

CAPTIVE BREEDING OF TWO RARE NON-MIGRATORY GALAXIIDS (TELEOSTEI: GALAXIIDAE) FOR SPECIES CONSERVATION

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ABSTRACT: Devising effective techniques for breeding and rearing rare non-migratory galaxiids is urgently required for conservation purposes where few animals exist in the wild for translocation or reintroduction. The development of such protocols is particularly pertinent in light of recent intense widespread bushfires and long-term drought in southeastern Australia, which have increased the likelihood of the need for captive maintenance to protect and recover remnant species. In this study, we promoted reproductive maturation via manipulation of day length and temperature, and produced viable offspring from two small, endemic freshwater galaxiids, *Galaxias fuscus* (Mack 1936) and *Galaxias longifundus* (Raadik 2014) using in vitro propagation techniques. Propagation trials resulted in 425 oocytes being stripped from four ripe *G. fuscus* females, and 1527 oocytes from three ripe *G. longifundus* females. Of these, 342 (80.5%) *G. fuscus* and 968 (63.4%) *G. longifundus* eggs hatched into larvae. Newly hatched *G. fuscus* and *G. longifundus* larvae were transparent, and 8.4–9.7 mm (mean 9.0 mm TL) and 7.1–8.9 mm (mean 8.3 mm TL) consecutively. Absorption of the yolk sac by *G. fuscus* larvae (1.5–2.0 mm diameter) was complete 6–7 days after hatching, and for *G. longifundus* (1.0–1.4 mm diameter) 12–13 days after hatching. One-month-old *G. fuscus* measured ~16 mm and two-month-old larvae ~22 mm, and one-month-old *G. longifundus* ~11 mm. Methods and techniques employed may aid broader galaxiid conservation efforts.

Keywords: galaxiids, captive breeding, reproduction, freshwater, conservation, Victoria

INTRODUCTION

The galaxioids are the dominant, most speciose group of freshwater fishes in the lands of the cool southern hemisphere, and among the most seriously threatened fishes worldwide (McDowall 2006). In total, 34 of the 44 described galaxiids in Australia and New Zealand are classified as threatened or as nearing extinction (Dunn et al. 2017; Linterman 2016). Although numerous anthropogenically driven threats are responsible for the decline, the most pertinent has been the widespread introduction of invasive species, particularly trout (*Salmo trutta*, *Oncorhynchus mykiss*). As a result, many species of galaxiids occupy increasingly isolated and vulnerable habitats which contain too few animals to supply individuals for translocation or reintroduction. Recent, ongoing bushfires, of a magnitude and expanse not previously known, have also burnt much of southeastern Victoria, a stronghold of threatened galaxiids in Australia (Raadik 2014). As a result, the risk of species extinction is considerable. Rescue, captive maintenance and breeding are therefore likely to become increasingly common to aid recovery of many threatened galaxiid species (Gaffney et al. 1992; Raadik et al. 2010; Raadik & Nicol 2012; Saddler et al. 2010).

Few incentives exist, however, for developing techniques to propagate rare, uneconomical and non-

recreationally important species (Rakes et al. 1999). Attempts at captive breeding of endangered non-migratory galaxiids have largely resulted in limited or no success due to the lack of resources, which is often compounded by a poor understanding of life-history requirements (Chilcott et al. 2013; Hamr 1992; Hardie et al. 2011; Hopkins 1979). However, where sufficient investment has occurred in developing techniques for the production of commercially important galaxiid species, considerable successes have been observed (Mardones et al. 2008; Wylie et al. 2016). Devising effective techniques for breeding and rearing rare galaxiid species is therefore undoubtedly achievable, and urgently required.

The barred galaxias *Galaxias fuscus* (Mack 1936) (Figure 1) and West Gippsland galaxias *Galaxias longifundus* (Raadik 2014) (Figure 2), are small (*G. fuscus* maximum 165 mm, 30 g; *G. longifundus* maximum 97 mm, 11 g), non-migratory, native fish, endemic to southeastern Australia (Raadik 2011; Raadik et al. 2010). The range and abundance of both species have declined since European settlement (Raadik 2011; Raadik et al. 2010), with *G. fuscus*, an upland species only known from 12 fragmented and geographically isolated sites (400–1600 m above sea level), and *G. longifundus*, a lowland species (195–275 m above sea level) from only a single site (Raadik 2014, 2011; Raadik et al. 2010).



Figure 1: An adult *Galaxias longifundus*. Photograph by Tarmo Raadik.

The *G. fuscus* National Recovery Plan calls for the development of methods to increase the number of populations and individuals within their natural range (Raadik et al. 2010), while evidence of rapid and ongoing decline of the only known *G. longifundus* population calls for immediate action to halt extinction of the species (Raadik unpub. data). Artificial breeding and successfully raising fish for supplementation and translocation were therefore seen as a feasible recovery action for these species.

Much of the ecology of *G. longifundus* remains unknown (Raadik 2011). It is likely, however, that the reproductive ecology of the species is similar to that of the closely (genetically) related *Galaxias olidus* Günther 1866 and *G. fuscus*. Both species occupy similar habitat and lay eggs on the underside of boulders in riffles from late winter to spring, at a time when day length and temperature are increasing (O'Connor & Koehn 1991; Raadik 1993, 2011; Shirley & Raadik 1997; Stoessel et al. 2011).

This study tested in vitro propagation techniques for *G. fuscus* and *G. longifundus*. We describe the approach and success of the attempts. Knowledge gained from the program provides new insight into the reproduction of both species and may be of value to the conservation of other threatened, non-migratory freshwater galaxiids.

MATERIALS AND METHODS

Adult broodstock *G. fuscus* were captured on the 23 August 2010 from Kalatha Creek (37°29' S, 145°32' E)

and adult *G. longifundus* on the 19 May 2014 from Rintoul Creek (38°02' S, 146°28' E), using a Smith Root® model LR20B backpack electrofisher operated at settings of 70 Hz and 500–1000 V. Reproductive maturation of captured fish was determined by observing the degree of gonadal development through the body wall, and categorising reproductive stage according to descriptors modified from Pollard (1972). Four reproductively mature female (range 88–97 mm TL) and four reproductively mature male (range 81–88 mm TL) *G. fuscus*, and three developing female (range 68–88 mm TL) and six developing male (range 56–78 mm TL) *G. longifundus* were selected for use in breeding experiments. All other fish collected were returned to the site of capture.

On return to the laboratory, *G. fuscus* were placed into outdoor glass aquaria and *G. longifundus* into an indoor glass aquarium (900 x 550 x 600 mm, 297 L). Aquaria were filled with aged (chlorine-free), carbon-filtered tap water (EC 550) to a depth of 300 mm. Water within aquaria was trickle-fed, recirculated, filtered (Eheim 2217 canister filter) and chilled (Teco TC20 water chiller).

To further promote final reproductive maturation in *G. fuscus*, water temperature within the aquaria in which they were housed was gradually increased from 9.5–11.5°C to mimic water temperatures present in the wild during the spawning period (Stoessel et al. 2015). Natural light was filtered using green shade cloth (70% shade factor) positioned approximately 1.5 m from the side of the tanks, and by green polycarbonate roofing (66% shade factor)



Figure 2: Several adult *Galaxias fuscus*. Photograph by Tarmo Raadik.

from above. Alternatively, water temperature within *G. longifundus* aquaria was gradually decreased from 15–7°C, and indoor photoperiod reduced from 9 h 59 minutes to 9 h 32 minutes over 12 days (to mimic the winter solstice), before temperature and photoperiod were gradually increased over 48 days to 12°C and 11 h 31 minutes to mimic the onset of spring, which is the species' likely spawning period (see O'Connor & Koehn 1991; Raadik 1993, 2011; Shirley & Raadik 1997; Stoessel et al. 2011, 2015).

All fish were monitored for reproductive status and fed live tubifex worms (*Tubifex* sp.) daily. Fish were considered reproductively ripe when ovaries were visually determined to fill approximately >90% of the body cavity (which was clearly distended), oocytes were large and extruded by applying gentle pressure on the body wall, and the spawning vent of males and females was enlarged and extended (Pollard 1972). Once fish were determined as reproductively ripe, fish were removed from the aquaria and hand-stripped without hormonal treatment or anaesthetic. The total oocytes produced by a female were divided as a single layer on the bottom of two or more plastic petri dishes lined with 1.5 mm plastic mesh. Within 30 seconds of eggs being stripped, the paired male was stripped, and the resultant milt spread over the oocytes using a soft fine brush.

Spent adult fish were treated in a saline solution (10 g/L) for 20 minutes immediately after being stripped, and then placed in a 40 L recovery aquarium containing aerated, chilled water treated with fungicide (Aquatopia®

fungus eliminator). After a further week, and if no adverse effects were noted, they were released back to their stream of origin.

Fertilised eggs were placed into individually labelled incubators within 20 L glass aquaria (Bacher & O'Brien 1989). Water within each tank was aerated and recirculated. Aquaria containing *G. fuscus* (an upland species) eggs were chilled to 9.0°C and those containing *G. longifundus* (a lowland species) eggs to 11.0°C. Egg batches were removed daily, photographed under a microscope-mounted digital camera (INFINITYX-21), checked for fungus, and sterilised in salt solution (10g/L) for 20 minutes before being returned. Exposure to air during photographing and fungus checks was minimised to <1 minute in total. The diameters of fertilised eggs were determined from digital images analysed using Image Pro® Express Version 5.0 software.

Newly hatched and non-free-swimming larvae were placed into static aerated 4 L aquaria. Larvae were fed twice daily on a diet progressing from a liquid feed (Aquasonic Pty Ltd Complete Fry Starter), encapsulated food (JBL NovoBaby 01), frozen brine shrimp and finally, artemia nauplii. Once larvae were observed freely swimming and feeding, they were transferred into 10 L aquaria until their yolk sac was absorbed, and then into 100 L aquaria at a maximum density of three fish per litre, until release. Following hatching, to replicate that occurring in the wild over the suspected spring spawning and subsequent larval development period, the temperature within *G. fuscus*

larval tanks was gradually increased (over 60 days) from 9.0–12.0°C, and in *G. longifundus* larval tanks (over 48 days) from 11.0–18.0°C. Growth was recorded using a subsample ($n=10$) of randomly chosen newly hatched, one-month-old and, where possible, two-month-old larvae, which were placed into a petri dish containing aquaria water, photographed using a microscope-mounted digital camera (INFINITYX-21), and returned to aquaria. The length of larvae was determined from digital images analysed using Image Pro® Express Version 5.0 software.

RESULTS

Broodstock of *G. fuscus* and *G. longifundus* were determined as reproductively ripe on the 4 October 2010 and 23 July 2014 respectively. Subsequent in vitro propagation trials (on the same days) resulted in the stripping of 425 oocytes from four ripe *G. fuscus* females (on average ~106 oocytes per female), and 1527 oocytes from three ripe *G. longifundus* females (on average 509 oocytes per female). On extrusion, oocytes from both species were adhesive, transparent and spherical. On contact with water, *G. fuscus* oocytes swelled from an approximate diameter of 2.0 mm to 3.0–4.1 mm (mean 3.5 mm) and *G. longifundus* oocytes from 1.5 mm to 2.0–2.5 mm (mean 2.2 mm). Fertile *G. fuscus* eggs eyed on day 16 (Figure 3) and hatched between 38 and 50 days (mean 44 days) after fertilisation, and *G. longifundus* eggs eyed on day 12 (Figure 4) and hatched between 26 and 30 days (mean 27 days) after fertilisation. The small number ($n=5$) of *G. fuscus* larvae that hatched prior to 41 days were developmentally immature and seldom free-swimming. Physical deformities were also noted in *G. fuscus* and *G. longifundus* larvae hatched beyond 48 days and 29 days respectively. Of the total number of oocytes stripped from female *G. fuscus*, 33 (7.8%) were removed from the experiment due to fungus infection; 11 (2.6%) remained underdeveloped or had significant deformities; 39 (9.2%) were infertile; and 342 (80.5%) hatched into larvae. In contrast, 25 (1.6%) *G. longifundus* oocytes were removed due to fungus infection; 78 (5.1%) remained underdeveloped or had significant deformities; 456 (29.9%) were infertile; and 968 (63.4%) hatched into larvae.

Newly hatched *G. fuscus* and *G. longifundus* larvae were transparent, and 8.4–9.7 mm (mean 9.0 mm TL) and 7.1–8.9 mm (mean 8.3 mm TL) in length consecutively. Larvae of both species were active swimmers and utilised the entire water column, excluding times when a portion of individuals within each aquarium (<5%) were seen to periodically lie motionless (for periods of <1 min) on the bottom of aquaria in the days immediately after hatching. Absorption of the yolk sac by *G. fuscus* larvae (1.5–2.0 mm diameter) was generally complete 6–7 days after hatching,

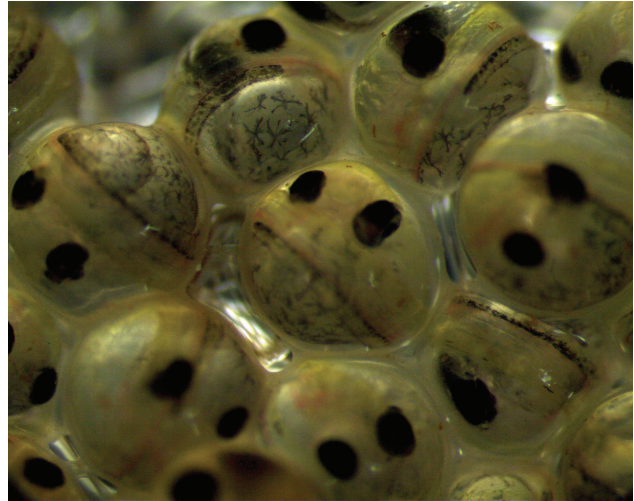


Figure 3: Unhatched larvae of *Galaxias longifundus*. Photograph by Daniel Stoessel.

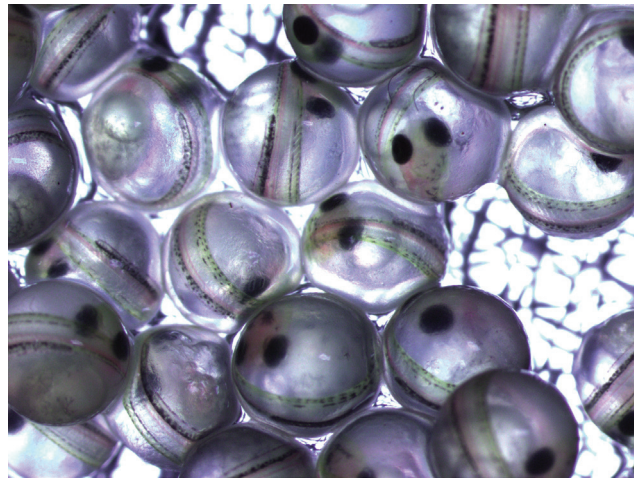


Figure 4: Unhatched larvae of *Galaxias fuscus*. Photograph by Daniel Stoessel.

and for *G. longifundus* (1.0–1.4 mm diameter) 12–13 days after hatching. Feeding commenced in *G. fuscus* larvae within 48 hours, and for *G. longifundus* within 72 hours after hatching. Larvae of both species fed throughout the water column, readily switched from one food source to another, and appeared only limited by gape size as to the type of prey ingested. Inspection of *G. longifundus* larvae under magnification on 9 September 2014 indicated that despite feeding being observed, a large proportion of individuals were emaciated. Feed type was immediately altered from liquid fry starter to encapsulated and live food; nevertheless, several hundred *G. longifundus* larvae died in the following week. One-month-old *G. fuscus* larvae measured ~16 mm and two-month-old larvae ~22 mm, and one-month-old *G. longifundus* larvae measured ~11 mm. Of the 342 *G. fuscus* and 968 *G. longifundus* larvae hatched, 304 (88.3%) *G. fuscus* were released back into Kalatha Creek on the 7 December 2010, and 73 (7.5%) *G. longifundus* were released back into Rintoul Creek on the 8 October 2014.

DISCUSSION

In this study, we successfully bred two non-migratory freshwater galaxiids, *G. fuscus* and *G. longifundus*, for the first time, produced offspring using in vitro propagation techniques, grew out larvae to between two months (*G. fuscus*) and six weeks (*G. longifundus*) of age, and released a small number of larvae of both species back to the wild. Knowledge gained in advancing the reproductive state of captive-held galaxiids will be useful in preparing individuals of similar species to a stage at which in vitro propagation can be undertaken. Similarly, techniques used to house, treat and grow out larvae will be useful in future efforts aimed at breeding endangered non-migratory galaxiids for conservation purposes. Such an approach, however, requires at least a basic understanding of the biology of a species, and therefore highlights the need for further research as to the life-history of many of the most endangered galaxiid species.

On average, broodstock of *G. fuscus* produced fewer oocytes per individual than *G. longifundus*. Nevertheless, eggs and larvae of *G. fuscus* were on average larger than that produced by *G. longifundus*, their larvae commenced feeding sooner, and egg sac absorption occurred over a shorter period. Traits described for these species therefore conform to those proposed by Humphries (1989) where galaxiids from colder climates (i.e. *G. fuscus*) produce fewer, larger eggs, while those from warmer climates (i.e. *G. longifundus*) produce more eggs which are comparatively smaller. The production of larger less numerous eggs by *G. fuscus*, which produced larvae that also commenced feeding sooner, is therefore undoubtedly a tactic which is advantageous to survival in colder upland streams which the species inhabits (Humphries 1989; Ware 1975; Wootton 1984).

Comparatively, a greater percentage of eggs produced by *G. fuscus* were deemed fertile and survived to hatch when compared with *G. longifundus*. The reason for this is unknown; however, performance of eggs and larvae derived from wild matured broodstock has been suggested, in some sections of the aquaculture industry, to be superior to that produced by captive matured animals (Browdy 1998). This is likely a response to artificial foods that are not specifically tailored to the nutritional requirements of a species, resulting in shifts in the biochemical composition of eggs produced, which in turn has implications for egg and larval quality (Izquierdo 2001; Sargent 1995). Future captive-breeding efforts should therefore consider nutrition to be an area that requires careful consideration, particularly if broodstock are to be induced to mature in captivity.

We emphasise that captive breeding should only ever be viewed as a last option for species recovery. It should not

be used as a prophylactic or long-term solution because of the unavoidable genetic and phenotypic changes that occur in captive environments (Ryman 1991; Snyder et al. 1996; Storfer 1999). Translocation is preferred (Philippart 1995) because it increases the number of separate populations, the total of which is usually far more important than the number of individuals (Maitland 1995). Translocation can, however, only occur if donor populations contain enough individuals to support such a strategy. The use of captive breeding as a management tool may therefore at times be unavoidable; thus research that assesses whether long-term captive held galaxiids are able to produce viable reproductive products and second-generation offspring should be considered.

Any such research should be carried out under strict conditions that guarantee the retention of morphological, physiological, ecological, behavioural and especially genetic characteristics of the species (Philippart 1995). For *G. fuscus*, the results of genetic investigations (Ayres et al. 2012) should be used to inform future captive breeding and translocation attempts, in order to limit loss of genetic integrity and to enable best practice management of the species. Furthermore, undertaking population viability analysis for these species may also provide a useful framework for collating key knowledge, identifying levels of uncertainty, and developing population models to assist management (Todd et al. 2017). Procedures, techniques and findings of the research, particularly in relation to artificial fertilisation, and larval grow-out may prove useful in the recovery, reestablishment and translocation of similar non-migratory galaxiid species elsewhere, many of which are in serious conservation crisis (McDowall 2006).

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