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Aerial exposure tolerance of a newly discovered galaxiid

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Laboratory experiments were conducted to investigate tolerance and physiological responses of *Galaxias* ‘nebula’, a newly discovered and widespread African galaxiid, to aerial exposure. This species can tolerate emersion for at least 36 h. Changes in water level and dewatering did not induce the fish to burrow into the substratum or find refugia, nor was there detectable mucus production following aerial exposure. Opercular movement, a proxy for gill ventilation rate, however, did vary with changes in water level. The initial steady ventilation rate increased significantly when the fish were partially emersed and ventilation ceased immediately upon total air exposure. When fish were re-immersed, there was first a period of hyperactivity with a corresponding inflated gill ventilation rate which was restored to pretreatment levels within 2 h. This is the first documented case of amphibious capabilities in an African galaxiid, which has implications for the interpretation of its widespread distribution pattern.

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Key words: behavioural adaptations; emersion tolerance; *Galaxias*; gill ventilation rate.

INTRODUCTION

Many fishes have the ability to extract oxygen from both air and water (Daxboeck & Heming, 1982; Martin *et al.*, 1993, 2004; Graham & Lee, 2004; Ip *et al.*, 2004; Sloman *et al.*, 2008). These fishes can be divided into two classes, amphibious and aquatic air-breathers (Graham, 1997). Amphibious air-breathers utilize atmospheric oxygen during periods of exposure to air (emersion) and many of them are capable of surviving periods without surface water (Martin *et al.*, 1993; Sayer, 2005; Taylor *et al.*, 2008). In contrast, aquatic air-breathers never leave water, but periodically break the water surface to gulp air (Gonzales *et al.*, 2006). These capabilities confer several advantages, which include providing alternatives to escape or evade predators and competitors (Sayer & Davenport, 1991), avoidance of hypoxic conditions and survival in temporary habitats (Eldon, 1979a; Graham, 1997; McPhail, 1999; Sayer, 2005). Studies on air-breathing fishes are crucial in the understanding of the evolution of vertebrate air-breathing and the fish-tetrapod transition (Brauner *et al.*, 2004; Graham & Lee, 2004). Knowledge of the patterns of air-breathing capabilities

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is also important to the understanding of the factors that shape the biogeography and evolutionary history of freshwater fishes (Main, 1989; Fraser *et al.*, 1995).

Air-breathing fishes use a combination of behavioural, structural and physiological adaptations to survive exposure to air (Sayer, 2005). Behavioural adaptations may involve burrowing into subterranean refugia or piles of vegetative debris and logs, where cool damp conditions may allow fishes to survive temporary cessation of surface flow, as observed in the mangrove killifish *Kryptolebias marmoratus* (Poey) (Taylor *et al.*, 2008) and the Australian salamanderfish *Lepidogalaxias salamandroides* Mees (Berra & Allen, 1989). Breathing out of water may be facilitated by possession of accessory respiratory structures, which include specialized lungs in the African lung fish *Protopterus* species (Maina & Maloiy, 1985), pharyngeal organs, such as the heavily vascularized buccopharyngeal cavity in the Gobiidae (Gee & Gee, 1995) and cutaneous respiration in the Canterbury mudfish *Neochanna burrowsius* (Phillipps) (Eldon, 1979b; Meredith *et al.*, 1982). Physiological adaptations to emersion include modification of gill function (Graham *et al.*, 1985; Ong *et al.*, 2007), metabolic adjustments (McPhail, 1999; Richards, 2010) and mechanisms to reduce toxic waste products in air (Ip *et al.*, 2004; Randall *et al.*, 2004). All these studies examined fish species known to be capable of air-breathing. It is much more difficult to establish, *via* non-lethal means, whether a newly discovered species has this capacity. The present study focuses on the amphibious survival capacity of a newly discovered galaxiid by assessing some of its behavioural and physiological adaptations for emersion survival.

The Galaxiidae consist of generally small osmeriform fishes confined to cool temperate regions in the southern hemisphere (McDowall, 1970, 1973). Several galaxiid species are known to be capable of air-breathing (Cadwallader & Backhouse, 1983; Koehan & Raadik, 1991; Thompson & Withers, 1999; O'Brien & Dunn, 2007), and research has addressed the ecological and evolutionary implications of this. For example, the amphibious air-breathing capabilities of *N. burrowsius* and its propensity to move over land could have resulted in its wide geographic distribution and the shallow genetic structuring of this species across the Canterbury Plains in New Zealand (Davey *et al.*, 2003). Paraphyletic relationships between isolated populations of the roundhead galaxias *Galaxias gollumoides* McDowall & Chadderton in New Zealand have been attributed to the hypoxia tolerance of this facultative swamp dweller, which could have allowed it to utilize intermittent wet connections to disperse between catchments (Burridge *et al.*, 2008). While knowledge of the ecology and air-breathing capabilities of galaxiids in New Zealand (Eldon, 1979a, b; Main, 1989; McDowall, 1999; McPhail, 1999; O'Brien & Dunn, 2007), Australia (Cadwallader & Backhouse, 1983; Koehan & Raadik, 1991; Thompson & Withers, 1999) and South America (Hickford *et al.*, 2010) is reasonably extensive, the current study is the first to address this potential in African galaxiid fishes.

Africa was thought to be represented by a single Galaxiidae species, the Cape Galaxias *Galaxias zebratus* (Castelnau). Recent phylogeographic studies, however, have revealed that *G. zebratus* is a species complex consisting of several unique lineages that are endemic to the Cape Floristic Region of South Africa (Waters & Cambray, 1997; Wishart *et al.*, 2006). In continuing research, many more lineages are being discovered and all of them are highly restricted geographically, except for one widespread lineage (hereafter *Galaxias* 'nebula') which is the subject of this study. African galaxiids appear to be confined to fresh water (McDowall, 1973).

Galaxias 'nebula' occurs in a range of habitats from fast-flowing mountain tributaries to coastal streams and swamps in river systems that are currently isolated by marine and terrestrial barriers.

In this study, a series of experiments were used to assess whether *G.* 'nebula' individuals can adjust their behaviour and physiology in response to emersion, and more importantly, reverse to a normal state after re-immersion. Specific predictions are that *G. nebula* (1) is tolerant of emersion, (2) can respond to declining water levels by actively seeking refugia and (3) has respiratory physiological responses to emersion. These intrinsic ecological and physiological adaptations may explain its wide distribution and utilization of diverse habitats.

MATERIALS AND METHODS

The research was conducted under permit from CapeNature (permit number: AAA-004-000205-0035) issued only after the approval of methods by a review panel. Specimens of *G.* 'nebula' were collected from the Hex River (33° 31' 44.2" S; 19° 32' 24.3" E) on 25 April 2009 for the emersion tolerance and refugia use experiment ($n = 60$) and on 29 April 2009 for assessing physiological responses to emersion ($n = 25$). The fish were transported to a nearby field laboratory and were kept overnight in aerated holding tanks. All the water used was collected from the same site and kept at ambient temperature. Sand for substratum and rocks and vegetation (*Juncus* sp.) for refugia were also collected at the same site.

To determine the tolerance of *Galaxias* 'nebula' to aerial exposure, 18 plastic trays (20 cm × 14 cm × 8 cm) were filled with 1.5 cm deep sand, one rock (7–10 cm length and 4–6 cm width), a clump of vegetation covering *c.* 40 cm² and 0.5 l of water. Two medium [3–4.5 cm total length (L_T)] and one large (5–6.5 cm L_T) fish were placed in each tray and allowed to acclimate for 6 h. The trays were covered with a net to reduce external disturbances. The experiment consisted of five phases: (1) pretreatment (P), (2) recession (R), (3) partial emersion (Pe), (4) emersion (E) and (5) re-immersion (Ri). Phases two (R), three (Pe) and four (E) were dewatering phases. With the exception of the emersion phase (E), the duration of each phase was 6 h. Each tray was observed at the start, midpoint and end of each phase, recording the number of fish in refugia (*i.e.* under the rock or vegetation) or visible in the open areas of the tray and noting any movement. The emersion (E) phase had four exposure periods (6, 12, 24 and 36 h) and observations were carried out at the beginning of this phase and after 3, 6, 12, 18, 24, 30 and 36 h of emersion (depending on the duration of the phase). Three randomly selected trays were designated as controls and left with water throughout the experiment. The remaining 15 trays were randomly assigned to an emersion exposure period such that there were three replicates for the 6 h exposure period, three replicates for the 12 h exposure period, three replicates for the 24 h exposure period and six replicates for 36 h. The ambient temperature ranged between 18 and 22° C throughout the experiment.

For the pretreatment phase (P), the trays were simply monitored to record the behaviour of the fish before experimental manipulations. For the recession phase (R), 0.2 l of water was drained from each of the treatment trays, such that the fish remained completely immersed in water. It took an average of 2 min to drain this volume of water from each tray. Another 0.2 l of water were drained from all treatment trays during the partial emersion phase (Pe) leaving *c.* 1 mm depth of water so that fish were partially exposed to air. For the emersion phase (E), all the surface water was drained from the treatment trays, leaving only the sand, vegetation and underside of rocks moist and fish completely exposed to air. Finally, for the re-immersion phase (Ri), 0.5 l of water was added to the respective trays at the end of each emersion period (*i.e.* after 6, 12, 24 and 36 h). The survival of the fish was confirmed and the time that the fish took to recover following re-immersion was noted. The fish were monitored for at least another 12 h to assess post-treatment behaviour and survival. Observations were conducted throughout these phases as set out above. Any dead fish encountered during treatment observations were immediately removed from the trays. Given that a combination of factors may influence the

subsequent survival of fish in laboratory experiments, the experiment was terminated after 36 h of emersion when two fish died between 30 and 36 h of exposure. Only four of the 54 fish used in this experiment died. In addition to the two above, one died during acclimation and another died after 24 h of emersion. At the end of the experiment, the fish were released alive at the site of collection.

In the second experiment, the respiratory physiological response of *Galaxias* 'nebula' to treatments (*i.e.* recession, partial emersion, emersion and re-immersion) was determined by visually quantifying opercular movement and calculating gill ventilation rate (GVR) as the number of opercular movements per minute. Twenty trays (10 controls and 10 treatments) were set up with 1.5 cm deep sand and 0.5 l of water. One medium-sized fish (3–4.5 cm L_T) was added to each tray and left to acclimate for 6 h. The same five phases as above (pretreatment, recession, partial emersion, emersion and re-immersion), each lasting 3 h, were carried out on all treatment trays. The control trays remained with water throughout the experiment. During each phase, three 1 min observations (*i.e.* immediately after the treatment, at midpoint of treatment and finally at the end of the treatment) were carried out to record the frequency of opercular movements (a measure of gill ventilation rate) for both control and treatment fish. No fish died during this experiment and these fish were also released at their exact collection site.

For experiment one, data were converted into proportions of fish in refugia, then, as sample sizes and variances were small, means of all the measurements per phase for each tray were taken and used as the dependent variable in analyses. As all treatment fish established their positions (either cover or open) by the end of partial emersion and remained in these positions throughout the emersion phase, only the data for the first 6 h of the emersion phase were used for all analyses to account for the varying duration of emersion. First, to establish whether there were any diurnal differences in use of refugia, the control data were subjected to a Friedman test with observations over time as the independent variable. Then, to assess whether exposure period affected refugia use, separate Kruskal–Wallis tests were carried out on the data from the emersion and re-immersion phases (four and five, the two phases likely to be affected) with exposure period as the independent variable. Finally, a Friedman test on the treatment trays with phase as the independent variable was used to assess differences in refuge use in different phases.

For experiment two, as data sets for operculum movement were normally distributed (assessed using Kolmogorov–Smirnov tests) parametric analyses were used. Levene's test was used to assess the homogeneity of variance (MANOVA) and where data were not spherical (repeated measures ANOVAs), a Greenhouse–Geisser correction was applied. Any diurnal effect on GVR was assessed using repeated measures ANOVA on the control data over all phases with time as the repeated measure and operculum movement as the dependent variable. To assess differences in GVR with phase, another repeated measures ANOVA was carried out with the three repeated measurements per phase nested within the repeated measure of phase. In the fourth phase (emersion), however, only zeros were recorded so this data set was omitted from this analysis. Then, as the interaction term was not significant, simple analyses were carried out. First, the data per phase for each tray were converted into means and used in a Friedman test with the five phases as the independent (repeated) factor to assess differences in GVR between phases, and *post hoc* Wilcoxon tests were used to find where these differences occurred. Finally, to assess differences over time within each phase, a repeated measures MANOVA was used with the three observations per phase as independent variables and operculum movements in each phase as dependent variables. For any phase in which significant differences were found, *post hoc* Tukey's tests were used to determine at what stage these differences occurred.

RESULTS

In the first experiment, *Galaxias* 'nebula' was able to withstand emersion for up to 36 h. There was no discernible pattern in the use of refugia among control fish (Friedman test, d.f. = 4, $P > 0.05$; Fig. 1). For the treatment fish, the exposure period did not affect refugia use in either the emersion (Kruskal–Wallis test, d.f. = 4,

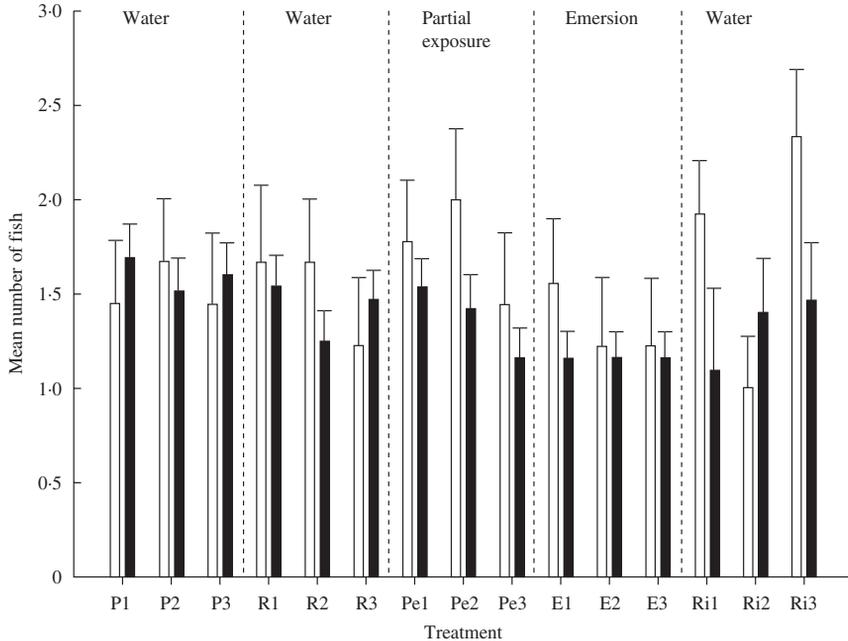


FIG. 1. Response of *Galaxias* 'nebula' to air exposure showing the mean number of fish in cover for control (□) and experimental (■) trays. The five phases are denoted by: P = pretreatment, R = recession, Pe = partial emersion, E = emersion and Ri = re-immersion. 1 = start (0 h), 2 = midpoint (3 h) and 3 = end (6 h) of phase. Only data for the first 6 h of the emersion phase are presented. Values are mean \pm s.e. $n = 3$ for control and 15 for experimental trays.

$P > 0.05$; Fig. 1) or re-immersion (Kruskal–Wallis test, d.f. = 4, $P > 0.05$; Fig. 1) phases, nor did refuge use change between phases (Friedman test, d.f. = 4, $P > 0.05$; Fig. 1). All fish immediately responded to partial exposure (Pe) by moving over the wet sand using serpentine movements. Some fish made excavations in the sand while moving, mainly around rocks and clumps of vegetation, but none of the fish burrowed into the substratum. Most fish became inactive while maintaining a dorso-ventral position within the first 2 h of emersion, but a few individuals occasionally moved laterally or rolled onto their dorsal side. No opercular movements or other visible respiratory behaviour was observed nor was there any visible mucus production. All fish responded rapidly to re-immersion. Approximately 25% of the fish were positively buoyant, suggesting that their gas bladders were inflated or they had taken an air bubble into the mouth and gill chamber. All fish expelled an air bubble through the mouth immediately following re-immersion. Many of the fish immediately started active swimming, *c.* 50% even swimming hyperactively, but all fish had calmed within the first 2 h of re-immersion.

In the second experiment, there was no diurnal difference in opercular movement in the control fish (repeated measures ANOVA, d.f. = 3,29, $P > 0.05$). The treatment fish did significantly differ in GVR over both phase (repeated measures ANOVA, d.f. = 2,16, $P < 0.05$) and time within phases (repeated measures ANOVA, d.f. = 2,16, $P < 0.05$). The interaction term, however, was not significant (repeated

measures ANOVA, d.f. = 4,32, $P > 0.05$). These factors were thus analysed separately. Mean opercular movement significantly differed between the five phases (Friedman test, d.f. = 4, $P < 0.001$) with *post hoc* pair-wise comparisons showing significant differences between the partial exposure and emersion phases (Wilcoxon, $n = 10$, $P < 0.05$) and between these phases and all others [Wilcoxon, partial emersion, $n = 10$, $P < 0.05$; emersion (all comparisons), $n = 10$, $P < 0.05$]. Partial exposure of fish to air resulted in a sharp increase in gill ventilation rate from an average of 67.1 to 93.5 beats min^{-1} and was maintained around this rate throughout this phase, while emersion caused an instant cessation of opercular movement (Fig. 2). A significant difference in opercular movement was found within the re-immersion phase, but no significant differences were detected within the other three phases (Table I). All fish immediately switched back to gill breathing when re-immersed, exhibiting an initial phase of hyperventilation (90 beats min^{-1}) with GVR declining and approaching pretreatment and control values within the first 2 h of re-immersion (Fig. 2).

DISCUSSION

The ability of *Galaxias* 'nebula' to tolerate emersion for a prolonged period of time suggests that this species has amphibious survival capabilities. They exhibited

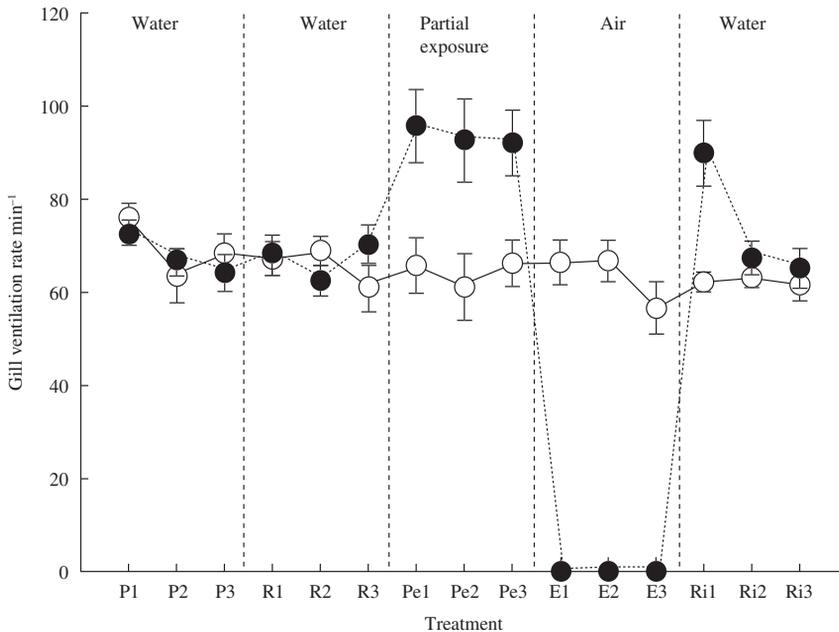


FIG. 2. Gill ventilation rates (mean \pm s.e.) of *Galaxias* 'nebula' for control (○) and experimental (●) trays during pretreatment (P), recession (R), partial emersion (Pe), emersion (E) and re-immersion (Ri) phases. 1 = start (0 h), 2 = midpoint (1.5 h) and 3 = end (3 h) of phase. For both control and experimental trays, $n = 10$ for each observation time for each phase. The initial pretreatment values were determined after 6 h of acclimation in river water.

TABLE I. Results from the repeated measures MANOVA comparing significance of differences in gill ventilation rates for experimental fish within pretreatment, recession, partial emersion and re-immersion phases

Phase	d.f.	<i>F</i>	<i>P</i>
Pretreatment	2,18	1.621	0.225
Recession	2,18	0.861	0.440
Partial emersion	2,18	0.141	0.870
Re-immersion	2,18	15.452	<0.001

both behavioural and physiological responses consistent with known amphibious adaptations in other fishes (Meredith, 1985; Sayer, 2005). The limited activity in *G. 'nebula'* when emersed probably indicates the suppression of metabolic rate corresponding to reduced energy and oxygen demand when exposed to an environment where respiration is constrained (Crocker & Cech, 1997; Richards, 2010). Maintenance of a dorso-ventral posture, facilitated by a low anguilliform body shape, is likely to enhance emersion tolerance by reducing pressure on the opercular region, which in turn may enable the fish to inflate their buccal cavities (Meredith, 1985). Species that are unable to maintain this position when removed from water, such as the common galaxias *Galaxias maculatus* (Jenyns), have low emersion tolerance (Meredith, 1985). The occasional rolling observed in *G. 'nebula'* may function to rehydrate the skin and expose different body surfaces, which may facilitate gas exchange, enhance waste excretion and prevent desiccation of gills through rehydration from residual water within the opercular cavity (Meredith, 1985). The rapid cessation of opercular ventilation in response to emersion is probably a crucial reflex to prevent desiccation of gills. The initial period of hyperventilation after re-immersion most likely functions to actively remove waste products accumulated during emersion from gill and body surfaces (Daxboeck & Heming, 1982; McPhail, 1999). Active swimming may also function to separate gill lamellae and force oxygenated water over the gill surfaces to enhance gaseous exchange.

Secretion of substantial amounts of mucus is considered to be a critical mechanism that enhances emersion tolerance, because it keeps the gills and skin surfaces moist for efficient gaseous exchange (Eldon, 1978; Meredith, 1985) and also facilitates retention of air bubbles in buccal and opercular cavities (Meredith, 1985). *Galaxias 'nebula'*, however, did not produce detectable mucus which could have affected their ability to retain air bubbles in the buccal cavity as none of the fish were observed to exhibit distinctly distended jaws or inflated opercular regions. Some amphibious fishes, such as *L. salamandroides*, do not produce large quantities of mucus when emersed, but can still respire aerially (Martin *et al.*, 1993). In the case of *G. 'nebula'*, it is probable that they cannot withstand considerable dehydration through complete drying of their habitat. Nevertheless, cutaneous respiration cannot be ruled out in this species, especially under very humid conditions or if the skin is kept moist for efficient gas exchange.

There are several alternative mechanisms by which *G. 'nebula'* may tolerate water loss. Gas bladders may function as accessory air-breathing organs (Brauner *et al.*, 2004). In this study, fish were positively buoyant when re-immersed, suggesting that their gas bladders may have been inflated. Galaxiids have been reported to survive

loss of surface water by burrowing into subterranean refugia (Eldon, 1979b; O'Brien, 2005; Davey *et al.*, 2006; Dunn & O'Brien, 2006; O'Brien & Dunn, 2007). Although the fish in this study did not attempt to burrow or actively seek refugia with decreasing water levels, it may be that the choice of substratum or the duration of water loss was influential. Both lowland longjaw galaxias *Galaxias cobitinis* McDowall & Waters (Dunn & O'Brien, 2006) and the common river galaxias *Galaxias vulgaris* Stokell (Davey *et al.*, 2006) burrow faster in coarser substrates. The small pore size of the sand used in this study may have inhibited subterranean burrowing. Alternatively, water decline may be more gradual under natural conditions. Here, the necessarily rapid water draw down may not stimulate a burrowing response. Finally, fishes may switch to anaerobic respiration (Sloman *et al.*, 2008), a possibility yet to be tested for *G. 'nebula'*.

Intrinsic ecological attributes such as emersion and hypoxia tolerance may facilitate interdrainage dispersal *via* intermittent wet connections in freshwater-restricted taxa (Hurwood & Hughes, 2001; Thacker *et al.*, 2007; BurrIDGE *et al.*, 2008). *Galaxias 'nebula'* has a relatively widespread distribution across several currently isolated river systems in the Cape Floristic Region. Many of these systems had common confluences during the lower sea levels of the last glacial maximum, forming palaeoriver systems that are now drowned. These connections along with river capture events played an important role in allowing wide distributions of some redfin *Pseudobarbus* (Cyprinidae) lineages (Swartz *et al.*, 2007, 2009). *Galaxias 'nebula'*, however, occurs across several of these palaeoriver systems indicating that historical river connections do not adequately explain the current distribution pattern of this lineage, especially since conventional river capture is a relatively rare event in the evolution of drainage networks (Bishop, 1995). The propensity to move over land is common in galaxiids and is well documented for mudfishes (*Neochanna* spp.) (Eldon, 1978). Field observations suggest that *G. 'nebula'* can move over damp soil. It is possible, therefore, that the amphibious adaptations exhibited by *G. 'nebula'* contributed to its wide distribution pattern.

While this is the first demonstration of emersion tolerance in an African galaxiid, the occurrence of air-breathing adaptations in galaxiids is well documented. For example, the blackstriped dwarf galaxias *Galaxiella nigrostriata* (Shipway) (Thompson & Withers, 1999) and the Australian mudfish *Neochanna cleaveri* (Scott) (Cadwallader & Backhouse, 1983; Koehan & Raadik, 1991) from Australia and *Neochanna* species from New Zealand (O'Brien & Dunn, 2007) all have amphibious adaptations similar to those described here, and one widespread species, *G. maculatus*, from South America, New Zealand and Australia (Berra *et al.*, 1996) has terrestrial egg development (Hickford *et al.*, 2010). The incidence of amphibious traits in galaxiids from all Gondwanan continents in the southern hemisphere suggests that it could be an ancestral phenomenon or that it evolved independently several times. It could also occur in many more galaxiids than presently known, but further research is required to assess whether these traits are homologous. Knowledge of intrinsic ecological attributes of freshwater limited taxa is crucial in explaining large-scale biogeographical and phylogeographical patterns (Hurwood & Hughes, 2001; Thacker *et al.*, 2007; BurrIDGE *et al.*, 2008).

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References

- Berra, T. M. & Allen, G. R. (1989). Burrowing, emergence, behaviour, and functional morphology of the Australian salamander fish, *Lepidogalaxias salamandroides*. *Fisheries* **14**, 2–10. doi: 10.1577/1548-8446(1989)014<0002:BEBAFM>2.0.CO;2
- Berra, T. M., Crowley, L. E. L. M., Ivantsoff, W. & Fuerst, P. A. (1996). *Galaxias maculatus*: an explanation of its biogeography. *Marine and Freshwater Research* **47**, 845–849. doi: 10.1071/MF9960845
- Bishop, P. (1995). Drainage rearrangement by river capture, beheading and diversion. *Progress in Physical Geography* **19**, 449–473. doi: 10.1177/030913339501900402
- Brauner, C. J., Matey, V., Wilson, J. M., Bernier, N. J. & Val, A. L. (2004). Transition in organ function during the evolution of air-breathing; insights from *Arapaima gigas*, an obligate air-breathing teleost from the Amazon. *Journal of Experimental Biology* **207**, 1433–1438. doi: 10.1242/jeb.00887
- Burridge, C. P., Craw, D., Jack, D. C., King, T. M. & Waters, J. M. (2008). Does fish ecology predict dispersal across a river drainage divide? *Evolution* **62**, 1484–1499. doi: 10.1111/j.1558-5646.2008.00377.x
- Cadwallader, P. L. & Backhouse, G. N. (1983). *A Guide to the Freshwater Fish of Victoria*. Melbourne: Victorian Government Printing Office.
- Crocker, C. E. & Cech, J. J. Jr. (1997). Effects of environmental hypoxia on oxygen consumption rate and swimming activity in juvenile white sturgeon, *Acipenser transmontanus*, in relation to temperature and life intervals. *Environmental Biology of Fishes* **50**, 383–389. doi: 10.1023/A:1007362018352
- Davey, A. J. H., Kelly, D. J. & Biggs, B. J. F. (2006). Refuge-use strategies of stream fishes in response to extreme low flows. *Journal of Fish Biology* **69**, 1047–1059. doi: 10.1111/j.1095-8649.2006.01180.x
- Davey, M. L., O'Brien, L. K., Ling, N. & Gleeson, D. M. (2003). Population genetic structure of the Canterbury mudfish (*Neochanna burrowsius*): biogeography and conservation implications. *New Zealand Journal of Marine and Freshwater Research* **37**, 13–21.
- Daxboeck, C. & Heming, T. A. (1982). Bimodal respiration in intertidal fish *Xiphister astropurpureus* (Kittlitz). *Marine and Freshwater Behaviour and Physiology* **9**, 23–33. doi: 10.1080/10236248209378581
- Dunn, N. R. & O'Brien, L. K. (2006). *Gravel Burrowing Ability in Galaxias cobitinis*. Wellington: Department of Conservation.
- Eldon, G. A. (1978). The life history of *Neochanna apoda* Günther (Pisces: Galaxiidae). In *Fisheries Research Bulletin No. 19*. Wellington: New Zealand Ministry of Agriculture and Fisheries.
- Eldon, G. A. (1979a). Habitat and interspecific relationships of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research* **13**, 111–119.
- Eldon, G. A. (1979b). Breeding, growth and aestivation of the Canterbury mudfish, *Neochanna burrowsius* (Salmoniformes: Galaxiidae). *New Zealand Journal of Marine and Freshwater Research* **13**, 331–346.
- Fraser, D. F., Gilliam, J. F. & Yip-Hoi, T. (1995). Predation as an agent of population fragmentation in a tropical watershed. *Ecology* **76**, 1461–1472. doi: 10.2307/1938148
- Gee, J. H. & Gee, P. A. (1995). Aquatic surface respiration, buoyancy control and the evolution of air-breathing in gobies (Gobiidae: Pisces). *Journal of Experimental Biology* **198**, 79–89.
- Gonzales, T. T., Katoh, M. & Ishimatsu, A. (2006). Air breathing of aquatic burrow-dwelling eel goby, *Odontamblyopus lacepedii* (Gobiidae: Amblyopinae). *Journal of Experimental Biology* **209**, 1085–1092. doi: 10.1242/jeb.02092
- Graham, J. B. (1997). *Air-breathing Fishes: Evolution, Diversity and Adaptation*. San Diego, CA: Academic Press.

- Graham, J. B. & Lee, H. J. (2004). Breathing air in air: in what ways might extant amphibious fish biology relate to prevailing concepts about early tetrapods, the evolution of vertebrate air breathing, and the vertebrate land transition? *Physiological and Biochemical Zoology* **77**, 720–731.
- Graham, J. B., Jones, C. B. & Rubinoff, I. (1985). Behavioural, physiological, and ecological aspects of the amphibious life of the pearl blenny *Entomacrodus nigricans* Gill. *Journal of Experimental Marine Biology and Ecology* **89**, 255–268. doi: 10.1016/0022-0981(85)90131-5
- Hickford, M. J. H., Cagnona, M. & Schiel, D. R. (2010). Predation, vegetation and habitat-specific survival of terrestrial eggs of a diadromous fish, *Galaxias maculatus* (Jenyns, 1842). *Journal of Experimental Marine Biology and Ecology* **385**, 66–72. doi: 10.1016/j.jembe.2010.01.010
- Hurwood, D. A. & Hughes, J. M. (2001). Nested clade analysis of the freshwater shrimp, *Caridina zebra* (Decapoda: Atyidae), from north-eastern Australia. *Molecular Ecology* **10**, 113–125. doi: 10.1046/j.1365-294X.2001.01175.x
- Ip, Y. K., Chew, S. F. & Randall, D. J. (2004). Five tropical air-breathing fishes, six different strategies to defend against ammonia toxicity on land. *Physiological and Biochemical Zoology* **77**, 768–782. doi: 10.1086/422057
- Koehan, J. D. & Raadik, T. A. (1991). The Australian mudfish, *Galaxias cleaveri* Scott, 1934 in Victoria. *Proceedings of the Royal Society of Victoria* **103**, 77–86.
- Main, M. R. (1989). Distribution and post-glacial dispersal of freshwater fishes in South Westland, New Zealand. *Journal of the Royal Society of New Zealand* **19**, 161–169.
- Maina, J. N. & Maloiy, G. M. O. (1985). The morphometry of the lung of the African lungfish (*Protopterus aethiopicus*): its structural-functional correlations. *Proceedings of the Royal Society* **224**, 399–420.
- Martin, K. L. M., Berra, T. M. & Allen G. R. (1993). Cutaneous aerial respiration during forced emergence in the Australian salamanderfish, *Lepidogalaxias salamandroides*. *Copeia* **1993**, 875–879.
- Martin, K. L. M., Van Winkle, R. C., Drais, J. E. & Lakisic, H. (2004). Beach spawning fishes, terrestrial eggs, and air breathing. *Physiological and Biochemical Zoology* **77**, 750–759.
- McDowall, R. M. (1970). The galaxiid fishes of New Zealand. *Bulletin of the Museum of Comparative Zoology, Harvard* **139**, 341–431.
- McDowall, R. M. (1973). The status of the South African galaxiid (Pisces, Galaxiidae). *Annals of the Cape Provincial Museums (Natural History)* **9**, 91–101.
- McDowall, R. M. (1999). Just hanging around for some fresh air, thanks! Survival adaptations of the mudfish. *Water and Atmosphere* **7**, 7–8.
- McPhail, J. D. (1999). A fish out of water: observations on the ability of black mudfish, *Neochanna diversus*, to withstand hypoxic water and drought. *New Zealand Journal of Marine and Freshwater Research* **33**, 417–424.
- Meredith, A. S. (1985). Metabolism and cutaneous exchange in an amphibious fish *Neochanna burrowsius* (Phillipps). PhD Thesis, University of Canterbury, Christchurch, New Zealand.
- Meredith, A. S., Davie, P. S. & Forster, M. E. (1982). Oxygen uptake by the skin of the Canterbury mudfish, *Neochanna burrowsius*. *New Zealand Journal of Zoology* **9**, 387–390.
- O'Brien, L. K. (2005). Conservation ecology of Canterbury mudfish (*Neochanna burrowsius*). PhD Thesis, University of Canterbury, Christchurch, New Zealand.
- O'Brien, L. K. & Dunn, N. R. (2007). *Mudfish (Neochanna Galaxiidae) Literature Review*. Wellington: Science and Technical Publishing, Department of Conservation.
- Ong, K. J., Stevens, E. D. & Wright, P. A. (2007). Gill morphology of the mangrove killifish (*Kryptolebias marmoratus*) is plastic and changes in response to terrestrial air exposure. *Journal of Experimental Biology* **210**, 1109–1115. doi: 10.1242/jeb.002238
- Randall, D. J., Ip, Y. K., Chew, S. F. & Wilson, J. M. (2004). Air breathing and ammonia excretion in the giant mudskipper, *Periophthalmodon schlosseri*. *Physiological and Biochemical Zoology* **77**, 783–788.
- Richards, J. G. (2010). Metabolic rate suppression as a mechanism for surviving environmental challenge in fish. In *Aestivation: Molecular and Physiological Aspects* (Navas, C. A.

- & Carvalho, J. E., eds), pp. 113–139. Berlin: Springer-Verlag. doi: 10.1007/978-3-642-02421-4_6
- Sayer, M. D. J. (2005). Adaptations of amphibious fish for surviving life out of the water. *Fish and Fisheries* **6**, 186–211. doi: 10.1111/j.1467-2979.2005.00193.x
- Sayer, M. D. J. & Davenport, J. (1991). Amphibious fish: why do they leave the water? *Reviews in Fish Biology and Fisheries* **1**, 159–181. doi: 10.1007/BF00157583
- Sloman, K. A., Mandic, M., Todgham, A. E., Fänge, N. A., Subrt, P. & Richards, J. G. (2008). The response of the tidepool sculpin, *Oligocottus maculosus*, to hypoxia in laboratory, mesocosm and field environments. *Comparative Biochemistry and Physiology A* **1490**, 284–292. doi: 10.1016/j.cbpa.2008.01.004
- Swartz, E. R., Skelton, P. H. & Bloomer, P. (2007). Sea-level changes, river capture and the evolution of populations of the Eastern Cape and fiery redbins (*Pseudobarbus afer* and *Pseudobarbus phlegethon*, Cyprinidae) across multiple river systems in South Africa. *Journal of Biogeography* **34**, 2086–2099. doi: 10.1111/j.1365-2699.2007.01768.x
- Swartz, E. R., Skelton, P. H. & Bloomer, P. (2009). Phylogeny and biogeography of the genus *Pseudobarbus* (Cyprinidae): shedding light on the drainage history of rivers associated with the Cape Floristic Region. *Molecular Phylogenetics and Evolution* **51**, 75–84. doi: 10.1016/j.ympev.2008.10.017
- Taylor, D. S., Turner, B. J., William, P., Davis, W. P. & Chapman, B. B. (2008). A novel terrestrial fish habitat inside emergent logs. *The American Naturalist* **171**, 263–266. doi: 10.1086/524960
- Thacker, C. E., Unmack, P. J., Matsui, L. & Rifenbark, N. (2007). Comparative phylogeography of five sympatric *Hypseleotris* species (Teleostei: Eleotridae) in south-eastern Australia reveals a complex pattern of drainage basin exchanges with little congruence across species. *Journal of Biogeography* **34**, 1518–1533. doi: 10.1111/j.1365-2699.2007.01711.x
- Thompson, G. G. & Withers, P. C. (1999). The metabolic response to hypoxia and emersion of aestivating fishes (*Lepidogalaxias salamandroides* and *Galaxiella nigrostriata*) and a non-aestivating fish (*Bostockia porosa*) from south-western Australia. *Australian Journal of Zoology* **47**, 295–305. doi: 10.1071/ZO99008
- Waters, J. M. & Cambray, J. A. (1997). Intraspecific phylogeography of the Cape galaxias from South Africa: evidence from mitochondrial DNA sequences. *Journal of Fish Biology* **50**, 1329–1338. doi: 10.1111/j.1095-8649.1997.tb01656.x
- Wishart, M., Hughes, J., Stewart, B. & Impson, D. (2006). Extreme levels of intra-specific divergence among Cape Peninsula populations of the Cape galaxias, *Galaxias zebratus* Castelnau 1861, reveals a possible species complex. *African Journal of Aquatic Science* **31**, 99–106.