Interaction of thermal tolerance and oxygen availability in the eurythermal gastropods *Littorina littorea* and *Nucella lapillus*

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ABSTRACT: Established hypothesis, derived predominantly from studies on wholly-aquatic polar organisms, indicates that the upper thermal limits of aquatic animals are defined by oxygen limitation. In this study *Littorina littorea* and *Nucella lapillus*, eurythermal temperate intertidal gastropods of different habit and life history, were investigated to test the generality of this hypothesis. Two separate hypotheses were tested on both species. Hypothesis 1 was that thermal tolerance in water will be increased under hyperoxic conditions and decreased under hypoxic conditions. Median upper lethal temperature/s (MULT) were established under normoxic (21 % O_2), hypoxic (10 % O_2) and hyperoxic (40 % O_2) conditions in seawater. For *Nucella lapillus* the hypothesis was supported, since MULT increased with increasing available oxygen in seawater (4.6°C difference between hypoxic and hyperoxic conditions). For *L. littorea* the hypothesis failed, since MULT did not differ significantly between different oxic conditions. Hypothesis 2 was that thermal tolerance in water-saturated air (normoxia) would be higher than in normoxic water due to the greater availability of oxygen in air. MULT was established for both species in water-saturated air (it had already been established in normoxic seawater). For *L. littorea* this hypothesis was supported by data, as MULT was 2.4°C higher in air than in normoxic seawater. However, the hypothesis was rejected for *N. lapillus*, in which MULT in air was depressed by 1.6°C by comparison with MULT in normoxic seawater. The departures from oxygen limitation theory can be explained by the species' habits, life history and respiratory capabilities in air and water.

KEY WORDS: Thermal tolerance · Oxygen · *Littorina* · *Nucella* · Cleft

INTRODUCTION

Temperature is regarded as the most important abiotic stress affecting ectothermic animals, since it has influences from population to molecular levels (e.g. Hochachka & Somero 2002). Relationships between environmental temperature and organismal distribution and temperature tolerance have been widely studied, especially as the impact of anthropogenically-driven climate change has focused attention on thermal biology (e.g. Taschler & Neuner 2004).

The seminal review of Pörtner (2001) demonstrates a close relationship between upper lethal temperature and the following attributes: an extended aerobic scope, the ability to switch to anaerobic metabolism and, *in extremis*, the ability to protect molecules by deployment of heat shock proteins and antioxidative defence. Pörtner (2001) postulated an intimate link between oxygen availability and critical temperatures for survival, and there is ample recent evidence to show that this relationship holds for polar invertebrates (e.g. Peck & Conway 2000, Peck et al. 2002), for which thermal windows are very narrow.

From this body of work it may be hypothesized that enhanced oxygen availability should allow animals to survive to higher environmental temperatures and that reduced levels of environmental oxygen should reduce critical temperatures. In confirmation of this hypothe-

sis, Pörtner et al. (2006) recently showed that doubling environmental oxygen tension raised upper thermal limits in the Antarctic bivalve *Laternula elliptica* by about 2.5°C. However, this work was again performed on a stenothermal polar species. In the study reported here 2 temperate intertidal invertebrate species were investigated. Both target species are eurythermal gastropod molluscs (Clarke et al. 2000, Davenport & Davenport 2005): the eulittoral (sensu McMahon 2001) herbivorous periwinkle *Littorina littorea* is distributed in Europe from northern Spain to the White Sea (northern Russia) (Jackson 2005); *Nucella lapillus*, a carnivore that occurs rather lower on the shore (lower eulittoral; sensu McMahon 2001), is found from the Straits of Gibraltar to the Arctic (Hayward & Ryland 1995). Both species occupy a wide range of microhabitats on rocky shores. They can be found in rock pools that can fluctuate between considerably hyperoxic conditions during the day (when temperatures are usually high) and markedly hypoxic conditions at night (e.g. Ganning 1971, Daniel & Boyden 1975, Morris & Taylor 1983). They tolerate emersion, during which they may close themselves off from the environment if the humidity is low (by retraction of the foot and closure of the operculum), or they may continue to maintain full contact with the environment if conditions are damp. Although both species have gills, they are air-breathers when emersed. *L. littorea* has haemocyanin in the haemolymph (Herskovits et al. 1985), as does *N. lapillus* (Leung et al. 2000, Tyler-Walters 2003). Following McMahon (2001) it is probable that the middle shore *L. littorea* is a more competent air breather than the lower shore *N. lapillus*. However, the concentration of oxygen in air is much greater than in air-saturated seawater (though the oxygen tensions will be near identical; see Spicer & Gaston (1999)), so a second hypothesis, that the gastropods should tolerate higher temperatures in air (even when evaporative cooling is not possible) than in air-saturated seawater, was also tested. Throughout our study we measured upper lethal temperatures (24 h exposure). These differ from the critical or pejus temperatures (sensu Pörtner 2001). Critical temperatures are those at which animals switch from aerobic to anaerobic mitochondrial metabolism and are then in a time/energy-limited tolerant state that cannot be sustained indefinitely. Thus acute upper lethal temperatures will be higher than critical temperatures, but the two values will converge the longer the period of high temperature exposure.

MATERIALS AND METHODS

Collection of species. Specimens of *Nucella lapillus* and *Littorina littorea* were collected from a single shore at Bullens Bay, Cork, Ireland (51° 38.584' N, 8° 33.052' W). For both species, animals were collected from the middle of their vertical distribution; Davenport & Davenport (2005) have recently shown significant differences in thermal tolerances of *N. lapillus* taken from the upper and lower distributional limits, perhaps reflecting the highly localized genetic substructuring already demonstrated for the species (Kirby 2000).

Assessment of thermal tolerances. Thermal tolerances were assessed following Davenport & Davenport (2005). For each target species, 20 freshly collected animals were exposed to each of a variety of temperature/oxygen level combinations, either in humid air or in seawater (34‰) (Table 1). Test temperatures (Table 2) were chosen pragmatically until at least 3 temperatures, which yielded mortality between 100 and 0%, were identified for each temperature/oxygen level combination. For *Nucella lapillus*, test temperatures ranged between 19.7 and 32.5°C; for *Littorina littorea,* the range was 31.8 to 38.9°C. The larger number of test temperatures for *L. littorea* was required, because the species has particularly small temperature differences between low and high survival (Davenport & Davenport 2005). Several temperature baths (accurate to ±0.1°C) were used simultaneously, so that all upper lethal temperatures were measured within a 3 d period (22 August 2004 to 24 August 2004 for *N. lapillus*; 9 June 2005 to 11 June 2005 for *L. littorea*).

Animals were held in lidded plastic vessels appropriate to their size. If they were to be held in air, they

Table 1. Treatments used during assessments of median upper lethal temperatures (MULT). RH = relative humidity

Expt	Treatment	Description
1	Humid air	normoxic: 100% RH: gas content 21% oxygen, 79% nitrogen
2	Normoxic seawater	gas content 21% oxygen, 79% nitrogen
3	Hypoxic seawater	gas content 10% oxygen, 90% nitrogen
4	Hyperoxic seawater	gas content 40% oxygen, 60% nitrogen

Table 2. Temperatures used to assess thermal tolerances

Species	Temperature (°C)
Nucella lapillus	19.7, 26.2, 28.6, 29.6, 30.3, 30.6, 31.4, 32.5
Littorina littorea	31.8, 31.9, 33.3, 34.2, 34.9, 35.2, 35.5, 35.7, 36.2, 36.3, 37.1, 37.2, 38.3, 38.4, 38.6, 38.9

were kept together with water-saturated paper tow-
elling to ensure 100% relative humidity (so that
evaporative cooling was impossible: cf. McMahon
1990). If they were to be held in seawater, the lid was
pierced so that a tube and air stone could be inserted
into the seawater. The tube was connected either to a
regulator supplying gas from a cylinder containing a
mixture of oxygen and nitrogen (see Table 1 for
details of gas mixtures used), or to an electrical aera-
tor that pumped air through the seawater. Trials
(using fibre-optic oxygen micro-sensors; PreSens)
were carried out to confirm that the intended oxygen
tension was attained within the test vessels when 20
animals were present. The vessels were placed in the
temperature baths. Animals were exposed to temper-
atures for 24 h, and then allowed to recover at ambi-
ent temperature for at least 2 h before assessment
for mortality using binocular microscopes, forceps
and needles to test for responsiveness. Median upper
lethal temperatures (MULT) with asymmetrical 95%
CI were established for each of the conditions shown
in Table 1 for each species using probit analysis
(Finney 1971). Throughout this paper, significant dif-
ferences between means ($p < 0.05$) are assumed if
95% CI do not overlap. An exposure period of 24 h
was chosen, partly because no single duration of
exposure is biologically relevant to animals that have
a substantial vertical range on the shore (as do
both dogwhelks and periwinkles). Pilot experiments
showed that both study species could tolerate emer-
sion in humid air for at least 2 d.

RESULTS

The results obtained are summarized in Table 3. It is
evident that there was no commonality in response
between the species. For *Nucella lapillus* held in
seawater, MULT was significantly affected by oxygen
tension/concentration, with MULT being higher the
greater the amount of oxygen available. The 3 MULT
values for hypoxic, normoxic and hyperoxic conditions
were significantly different from each other. However,
in humid air the MULT was significantly lower (by
1.6°C) than in normoxic seawater, even though far
more oxygen was available in air and the oxygen
tensions were equal.

In contrast, for *Littorina littorea* held in seawater,
MULT was unaffected by oxygen tension/concentra-
tion over the range tested (10 to 40% oxygen) with the
3 MULT values for hypoxic, normoxic and hyperoxic
conditions being virtually identical. On the other hand,
in humid air, periwinkles survived to a MULT sig-
nificantly higher (by 2.4°C) than they did in normoxic
seawater.

Table 3. Median upper lethal temperatures (MULT) of dog-
whelks *Nucella lapillus* and periwinkles *Littorina littorea* un-
der different oxygen tensions/concentrations. LC/UC = lower
and upper 95% confidence limits (CL) for MULT respectively

Expt	Oxygen %	MULT (°C)	LC (°C)	UC (°C)
Nucella lapillus				
1	21	28.9	28.3	29.2
2	10	26.4	25.9	26.9
3	21	30.5	30.4	30.6
4	40	31.0	30.8	31.2
Littorina littorea				
1	21	37.5	37.2	37.9
2	10	35.1	34.8	35.4
3	21	35.1	34.9	35.3
4	40	35.0	34.8	35.3

DISCUSSION

MULT (24 h) temperatures of *Littorina littorea* in sea-
water clustered around 35°C. No values for critical/
pejus temperature (sensu Pörtner 2001) for the species
are available, though Clarke et al. (2000) published
heat coma temperatures (HCT) for geographically sep-
arated populations of the species; these were derived
from experiments in which animals were warmed at
0.2°C min^{-1}. HCT and critical temperatures are be-
lieved to be similar. HCT values for *L. littorea* given by
Clarke et al. (2000) clustered around 30°C, confirming
that MULT values exceed critical temperatures but
also implying that survival beyond critical tempera-
tures is possible for many hours in this species. No such
comparisons are possible for *Nucella lapillus*.

The results presented here demonstrate that oxygen
availability and upper lethal temperature are not neces-
sarily closely coupled in eurythermal temperate gas-
tropods as they are in stenothermal polar molluscs (Peck
et al. 2004, Pörtner et al. 2006). While the slow moving,
low activity (Hughes et al. 1992) dog whelk *Nucella
lapillus* does show such coupling in that there was a
4.6°C difference between MULT in hypoxic and hyper-
oxic conditions (in seawater), the highly mobile, active
browser *Littorina littorea* showed no such difference.
The 2 species also differ in their thermal tolerance pat-
tern when in water-saturated air. *N. lapillus* tolerated a
significantly lower MULT (by about 1.6°C) in air than
in air-saturated seawater, but *L. littorea* survived to a
MULT 2.4°C higher when in air than in air-saturated
seawater. Tomanek & Helmuth (2002) point out that the
relationship between intertidal distribution and eco-
physiology of rocky intertidal organisms is extremely
complex and that simple models are inadequate to
encompass all species; our study reinforces this view.

Can the data be explained in the context of the Pörtner
(2001) synthesis? We believe that they can to some ex-

tent, if the different physiological characteristics of the 2 species are taken into account. *Nucella lapillus* is of the group of lower shore gastropods that is predicted to have a limited ability to take up oxygen from air (McMahon 2001), so this may explain why dogwhelks cannot survive to higher temperatures in humid air than in air-saturated seawater. At high temperature they will exceed aerobic scope, even though plenty of oxygen is present in the surrounding medium. Conversely, *Littorina littorea* is a competent air breather that can take advantage of the ready availability of oxygen in air to maintain aerobic scope to a higher temperature than in seawater.

When in seawater the thermal tolerance of *Littorina littorea* was unaffected over the hypoxic-hyperoxic range tested (unlike *Nucella lapillus*). This presumably reflects an efficient haemolymph circulation (Andrews & Taylor 1988) and haemolymph-haemocyanin carriage of oxygen to the tissues. It should be noted that in the experimental conditions used in this study, the seawater was always in motion because of aeration by the test gas mixtures. Irwin & Davenport (2006) have recently demonstrated that *L. littorea* cannot disperse unstirred layers by its gill ciliary activity, so that hypoxic conditions prevail around its tissues if it is held in static normoxic seawater. It is therefore likely that the species is more susceptible to high temperature when in unstirred rock pools/puddles than in situations where wind or wave-driven circulation disperses unstirred layers.

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