Can the Antarctic terrestrial midge, *Eretmoptera murphyi*, tolerate life in water?

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**Abstract**

Early season flooding and ice entrapment at sub-zero temperatures pose significant challenges to any polar terrestrial invertebrate. The chironomid midge, *Eretmoptera murphyi*, is native to the sub-Antarctic island of South Georgia and has been introduced to the maritime Antarctic (Signy Island). While the majority of its two year life cycle is spent as a terrestrial larva, it is found in habitats exposed to prolonged flooding. The current study explored the tolerance of the larvae to extended submergence, demonstrating survival for at least 28 d, underlain by their ability to respire (oxy-regulate) whilst submerged. To date, this ability is not known to be shared by any other terrestrial midge. Larvae also demonstrated notable anoxia tolerance whilst encased in ice, surviving for up to 28 d. These data indicate a capacity to survive ecologically relevant periods of submergence and/or ice entrapment, such as may be experienced in their natural habitats.

Key words: Respiration; Anoxia; Survival; Chironomid; Submergence

**1. Introduction**

*Eretmoptera murphyi*, an endemic midge of sub-Antarctic South Georgia (55°S, 37°W), was introduced to Signy Island (maritime Antarctic, 60°S, 45°W) in the 1960s and has successfully established and spread (Block *et al.*, 1984; Worland, 2010).On South Georgia, larvae are commonly found in vegetation adjacent to meltwater streams (Cranston, 1985), while on Signy Island they occur within a sloping soil/moss habitat (Convey, 1992).

During the summer in sub- and maritime Antarctic habitats, freshwater from snow and ice melt often saturates the soil and overlying vegetation. This enforced switch between a terrestrial and an aquatic lifestyle poses a number of challenges to the resident invertebrates which predominantly respire, feed and develop in a terrestrial environment. These challenges are exacerbated further under sub-zero conditions, when invertebrates are at risk from ice entrapment and subsequent anoxia (Sømme and Block, 1982). It is unknown whether *E. murphyi* can respire while submerged or whether it can tolerate extended hypoxia/anoxia.

There are three known respiration strategies in freshwater habitats - oxy-regulation, oxy-conformation and oxy-stressor (see Berg *et al.*, 1962). Organisms which oxy-regulate maintain a constant level of oxygen consumption as the level of ambient oxygen decreases until they reach a critical point of oxygen saturation, when regulation fails and respiration switches from aerobic to anaerobic (Berg *et al.*, 1962; Bridges and Brand, 1980). Oxy-regulators are generally found in low oxygen environments, where maximisation of oxygen consumption is critical (Brodersen and Quinlan 2006). In contrast, organisms which are found in well-oxygenated environments tend to oxy-conformand show rates of oxygen consumption that parallel the level of ambient oxygen until a critical point is reached (Berg *et al.*, 1962). Oxy-stressors are less common and are characterised by an initial increase in oxygen consumption at low ambient levels prior to the critical point (Brodersen *et al.*, 2008).

In this study, we investigated (i) the tolerance of *E. murphyi* to submergence in water, (ii) its capacity for aquatic respiration, and (iii) survival of ice entrapment and hence of anoxia.

**2. Materials and methods**

*2.1. Insect collection and storage conditions*

Summer acclimatised larvae of *E. murphyi* were collected from Signy Island between January and March 2013. They were transported to the University of Birmingham in plastic boxes containing substratum and maintained at 4-6°C. Upon arrival samples were held at 5°C under constant darkness (DD).

*2.2. Tolerance of water submergence*

Larvae were submerged in groups (N = 10) in tap water inside small plastic containers and held at 5°C (DD). Survival was assessed after 7, 14 and 28 d in water, either immediately following removal from water, or following 72 h recovery in plastic containers with moist substratum. Three replicates were used for each treatment duration. Larvae were also held in deionised water as a comparison, but limited numbers of larvae only permitted assessment after 7 d (one replicate, N=10 larvae). Survival was defined by individuals moving either spontaneously or in response to gentle contact stimulus.

*2.3. Respirometry*

Oxygen consumption of individual midge larvae was measured using the Unisense respirometry system (MicroResp, Unisense, Denmark) (see Brodersen *et al.*, 2008). The experiment was carried out at 10°C on five larvae. Following measurement of respiration, larval dry mass (DM) was determined by weighing individual larva on a microbalance after drying on paper and then for 36 h at 50°C to constant mass in a drying oven. Oxygen consumption data were subsequently corrected for each larva based on their individual mass. Respiration rates (R) were expressed as µmol O2 g DM-1 h-1.

*2.4. Ice entrapment*

Larvae were submerged in tap water in Eppendorf tubes and held at -2°C (DD) for up to 28 d. Within a few hours of being placed at -2°C, the water froze. Encasement within ice was assumed equivalent to an anoxic environment, though metabolism at -2°C would have been slow and tissues may not have experienced anoxia immediately. Survival was assessed 72 h after each treatment (7, 14 and 28 d) as described previously.

*2.5. Statistical analysis*

The Kolmogorov-Smirnov test was used to determine whether data were normally distributed. Normally distributed data were analysed using analysis of variance (ANOVA) and Tukey’s multiple range test, and non-normally distributed data were analysed using the Kruskal-Wallis test.

**3. Results and discussion**

*3.1. Submergence in water*

Many polar terrestrial habitats are vulnerable to large seasonal fluctuations in water availability, often (but not only) timed with the spring melt. *E. murphyi*’s habitats on South Georgia and Signy Island are frequently vulnerable to waterlogging during the summer months (Sømme and Block, 1982). The current study shows the larvae to be capable of tolerating submergence in water for at least 28 d (Fig. 1). The ability to survive such a prolonged exposure suggests that the midge is either able to tolerate anoxia or is able to respire while submerged. The respirometry approach used here demonstrated that the larvae can respire in an aquatic environment (Fig. 2), and that rates are comparable with wholly aquatic midge larvae (e.g. Brodersen and Quinlan, 2006). Our demonstration of respiration in *E. murphyi* appears to be the first in any terrestrial midge species.

Very few insects are currently considered to be amphibious, and there is only one example of a terrestrial genus (*Hyposmocoma*, Lepidoptera) whose larvae have evolved an ability to forage and possibly pupate underwater as well as in the terrestrial environment (Rubinoff and Schmitz 2010). Larvae of *E. murphyi*’s close relative *Belgica antarctica* are also known to survive well (~100% survival) for at least 10 d in ‘field’ water (Elnitsky *et al.*, 2009), though whether this represents anoxia tolerance or an ability to respire under water remains unexplored.

*Eretmoptera murphyi* larvae generally maintained consistent levels of oxygen consumption with decreasing oxygen saturation (Fig. 2), which is indicative of oxy-regulation (Berg *et al.*, 1962). An ability to regulate oxygen consumption may be critical to their fitness underwater, especially as they are likely to inhabit oxygen-poor, stagnant water.

While larvae of *E. murphyi* were able to survive for 28 d in tap water, only 31% did so and there was already considerable mortality after 7 d (Fig. 1). The data for deionised water, 100% survival after 7 d submergence, suggests that tap water may have had a negative effect on larvae. The 100% survival of *B. antarctica* reported after 10 d submergence in ‘field’ water (Elnitsky *et al*., 2009) further supports the proposition that Antarctic midge larvae are able to tolerate flooding of their usually terrestrial environments.

*3.2. Ice entrapment*

As a result of encasement in ice at sub-zero temperatures, periods of hypoxia and/or anoxia are commonly experienced in polar and alpine regions (Sømme and Block, 1982). Some invertebrates have consequently evolved considerable capacity for anoxia tolerance (e.g. Conradi-Larsen and Somme, 1973; Sømme and Block, 1982, 1984). Larvae of *E. murphyi* are able to respire in water and are therefore unlikely to be exposed to anoxia while submerged during early season flooding. However, there is a clear possibility that they may become trapped, and experience anoxia, during periods of ice formation, both in short-term freezing events and at the onset of winter conditions. Our data indicate that larvae were able to survive anoxia for at least 28 d (Fig. 1). This level of tolerance is comparable with that reported in other invertebrates from Signy Island, including the collembolan, *Cryptopygus antarcticus*, and mite, *Alaskozetes antarcticus* (Block and Sømme, 1982; Sømme and Block, 1982).

While larvae of *E. murphyi* are likely to be able to tolerate the short periods of anoxia of hours or days which may occur during the spring, summer and autumn, based on our data they would struggle to survive months of anoxia, as may occur in winter. Acclimation to low temperatures, as occurs in the approach to winter (Worland, 2010), may confer additional anoxia tolerance, but this remains to be tested. The physiological symptoms and injuries resulting from low temperature and anoxia are similar, so any response to low temperature may provide cross tolerance, as has been demonstrated in other insects (Yoder *et al.*, 2006).

**4. Conclusion**

To date, the success of *E. murphyi* in its native and introduced Antarctic terrestrial habitats has been attributed to its life cycle traits (Convey, 1992), low temperature tolerance (Block *et al.*, 1984; Everatt *et al.*, 2012; Worland, 2010), desiccation tolerance and cross-tolerance (Everatt *et al.* in review). We provide evidence here that this terrestrial midge also possesses a unique ability to respire in water and tolerate prolonged submergence, and to tolerate anoxia under ice entrapment.

**Acknowledgements**

MJE was funded by the Natural Environment Research Council (RRBN15266) and supported by the British Antarctic Survey and the University of Birmingham. The NERC Antarctic Funding Initiative Collaborative Gearing Scheme (CGS-73) supported the fieldwork at Rothera Research Station. PC is supported by NERC core funding to the BAS ‘Ecosystems’ programme. Two anonymous referees are thanked for their helpful comments. This paper also contributes to the SCAR ‘Antarctic Thresholds – Ecosystem Resilience and Adaptation’ research programme.

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