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Hatching Success of Rainbowfish Eggs Following Exposure to Air

Lois J. Oulton
Macquarie University

Penelope Carbia
Macquarie University

Culum Brown
Macquarie University

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Lois Oulton, Penelope Carbia, and Culum Brown
Macquarie University

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ABSTRACT

Translocation of fishes within and between drainage basins is widely recognised as a threatening process to Australian native fishes. While many translocations are deliberate, for example for fisheries enhancement, it is possible that translocation can occur naturally. In the Wet Tropic region of Australia, the widespread eastern rainbowfish, Melanotaenia splendida, has begun to colonise the Atherton tablelands. This is of particular concern because the area is home to several endangered endemic species such as the Lake Eacham rainbowfish, M. eachamensis, and its allies. It is likely that some of the translocations have occurred through the use of this species as bait, but the recent invasion of Lake Eacham may have occurred naturally via the movement of eggs between nearby streams running into Lake Tinaroo. Here we determine whether rainbowfish eggs could be transported over land by examining their desiccation tolerance. In the first experiment we plucked eggs from spawning media and exposed them to air for varying amounts of time. The results showalmost100%mortality after just 15 min. Rainbowfish eggs have a web-like projection that enables them to adhere to aquatic vegetation. In the second experiment, therefore, eggs were exposed to air attached to simulated weed (wet acrylic wool). Around 20% of eggs on the simulated weed where still viable after an hour. The results show that rainbowfish eggs could readily be transported between catchments by aquatic birds or human leisure activities such as kayaking or swimming. The implications for conservation management of Australian freshwater species is discussed.

Introduction

Australia has a long history of translocation of fishes between catchments. To date, translocation of some 76 species from 28 families has been documented, but many more have undoubtedly occurred (Harris 2013). In most cases the introduction of fishes outside their natural ranges has unpredictable and often devastating impact on the indigenous fauna (Keller and Brown 2008; Strayer 2010; Bool *et al*. 2011). Despite being recognized as one of the principal threatening processes to Australian native fishes, translocations are often government-sanctioned largely to support or promote recreational fishing activities. However, not all translocations occur at the hands of man (Lintermans 2004). Some may occur naturally, for example via unusual dispersal events, although there is seldom supporting evidence. For example, Australian inland waters are prone to inundation that can temporarily join river drainages, especially in regions of low topography. It is likely that the broad distribution of spangled perch is a testament to its ability to colonise new habitats during flood events (Humphries *et al*. 1999). Similarly, river plumes displace marine waters following heavy rainfall and may allow freshwater fish to move between river mouths (Grimes and Kingsford 1996). There is also speculation that plants and animals can be moved between catchments by aquatic birds (Figuerola and Green 2002).

Rainbowfishes are ubiquitous small fish found in virtually every freshwater body in Australia from the Murray–Darling basin to the tip of Cape York (Tappin 2010). There are currently 13 species described in Australia but the number continues to grow as new species are identified. The Atherton Tableland region in the hinterland of Cairns is a particularly speciose region for rainbowfish diversity. Here, two widely divergent clades are coming into secondary contact, with *Melanotaenia splendida* colonising the tablelands region from its largely coastal distribution (McGuigan *et al*. 2000). The endemic clade in the region contains several species that now have highly restricted distributions (eg *M. eachamensis* and *M. utcheensis*) and there is evidence of hybridisation and introgression between the two clades (McGuigan *et al*. 2000). Translocation of predators and competitors continues to be a major threat to the conservation status of these small-bodied fishes.

Recently, we described the presence of a new population of the eastern rainbowfish in Lake Eacham and used molecular markers to identify potential source populations (Brown *et al*. 2013). Lake Eacham is the type locality for the endangered Lake Eacham rainbowfish, but the fish was extirpated following multiple systematic translocations of native fishes from surrounding catchments (Barlow *et al*. 1987; Brown and Warburton 1997). The appearance of another rainbowfish species in the lake begs the question as to how the fish colonised the isolated crator lake. Brown *et al*. (2013) speculated that the colonisation from an adjacent catchment could have occurred by the transfer of fertilised eggs. Previous researchers have suggested that transfer of eggs via natural means, such as wading birds, is reasonably unlikely to occur for most freshwater species (Unmack 2013). However, rainbowfish eggs contain sticky weblike projections that fasten to aquatic vegetation, preventing downstream drift to potentially unfavourable habitats (Humphrey *et al*. 2003). They readily attach to anything with which they come in contact, which would include wading birds, but also human swimmers or recreational devices (snorkelling equipment, kayaks, etc). Both Lake Eacham and the neighbouring Lake Tinaroo are popular tourist attractions and there is a constant flow of tourists between the two lakes. It is highly likely that wading birds also move between the two water bodies on a regular basis. Moverover, propogule size and desiccation resistance are important factors when considering transport by birds and there is evidence that both plants and invertebrates can be transported and colonise new habitats in this fasion (Figuerola and Green 2002; Figuerola *et al*. 2005). No data relating to fish transport via this means are currently available.

Whilst the attachment of rainbowfish eggs to objects seems likely, less is known about how long these eggs might survive out of water. If they desiccate rapidly, then colonisation of new water bodies over land barriers seems highly unlikely. If, on the other hand, the eggs show signs of desiccation resistance, then a window of opportunity exists for eggs to colonise new water bodies before the embryos die. Tappin (1983) conducted a preliminary study using *Melanotaenia fluviatilis* and reported 40% hatching success of eggs stored in a sealed plastic bag at 19°C for 10 days. While he conducted only a single replicate, the results suggest that the eggs might survive prolonged exposure to air under certain conditions. Rainbowfish eggs are very small (~1mm in diameter) and the membrane is highly permeable (Oulton *et al*. 2013). Both of these factors suggest that they would not last long once directly exposed to air. Should they be transported in association with aquatic weeds or any other material that retains water, however, egg survival could be greatly prolonged.

Here we examined the hatching success of crimson spotted rainbowfish, *Melanotaenia duboulayi*, embryos following prolonged exposure to air. *M. duboulayi* is part of the 'australis' clade that includes *M.*

eachamensis, *M. fluviatilis* and *M. utcheensis* (McGuigan *et al*. 2000) and has a coastal distribution ranging from Coffs Harbour to Bundaberg. In the first treatment we simply harvested eggs and exposed them to air directly for varying lengths of time. In the second treatment, we attached the eggs to wet acrylic wool to simulate aquatic vegetation in the expectation that contact with the wool would greatly reduce desiccation times and thus provide a broader window of opportunity for cross-catchment colonisation.

Methods

Adult rainbowfish, *M. duboulayi*, were collected from the Pine River system at Juff's Crossing (27°12.378S, 152°48.486E), Queensland, Australia, in 2010. They were maintained in captivity in mixed-sex groups in 100-L aquaria furnished with river gravel and a filter. Light was provided on a 14 : 10 light : dark cycle and room temperature was maintained at 26°C. Fish were maintained on standard flake food, but two days before spawning their diet was supplemented with high-protein items, including blood worms (chironomid larvae) and brine shrimp (*Artemia* spp.). Sterilised spawning mops constructed out of acrylic wool were introduced to the tank as an egg deposition substrate in the evening and left for two days. Following this, the mops were removed and lightly disinfected in a methylene blue solution (0.25mLL^{-1}; Aqua Master) before being placed in small tanks (20×20×38 cm) filled with aged tap water and lightly aerated. Four days after fertilisation eggs were harvested from the mops and placed in the treatment Petri dishes (5 cm diameter, n = 5 eggs per dish). For the first treatment, eggs were placed in an empty plastic Petri dish for the allocated period (Table 1) and then inundated with 14mL of aged tap water. In the second treatment, 30-cm strings of acrylic wool were soaked in aged tap water and curled into the Petri dishes. The wool was soaked in boiling water for 5 min before use to remove any dye that may leach out and also to sterilise it. Harvested eggs were placed directly onto the wool. Once again the eggs were inundated with aged tap water at the allocated time interval (Table 1). Pilot studies suggested that eggs attached to wool were still viable after 15 min, so, for ethical reasons, we restricted our exposure times to concentrate on survival after 15 min. The eggs were allowed to sit for the designated period and the number of larvae that hatched in each dish was recorded.

The data were analysed using ANOVA (fixed variables: length of air exposure and treatment; dependent variables: number of eggs that hatched in each Petri dish). Our initial analysis examined the time points that the two treatments had in common (0, 15 and 30 min). We then examined each treatment in isolation to isolate the effects of time.

Rainbowfish were housed under licence from the Macquarie University Animal Ethics Committee (ARA: 2011/024).

Results

Survival of rainbowfish embryos dramatically declined following prolonged exposure to air. While most eggs hatch under control conditions (0 min exposure to air), hatching success declined rapidly, and very few embryos survive more than 7.5 min exposure. In contrast, when eggs were deposited on simulated aquatic plants (acrylic wool soaked in water) eggs remained viable even after 60 min (Fig. 1).

Analysis of the three common time points (0, 15 and 30 min) revealed a significant interaction between time and treatment ($F_{2,45}$ = 69.945, P < 0.001). *Post hoc* tests revealed a highly significant effect of air exposure time on hatching success in the Petri dishes lacking wool ($F_{2,21}$ = 124.324, P < 0.001). By comparison, analysis of the treatment containing wool showed no decrease during this period ($F_{2,24}$ = 1.939, P = 0.166) (Fig. 1).

Table 1. The amount of time that eggs from each treatment were exposed to air before inundation

Treatment	Air exposure times for each treatment					
No wool	0	5	7.5	10	15	30
Wool	0	15	30	45	60	---

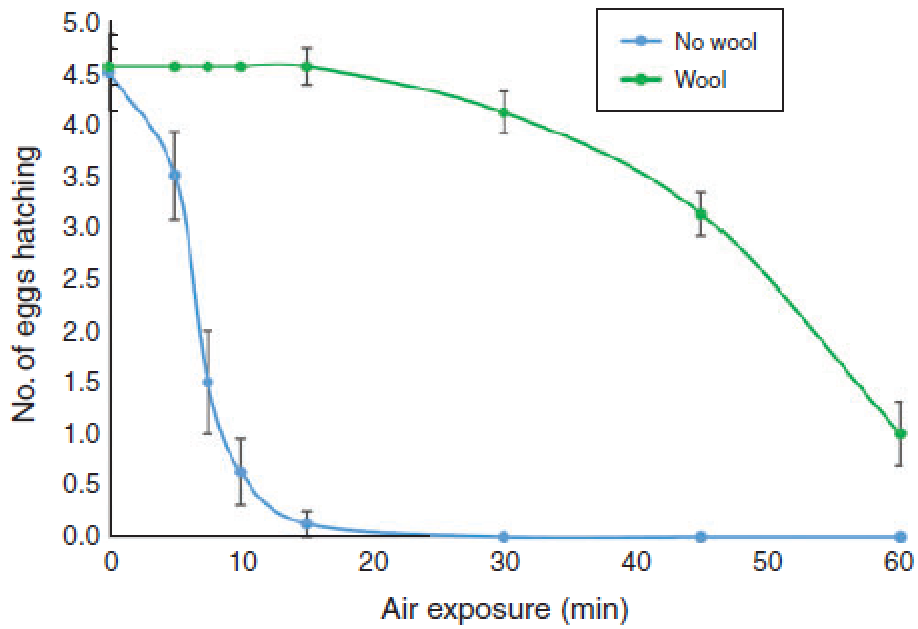

Fig. 1. The mean (±s.e.) number of embryos hatching in each Petri dish following exposure to air. Petri dishes were either bare (no wool) or contained a damp 30-cm piece of acrylic wool (wool) to simulate aquatic weeds.

Analysis of each treatment in isolation showed highly significant decreases in egg survival over time in both treatments ($F_{5,42}$ = 30.601 and $F_{4,38}$ = 43.660 in the no-wool and wool treatments respectively, P < 0.001 in both cases). In the absence of any substrate, embryo survival showed significant declines from 0 to 5 min (P = 0.044) and from 5 to 7.5 min (P < 0.001). In the wool treatment, significant declines in survival occurred from 30 to 45 min (P = 0.004) and from 45 to 60 min (P < 0.001). Examination of Fig. 1 suggests that the half life without substrate was ~7 min, whereas in the presence of wool it was 50 min.

Discussion

Rainbowfish eggs are clearly prone to desiccation in the absence of a damp substrate, which limits their dispersal potential. Our results show that, in the absence of a damp substrate, most eggs do not survive out of water for longer than 15 min. In comparison, if the eggs are attached to a substrate that retains moisture, such as aquatic plants, survival of embryos is greatly prolonged. Here, some larvae were still alive and hatched successfully after 60 min of exposure to air. Clearly, the extent to which the embryos survive is dependent on the ability of the substrate to retain water and, to some extent, on the relative humidity of the environment. While these results are directly relevant to the transportation of eggs over land barriers, they are also interesting in their own right, since few studies have examined egg desiccation in freshwater fishes.

The fact that rainbowfish eggs can survive for extended periods if attached to aquatic weeds clearly opens up opportunities for them to travel over land barriers either naturally (e.g. by wading birds) or artificially (by human intervention). Our previous study found that the source of a new population of rainbowfish in Lake Eacham likely came from a population just 500m away (Brown *et al*. 2013). Wright Creek is a tributary of Lake Tinarroo and the lake was stocked with *M. splendida*, probably in the form of bait fish, which subsequently hybridized with the endemic *M. eachamensis* (McGuigan *et al*. 2000). Wading birds could readily move between these catchments in a matter of minutes and there is ample time for eggs to move from Lake Tinaroo itself if the eggs were attached to aquatic weeds. Birds have been implicated in the transfer of both aquatic plant and invertebrate propagules, but there are almost no data on fish egg transfer (Figuerola and Green 2002; Figuerola *et al*. 2005). Such translocation could also readily occur through the movement of aquatic leisure craft or bathers that frequent both locations. It is important to note that several viable embryos would have to complete the journey for the founding event to be successful. However, female rainbowfish often spawn their eggs *en masse* and they can be regularly found in chains amongst the spawning media. Moreover, while many temperate-zone rainbowfish survive for only 12 months, in tropical locations they may successfully overwinter and thus become multiyear populations (Pusey *et al*. 2001). In this latter scenario, several translocations could occur over a reasonably lengthy time frame, which dramatically increases the opportunity to found a self-sustaining population.

The implications of this study for the conservation management of rainbowfishes (or other small-bodied fishes) are rather interesting. While undescribed species of rainbowfish exist in the Atherton Tablelands region, many of these are already threatened by invasion from *M. splendida* (McGuigan *et al*. 2000; Brown, unpubl. data). We previously assumed that the extent of the invasion would be impeded by natural and artificial barriers (e.g. waterfalls and weirs), but it is apparent that this may not be the case. The ability to cross barriers by attachment may be a trait limited to rainbowfishes and the closely related hardyheads (*Craterocephalus* spp.), given the web-like projections on the eggs. Such observations may be important to understanding the biogeography of this family and its potential to invade (Unmack *et al*. 2013). Population genetic studies, however, often show discrete populations within catchments, thus these colonization events may be relatively rare unless assisted by humans.

On a final note, despite the fact that freshwater fishes are prone to laying their eggs in the shallow weedy regions of rivers and lakes, few have considered the impact this might have on the survival chances of the embryos. Spawning during times of flood obviously increases the risk of the embryos becoming stranded above the water where they are prone to a range of new selection pressures, including, but not restricted to, desiccation. To our knowledge there have been no studies of desiccation tolerance of the embryos of Australian freshwater fishes despite the unpredictability of the flows in most catchments. Interestingly, some tropical rainbowfish species have a peak spawning period during the dry season (Pusey *et al*. 2001), which may prevent eggs becoming stranded after flooding, but this is by no means universal. In tropical regions, such as Panama, some fish species seem to deliberately lay eggs in damp environments out of water. Even though several species are known to adopt this unusual strategy, very little is known about why they do it or what the consequences might be (Martin *et al*. 2004). *Rivulus marmoratus*, for example, has terrestrial egg deposition (Abel *et al*. 1987), and the eggs of *Brycon petrous* can be found on the river bank, vegetation and wet stones (Kramer 1978*a*, 1978*b*). Pollard (1971) reports that some land-locked populations of *Galaxias maculatus* lay their eggs among weeds during floods and the embryos develop out of water. Once development is complete, hatching is stimulated by the next flood event up to a month later. Perhaps just as important is the implications for those embryos that are exposed to air for prolonged periods. Air exposure may stress the developing embryo, resulting in death, or induce long-term consequences on behaviour and development. This avenue remains an exciting opportunity for future research.

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References

Abel, D. C., Koenig, C. C., and Davis,W.P. (1987). Emersion in the mangrove forest fish *Rivulus marmoratus*: a unique response to hydrogen sulfide. *Environmental Biology of Fishes* **18**, 67–72. doi:10.1007/BF00002329

Barlow, C. G., Hogan, A. E., and Rogers, L. J. (1987). Implication of translocated fishes in the apparent extinction in the wild of the Lake Eacham rainbowfish, *Melanotaenia eachamensis. Australian Journal of Marine and Freshwater Research* **38**, 897–902. doi:10.1071/MF9870897

Bool, J. D., Witcomb, K., Kydd, E., and Brown, C. (2011). Learned recognition and avoidance of invasive mosquitofish by the shrimp, *Paratya australiensis. Marine and Freshwater Research* **62**, 1230–1236. doi:10.1071/MF11140

Brown, C., and Warburton, K. (1997). Predator recognition and anti-predator responses in the rainbowfish *Melanotaenia eachamensis. Behavioral Ecology and Sociobiology* **41**, 61–68. doi:10.1007/s002650050364

Brown, C., Aksoy, Y., Varinli, H., and Gillings, M. (2013). Identification of the rainbowfish in Lake Eacham using DNA sequencing. *Australian Journal of Zoology* **60**, 334–339. doi:10.1071/ZO12106

Figuerola, J., and Green, A. J. (2002). Dispersal of aquatic organisms by waterbirds: a review of past research and priorities for future studies. *Freshwater Biology* **47**, 483–494. doi:10.1046/j.1365-2427.2002.00829.x

Figuerola, J., Green, A. J., and Michot, T. C. (2005). Invertebrate eggs can fly: evidence of waterfowl-mediated gene flow in aquatic invertebrates. *American Naturalist* **165**, 274–280. doi:10.1086/427092

Grimes, C. B., and Kingsford, M. J. (1996). How do riverine plumes of different sizes influence fish larvae: do they enhance recruitment? *Marine and Freshwater Research* **47**, 191–208. doi:10.1071/MF9960191

Harris, J. H. (2013). Fishes from elsewhere. In 'Ecology of Australian Freshwater Fishes'. (Eds P. Humphries and K. Walter.) pp. 259–282. (CSIRO Publishing: Melbourne.)

Humphrey, C., Klumpp, D. W., and Pearson, R. (2003). Early development and growth of the eastern rainbowfish, *Melanotaenia splendida splendida* (Peters). I. Morphogenesis and ontogeny. *Marine and Freshwater Research* **54**, 17–25. doi:10.1071/MF02037

Humphries, P., King, A., and Koehn, J. (1999). Fish, flows and flood plains: links between freshwater fishes and their environment in the Murray–Darling river system, Australia. *Environmental Biology of Fishes* **56**, 129–151. doi:10.1023/A:1007536009916

Keller, K., and Brown, C. (2008). Behavioural interactions between the introduced plague minnow *Gambusia holbrooki* and the vulnerable native Australian ornate rainbowfish *Rhadinocentrus* *ornatus*, under experimental conditions. *Journal of Fish Biology* **73**, 1714–1729. doi:10.1111/j.1095-8649.2008.02045.x

Kramer, D. L. (1978*a*). Reproductive seasonality in the fishes of a tropical stream. *Ecology* **59**, 976–985. doi:10.2307/1938549

Kramer, D. L. (1978*b*). Terrestrial group spawning of *Brycon petrosus* (Pisces: Characidae) in Panama. *Copeia* **1978**, 536–537. doi:10.2307/1443624

Lintermans, M. (2004). Human-assisted dispersal of alien freshwater fish in Australia. *New Zealand Journal of Marine and Freshwater Research* **38**, 481–501. doi:10.1080/00288330.2004.9517255

Martin, K. L., Van Winkle, R. C., Drais, J. E., and Lakisic, K. (2004). Beach spawning fishes, terrestrial eggs and air breathing. *Physiological and Biochemical Zoology* **77**, 750–759. doi:10.1086/421755

McGuigan, K., Zhu, D., Allen, G. R., and Moritz, C. (2000). Phylogenetic relationships and historical biogeography of melanotaeniid fishes in Australia and New Guinea. *Marine and Freshwater Research* **51**, 713–723. doi:10.1071/MF99159

Oulton, L. J., Haviland, V., and Brown, C. (2013). Predator recognition in rainbowfish, *Melanotaenia duboulayi*, embryos. *PLoS ONE* **8**(10), e76061. doi:10.1371/journal.pone.0076061

Pollard, D. (1971). The biology of a landlocked form of the normally catadromous salmoniform fish *Galaxias maculatus* (Jenyns). I. Life cycle and origin. *Marine and Freshwater Research* **22**, 91–124. doi:10.1071/MF9710091

Pusey, B. J., Arthington, A. H., Bird, J. R., and Close, P. G. (2001). Reproduction in three species of rainbowfish (Melanotaeniidae) from rainforest streams in northern Queensland, Australia. *Ecology of Freshwater Fish* **10**, 75–87. doi:10.1034/j.1600-0633.2001.100202.x

Strayer, D. L. (2010). Alien species in fresh waters: ecological effects, interactions with other stressors, and prospects for the future. *Freshwater Biology* **55**, 152–174. doi:10.1111/j.1365-2427.2009.02380.x

Tappin, A. R. (1983). Drying effects on the eggs of the rainbow fishes. *Fishes of Sahul* **1**, 20–21.

Tappin, A. R. (2010). ‘Rainbowfishes: Their Care and Keeping in Captivity.’ (Art Publications.)

Unmack, P. (2013). Biogeography. In ‘Ecology of Australian Freshwater Fishes’. (Eds P. Humphries and K. Walter.) pp. 25–48. (CSIRO Publishing: Melbourne.)

Unmack, P. J., Allen, G. R., and Johnson, J. B. (2013). Phylogeny and biogeography of rainbowfishes (Melanotaeniidae) from Australia and New Guinea. *Molecular Phylogenetics and Evolution* **67**, 15–27. doi:10.1016/j.ympev.2012.12.019