Spawning of Threatened Barred Galaxias, *Galaxias fuscus* (Teleostei: Galaxiidae)

Daniel J. Stoessel , Tarmo A. Raadik and Renae M. Ayres

Department of Environment, Land, Water and Planning, Arthur Rylah Institute for Environmental Research,
123 Brown Street, Heidelberg, Victoria, 3084 Australia

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Barred galaxias *Galaxias fuscus* is an endangered freshwater fish endemic to south-eastern Australia. Little is known of the species' ecology. We investigated spawning biology of *G. fuscus* in three headwater streams and found spawning to occur mid-August to late September when photoperiod was 10 h 39 min – 12 h 25 min. Spawning sites were in fresh (range 35.3 – 56.6 EC, mean 44.7 EC), slightly acidic (range 5.7 – 7.1 pH, mean 5.9 pH), moderate to fast flowing (range 0.4 – 2.0 m/s, mean 1.0 m/s), shallow (range 70 – 310 mm, mean 174 mm), well oxygenated (range 10.8 – 12.4 mg/l, mean 11.3mg/l), clear (range 1.2 – 6.3 NTU, mean 3.8 NTU), cool waters (range 8.4 – 10 °C, mean 9.1°C) immediately upstream of pools. Multi-layered clusters of up to 218 eggs were generally adhered close to the stream bed on the downstream side of cobbles greater than 180 mm diameter.

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INTRODUCTION

The barred galaxias, *Galaxias fuscus* is a small (maximum 160 mm TL, 40 g), endemic, scaleless, non-migratory fish (Raadik et al. 1996). Remnant populations are restricted to 12 geographically isolated headwater streams above 400 m in elevation in the Goulburn River system in south-eastern Australia (Raadik et al. 1996; Koehn and Raadik 1995; Allen et al. 2003). This range is likely to represent fragmentation of a much wider and continuous historic distribution within headwater streams within the catchment (Raadik et al. 2010). Predation by alien rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) (Salmonidae) is the primary cause of the decline (Raadik et al. 1996; Raadik et al. 2010). Changed water regimes, genetic isolation and deleterious stochastic events including wildfire and drought also represent significant long-term threats to *G. fuscus* (Raadik et al. 2010). The species is listed as Endangered under the Commonwealth Environment Protection and Biodiversity Conservation Act 1999 (EPBC Act) and the Australian and New Zealand Environment Conservation Council (ANZECC), and as Critically Endangered Internationally (Wager 1996).

Knowledge of *G. fuscus* biology is limited. Preliminary observations suggest that spawning occurs in late winter-early spring, and is likely to be triggered by increasing day length and water temperature (Raadik 1993, Shirley and Raadik 1997). Fecundity is low (~500 ova), and eggs are adhesive and large (~2.2 mm; Raadik 1993). Limited observations of two nest sites, suggest multi-layered clusters of eggs are laid underneath and on the downstream side of large rocks in fast-flowing, shallow, cold (1 – 5 °C) water (Raadik et al. 2010), and incubation time of eggs is approximately 30 days in water at 7 °C in an aquaria (Raadik, T. unpublished data).

This paper further investigates the spawning of *G. fuscus*, and includes data on habitat, spawning season and site, egg description, incubation period, and description of larvae. The biological information obtained is vital for the preparation of management strategies to maintain, enhance or restore processes fundamental to survival, reproduction and viability of remnant populations.

MATERIALS AND METHOD

Study area and sites

The study was conducted in three geographically closely associated headwater streams (S Creek, Kalatha Creek and Luke Creek) of the Goulburn River in south-eastern Australia (37º 28´ S, 145º 28´E) (Fig. 1). Considerable bushfires in the region in February 2009, had resulted in varying loss of riparian cover. At the S Creek reach riparian vegetation and tree canopy cover was non-existent, while at Luke Creek approximately 50 % of tree canopy cover remained, and at Kalatha Creek the area remained unburnt. Coarse sand within the stream channel was most prevalent in the S Creek study reach and least noticeable at Kalatha Creek.

G. fuscus was the only fish species present in the study reaches (Raadik et al. 2010), which were at elevations above 400 m (Australian Height Datum) and located 1 – 2 km upstream of large natural instream barriers which had prevented the headwater colonisation by other native fish and, importantly, by alien salmonids. The freshwater streams were clear, well-oxygenated, cool, narrow (1 – 4 m wide), had moderate to fast flow and alternating sequences of pools and riffles. The substrate was typically composed of boulder, pebble, gravel and sand (Raadik et al. 1996).

Within each reach, a 100 m long monitoring site was established and surveyed repeatedly during the study to assess for the presence of reproductively ripe females. A second site, 200 m in length, and located immediately upstream was later searched for newly laid eggs.

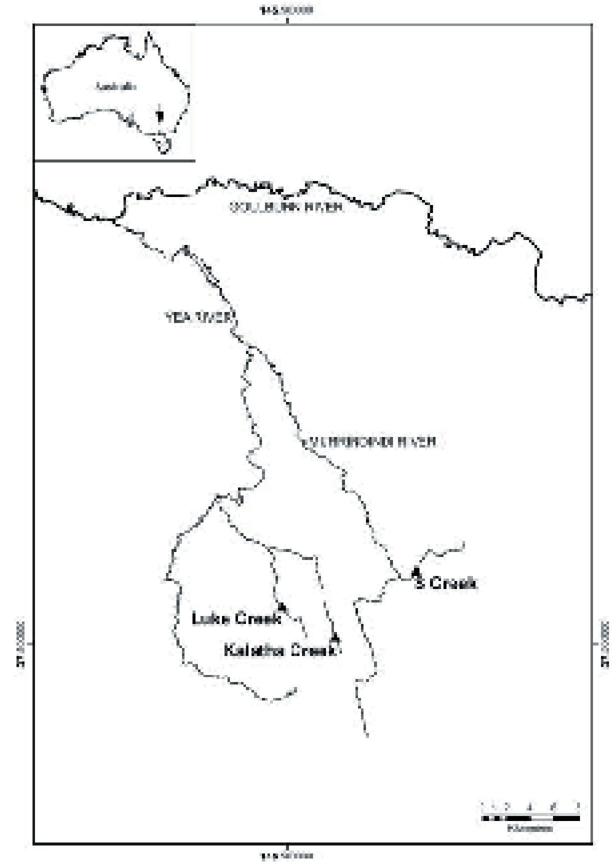

Figure 1. Location of barred galaxias study sites in Kalaltha, Luke and S creeks in south-eastern Australia.

Monitoring of fish spawning condition

G. fuscus were surveyed weekly from July to September 2010 (mid austral winter to early austral spring) at the monitoring site in each study reach, using a Smith Root® model LR20B portable electrofishing backpack unit operated at settings of 70 Hz and 500 to 1000 V. Fish caudal fork lengths (mm) and weights (g) were recorded. Females were determined as ripe, when ovaries filled >90 % of the body cavity, eggs were large, body cavity clearly distended, and eggs could be extruded by gentle pressure on the body wall. Spawning vent in males and females in addition was enlarged and extended (see Pollard 1972). All fish collected were released once processed.

Spawning habitat search

Once ripe females were no longer observed at all monitoring sites, searches to locate eggs were conducted. All instream structures, including timber debris, undercut banks, and closely associated riparian habitat, were examined for the presence of eggs. Where eggs were found, they were left instream and their location marked with flagging tape so the site could be avoided during kick sampling (see below).

A drift net (500 mm mouth opening, 150 μm mesh) was deployed downstream of each site within each stream during the search period to capture drifting eggs or newly hatched larvae. The contents of the drift net were sorted at the completion of the search period. In addition, substrate kick sampling was undertaken over multiple, randomly chosen stream sections (1 x 1 m) at each site to search for eggs potentially deposited on sand or gravel beds. This involved gently disturbing an area of stream bed immediately upstream of a dip-net (250 x 300 x 20 mm with a 400 mm long x 1 mm multifilament mesh bag attached) for approximately 10 seconds.

Where eggs were located, water depth, flow (Hydrological Services Current Meter Counter Model CMC-20), the type and dimensions of the spawning structure, and the characteristics of the placement of eggs on the structure, were recorded. In addition, water electrical conductivity (EC standardized to 25ºC µS.cm^{-1}), pH, dissolved oxygen (mg/L and % saturation), turbidity (NTU) and temperature (ºC) were measured in situ at a maximum depth of 0.2 m below the water surface during each spawning condition monitoring event, and immediately adjacent to egg nest sites using a TPS 90FL-T Field Lab Analyser.

Egg Incubation

On completion of recording habitat attributes at nest sites, eggs were transferred to aquarium facilities and placed into indoor 20 l aquaria. Each aquarium contained a Perspex holder housing eight egg hatching baskets (see Bacher and O'Brien 1989), into each of which was placed a single batch of eggs. Aquarium water was aerated, recirculated and kept chilled to between 9.5 – 10.5 °C. Hatching baskets were removed each day from aquaria, placed under a microscope and eggs inspected for fungus. Any found to be infected or killed by fungus were removed using sterilised tweezers. The presence and degree of embryo development was visually inspected and the time and date of any hatching recorded. Following inspection all hatching baskets (along with eggs) were placed into a salt solution (10 g/l) for 20 min to minimise the possibility of fungus infection, before being returned to the aquarium.

RESULTS

Spent female *G. fuscus* were present at all sites in surveys conducted 21 Sept 2010. Subsequent egg searches undertaken 28 – 30 Sept 2010, located 13 egg clusters: four in Kalatha Creek (the least sediment and fire affected site); eight in Luke Creek (the moderately sediment and fire affected site); and one in S Creek (the most severely sediment and fire affected site). Individual clusters were adhered to the downstream side of cobbles (115 – 280 mm, mean 180 mm) close to the stream bed, in riffles immediately upstream of pools, in moderately to fast flowing (0.4 – 1.9 m/s, mean 1.0 m/s), shallow (20 – 310 mm, mean 174 mm), cool (8.4 – 10.0 ºC, mean 9.1 ºC), fresh (35.3 – 56.6 EC, mean 44.7 EC), slightly acidic (5.7 – 7.1 pH, mean 5.9 pH), well oxygenated clear water (10.8 – 12.4 mg/l, mean 11.3 mg/l). Clusters were composed of up to 218 (mean = 78) eggs, were multi-layered (up to three layers), and coated with sand and fine gravel particles. Eggs were not found attached to timber debris, aquatic plants, or moss. No eggs or larvae were collected in kick samples or larval drift nets.

Water-hardened, fertilised eggs were spherical, approximately 3 – 4 mm in diameter, adhesive, demersal, and transparent to relatively opaque. Embryos in approximately half of the egg clusters from Kalatha Creek, and the majority from Luke Creek, were sufficiently developed to clearly distinguish their eyes when visually inspected in the field. Embryos in the egg cluster from S Creek were fully developed and hatched within 30 minutes of being located and removed from the creek 29 Sept 2010.

Eggs from Luke Creek placed into the aquarium facility hatched 6 Oct 2010 –11 Nov 2010, those from Kalatha Creek 1 – 17 Nov 2010, and those from S Creek 29 Sept 2010 – 5 Oct 2010. Ninety percent of eggs hatched within 44 days of being brought into captivity, with the last eggs hatching by day 48.

Newly hatched larvae were transparent, 8.4 – 9.7 mm in length (mean 9.0 mm, n = 10) and were active swimmers which utilised the entire water column excluding times when they were seen to periodically lay motionless on the bottom of aquaria in the days immediately after hatching. Yolk sac (1.5 – 2.0 mm in diameter) absorption was generally complete within 3 days of hatching, and feeding commenced within 24 – 48 hours of hatching. Larvae appeared to use the entire water column for feeding, were only limited by gape size as to what they were feeding, and readily switched from one feed to another.

DISCUSSION

This study confirms that *G. fuscus* are a demersal egg layer, preferring to use nest sites on cobble substrates located in moderate to fast flowing water. Eggs are relatively large and generally laid in a tight multi-layered cluster, spawning occurs during late austral winter to early spring, and the time of larval development is relatively long. As the only other nest sites located (n=2) prior to this study were found attached to boulders (Raadik, T. unpublished; Raadik 1993; Raadik et al. 1996; Raadik et al. 2010), a substrate size that was lacking in our study streams, the combined findings suggest that *G. fuscus* prefer to lay eggs on larger in stream rock substrates, and to avoid pebbles and gravels.

Despite the average fecundity of mature females suggested as being ~500 (Raadik et al. 1996), individual nest sites found in this study had an average of ~80 eggs present. This suggests that females may spawn at multiple sites, laying many, small clusters of eggs, thereby reducing the risk of potential loss of all eggs deposited if laid in a single cluster. This strategy is uncommon in the Galaxiidae, having only been documented in the flat-headed galaxias (*Galaxias rostratus*) (Llewellyn 1971), which is comparatively highly fecund, and lays batches of eggs over an extended spawning period of up to a month (Llewellyn 1971). The spawning period of *G. fuscus* is alternatively likely to be relatively short, as we found the proportion of mature/ripe females declined rapidly once spawning began at individual reaches. Therefore if *G. fuscus* undertake spawning at multiple nest-sites, it is likely that this occurs over a period of days, rather than weeks.

Eggs collected from the wild took a maximum of 48 days to hatch in captivity. Assuming eggs which hatched last were spawned just prior to collection, this finding extends the suggested incubation period by at least 18 days (Raadik 1993; Raadik et al. 1996; Raadik et al. 2010). However, a strong relationship between development of larvae and ambient water temperature exists for many fish species (Pauly and Pullin 1988; Pepin 1991; Pepin et al. 1997), and therefore annual differences in stream temperatures would likely alter the incubation period of eggs of the species. Back-calculating by the egg incubation period of 30 – 48 days suggests a spawning period for *G. fuscus* lasting from about mid-August to the end of September (late austral winter to early spring).

Differences in the stage of maturation, and in the subsequent date of hatching, of eggs between the three study streams indicates spawning was not synchronous across the populations within a small geographic area (~10 km^2). The S Creek population probably spawned several weeks prior to the Luke Creek population, which in turn spawned one to two weeks earlier than the population in Kalatha Creek. Similar variation in the time of breeding in other galaxiid species has been attributed to water temperature (O'Connor and Koehn 1991; Allibone and Townsend 1997) and changes in stream levels (Moore et al. 1999). However, environmental cues that initiate spawning were not obvious in this study and could not be directly associated with changes in water flow or water temperature, although spawning did occur at a time when water temperature was increasing. Photoperiod may also be influential (Shirley and Raadik 1997). However, the lack of synchronicity across the populations in the current study suggests additional stimuli could be responsible. As fire had recently removed much of the riparian vegetation and over-storey canopy cover at S Creek, and to a lesser extent at Luke Creek, it is possible that such differences may be attributed to increases in light intensity, and resident fish perception of photoperiod at these sites. Similar changes in the time of spawning as a consequence of photoperiod alterations, often independent of temperature, have also been shown in other fish species (see Björrnsson et al. 1998; Davies and Bromage 2002; Elliot et al. 2003; Howell et al. 2003).

Nest-site characteristics of *G. fuscus* are similar to that described for the ornate mountain galaxias (*G. ornatus*; see Raadik 2014). Both lay a small number of relatively large, adhesive eggs in a protected site, usually on rock (O'Connor and Koehn 1991). In addition, both barred and ornate mountain galaxias lay their eggs predominantly in riffles, where the surrounding water is relatively fast-flowing and well-oxygenated (O'Connor and Koehn 1991). Adhering eggs to large stone substrates can be advantageous as the substrate is relatively stable and thus eggs remain within the area chosen by the parent. However, demersal egg-laying may result in eggs being susceptible to environmental disturbances to streambeds, such as siltation (Growns 2004). In addition, reduced water levels during the breeding season may expose spawning habitat or eggs at nest sites, thereby limiting spawning habitat availability and reducing egg survival and overall spawning success (Moore et al. 1999). Similarly, post-fire sedimentation can reduce the availability of spawning habitat thus limiting spawning potential, or smother eggs at nest sites causing egg mortality and decline in spawning success (O'Connor and Koehn 1991).

The study reconfirms the importance of larger loose rock substrates for *G. fuscus* reproduction (spawning), and highlights that the loss of such habitat could result in the decline of remnant populations of the species. Similar loss of habitat has been implicated in the decline of a number of fish species worldwide (see Scott and Helfman 2001; Pillar et al. 2004; Wyatt et al. 2010). Much of this habitat has been lost due to anthropogenic degradation of riverine habitats, primarily through siltation. Rehabilitation of streams is today common, however, the strategy is often tailored towards improving habitat of larger, auspicious, recreational fish species. To date, little augmentation of suitable substrates for smaller bodied, demersal egg laying freshwater fish has occurred. The introduction of rock substrates to streams affected by siltation may be of particularly value where threatened demersal egg laying fish exist, and where remediation works (such as fencing and replanting of the riparian zone) has occurred.

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