Comparative Biochemistry and Physiology, Part A 176 (2014) 49–58

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part A

journal homepage: www.elsevier.com/locate/cbpa

Physiological and biochemical strategies for withstanding emersion in two galaxiid fishes

Mauricio A. Urbina [a,b,*], Patrick J. Walsh [c], Jonathan V. Hill [a], Chris N. Glover [a]

[a] School of Biological Sciences, University of Canterbury, Christchurch, New Zealand
[b] Department of Biosciences, College of Life and Environmental Sciences, University of Exeter, United Kingdom
[c] Department of Biology, University of Ottawa, Ottawa, Canada

ARTICLE INFO

Article history:
Received 6 February 2014
Received in revised form 30 June 2014
Accepted 3 July 2014
Available online 12 July 2014

Keywords:
Aerial gas exchange
Aestivation
Ammonia
Galaxiid
Lactate
Lipid metabolism
Mudfish
Nitrogen excretion
Sodium/potassium ATPase
Urea

ABSTRACT

The galaxiid fishes of the Southern hemisphere display variable tolerance to aerial exposure. Brown mudfish (*Neochanna apoda*), for example, pseudoaestivate, inhabiting moist soil for months at a time, whereas inanga (*Galaxias maculatus*) emerse under unfavourable water conditions, but only for periods of a few hours. This study sought to identify the physiological and biochemical strategies that determine emersion tolerance in these species. Nitrogenous waste excretion was measured before and after an experimental emersion period (14 days for mudfish, 6 h for inanga). Both species showed significantly elevated ammonia "washout" upon return to water, but no increase in plasma or muscle ammonia. Post-emersion urea levels were elevated in plasma and muscle in both fish, however the extent of the accumulation did not indicate significant de novo urea production. This was supported by the lack of carbamoyl phosphate synthetase activity in tissues. Consequently, mudfish metabolism was examined to determine whether changes in parameters such as oxygen consumption, carbon dioxide excretion, and/or altered metabolic costs (represented by the key ionoregulatory enzyme Na^+, K^+-ATPase; NKA) could explain emersion tolerance. Oxygen consumption rates, already very low in immersed mudfish, were largely maintained over the course of emersion. Carbon dioxide excretion decreased during emersion, and a small, but significant, decrease in NKA was noted. These data suggest that the extended emersion capacity of mudfish may result from a generally low metabolic rate that is maintained throughout aerial exposure via cutaneous gas exchange, and which limits the production of potentially toxic nitrogenous waste.

1. Introduction

A number of fish species are capable of spending extended periods out of water, be it in response to drought (e.g. lungfish; Smith, 1931; Janssens, 1964), tidal stranding (e.g. blennies; Shimizu et al., 2006), an escape from unfavourable water conditions (e.g. triplefins; Hill et al., 1996), or to exploit land-based resources (e.g. mudskippers, Gordon et al., 1985). Life on land, however, comes at a cost. Fish that utilise terrestrial habitats must employ physiological and biochemical mechanisms that allow them to deal with the challenges posed by the absence of water.

Among the challenges faced by an aerially-exposed fish are the key processes of gas exchange and nitrogenous waste elimination. In water these roles are primarily performed by the gill, which by virtue of its small diffusive distances and large surface area is ideally suited for exchange with the environment (Evans et al., 2005). In air the delicate nature of the gill can lead to lamellar collapse, reducing the surface area available for gas exchange and/or ammonia excretion (Graham, 1997). Furthermore, in the case of ammonia excretion, immersion in water is critical for the maintenance of the diffusive gradient that facilitates ammonia elimination (Wright and Wood, 2009). In the absence of a medium that facilitates diffusion, air exposure can result in the accumulation of ammonia in the fish, leading to toxicity (Randall and Tsui, 2002). Thus an emersed fish requires mechanisms that mitigate ammonia toxicity. The exact mechanisms employed depend on a number of factors, including the length of time the fish will spend out of the water (Chew and Ip, 2014).

Galaxiid fish are the most widespread and speciose of Southern hemisphere fish groupings (McDowall, 2006). They are characterised by slender, cylindrical body forms and a lack of scales, and within this group there are a number of species known to tolerate aerial exposure. The most terrestrial of the galaxiids are the mudfish, including the brown mudfish (*Neochanna apoda*). Brown mudfish most commonly inhabit low oxygen, low pH, temporary pools in New Zealand temperate rainforests (White et al., in press). The ephemeral nature of their habitat

---

* Corresponding author at: Biosciences, College of Life and Environmental Sciences, Geoffrey Pope Building, University of Exeter, Exeter EX4 4QD, United Kingdom. Fax: +44 1392 723434.
E-mail address: m.a.urbina-foneron@exeter.ac.uk (M.A. Urbina).

http://dx.doi.org/10.1016/j.cbpa.2014.07.006

means that they may be subjected to months without access to free water (Eldon, 1978). During this time mudfish burrow into moist soil, where they are largely quiescent, but remain responsive to stimuli (Eldon, 1978), a condition best described as pseudoaestivation. This phenomenon is also characteristic of other, closely-related mudfish species (McDowall, 2006), such as the Canterbury mudfish (*Neochanna burrowsius*). Very little is known regarding the mechanisms mudfishes employ to withstand extended aerial exposures, although recent research has shown that the Canterbury mudfish is capable of utilising the skin as a pathway of oxygen uptake during terrestrialisation (Urbina et al., 2014).

At the opposite end of the temporal spectrum of emersion in galaxiid fish is the inanga (*Galaxias maculatus*). This species inhabits near-coastal freshwater streams, which may be prone to eutrophication and hypoxia (Urbina and Glover, 2012). It was recently shown that this species will emerse from water in response to severe hypoxia (Urbina et al., 2011), leaping into riparian vegetation that likely maintains humidity and limits desiccation. However, this is a strategy that will be effective for only a few hours, and eventually the fish must return to the water or mortality will occur. During emersion inanga are able to meet their metabolic requirements, at least in part, by aerial gas exchange across the scaleless integument (Urbina et al., 2014). Although it is known that inanga are relatively tolerant to waterborne ammonia (Richardson, 1997), the nitrogenous waste handling of this species, in either aquatic or aerial environments, has not been studied to date.

The brown mudfish and inanga are related species that display distinct emersion tolerances. Given their shared phylogeny we hypothesised that they would share similar mechanisms for withstanding aerial exposure, but given their different degrees of tolerance to emersion, we predicted that the magnitude of their adaptive responses to emersion would differ. Initially we proposed that these fish would employ specialised mechanisms for nitrogen waste handling, a hypothesis tested by investigating ammonia and urea handling following emersion exposures representative of those that occur in the natural environment (6 h for inanga; 14 days for brown mudfish). Additionally, based upon previous findings of a low metabolic rate in other mudfish species (e.g. Canterbury mudfish; Urbina et al., 2014), we investigated oxygen consumption, carbon dioxide excretion, and activities of a key ionoregulatory enzyme (Na^+/K^+-ATPase; NKA) to determine whether changes in metabolism were factors facilitating prolonged emersion in brown mudfish.

2. Materials and methods

2.1. Fish and rearing conditions

Adult brown mudfish (*N. apoda*; wet mass 3.6 ± 0.4 g) were collected using minnow traps from small, temporary, low pH (5.3), "brown water" streams in a native forest on the West Coast of New Zealand's South Island. After capture, fish were placed in plastic tanks with water from the stream and covered with sphagnum moss collected nearby to provide a refuge for the fish. Constant aeration was also provided. Adult inanga (*G. maculatus*; wet mass 4.2 ± 0.4 g), were caught by seine net from permanent freshwater streams located in Canterbury, New Zealand. Fish were placed in plastic tanks with water from the stream and constant aeration. Both fish species were transported to the aquarium facility in the School of Biological Sciences, University of Canterbury. Brown mudfish were maintained in 30-L aquaria (fish density ~1.4 kg m^3) in static conditions with gentle aeration for 10 days prior to experimentation. Over this period fish were gradually acclimated to naturally chlorine-free well water (composition (in μM): 375 Na^+, 574 Ca^{2+}, 119 Mg^+, 29 K^+, 310 Cl^-; total hardness: 70 mg L^{-1}; pH: 6.7) by daily 25% renewal of the initial water (from the collection site) with well water. Inanga were maintained in a 500 l aquarium, at a similar density to that of brown mudfish, with flow-through well water (identical to that described above) and gentle aeration for one month prior to experimentation. Brown mudfish were fed once a day to satiation using *Tubifex* bloodworm, while inanga were fed twice daily to satiation using a commercial flake food (NutrafinMax, USA). Temperature (14 °C) and light (12 h L:12 h D) were held constant throughout both holding and experimentation. Feeding was stopped two days before the experiments started. All procedures were approved by the University of Canterbury Animal Ethics Committee.

Throughout the paper animals that were not emersed are referred to as either "pre-emersion" or "control" fish. A "pre-emersion" treatment is one that was analysed prior to being removed from water, and subsequently underwent emersion. A "control" group is one that did not undergo emersion but was held unfed in aquatic conditions for the duration of the emersion and was then sampled in parallel with the emersed group (i.e. a time-matched control).

2.2. Emersion

Brown mudfish ($n = 8$) were transferred to an emersion chamber, and subjected to conditions designed to mimic the conditions that trigger pseudoaestivation in this species in natural settings. The chamber consisted of a plastic bucket (diameter 30 cm, depth 35 cm) filled to a depth of ~ 15 cm with a thick slurry of mud sourced from the collection site, mixed with well water. The slurry was such that upon settling (approximately 1 h) there was a small amount of water (~0.2–0.5 cm) overlying the mud substrate. All eight fish were then released into the chamber, where they quickly began to burrow superficially into the mud. The mud and the mudfish were then covered with sphagnum moss collected from the capture site. After 4 h a hole at the bottom of the chamber was used to slowly drain excess water over an ensuing 4 h period. Moss was removed and the mud was further dried for 24 h via convection of air across the top of the chamber using a fan. At the conclusion of this period there was no evidence of excess water, and the mud had formed into a cohesive, but soft and moist, substrate. This point was considered the start of the 14 day emersion period. The mud and the mudfish were again covered with the sphagnum moss which ensured that the mud remained moist for the duration of emersion. At the end of the emersion period, the block of mud was excised from the chamber, and the superficially-burrowing mudfish were gently removed by hand. The block of mud was then pulled apart to reveal mudfish that had buried slightly deeper and again these were easily removed by hand.

Inanga ($n = 8$) were emersed as described previously (Urbina and Glover, 2012), with slight modifications. Briefly, fish were placed in a shallow glass chamber (diameter 15 cm, depth 10 cm) where the bottom 2–3 cm was covered by wet tissue paper mimicking the natural emersion conditions of this species. Inanga remained emersed for 6 h.

2.3. Ammonia and urea fluxes

Ammonia and urea excretion rates were assessed in both mudfish and inanga prior to emersion, and at periods following reimmersion (0–4, 4–8, 8–24, 24–48, 48–72 h; plotted as 2, 6, 24, 48 and 72 h; only mudfish excretion was assessed over the 48–72 h period). Fish ($n = 8$) were placed individually in 1 l plastic chambers filled with 500 mL well water at 14 °C. Duplicate samples (3 mL) were taken at the beginning of each assay interval, and then again at the end. Well water was exchanged every 24 h in the post-emersion assays. Three chambers lacking fish were used as controls to account for any potential bacterial influence. Water ammonia and urea concentration were determined using

Fig. 1. Ammonia (A), urea (B) and total nitrogenous waste (C) excretion before and after a 14 days (brown mudfish, $n = 7–8$; grey bars) or 6 h (inanga, $n = 7–8$; white bars) emersion. Data are presented as means ± SEM. Asterisks represent significant ($p < 0.05$; Friedman one-way RM ANOVA on ranks) differences between the reimmersion and pre-emersion values. Daggers indicate significant ($p < 0.05$; *t*-test) differences between species under pre-emersion conditions (aquatic normoxia).