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Variation in oxygen consumption among 'living fossils' (Mollusca: Polyplacophora)

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Polyplacophoran molluscs (chitons) are phylogenetically ancient and morphologically constrained, yet multiple living species are often found co-occurring within widely overlapping ecological niches. This study used two sets of experiments to compare interspecific variation among co-occurring species in the North Atlantic (Ireland) and separately in the North Pacific (British Columbia, Canada) chiton faunas. A complementary review of historical literature on polyplacophoran physiology provides an overview of the high level of metabolic variability in this group of 'living fossils'. Species examined in de novo *experiments showed significant variation in oxygen consumption both under air-saturated water conditions (normoxia), and in response to decreasing oxygen availability (hypoxia). Some species demonstrate an ability to maintain constant oxygen uptake rates despite hypoxia (oxyregulators), while others oxyconform, with uptake rate dependent on ambient oxygen tension. These organisms are often amalgamated in studies of benthic communities, yet show obvious physiological difference that may impact their response or tolerance to environmental change.*

Keywords: chiton, basal metabolic rate, physiology, oxyregulation

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INTRODUCTION

Studies of marine ecology and ecosystem functioning naturally depend on the simplification of complex, dynamic real-world systems. An ecological approach to define the true place of organisms within marine communities is, understandably, often constrained by a lack of baseline data, methodological limitations, or even because of a bias towards study of more 'attractive' species. Gaps in knowledge about the precise trophic positions of, or intra-guild relationships between, common taxa often cause notable biases in ecosystem models (Pauly *et al.*, 2009; Bolnick *et al.*, 2011), and such organisms are sometimes amalgamated or omitted altogether. Some taxa, especially those that are morphologically similar, may be placed together within model units that reduce the true complexity of their ecology, life history or physiology (Padilla & Allen, 2000). As these models often form the basis of predictions of the impacts of environmental change, undocumented and unconsidered variations in basic relationships and physiological traits may significantly affect such predictions (Boero *et al.*, 2008; Pauly *et al.*, 2009; Bolnick *et al.*, 2011).

Polyplacophoran molluscs (or chitons) are one such group sometimes regarded as homogeneous in studies of marine benthic communities (e.g. Steneck & Watling, 1982; Ortiz & Wolff, 2002; Bishop, 2003; Hetherington & Reid, 2003), or often omitted altogether (e.g. Poloczanska *et al.,* 2011). Chitons are characterized by their eight articulating shells, or valves, and usually feed as generalist grazers on hard substrates in the intertidal and shallow subtidal, with many species also present in deep-sea habitats. They are often present in high densities with numerous species co-occurring, and can form a significant component of marine benthic communities (Horn, 1982; Paine, 1992; Littler *et al.*, 1995; Barbosa *et al.*, 2008), often controlling algal biomass and thus community structure (Dethier & Duggins, 1984, 1988). In some regions chiton species may be amongst the most abundant shore molluscs (Boyle, 1970). Numerous chiton species apparently co-occur within ranges and habitats, and phylogeographical studies have addressed the recent and continuing radiation of species in apparently widely-overlapping ecological niches (Kelly & Eernisse, 2008).

Organisms living in the intertidal are likely to experience natural extremes of oxygen availability, whether within hypoxic sediments, in adverse water chemistry conditions, or through factors such as predator harassment causing valve closure and subsequent hypoxia within shells (Larade & Storey, 2002). On rocky shores, tidepools and other microhabitats often experience extremes of conditions, from severe hypoxia at night to ambient oxygen tensions higher than air-saturation in daylight (Truchot & Duhamel-Jouve, 1980; Hagerman, 1998). Under the predicted impacts of climate change it is likely that all marine communities will experience conditions of decreased dissolved oxygen in the future (Brewer & Peltzer, 2009; Keeling *et al.*, 2010). Physiological traits that confer better survival in oxygen-depleted conditions

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may have considerable impact on the composition of marine communities in such conditions.

Physiological traits are important factors in determining an organism's success in a dynamic environment; the ability to metabolize oxygen may determine fitness, spatial distribution, and the capacity of a species to adapt to changing conditions. Physiological flexibility is necessary for organisms to survive in habitats that exhibit extremes of oxygen availability, especially those species that lack the locomotory ability to move to another area (e.g. Hagerman, 1998). Such flexibility in physiology has been demonstrated in many organisms, including bivalves (de Zwaan *et al.*, 1991; Shumway *et al.*, 1993), gastropods (Eberlee & Storey, 1988; Pannunzio & Storey, 1998), and crustacean zooplankton (McAllen *et al.*, 1999).

Responses to hypoxic conditions differ between invertebrates. Motile species such as littorinid snails may simply relocate to escape hypoxia, while more sedentary species such as bivalves instead rely on a reduction in metabolic rate (Livingstone, 1991). One possible physiological response to hypoxia is a compensatory increase in the uptake rate of oxygen in order to maintain adequate supply (Mangum & Van Winkle, 1973). Many species lack the physiological mechanisms necessary to maintain oxygen uptake under hypoxic conditions and as a result, oxygen uptake is proportional to ambient oxygen tension (oxyconformers) (e.g. Wilson & Davis, 1984; Spicer *et al.*, 2002). However some species, often those found where large variations in oxygen tensions are common, can maintain routine oxygen uptake rates despite declining oxygen tension (oxyregulators) (e.g. Taylor & Moore, 1995; Strahl *et al.*, 2011). Typically this occurs over a range of oxygen tensions to a critical tension (P_{crit}) below which they display oxyconforming behaviour. The mechanisms by which this increase may occur are varied and may include increases in ventilation of the gills (Taylor & Moore, 1995), or in species with active circulatory systems an increase in heart rate or heart stroke volume (Bayne, 1971; DeFur & Mangum, 1979). Gastropods such as the limpets *Patella granularis* Linnaeus, 1758 and *Siphonaria capensis* Quoy & Gaimard, 1833 may induce bradycardia as a response in order to decrease short-term oxygen consumption (Marshall & McQuaid, 1993).

In chitons, variation in oxygen consumption has been observed within a single species (*Chiton pelliserpentis* Quoy & Gaimard, 1835) related to size, temperature and shore position (Horn, 1985). Another species, *Chiton stokesii* Broderip & Sowerby, 1832 appears to possess physiological adaptations to extended periods of emersion to better exploit the intertidal habitat (McMahon *et al.*, 1991). Only one previous study has examined the variation in oxygen metabolism between co-occurring species of chiton (Murdoch & Shumway, 1980). Chitons make a good candidate to examine how a single physiological trait may vary between species that are often assumed to have identical roles in ecosystem functioning, and so demonstrate how intra-guild complexity is often neglected at larger scales. Here we present a case study to examine the variation in oxygen uptake within a morphologically constrained group of common, co-occurring intertidal molluscs, within and between Atlantic and Pacific temperate ecosystems.

MATERIALS AND METHODS

Two sets of experiments were conducted to examine standard respiration rates and responses to declining oxygen tensions in Atlantic (Ireland) and Pacific (British Columbia, Canada) species of chiton.

Atlantic experiments

Three species of chiton, *Lepidochitona cinerea* Linnaeus, 1767 (N = 10), *Acanthochitona crinita* Pennant, 1777 (N = 15) and *Leptochiton asellus* Gmelin, 1791 (N = 5) (Figure 1), were collected from the low intertidal zone at six sites in Strangford Lough, Northern Ireland, October 2010. Some *Leptochiton asellus* specimens were collected by SCUBA divers from subtidal *Modiolus modiolus* Linnaeus, 1758 beds at 10–20 m depth. Specimens were housed in aerated seawater obtained from Strangford Lough (14°C, salinity = 33.5), and kept attached to bare rocks but otherwise not actively fed. Experiments took place from October to December 2010.

On the day of each experiment specimens were transferred to a holding vessel containing 22 μm-filtered seawater at 14°C and cleaned gently with a fine, soft brush to remove epibiota. They were then individually transferred to glass respirometers fitted with a Clark oxygen electrode and a water jacket kept at a temperature of 14°C. A stir bar in the base of the chamber was used to ensure adequate mixing in the respirometer, and was guarded by a plastic barrier to prevent interference by the specimen. The chamber was sealed with a Perspex stopper possessing a narrow (<1 mm) central aperture,

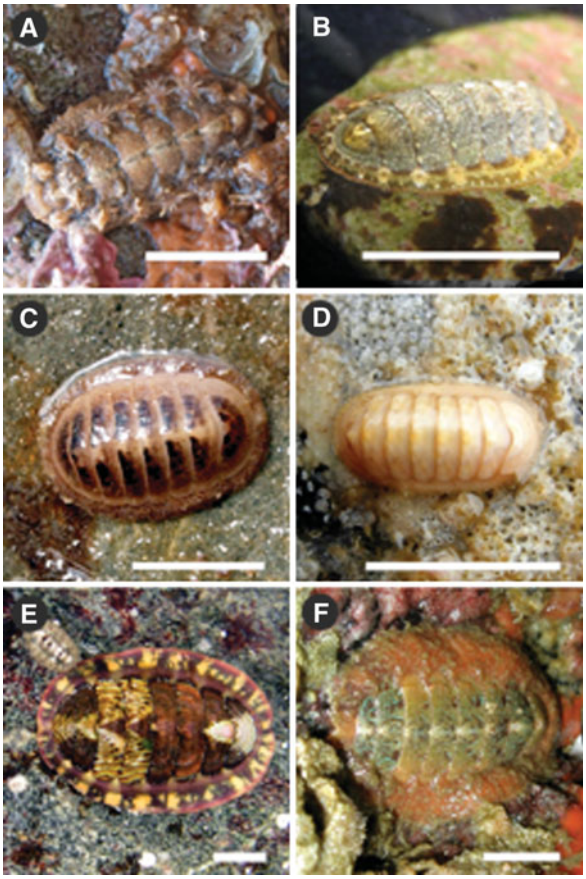

Fig. 1. Chitons examined for oxygen uptake rates in this study. Atlantic species: (A) *Acanthochitona crinita*; (B) *Lepidochitona cinerea*; (C) *Leptochiton asellus*. Pacific species: (D) *Leptochiton rugatus*; (E) *Tonicella lineata*; (F) *Mopalia ferreirai*. All animals are shown with anterior to the right; all scale bars are 10 mm.

allowing the pressure within the experimental chamber to remain equalized with the external atmospheric pressure. Changes in oxygen tension were monitored by a PC recording module at a sampling interval of 100 ms. Trials were continued until oxygen was exhausted or uptake had noticeably levelled off.

After removal from the chamber, specimens were blotted dry on tissue paper, and wet mass determined. Before each trial the Clark electrode was calibrated for zero and saturated oxygen concentrations using anoxic and fully air-saturated seawater. After experiments the chamber was cleaned with filtered seawater and the calibrations checked, with any drift of sensitivity noted, and the apparatus recalibrated if necessary for the next experimental trial. In the interests of data integrity, experimental runs with a drift of more than 10% in sensitivity were discarded. Drift in probe sensitivity below this threshold was assumed to be linear over the course of the experiment, and the probe recordings adjusted as such.

Specimens were dried at 60°C until constant mass was achieved to obtain total dry mass (TDM). They were then incinerated at 500°C for two hours in a muffle furnace to obtain shell and ash mass, and this subtracted from TDM to obtain the ash-free dry mass of tissue (AFDM). All masses are reported in grams.

Pacific experiments

All specimens were collected intertidally in British Columbia, Canada, June 2008; *Leptochiton rugatus* Carpenter in Pilsbry, 1892 (N = 10), at Whiffen Spit, Sooke, Vancouver Island; *Tonicella lineata* Wood, 1815 (N = 8) and *Mopalia ferreirai* Clark, 1991 (N = 3) at Walker's Hook, Salt Spring Island (Figure 1). Specimens were transported to University of British Columbia (Vancouver) aquarium facilities and maintained in aerated, filtered seawater (12°C, salinity = 32) for eight days before experiments and not fed during this time. On the day of each trial four specimens were moved to a water-bath with the same salinity and temperature as the acclimation aquarium. Chitons were individually placed in glass respirometers of a volume matching roughly 20 times that of the chiton, with a magnetic stir bar. The respirometry chambers were sealed with a rubber stopper, fixed with parafilm, and fitted with a fibre-optic oxygen probe (FOXY systems, Ocean Optics, Dunedin, Florida) connected to a PC recording module. Four trials were run simultaneously and oxygen readings recorded at intervals of 15 seconds. Each trial was run for a minimum of three hours and at the end of each trial the wet mass of the chiton and the volume of the respirometer recorded, with additional trials that demonstrated leakage discarded.

Data analysis

Data were analysed using Microcal Origin 8.0 and probe recordings smoothed using Savitzky–Golay smoothing. Statistical tests were performed using *R: a language and environment for statistical computing* (R Core Development Team, 2012). Calibrations for zero and air-saturated oxygen tensions were used to convert oxygen probe recordings to oxygen concentrations using calculated air-saturated concentrations of 8.38 mg l^{-1} for the Atlantic seawater and 8.88 mg l^{-1} for the Pacific seawater. Air-saturated concentrations were determined according to the methods of Benson & Krause (1984).

Initial mass-specific basal oxygen uptake rates (VO_2) ($\mu gO_2\ min^{-1}\ g^{-1}$) were calculated for each specimen, based on the mean rate at which oxygen was depleted from fully air-saturated to 90% air-saturated, per time (minutes) and mass (AFDM) (Table 1). For Pacific specimens, only wet tissue mass was determined (by subtracting dry shell mass from total wet mass), and basal mass-specific uptake rates based on these masses will be used for analysis.

For further analysis in conditions of progressive hypoxia, uptake rates were standardized to a percentage, with basal VO_2 of each specimen assigned a value of 100% and a zero uptake value assigned a value of 0% (standardized VO_2 or $^{S}VO_2$). Rates of half ($^{S}VO_2^{50\%}$) and one-quarter ($^{S}VO_2^{25\%}$) these standardized basal rates were determined by plotting the rate of oxygen uptake against declining oxygen concentrations for each specimen and determining the concentrations at which $^{S}VO_2^{50\%}$ and $^{S}VO_2^{25\%}$ occurred. Where $^{S}VO_2^{50\%}$ and $^{S}VO_2^{25\%}$ occurred over a range of concentrations a mean of these was taken.

To quantify the degree of oxygen dependence of the different species we use the method described by Alexander & McMahon (2004) to determine the 'regulation value', *R*. Here, the integrated sum of each proportional value of VO_2 at each 5% decrease in oxygen concentration is expressed as a percentage of that which would be expected for an organism that exhibits perfect oxygen uptake regulation. An idealized oxyregulator would have an *R* value of 100%, while an idealized oxyconformer (as represented by the solid lines in Figure 2) would have an *R* value of 50%. Values of *R* within this range represent

Table 1. Mean values (±SE) for total wet mass (WM), wet tissue mass (WT), and basal oxygen uptake rates (VO_2) for each species, standardized by each wet body mass metric. These are presented for comparisons of Pacific species examined here with historical data (Figure 5). VO_2 for Atlantic species standardized by ash-free dry tissue mass are shown in Figure 4.

	Mean total wet mass (WM) (g)	Mean wet tissue mass (WT) (g)	VO_2 ($\mu gO_2\ min^{-1}\ gWM^{-1}$)	VO_2 ($\mu gO_2\ min^{-1}\ gWT^{-1}$)
Atlantic species				
Leptochiton asellus	0.125 ± (0.028)	0.075 ± (0.018)	0.994 ± (0.125)	1.657 ± (0.211)
Acanthochitona crinita	0.166 ± (0.026)	0.124 ± (0.019)	1.299 ± (0.091)	1.749 ± (0.124)
Lepidochitona cinerea	0.070 ± (0.011)	0.044 ± (0.007)	1.995 ± (0.194)	3.195 ± (0.335)
Pacific species				
Leptochiton rugatus	0.017 ± (0.002)	0.017 ± (0.002)	1.035 ± (0.116)	1.380 ± (0.154)
Tonicella lineata	0.832 ± (0.278)	0.832 ± (0.278)	0.712 ± (0.072)	1.063 ± (0.108)
Mopalia ferreirai	3.847 ± (2.056)	3.847 ± (2.056)	0.680 ± (0.164)	0.933 ± (0.225)

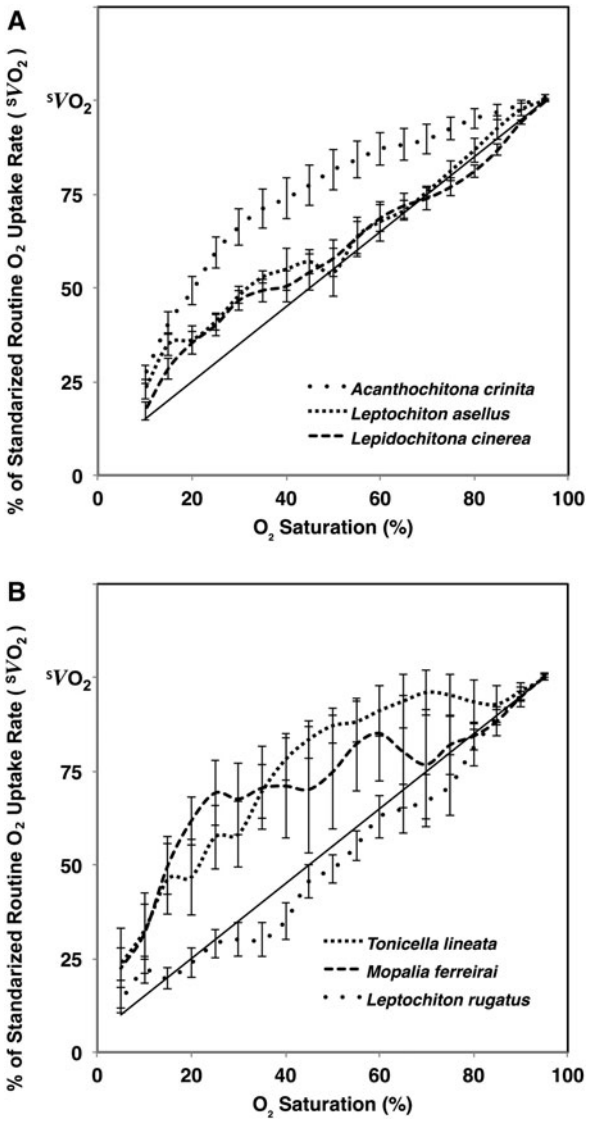

Fig. 2. Standardized basal oxygen uptake rates for Atlantic (Figure 2A) and Pacific (Figure 2B) species in conditions of decreasing oxygen tensions. Vertical axis indicates basal rate of uptake for each specimen standardized to 100% ($^{S}VO_2$), with subsequent uptake rates calculated as a percentage of this at each 5% decrease in oxygen concentration ($\pm$SE). The metrics $^{S}VO_2^{50\%}$ and $^{S}VO_2^{25\%}$ are used in the text to indicate rates of half- and one-quarter basal mass-specific uptake rates respectively. Solid diagonal lines represent idealized oxyconforming behaviour in which oxygen uptake would be directly proportional to concentration. Deviation above this line indicates different degrees of oxyregulation ability (deviation below the line would indicate an organism with VO_2 highly constrained by hypoxia). An idealized oxyregulator would be represented by a horizontal line at $^{S}VO_2$ across all concentrations, indicating an ability to maintain the same rate of uptake at any concentration of oxygen.

different degrees of oxyregulation ability in a particular specimen; the closer these R values are to 100% the greater this ability (Alexander & McMahon, 2004; Lencioni *et al.*, 2008). In addition we calculated mean $R_{25\%}$ for each species, which is the respiration rate at oxygen concentrations 25% that of air-saturated concentrations, expressed as a percentage of the $^{S}VO_2$. Typical values for defining oxyregulating organisms are $R > 50\%$, and $R_{25\%} > 37\%$ (Alexander & McMahon, 2004; Brodersen *et al.*, 2004; Lencioni *et al.*, 2008).

We also compare our results with those in published literature for chiton oxygen uptake rates. When necessary, data were extracted from appropriate figures using PlotDigitizer 2.5.1 for Mac OS X, and O_2 concentration and uptake rate units converted to $\mu gO_2\ min^{-1}\ g^{-1}$ according to the methods of García & Gordon (1992). Mass-specific VO_2 recorded in this study were recalculated and standardized by total wet mass, total wet tissue mass, or total dry tissue mass for comparisons with historical data where appropriate. In chitons, ratios of the contribution of shell, tissue and water content to total mass vary considerably between species (McMahon *et al.*, 1991). Previous studies have used a variety of mass measurements for calculating VO_2, and we are careful to only make comparisons with historical data where mass measurements are comparable.

All tests of significance are one-way analyses of variance (ANOVAs) ($\alpha = 0.05$), and all measurements of variability are standard error (SE). *Post-hoc* tests are Tukey multiple comparisons with 95% confidence intervals. Data were tested to ensure they meet the assumptions of the ANOVA test (i.e. independence, normality and homogeneity of variances).

RESULTS

The chiton species examined showed substantial variation in oxygen consumption rate, both in basal uptake under initial normoxic conditions, and in response to decreasing oxygen availability.

Changes in VO_2 in response to decreasing oxygen concentrations were different amongst both the Atlantic and Pacific faunas (Figure 2). Among the Atlantic species, *A. crinita* was the strongest oxyregulator, with *L. cinerea* and *Leptochiton asellus* having mean regulation values of R and $R_{25\%}$ much closer to typical oxyconformer values (Table 2). Among the Pacific species, *Leptochiton rugatus* can be considered to be a typical oxyconformer, while *M. ferreirai* and *T. lineata* both showed strong oxyregulatory ability (Table 2).

In order to demonstrate the degree of variation between species in basic respiratory patterns independent of body mass, the mean oxygen concentrations at which $^{S}VO_2$ had declined to half ($^{S}VO_2^{50\%}$) and one-quarter ($^{S}VO_2^{25\%}$) of basal initial rates were determined (Figure 3). These metrics are mass-independent and so can be used to compare respiration patterns between individuals of different sizes (Spearman rank

Table 2. Quantification of oxyregulatory ability for each species; mean R values and mean $R_{25\%}$ values ($\pm$SE). R values were determined according the method described by Alexander & McMahon (2004). $R_{25\%}$ indicates the respiration rate at 25% of air-saturated oxygen concentration as a percentage of $^{S}VO_2$. Typical values for defining oxyregulating organisms are $R > 50\%$, and $R_{25\%} > 37\%$ (Alexander & McMahon, 2004; Brodersen *et al.*, 2004; Lencioni *et al.*, 2008).

	Mean R value	Mean $R_{25\%}$ values
Atlantic species		
Leptochiton asellus	63.2% $\pm$ (5.3%)	41.0% $\pm$ (2.3%)
Acanthochitona crinita	76.7% $\pm$ (5.0%)	59.4% $\pm$ (4.1%)
Lepidochitona cinerea	60.9% $\pm$ (5.4%)	40.0% $\pm$ (2.8%)
Pacific species		
Leptochiton rugatus	51.9% $\pm$ (6.2%)	28.9% $\pm$ (3.7%)
Mopalia ferreirai	75.3% $\pm$ (4.9%)	57.4% $\pm$ (8.6%)
Tonicella lineata	71.7% $\pm$ (3.8%)	69.2% $\pm$ (8.6%)

correlations: ${}^{S}VO_2^{50\%}$ to gWT $r_s = -0.54$, $N = 51$, $P < 0.0001$; ${}^{S}VO_2^{25\%}$ to gWT $r_s = -0.52$, $N = 49$, $P = 0.0001$). These metrics again demonstrated differences in oxyregulatory behaviour with decreasing oxygen concentration. ${}^{S}VO_2^{50\%}$ differed significantly between the six study species ($F_{5,45} = 12.21$, $P < 0.0001$: Figure 3A). The three species with high *R* values, *A. crinita, M. ferreirai* and *T. lineata,* showed different behaviour than the other species, as indicated by the lower mean concentrations at which ${}^{S}VO_2^{50\%}$ occurred, again indicating these three species were able to maintain higher relative oxygen uptake rates at lower concentrations than the other species, and so demonstrated stronger oxyregulatory ability.

Analysis within the respective geographical species groups also showed significant differences in the ${}^{S}VO_2^{50\%}$ metric (Atlantic species $F_{2,27} = 12.59$, $P = 0.0001$; Pacific species $F_{2,18} = 12.91$, $P = 0.0003$). *Post-hoc* tests reveal support for the categorization of the different species into groups with differing degrees of oxyregulatory ability. Tests show the single species with the strongest oxyconforming behaviour, *Leptochiton rugatus* to be significantly different from all other species ($P < 0.0001$). There were no significant differences between the species that apparently demonstrated oxyregulation: *A. crinita, T. lineata* and *M. ferreirai* ($P = 0.45$).

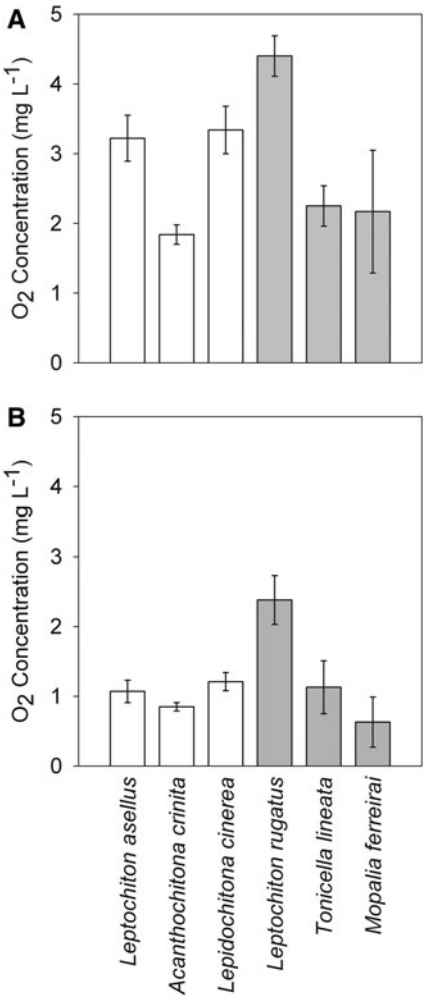

Fig. 3. Mean concentrations of O_2 (mg l^{-1}) at which ${}^{S}VO_2^{50\%}$ (Figure 3A) and ${}^{S}VO_2^{25\%}$ (Figure 3B) occur for Atlantic (white columns) and Pacific (grey columns) species examined in this study. The metrics ${}^{S}VO_2^{50\%}$ and ${}^{S}VO_2^{25\%}$ indicate rates of 50% and 25% of initial standardized basal rates respectively. Error bars indicate standard error.

The ${}^{S}VO_2^{25\%}$ metric also differed significantly between the study species ($F_{5,43} = 6.79$, $P = 0.0001$: Figure 3B), though this was clearly a result of the relatively higher mean concentration at which ${}^{S}VO_2^{25\%}$ occurs for *Leptochiton rugatus*. *Post-hoc* Tukey tests revealed significant differences only involving *Leptochiton rugatus,* and with the exception of this species there was not a significant difference in ${}^{S}VO_2^{25\%}$ between the remaining species.

Basal mass-specific oxygen uptake rates under normoxic conditions (VO_2) were significantly different between all six species examined using wet tissue mass (WT) as the standardizing mass denominator ($F_{5,45} = 14.57$, $P < 0.0001$). *Lepidochitona cinerea* in particular showed a markedly greater mean VO_2 than any other species, the next closest being *A. crinita* (Table 1). *Post-hoc* Tukey tests indicate differences were due to the greater VO_2 demonstrated by *L. cinerea*.

Within the Atlantic fauna there were significant differences when both wet tissue mass (WT) and ash-free dry tissue mass (AFDM) were used as the standardizing mass denominator; ($F_{2,27} = 13.74$, $P = 0.0001$) and ($F_{2,27} = 16.19$, $P < 0.0001$) respectively. Again, *post-hoc* Tukey tests reveal differences were driven by the greater VO_2 demonstrated by *L. cinerea,* with no significant differences between the other two species.

The basal VO_2 standardized by dry tissue mass of the Atlantic species examined in this study were compared to that of six New Zealand species (data extracted from Murdoch & Shumway (1980, Figure 3) and Horn (1985, figures 1&3)). The New Zealand species demonstrate a similar level of variation in VO_2 between species as was observed in this study (Figure 4). There was a significant difference in the VO_2 within the New Zealand fauna ($F_{6,124} = 35.98$, $P < 0.0001$) and between all nine species ($F_{9,151} = 31.52$, $P < 0.0001$). *Post-hoc* Tukey tests show significant differences to be associated with *L. cinerea* and *O. neglectus*, which are different from all other species except for each other and other significant differences are associated with *A. crinita* and *C. pelliserpentis* (Figure 4).

There was no significant difference in basal VO_2 between the three Pacific species examined in this study (Table 1). Comparisons with historical data from other Pacific species are presented in Figure 5, using data extracted from appropriate tables, figures or text. However none of these studies provide data with which to further test statistical differences in VO_2 between Pacific species.

DISCUSSION

Simplification and abstraction of complex and dynamic environmental systems is necessary to make sense of marine ecology. However, such simplification must strike a balance between practicality and true reflection of real-world complexity (Warwick, 1993), and seek to fully incorporate previously neglected aspects of the ecology and biology of component taxa (Boero *et al.*, 2008). Given the morphological and ecological similarity of chitons, there is a surprisingly large variation in uptake rates and behaviour among species globally, and co-occurring species in separate faunas demonstrate substantial differences in physiology.

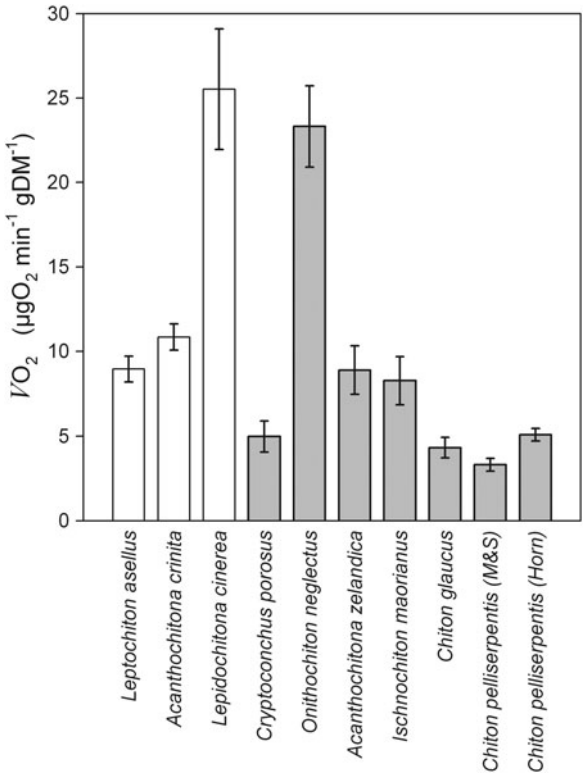

Fig. 4. Mean basal mass-specific VO_2 (μgO_2 min^{-1} gDM^{-1}) for three Atlantic species in this study (white columns) and six New Zealand species examined by Murdoch & Shumway (1980, figure 3), one of which was also examined by Horn (1985, figures 1&3) (grey columns). For Atlantic species ash-free dry tissue mass (AFDM) was used as the mass-standardizing denominator (DM). For DM, Murdoch & Shumway (1980) used dry tissue mass as determined through subtraction of KOH-dissolved mass from total dry mass; Horn (1985) does not specify a method for determining DM. This study used the period over which oxygen concentration was reduced to 90% of air-saturated to determine basal VO_2. Horn (1985) determined VO_2 by averaging rates over a 2-hour period. Murdoch & Shumway (1980) do not specify a methodology or time period over which uptake rates were determined. Temperatures at which species were examined are: this study 14°C; Murdoch & Shumway (1980) 15°C; Horn (1985) 15.5°C. Columns are arranged with low shore or subtidal species on the left, with those to the right occurring progressively higher in the intertidal (within the respective geographical groups). Error bars indicate standard error.

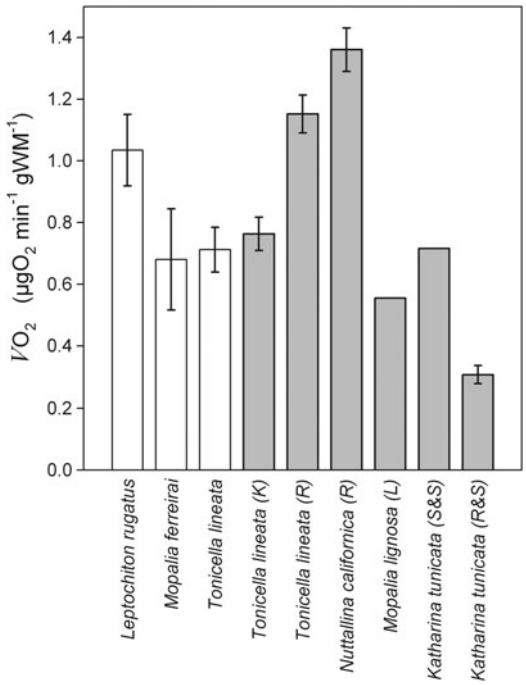

Fig. 5. Mean basal mass-specific VO_2 standardized by total wet mass (μgO_2 min^{-1} gWM^{-1}) for three Pacific species in this study (white columns), and additional Pacific species taken from existing literature (grey columns). K, Kincannon (1975, figures 3&4: T, 13°C); R, Robbins (1975, table 1: T, 13.5°C); L, Lebsack (1975, figure 1: T, 13.5°C); S&S, Stickle & Sabourin (1979, p. 266: T, 13°C); R&S, Rostal & Simpson (1988, p.124: T, 11°C). Species in this study were examined at 12°C. Error bars indicate standard error and are presented only when they could be extracted from the historical data.

Comparative data

A small number of other studies have quantified respiratory physiology in chitons. Each of these employed different experimental and analytical methods, were conducted at different times in the seasonal cycle or at different temperatures, and mostly were restricted to manipulations of one or two taxa. Variations in metabolic rates due to extrinsic factors such as time of year and temperature are to be expected, so direct comparisons of uptake rates should be considered with this caveat in mind. The two groups of species examined in the present study were examined at different times of year and under slightly different laboratory conditions, which may account for some differences in respiratory rates. However, basic physiological traits such as oxyregulatory ability are likely to be inherent (Mangum & Van Winkle, 1973). Nagabhushanam & Murti (1972) reported effects of body size and salinity on respiration in *Chiton granoradiatus* Leloup, 1937 in India. Petersen & Johansen (1973) measured metabolism in a range of body sizes and temperatures of adult *Cryptochiton stelleri* von Middendorff, 1847 in the north-east Pacific. Kincannon (1975) reported oxygen consumption in *T. lineata* (included here as one of our Pacific species) from intertidal and subtidal populations, and at different temperatures. Robbins (1975) also examined aerial and aquatic respiration in *T. lineata*, as well as *Nuttallina californica* Nuttall MS, Reeve, 1847. Lebsack (1975) and Stickle & Sabourin (1979) reported data for respiration varying with temperature and salinity in two common species in the north-east Pacific (*Mopalia muscosa* Gould, 1846 and *Katharina tunicata* Wood, 1815, respectively). Rostal & Simpson (1988) also examined *K. tunicata* in different salinities. Horn (1985) recorded temperature sensitivity in the respiratory rates of *Chiton pelliserpentis* at varying shore heights, but no overall difference in rates. This was also one of the species examined by Murdoch & Shumway (1980), who tested six co-occurring species of chitons in New Zealand from different shore heights, in terms of their oxygen uptake and oxyregulatory function. McMahon *et al.* (1991) measured oxygen uptake in the tropical species *Chiton stokesii* in Panama. Not all of these publications include data that could be directly compared to our present results, but quantitative comparisons were made wherever appropriate conversions were determinable (Figures 4 & 5). Studies that used tropical study organisms (Nagabhushanam & Murti, 1972; McMahon *et al.*, 1991) show much higher metabolism, as would be expected of ectothermic organisms in higher ambient temperatures. The more meaningful comparisons with our results are the global trends in animals at temperate latitudes.

Various approaches have been proposed to quantify the degree of oxyregulation an organism exhibits over a range of oxygen tensions; the ongoing methodological development being one aspect that confounds direct comparison with historical data. Categorization of organisms as either oxyconformers or oxyregulators is highly simplistic, as there is a continuum of oxyregulatory responses by species (Taylor & Brand, 1975; Alexander & McMahon, 2004). Tang (1933) and Bayne (1973) show how the intercept (K_1) and slope (K_2) of a linear regression of VO_2 against oxygen concentration may be used to obtain an oxygen independence index (K_1/K_2) for comparison of regulatory ability between species. This method was used by Murdoch & Shumway (1980) to demonstrate that the degree of oxyregulation in the chitons they examined appears to be greater the higher the species is found in the intertidal. Other methods use semi-logarithmic, exponential, or hyperbolic regressions (Mangum & Van Winkle, 1973; Taylor & Brand, 1975; Herreid, 1980) to obtain coefficients with which to determine the degree of oxygen regulation. However, such calculations are often based on relatively few data points, and as such are not particularly suited to dense, high-resolution data (such as recorded in this study) where changes in uptake rates may show a strong trend but fluctuate significantly over short time periods. As Alexander & McMahon (2004) point out, some species are particularly adept at oxyregulation and in such cases regressions on which to base these metrics are difficult to fit. In some species uptake may vary unpredictably with progressive hypoxia, and may actually increase initially (Alexander & McMahon, 2004). This was observed here in some *A. crinita* and *T. lineata* specimens (both oxyregulators) which showed initial increases in uptake rates as oxygen concentrations declined. While oxyregulatory ability is likely to be inherent in species (Mangum & Van Winkle, 1973), the ability may not necessarily be observed in particular individuals for a number of reasons, and several studies have demonstrated that some individuals show strong oxyregulation while others of the same species act as conformers under similar conditions (Bayne, 1971; Herreid, 1980; Duke & Ultsch, 1990). Some *A. crinita* specimens in this study (both small and large individuals within the sample set) showed strong oxyregulation while others did not (R values ranged from 87% to 61%). The strongly oxyregulating *A. crinita* specimens were able to maintain basal VO_2 in ambient oxygen concentrations ranging from fully air-saturated (8.38 mg l^{1-}) to approximately 35% of air-saturated (3.0 mg l^{-1}). Anecdotally, we have also observed *A. crinita* specimens that were revived and apparently healthy after prolonged periods (over 36 hours) of exposure to almost totally anoxic conditions (Carey & Sigwart, unpublished observation). Other individual specimens of the same taxon showed generally linear, oxyconforming relationships to ambient oxygen tensions, similar to those observed in *L. cinerea* and *Leptochiton asellus*. No *L. cinerea* or *Leptochiton asellus* specimens showed evidence of substantial oxyregulation ability.

McMahon *et al.* (1991) showed increases in haemoconcentration and haemolymph pressure of *C. stokesii* associated with air exposure, and similar mechanisms may be utilized by *A. crinita* and the other oxyregulating species to increase oxygen uptake in depleted conditions. Our data measured at the organismal level however, cannot indicate how or at what stage in the metabolic pathway oxyregulation is implemented.

Few other studies have quantified VO_2 in chitons in a manner that can be directly compared to the species included here. Kincannon (1975, figures 3 & 4) quantified mean mass-specific VO_2 in *T. lineata* ($T = 13^{\circ}C$), with a result comparable with that found in this study given that our experiments were conducted at a slightly lower temperature (Figure 5). Robbins (1975, table 1) however, found a higher mass-specific VO_2 in *T. lineata* ($T = 13.5^{\circ}C$) (Figure 5). The disparity in this result with that presented here and with Kincannon (1975) is too great to be explained solely by the slightly higher temperature. Robbins (1975) found another co-occurring species *N. californica* to have a greater VO_2 than recorded in any other temperate Pacific species. Lebsack (1975) and Stickle & Sabourin (1979) found VO_2 in two other Pacific species more comparable to those recorded in this study (Figure 5). Within the New Zealand fauna, Murdoch & Shumway (1980) and Horn (1985) independently measured VO_2 in *C. pelliserpentis* with results not significantly different (Figure 4). While there was no significant difference in VO_2 between the Pacific species in this study, the historical data (Figure 5) suggests some variation may exist.

These existing studies illustrate the issues with comparisons of historical data. With the small number of datasets available it is not always clear whether there are unknown variables that complicate the results, or if one study is anomalous. In the case of chitons, several species complexes, including some of those examined in the present study such as *T. lineata* and several *Mopalia* species, have been redescribed since historical experiments were published (Eernisse *et al.*, 2007). There is no way of checking the modern identity of the species used in these historical studies.

Below we consider several specific potential factors (body size, shore height, local environmental conditions and phylogeny) that may control the variation observed among our study species and the few previously published observations.

Body size

The influence of body mass on oxygen consumption has been well studied in many marine species (Newell & Roy, 1973; Fisher, 1976; Bridges & Brand, 1980; Katsanevakis *et al.*, 2007; Seibel, 2007). The greater the body mass of an organism, the greater its oxygen consumption with this relationship following generalized mass-dependent scaling factors (West *et al.*, 1997; Gillooly *et al.*, 2001). In our study, the species that showed oxyregulatory ability (*A. crinita* (Atlantic), and *M. ferreirai*, and *T. lineata* (Pacific)) are also those with generally greater adult body mass of the sampled fauna, both observationally and among the individual specimens included in our experiments (Table 1). The species we examined that are typically smaller in body mass showed general oxyconforming behaviour, suggesting direct diffusion processes may dominate uptake, rather than active responses such as increased ventilation of gills or haemolymph circulation or pressure modifications. Those species with larger body mass may not be able to rely on diffusion alone to supply body tissues with oxygen, and so may possess additional mechanisms to regulate respiration. However, *Cryptoconchus porosus* Blainville MS, Burrow, 1815 in New Zealand, the largest species examined by Murdoch & Shumway (1980) was also one of the strongest oxyconformers in their study.

Body mass is clearly a factor in the observed differences in total oxygen consumption between the species studied here.

However, in our chosen metrics ($^{S}VO_2^{50\%}$ and $^{S}VO_2^{25\%}$) there was no significant correlation with body mass, demonstrating that interspecific differences in oxygen physiology are a much greater factor than differences between individuals or species of different mass.

Shore height

Murdoch & Shumway (1980, p. 132) state that oxygen demand is inversely related to shore height, and the species occurring highest on the shore have a lower rate of aquatic oxygen consumption than those lower down. While this is broadly true in their results, it takes no account of mass-specific differences in consumption. All of the species Murdoch & Shumway (1980) examined were in approximately the same size-range, with the exception of *C. porosus*, which is generally larger, and had little overlap in mass with the other species they examined. The mass-specific uptake of *C. porosus* is actually among the lowest of those they examined (Figure 4), yet it occurs lower on the shore than the other species.

In order to relate shore position (or other abiotic factors) to oxygen consumption one must take account of relative body mass, which is an important confounding variable. The hypothesis put forward by Murdoch & Shumway (1980) is not supported by the results described here. The highest occurring chiton species in the Atlantic fauna *L. cinerea*, had by far the greatest VO_2 among those examined, and *Leptochiton asellus*, which is almost exclusively subtidal, had the lowest (Figure 4).

Similar to this study, Murdoch & Shumway (1980) found one species, *Onithochiton neglectus* Rochebrune, 1881 to have a greatly higher mass-specific VO_2 than the other species in the local fauna, and within the same general range of values that we observed in *L. cinerea* (Figure 4). In contrast however, *O. neglectus* is a low shore species, while *L. cinerea* is the highest occurring chiton species in the Atlantic fauna. Kincannon (1975) reported no significant difference in respiratory rates between sub- and intertidal *T. lineata*. Similarly Horn (1985) reported no difference in aquatic respiration rates between low and high shore *C. pelliserpentis*. Shore height is only one factor among many that may explain chiton respiratory behaviour and ability.

Murdoch & Shumway (1980) also observed variations in oxyregulation between their species correlated with shore position, with species occurring higher up the shore possessing greater oxyregulatory ability. This agrees with findings in work on other taxa, which suggests a correlation between species possessing oxyregulatory ability and the possibility of experiencing hypoxia, such as those occurring higher in the intertidal (Sassaman & Mangum, 1972; Bayne, 1973). These results however, were not replicated here. Within the Atlantic species, *A. crinita* showed the greatest oxyregulatory ability, but it occurs lower on the shore than *L. cinerea*, which showed typical oxyconforming behaviour. Within our Pacific species there is broader agreement with the previous studies, with the two intertidal species possessing oxyregulatory ability, and the subtidal species being a typical oxyconformer.

Local environment

There was no significant difference in mass-specific VO_2 among our Pacific species (Table 1), despite differences in shore height and also body mass. The total fauna of chiton species in the north-east Pacific is much more speciose than in the Atlantic and they also exhibit a much greater disparity in body mass (Kaas & Van Belle, 1985; Slieker, 2000; Eernisse *et al.*, 2007). A lack of variation in mass-specific VO_2 might be indicative of an increase in niche specialization through other adaptations. Body mass modification may prove a more beneficial adaptation in exploiting new niche opportunities among a more competitive community than changes in basal physiological traits.

In the north-east Atlantic fauna, where there are fewer species, *L. cinerea* is by far the most common and widespread (Poppe & Goto, 1991), yet its mass-specific VO_2 far exceeds that demonstrated by the other two Atlantic species in this study (Figure 4). This higher consumption may be indicative of an adaptation to a more active lifestyle unconstrained by competitors that has allowed it to exploit niche opportunities unavailable to the other species.

The two most common chitons on north-east Atlantic shores are *A. crinita* and *L. cinerea*, and while there are areas where one or other species may be more abundant, they are frequently found together in mixed communities. The ability of *A. crinita* to oxyregulate with increasing hypoxia is more typical of the physiological capacity required to survive the stresses of the intertidal zone, yet *L. cinerea* is much more widely distributed on Atlantic coasts and is found higher in the intertidal (Nichols, 1900; Poppe & Goto, 1991; Hayward & Ryland, 1996). The greatly higher basal VO_2 observed in *L. cinerea* might be indicative of a higher general metabolism and more active lifestyle. The microhabitat where *L. cinerea* is characteristically found is within cobble, which lacks the wide variety and abundance of biota found on the larger stationary boulders *A. crinita* frequents (Carey & Sigwart, unpublished observations). Many molluscs, including chitons, have a home 'range' over which they forage before returning to a home scar (e.g. Chelazzi *et al.*, 1988). We speculate that the higher metabolism of *L. cinerea* may be related to the need to cover a greater range of this less productive habitat in order to maintain adequate nutrition. Wide variations in metabolic rate related to locomotory capacity have previously been observed within molluscs, albeit in the highly morphologically varied Cephalopoda (Seibel, 2007).

The seeming inability of *L. cinerea* to oxyregulate as effectively as other species might be related to this relatively higher VO_2. *Lepidochitona cinerea* has a higher mass-specific VO_2 of any species examined here or in any previous studies of temperate-latitude chitons (Kincannon, 1975; Robbins, 1975; Murdoch & Shumway, 1980; Horn, 1985), and has been found to occur in extremes of physical conditions not tolerated by any other chiton species, such as the extremely low salinity of the Baltic (Kaas & Van Belle, 1985). If a part of the physiological pathway is functioning close to its limit, there would be less capacity for the increases required in order to maintain uptake in depleted conditions. There may be some physiological trade-off between the higher activity rates that foraging in a resource-poor microhabitat requires, and the ability to regulate uptake in depleted conditions. The fact that *L. cinerea* is much more common and widespread than any other Atlantic chiton species suggests lack of oxyregulatory ability may not be a critical factor in its survival. Murdoch & Shumway (1980) similarly found *O. neglectus*, the species operating at VO_2 close to that observed here in *L. cinerea*, to have little oxyregulatory ability. However, they

also observed *C. porosus* to be one of the weakest oxyregulators, but it had one of the lowest mass-specific VO_2, suggesting that constraints caused by operating at higher metabolic rates are not solely responsible for a lack of oxyregulatory ability.

Phylogeny

There were no clear patterns in physiology correlated to membership of the two major polyplacophoran clades: Lepidopleurida and Chitonida. The term 'living fossil' is often applied to groups (like Polyplacophora) that have a deep fossil record and constrained morphology in extant taxa (Sirenko, 2006). Although most chitons are superficially very similar in their body morphology, the extant diversity represents two separate radiations; the more recently derived order Chitonida (represented here by *A. crinita*, *L. cinerea*, *M. ferreirai* and *T. lineata*) and the more 'primitive' order Lepidopleurida (represented here by two *Leptochiton* spp.) (Sirenko, 2006). The two clades can be distinguished anatomically by features of the gill arrangement, with Lepidopleurida typically having fewer gills (Yonge, 1939; Sirenko, 1993; Sigwart, 2008). Lepidopleurida are often found in the deep sea and are almost exclusively subtidal, while species occurring in the intertidal are almost always in Chitonida (Sigwart *et al.*, 2011). Both of the 'primitive' *Leptochiton* species studied here demonstrated clear oxyconforming behaviour, suggesting lepidopleuran species may lack adaptations to oxyregulation possessed by some members of Chitonida. A lack of oxyregulatory ability may have constrained lepidopleuran chitons from colonizing the dynamic intertidal habitat where fluxes in oxygen availability are common (Eernisse & Reynolds, 1994). However, this has not prevented *L. cinerea* (in the more recently derived Chitonida) from extensively colonizing the intertidal and being found at the highest shore level of any north-east Atlantic chiton.

Conclusions

Factors such as microhabitat structure, body mass and phylogenetic position are among the main drivers that collectively underpin differences in physiological traits between species. The physiological variability observed here and in earlier studies (e.g. Murdoch & Shumway, 1980) is likely to be common across other taxa, and demonstrates how basal physiological traits can show significant complexity even within a highly morphologically constrained group. Such physiological variability has implications for studies of the ecology of such communities (Padilla & Allen, 2000).

In marine ecology, morphologically similar, co-occurring groups of species such as chitons may often be reported within single functional or taxonomic units without further detail (Steneck & Watling, 1982; Ortiz & Wolff, 2002; Bishop, 2003; Hetherington & Reid, 2003). The predicted impacts of environmental change, such as changes in physical conditions, may be presumed to have equal influence on species grouped together within these guilds. Variation in physiology among commonly amalgamated taxa, such as those observed here, means this is unlikely to be the case, and impacts may be unpredictable. Another implication is that the full role or contribution of component groups to ecosystem functioning may be significantly understated (Padilla & Allen, 2000; Boero *et al.*, 2008). While studies of marine ecology necessarily involve a level of abstraction of complex real world systems into more easily studied guilds, these findings demonstrate that such research must give due consideration to the extent of intra-guild variation at a finer taxonomic level.

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REFERENCES

Alexander J.E. and McMahon R.F. (2004) Respiratory response to temperature and hypoxia in the zebra mussel *Dreissena polymorpha*. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 137, 425–34.

Barbosa S.S., Byrne M. and Kelaher B.P. (2008) Bioerosion caused by foraging of the tropical chiton *Acanthopleura gemmata* at One Tree Reef, southern Great Barrier Reef. *Coral Reefs* 27, 635–639.

Bayne B. (1971) Ventilation, the heart beat and oxygen uptake by *Mytilus edulis* L. in declining oxygen tension. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 40, 1065–1085.

Bayne B. (1973) The responses of three species of bivalve mollusc to declining oxygen tension at reduced salinity. *Comparative Biochemistry and Physiology Part A: Molecular and Integrative Physiology* 45, 793–806.

Benson B.B. and Krause D. (1984) The concentration and isotopic fractionation of oxygen dissolved in freshwater and seawater in equilibrium with the atmosphere. *Limnology and Oceanography* 29, 620–632.

Bishop G. (2003) *The ecology of the rocky shores of Sherkin Island: a twenty year perspective*. Cork, Ireland: Sherkin Island Marine Station Publications.

Boero F., Bouillon J., Gravili C., Miglietta M., Parsons T. and Piraino S. (2008) Gelatinous plankton: irregularities rule the world (sometimes). *Marine Ecology Progress Series* 356, 299–310.

Bolnick D.I., Amarasekare P., Araújo M.S., Bürger R., Levine J.M., Novak M., Rudolf V.H.W., Schreiber S.J., Urban M.C. and Vasseur D.A. (2011) Why intraspecific trait variation matters in community ecology. *Trends in Ecology and Evolution* 26, 183–192.

Boyle P.R. (1970) Aspects of the ecology of a littoral chiton, *Sypharochiton pelliserpentis* (Mollusca: Polyplacophora). *New Zealand Journal of Marine and Freshwater Research* 4, 364–384.

Brewer P.G. and Peltzer E.T. (2009) Limits to marine life. *Science* 324, 347–348.

Bridges C.R. and Brand A.R. (1980) Oxygen consumption and oxygen-independence in marine crustaceans. *Marine Ecology Progress Series* 2, 133–141.

Brodersen K.P., Pedersen O., Lindegaard C. and Hamburger K. (2004) Chironomids (Diptera) and oxy-regulatory capacity: an experimental approach to paleolimnological interpretation. *Limnology and Oceanography* 49, 1549–1559.

Chelazzi G., Focardi S. and Deneubourg J.-L. (1988) Analysis of movement patterns and orientation mechanisms in intertidal chitons and gastropods. In Chelazzi G. and Vannini M. (eds) *Behavioural adaptation to intertidal life*. New York: Plenum Press, pp.173–184.

De Fur P. and Mangum C.P. (1979) The effects of environmental variables on the heart rates of invertebrates. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 62, 283–294.

Dethier M.N. and Duggins D.O. (1984) An indirect commensalism between marine herbivores and the importance of competitive hierarchies. *American Naturalist* 124, 205–219.

Dethier M.N. and Duggins D.O. (1988) Variation in strong interactions in the intertidal zone along a geographical gradient: a Washington–Alaska comparison. *Marine Ecology Progress Series* 50, 97–105.

de Zwaan A., Cortesi P., Thillart G., Roos J. and Storey K.B. (1991) Differential sensitivities to hypoxia by two anoxia-tolerant marine molluscs: a biochemical analysis. *Marine Biology* 111, 343–351.

Duke J.T. and Ultsch G.R. (1990) Metabolic oxygen regulation and conformity during submergence in the salamanders *Siren lacertina, Amphiuma means*, and *Amphiuma tridactylum*, and a comparison with other giant salamanders. *Oecologia* 84, 16–23.

Eberlee J. and Storey K.B. (1988) Tissue-specific biochemical responses during anoxia and recovery in the channelled whelk. *Journal of Experimental Marine Biology and Ecology* 121, 165–176.

Eernisse D.J., Clark R.N. and Draeger A. (2007) Polyplacophora. In Carlton J.T. (ed.) *The Light and Smith Manual: intertidal invertebrates from central California to Oregon*. Berkeley, CA: University of California Press, pp. 701–713.

Eernisse D.J. and Reynolds P.D. (1994) Polyplacophora. In Harrison F.W. and Kohn A.J. (eds) *Microscopic anatomy of invertebrates, Volume 5, Mollusca*. New York: Wiley, pp. 56–110.

Fisher T.R. (1976) Oxygen uptake of the solitary tunicate *Styela plicata*. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 151, 297–305.

García H.E. and Gordon L.I. (1992) Oxygen solubility in seawater: better fitting equations. *Limnology and Oceanography* 37, 1307–1312.

Gillooly J.F., Brown J.H., West G.B., Savage V.M. and Charnov E.L. (2001) Effects of size and temperature on metabolic rate. *Science* 293, 2248–2251.

Hagerman L. (1998) Physiological flexibility: a necessity for life in anoxic and sulphidic habitats. *Hydrobiologia* 375–76, 241–254.

Hayward P.J. and Ryland J.S. (1996) *Handbook of the marine fauna of North-West Europe*. Oxford: Oxford University Press.

Herreid C.F. (1980) Hypoxia in invertebrates. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 67, 311–320.

Hetherington R. and Reid R.G.B. (2003) Malacological insights into the marine ecology and changing climate of the late Pleistocene—early Holocene Queen Charlotte Islands archipelago, western Canada, and implications for early peoples. *Canadian Journal of Zoology* 81, 626–661.

Horn P.L. (1982) Adaptations of the chiton *Sypharochiton pelliserpentis* to rocky and estuarine habitats. *New Zealand Journal of Marine and Freshwater Research* 16, 253–261.

Horn P.L. (1985) Respiration in air and water of the chiton *Chiton pelliserpentis* from high and low zones of a sheltered shore. *New Zealand Journal of Marine and Freshwater Research* 19, 11–19.

Kaas P. and Van Belle R.A. (1985) *Monograph of living chitons: Volume 1. Order Neoloricata: Lepidopleurina*. Leiden, The Netherlands: Brill.

Katsanevakis S., Xanthopoulos J., Protopapas N. and Verriopoulos G. (2007) Oxygen consumption of the semi-terrestrial crab *Pachygrapsus marmoratus* in relation to body mass and temperature: an information theory approach. *Marine Biology* 151, 343–352.

Keeling R.F., Koertzinger A. and Gruber N. (2010) Ocean deoxygenation in a warming world. *Annual Review of Marine Science* 2, 199–229.

Kelly R.P. and Eernisse D.J. (2008) Reconstructing a radiation: the chiton genus *Mopalia* in the North Pacific. *Invertebrate Systematics* 22, 17–28.

Kincannon E.A. (1975) The relations between body weight and habitat temperature and the respiratory rate of *Tonicella lineata* (Wood, 1815) (Mollusca: Polyplacophora). *Veliger* 18 (Supplement), 87–93.

Larade K. and Storey K.B. (2002) A profile of the metabolic responses to anoxia in marine invertebrates. In Storey K.B. and Storey J.M. (eds) *Cell and molecular response to stress. Volume 3: sensing, signaling and cell adaptation*. Amsterdam: Elsevier, pp. 27–46.

Lebsack C.S. (1975) Effect of temperature and salinity on the oxygen consumption of the chiton *Mopalia lignosa*. *Veliger* 18 (Supplement), 94–97.

Lencioni V., Bernabò P., Vanin S., Di Muro P. and Beltramini M. (2008) Respiration rate and oxy-regulatory capacity in cold stenothermal chironomids. *Journal of Insect Physiology* 54, 1337–1342.

Littler M.M., Littler D.S. and Taylor P.R. (1995) Selective herbivore increases biomass of its prey: a chiton–coralline reef-building association. *Ecology* 76, 1666–1681.

Livingstone D.R. (1991) Organic xenobiotic metabolism in marine invertebrates. In Gilles R. (ed.) *Advances in comparative and environmental physiology. Volume 7*. Berlin: Springer, pp. 45–185.

Mangum C.P. and Van Winkle W. (1973) Responses of aquatic invertebrates to declining oxygen conditions. *American Zoologist* 13, 529–541.

Marshall D. and McQuaid C. (1993) Effects of hypoxia and hyposalinity on the heartbeat of the intertidal limpets *Patella granvlaris* (prosobranchia) and *Siphonaria capensis* (pulmonata). *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 106, 65–68.

McAllen R., Taylor A.C. and Davenport J. (1999) The effects of temperature and oxygen partial pressure on the rate of oxygen consumption of the high-shore rock pool copepod *Tigriopus brevicornis*. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 123, 195–202.

McMahon B.R., Burggren W.W., Pinder A.W. and Wheatly M.G. (1991) Air exposure and physiological compensation in a tropical intertidal chiton, *Chiton stokesii* (Mollusca: Polyplacophora). *Physiological Zoology* 64, 728–747.

Murdoch R.C. and Shumway S.E. (1980) Oxygen consumption in six species of chitons in relation to their position on the shore. *Ophelia* 19, 127–144.

Nagabhushanam R. and Murti K.G. (1972) The influence of body size, salinity and temperature on the respiration of *Chiton granoradiatus*. *Marathwada University Journal of Science, Section B, Biological Sciences* 11, 79–82.

Newell R.C. and Roy A. (1973) A statistical model relating the oxygen consumption of a mollusk (*Littorina littorea*) to activity, body size, and environmental conditions. *Physiological Zoology* 46, 253–275.

Nichols A.R. (1900) *Marine Mollusca of Ireland*. Dublin: Royal Irish Academy.

Ortiz M. and Wolff M. (2002) Application of loop analysis to benthic systems in northern Chile for the elaboration of sustainable management strategies. *Marine Ecology Progress Series* 242, 15–27.

Padilla D.K. and Allen B.J. (2000) Paradigm lost: reconsidering functional form and group hypotheses in marine ecology. *Journal of Experimental Marine Biology and Ecology* 250, 207–221.

Paine R.T. (1992) Food-web analysis through field measurement of per capita interaction strength. *Nature* 355, 73–75.

Pannunzio T.M. and Storey K.B. (1998) Antioxidant defenses and lipid peroxidation during anoxia stress and aerobic recovery in the marine gastropod *Littorina littorea*. *Journal of Experimental Marine Biology and Ecology* 221, 277–292.

Pauly D., Graham W., Libralato S., Morissette L. and Deng Palomares M.L. (2009) Jellyfish in ecosystems, online databases, and ecosystem models. *Hydrobiologia* 616, 67–85.

Petersen J.A. and Johansen K. (1973) Gas exchange in the giant sea cradle *Cryptochiton stelleri* (Middendorff). *Journal of Experimental Marine Biology and Ecology* 12, 27–43.

Poloczanska E.S., Smith S., Fauconnet L., Healy J., Tibbetts I.R., Burrows M.T. and Richardson A.J. (2011) Little change in the distribution of rocky shore faunal communities on the Australian east coast after 50 years of rapid warming. *Journal of Experimental Marine Biology and Ecology* 400, 145–154.

Poppe G. and Goto Y. (1991) *European seashells. Volume 1 (Polyplacophora, Caudofoveata, Solenogastra, Gastropoda)*. Wiesbaden, Germany: Verlag Christa Hemmen.

R Core Development Team (2012) *R: a language and environment for statistical computing. An introduction to R notes on R a programming environment for data analysis and graphics R core team version*. Vienna: R Foundation for Statistical Computing.

Robbins B.A. (1975) Aerial and aquatic respiration in the chitons *Nuttallina californica* and *Tonicella lineata*. *Veliger* 18 (Supplement), 98–102.

Rostal D.C. and Simpson L. (1988) The influence of salinity on the distribution of two Oregon chiton species (*Katharina tunicata* Wood and *Mopalia hindsii* Reeve). *Veliger* 31, 120–126.

Sassaman C. and Mangum C.P. (1972) Adaptations to environmental oxygen levels in infaunal and epifaunal sea anemones. *Biological Bulletin. Marine Biological Laboratory, Woods Hole* 143, 657–678.

Seibel B.A. (2007) On the depth and scale of metabolic rate variation: scaling of oxygen consumption rates and enzymatic activity in the Class Cephalopoda (Mollusca). *Journal of Experimental Biology* 210, 1–11.

Shumway S.E., Scott T.M. and Shick J.M. (1993) The effects of anoxia and hydrogen sulphide on survival, activity and metabolic rate in the coot clam, *Mulinia lateralis* (Say). *Journal of Experimental Marine Biology and Ecology* 71, 135–146.

Sigwart J.D. (2008) Gross anatomy and positional homology of gills, gonopores, and nephridiopores in 'basal' living chitons (Polyplacophora: Lepidopleurina). *American Malacological Bulletin* 25, 43–49.

Sigwart J.D., Schwabe E., Saito H., Samadi S. and Giribet G. (2011) Evolution in the deep sea: a combined analysis of the earliest diverging living chitons (Mollusca: Polyplacophora: Lepidopleurida). *Invertebrate Systematics* 24, 560–572.

Sirenko B.I. (1993) Revision of the system of the order Chitonida (Mollusca: Polyplacophora) on the basis of correlation between the type of gills arrangement and the shape of the chorion processes. *Ruthenica* 3, 93–117.

Sirenko B.I. (2006) New outlook on the system of chitons (Mollusca: Polyplacophora). *Venus* 65, 27–49.

Slieker F.J.A. (2000) *Chitons of the world*. Ancona, Italy: L'Informatore Piceno Edito.

Spicer J.I., Dando C.L. and Maltby L. (2002) Anaerobic capacity of a crustacean sensitive to low environmental oxygen tensions, the freshwater amphipod *Gammarus pulex* (L.). *Hydrobiologia* 477, 189–194.

Steneck R.S. and Watling L. (1982) Feeding capabilities and limitation of herbivorous molluscs: a functional group approach. *Marine Biology* 68, 299–319.

Stickle W.B. and Sabourin T.D. (1979) Effects of salinity on the respiration and heart rate of the common mussel, *Mytilus edulis* L., and the black chiton, *Katharina tunicata* (Wood). *Journal of Experimental Marine Biology and Ecology* 41, 257–268.

Strahl J., Dringen R., Schmidt M.M., Hardenberg S. and Abele D. (2011) Metabolic and physiological responses in tissues of the long-lived bivalve *Arctica islandica* to oxygen deficiency. *Comparative Biochemistry and Physiology, Part A: Molecular and Integrative Physiology* 158, 513–519.

Tang P.S. (1933) On the rate of oxygen consumption by tissues and lower organisms as a function of oxygen tension. *Quarterly Review of Biology* 8, 260–274.

Taylor A.C. and Brand A.R. (1975) Effects of hypoxia and body size on the oxygen consumption of the bivalve *Arctica islandica* (L.). *Journal of Experimental Marine Biology and Ecology* 19, 187–196.

Taylor A.C. and Moore P.G. (1995) The burrows and physiological adaptations to a burrowing lifestyle of *Natatolana borealis* (Isopoda: Cirolanidae). *Marine Biology* 123, 805–814.

Truchot J.P. and Duhamel-Jouve A. (1980) Oxygen and carbon dioxide in the marine intertidal environment: diurnal and tidal changes in rockpools. *Respiration Physiology* 39, 241–254.

Warwick R.M. (1993) Environmental impact studies on marine communities: pragmatical considerations. *Australian Journal of Ecology* 18, 63–80.

West G.B., Brown J.H. and Enquist B.J. (1997) A general model for the origin of allometric scaling laws in biology. *Science* 276, 122–126.

Yonge C. (1939) Memoirs: on the mantle cavity and its contained organs in the Loricata (Placophora). *Quarterly Journal of Microscopical Science* 81, 367–390.

and

Wilson J.G. and Davis J.P. (1984) The effect of environmental variables on the oxygen consumption of the protobranch bivalve *Nucula turgida* (Leckenby and Marshall). *Journal of Molluscan Studies* 50, 73–77.

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