shock proteins. *Physiological and Biochemical Zoology* **78**, 133–144.

Tomanek, L. 2010. Variation in the heat shock response and its implication for predicting the effect of global climate change on species' biogeographical distribution ranges and metabolic costs. *Journal of Experimental Biology* **213**, 971–979.

Tomanek, L. & Somero, G.N. 1999. Evolutionary and acclimation-induced variation in the heat-shock responses of congeneric marine snails (genus *Tegula*) from different thermal habitats: Implications for limits of thermotolerance and biogeography. *Journal of Experimental Biology* **202**, 2925–2936.

Tomanek, L. & Somero, G.N. 2002. Interspecific- and acclimation-induced variation in levels of heat-shock proteins 70 (hsp70) and 90 (hsp90) and heat-shock transcription factor-1 (HSF1) in congeneric marine snails (genus *Tegula*): Implications for regulation of *hsp* gene expression. *Journal of Experimental Biology* **205**, 677–685.

Torson, A.S., Dong, Y. & Sinclair, B.J. 2020. Help, there are 'omics' in my comparative physiology! *Journal of Experimental Biology* **223**, doi:10.1242/jeb.191262.

Turko, A.J., Doherty, J.E., Yin-Liao, I., Levesque, K., Kruth, P., Holden, J.M., Earley, R.L. & Wright, P.A. 2019. Prolonged survival out of water is linked to a slow pace of life in a self-fertilizing amphibious fish. *Journal of Experimental Biology* **222**, doi:10.1242/jeb.209270.

Turko, A.J., Robertson, C.E., Bianchini, K., Freeman, M. & Wright, P.A. 2014. The amphibious fish *Kryptolebias marmoratus* uses different strategies to maintain oxygen delivery during aquatic hypoxia and air exposure. *Journal of Experimental Biology* **217**, 3988–3995.

Turko, A.J., Tatarenkov, A., Currie, S., Earley, R.L., Platek, A., Taylor, D.S. & Wright, P.A. 2018. Emersion behaviour underlies variation in gill morphology and aquatic respiratory function in the amphibious fish *Kryptolebias marmoratus*. *Journal of Experimental Biology* **221**, doi:10.1242/jeb.168039.

Turra, A. & Denadai, M.R. 2001. Desiccation tolerance of four sympatric tropical intertidal hermit crabs (Decapoda, Anomura). *Marine and Freshwater Behaviour and Physiology* **34**, 227–238.

Underwood, A.J. 2000. Experimental ecology of rocky intertidal habitats: What are we learning? *Journal of Experimental Marine Biology and Ecology* **250**, 51–76.

Viña, N., Bascur, M., Guzmán, F., Riera, R., Paschke, K. & Urzúa, Á. 2018. Interspecific variation in the physiological and reproductive parameters of porcelain crabs from the Southeastern Pacific coast: Potential adaptation in contrasting marine environments. *Comparative Biochemistry and Physiology Part A: Molecular & Integrative Physiology* **226**, 22–31.

Völkel, S. & Grieshaber, M.K. 1997. Sulphide oxidation and oxidative phosphorylation in the mitochondria of the lugworm. *Journal of Experimental Biology* **200**, 83–92.

Waller, C.L., Worland, M.R., Convey, P. & Barnes, D.K.A. 2006. Ecophysiological strategies of Antarctic intertidal invertebrates faced with freezing stress. *Polar Biology* **29**, 1077–1083.

Weihe, E. & Abele, D. 2008. Differences in the physiological response of inter- and subtidal Antarctic limpets *Nacella concinna* to aerial exposure. *Aquatic Biology* **4**, 155–166.

West-Eberhard, M.J. 1989. Phenotypic plasticity and the origins of diversity. *Annual Review of Ecology and Systematics* **20**, 249–278.

Whitear, M. & Mittal, A.K. 1984. Surface secretions of the skin of *Blennius* (*Lipophrys*) *pholis* L. *Journal of Fish Biology* **25**, 317–331.

Widdows, J. & Shick, J.M. 1985. Physiological responses of *Mytilus edulis* and *Cardium edule* to aerial exposure. *Marine Biology* **85**, 217–232.

Williams, G.A., De Pirro, M., Cartwright, S., Khangura, K., Ng, W.-C., Leung, P.T.Y. & Morritt, D. 2011. Come rain or shine: The combined effects of physical stresses on physiological and protein-level responses of an intertidal limpet in the monsoonal tropics. *Functional Ecology* **25**, 101–110.

Williams, S.E., Shoo, L.P., Isaac, J.L., Hoffmann, A.A. & Langham, G. 2008. Towards an integrated framework for assessing the vulnerability of species to climate change. *PLoS Biology* **6**, doi:10.1371/journal.pbio.0060325.

Wilson, W.J. 1970. Osmoregulatory capabilities in isopods: *Ligia occidentalis* and *Ligia pallasii*. *The Biological Bulletin* **138**, 96–108.

Wilson, J.M., Randall, D.J., Donowitz, M., Vogl, A.W. & Ip, A.K. 2000. Immunolocalization of ion-transport proteins to branchial epithelium mitochondria-rich cells in the mudskipper (*Periophthalmodon schlosseri*). *Journal of Experimental Biology* **203**, 2297–2310.

Wolcott, T.G. 1973. Physiological ecology and intertidal zonation in limpets (*Acmaea*): A critical look at "limiting factors". *The Biological Bulletin* **145**, 389–422.

Wolcott, T.G. & Wolcott, D.L. 2001. Role of behavior in meeting osmotic challenges. *American Zoologist* **41**, 795–805.

Wright, J.C. & Pena-Peralta, M. 2005. Diel variation in ammonia excretion, glutamine levels, and hydration status in two species of terrestrial isopods. *Journal of Comparative Physiology B* **175**, 67–75.

Wright, P.A. & Turko, A.J. 2016. Amphibious fishes: Evolution and phenotypic plasticity. *Journal of Experimental Biology* **219**, 2245–2259.

Wright, P.A. & Wood, C.M. 1995. Ammonia and urea excretion in the tidepool sculpin (*Oligocottus maculosus*): Sites of excretion, effects of reduced salinity and mechanisms of urea transport. *Fish Physiology and Biochemistry* **14**, 111–123.

Yancey, P.H. 2005. Organic osmolytes as compatible, metabolic and counteracting cytoprotectants in high osmolarity and other stresses. *Journal of Experimental Biology* **208**, 2819–2830.

Yancey, P.H., Clark, M.E., Hand, S.C., Bowlus, R.D. & Somero, G.N. 1982. Living with water stress: Evolution of osmolyte systems. *Science* **217**, 1214–1222.

Yokoya, S. & Tamura, O.S. 1992. Fine structure of the skin of the amphibious fishes, *Boleophthalmus pectinirostris* and *Periophthalmus cantonensis*, with special reference to the location of blood vessels. *Journal of Morphology* **214**, 287–297.

Yoshiyama, R.M. & Cech, J.J., Jr. 1994. Arial respiration by rocky intertidal fishes of California and Oregon. *Copeia* **1994**, 153–158.

You, X., Bian, C., Zan, Q., Xu, X., Liu, X., Chen, J., Wang, J., Qiu, Y., Li, W., Zhang, X. & Sun, Y. 2014. Mudskipper genomes provide insights into the terrestrial adaptation of amphibious fishes. *Nature Communications* **5**, doi:10.1038/ncomms6594.

You, X., Sun, M., Li, J., Bian, C., Chen, J., Yi, Y., Yu, H. & Shi, Q. 2018. Mudskippers and their genetic adaptations to an amphibious lifestyle. *Animals* **8**, doi:10.3390/ani8020024.

Zadunaisky, J.A., Cardona, S., Au, L., Roberts, D.M., Fisher, E., Lowenstein, B., Cragoe, E.J. & Spring, K.R. 1995. Chloride transport activation by plasma osmolarity during rapid adaptation to high salinity of *Fundulus heteroclitus*. *Journal of Membrane Biology* **143**, 207–217.

Zamer, W.E. 1986. Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. I. Prey capture, absorption efficiency and growth. *Marine Biology* **92**, 299–314.

Zamer, W.E. & Shick, J.M. 1987. Physiological energetics of the intertidal sea anemone *Anthopleura elegantissima*. II. Energy balance. *Marine Biology* **93**, 481–491.

Zamzow, J.P. 2003. Ultraviolet-absorbing compounds in the mucus of temperate Pacific tidepool sculpins: Variation over local and geographic scales. *Marine Ecology Progress Series* **263**, 169–175.

Zhang, G., Li, L., Meng, J., Qi, H., Qu, T., Xu, F. & Zhang, L. 2016. Molecular basis for adaptation of oysters to stressful marine intertidal environments. *Annual Review of Animal Biosciences* **4**, 357–381.

Zhao, X., Li, Q., Meng, Q., Yue, C. & Xu, C. 2017. Identification and expression of cysteine sulfinate decarboxylase, possible regulation of taurine biosynthesis in *Crassostrea gigas* in response to low salinity. *Scientific Reports* **7**, doi:10.1038/s41598-017-05852-6.

Zippay, M.L. & Helmuth, B. 2012. Effects of temperature change on mussel, *Mytilus*. *Integrative Zoology* **7**, 312–327.