A captive spawning and rearing trial of river blackfish (*Gadopsis marmoratus*).

Efforts towards savings local genetic assets with recognised conservation significance from the South Australian Murray-Darling Basin.

Simon Westergaard and Qifeng Ye

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Cover: River blackfish hiding in PVC tube.

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EXECUTIVE SUMMARY

Extended low rainfall conditions across south eastern Australia, including the Murray-Darling Basin (MDB) and south east (SE) of South Australia (Murphy and Timbal 2008), have lead to significant reductions in the availability and quality of aquatic habitat (Hammer 2007a; Slater and Hammer, 2009). The drought conditions have the potential to severely impact upon native fish populations, especially those which are already restricted and threatened with either local or state wide extinction (Hammer 2007b).

For the population of river blackfish in Rodwell Creek (Bremer River catchment, eastern Mount Lofty Ranges (EMLR)), the situation was deemed critical in March 2008 when one pool dried and the population became restricted to a single pool. Prior to habitats deteriorating and potentially drying, 9 fish were rescued and transported to SARDI Aquatic Sciences for captive maintenance during 2008/2009. The primary goal was to achieve short-term population survival with view to eventual restocking when and where possible.

For successful reintroductions or indeed to simply preserve the currently held captive population, the development of techniques for propagating the species is crucial. To date scant data exists on the captive spawning and subsequent rearing of river blackfish. In 2009/2010 a project was established by SARDI Aquatic Sciences, with request from SA Department for Environment and Heritage, to trial captive spawning and rearing techniques and develop protocols for river blackfish.

This report describes the first account of captive spawning of river blackfish and 'natural inducement' methods were successfully used in this study. Fish responded well to tank conditions with viable eggs and larvae produced. In this first attempt, offspring were successfully raised through to a juvenile stage, however, production was limited by a low male: female ratio of brood stock and fungal infections during egg incubation. To combat this for future production it is suggested that an increased number of fish are collected to achieve a paired collection of broodstock as well as chillers are used to facilitate better temperature control in broodstock tanks.

1 INTRODUCTION

Across south eastern Australia, including the Murray-Darling Basin (MDB) and south east (SE) of South Australia (Murphy and Timbal 2008) extended low rainfall conditions have lead to significant reductions in the availability and quality of aquatic habitat (Hammer 2007a; Slater and Hammer, 2009). The potential for severe impacts upon native fish populations under such drought conditions exists, especially those which are already restricted and threatened with either local or state wide extinction (Hammer 2007b). These include the following native fish all listed as endangered in the SA action plan:

- Murray hardyhead (*Craterocephalus fluviatilis*),
- **River blackfish** (*Gadopsis marmoratus*)**,**
- Southern purple spotted gudgeon (*Mogurnda adspersa*),
- Southern pygmy perch *(Nannoperca australis*),
- Yarra pygmy perch (*Nannoperca obscura*) and
- Variegated pygmy perch (*Nannoperca variegata*).

Murray hardyhead and Yarra pygmy perch also have a national status of vulnerable under the Environment protection and biodiversity conservation act 1999. River blackfish are protected by Fisheries Management (General) Regulations 2007 under the Fisheries Management Act 2007. The Drought Action Plan for South Australian Freshwater Fishes by Hall *et al.* (2009) identified 27 sites with populations of the above six species that were at risk of extirpation due to the prevailing drought conditions. This included four sites where river blackfish occur in the SA MDB. These sites are monitored to gauge population trends and habitat quality.

River blackfish (*Gadopsis marmoratus*) have a widespread distribution in south eastern Australia, (Merrick and Schmida 1984; McDowell *et al* 1996; Allen *et al* 2002) occurring in suitable habitats in Victoria, NSW, Queensland, South Australia and Tasmania. This includes coastal drainages in Victoria, Tasmania, South Australia, and also in the MDB within Victoria, NSW, Queensland, and South Australia. Nevertheless, the abundance of this species has decreased considerably in the last century; apparently a result of susceptibility to siltation and overfishing (Allen *et al.* 2000).

In South Australia, river blackfish have undergone extensive range reduction since European settlement (Loyd and Walker 1986; Lloyd 1987; Hammer 2004; McNeil and Hammer 2007) with well documented population reductions and evidence for the local extinction of one isolated population in recent years (Hammer 2002, 2005, 2006 and 2007a; McNeil and Hammer 2007). Declines are likely due to the effects of anthropogenic change upon the landscape compounded by a recent period of extended low rainfall (Hammer 2007a; Hammer 2007b; Slater and Hammer, 2009). Populations currently remain in the eastern Mount Lofty Ranges (EMLR) within the lower MDB and in the SE. The Drought Action Plan for South Australian Freshwater Fishes (Hall *et al.* 2009) identified five sites with populations of river blackfish in the SA MDB that were deemed to be at risk of extirpation due to the prevailing drought conditions. These four sites are within the Marne River, Bremer River, Angas River and Tookayerta Creek catchments. A review of monitoring data by Hammer (2006) indicated that the Marne and Bremer populations are at greatest risk of being isolated and restricted to a string of pools.

River blackfish tend to show localized biodiversity. Miller *et al* (2004) recently suggested the occurrence of at least three distinct species of *Gadopsis* in south eastern Australia with four evolutionary significant units in Victoria alone, an indication of localised biodiversity for the species. Biodiversity between those populations in SA MDB and those in victoria and south eastern Australia are yet to be investigated but are suspected to be significant. Never the less, Moore *et al* (2010) recently recognized the SA MDB river blackfish as a genetic asset of conservation significance.

In 2008, following low rainfall and low inflows, water levels in Rodwell Creek (Bremer River catchment, EMLR) receded and the river blackfish population became restricted to a single pool. Furthermore, in March 2008, water quality and in-stream habitat integrity deteriorated to a point where a significant risk to population survival was deemed to exist. Given that for other species oxygen concentrations below 1.0 mg O_2 L^{-1} have been shown to dramatically effect fish behaviour (McNeil and Closs 2007), causing fish to surface increasing predation risk and that lower concentrations death presumably cause death. Before dissolved oxygen (DO) concentrations dropped to levels believed to be too low for species survival (i.e. 0.8 ppm: Michael Hammer, unpublished data) a number of the fish (n = 9) were captured and transferred to the SA Research and Development Institute's (SARDI) Aquatic Sciences Centre at West Beach in late March 2008.

Similarly populations of river blackfish in the South East of South Australia are also at risk with significant range contraction and population declines. A single specimen in Henry Creek in 2002 without subsequent recordings (Hammer 2002 and 2007a), indicates the likely loss of a population at this site. A status assessment by Hammer (2009), reported a 90% reduction in abundance and 60% reduction in occupied sites for river blackfish within Mosquito Creek between 2006-2008. With only five fish located in Mosquito creek in summer 08 sampling, and concerns of local extinction, these fish were placed into captive maintenance at Kingston Community School (Slater and Hammer, 2009).

As such the successful development of captive spawning and rearing techniques for blackfish are immediately relevant for population conservation of river blackfish from Rodwell Creek in the EMLR and also Mosquito Creek in the state's SE.

River blackfish were rescued from deteriorating habitat in Rodwell Creek with the primary goal of achieving short-term population survival with view to eventual restocking where possible; a plan suggested for endangered fish species by a number of authors (i.e. Philippart 1995: Maitland 1995; Crook and Sanger 1997). For successful reintroductions or indeed to simply preserve the fish currently held in captivity, developing techniques for propagating the species are crucial. Some information on egg or larval development is provided by Jackson (1978), however no data exists on the captive spawning and subsequent rearing of river blackfish. Nonetheless it is desirable for future conservation measures that the reproductive characteristics of river blackfish be documented as has been done for other native freshwater species such as spangled perch, southern pygmy perch, southern purple spotted gudgeon and crimson spotted rainbowfish (i.e. Llewellyn, 1972, 1974 and 2006: Reid, *et al* 1995).

The aim of the current project is to adapt captive spawning and rearing techniques used for other species and trial these for use with river blackfish. The study will address key knowledge gaps that limit current outcomes from captive maintenance and provide opportunities for artificial spawning and recruitment. It will thereby contribute towards the effective management and conservation of threatened native fish by developing and setting protocols for captive breeding. These protocols will guide future propagation operations and allow restocking and other emergency conservation measures which reduce the likelihood of protected species such as river blackfish from being lost in the state.

The specific objectives of this study were:

- To trial natural spawning cues, by manipulating temperature and photoperiod to induce captive spawning of river blackfish.

- To rear any subsequent offspring from currently held stock of river blackfish from the threatened Rodwell creek population and assess techniques used.

These objectives have been targeted to provide essential knowledge to meet recommendations from the Drought Action Plan for South Australian Freshwater Fishes (Hall *et al* 2009).

2 REPRODUCTIVE BIOLOGY BACKGROUND

River blackfish spawn in spring and early summer, reportedly when water temperatures reach around 16°C (McDowell 1996). Fish form pairs in days prior to spawning (McDowell 1996; pers. obs.) and occupy a potential spawning site, which is often a hollow log (Jackson 1978). (O'Connor and Zampatti 2006) observed the use of rocky crevices as a spawning substrate by the Two-spined blackfish (*Gadopsis bispinosus*) indicating that river blackfish (*G.marmoratus)* may also adapt to use a variety of in stream structures as spawning sites. Fecundity is low, with Jackson (1978) reporting a linear increase with body length. McDowell (1996) reported fecundity in the order of 40 eggs from a 120 mm fish, 300 eggs from a 250 mm fish and 750 eggs from a 320mm fish. Eggs are large (approximately 4 mm in diameter) and laid attached to a surface in a patch guarded by the male. Similar to many other nest brooding fish (speleophils, Balon 1981) (or 'C2 guild', Growns, 2004), the eggs are gently fanned by the male.

Duration of egg development prior to hatching is temperature dependant, however McDowell (1996) reported 14 days at 15°C and Jackson (1978) similarly 16 days at 12 to 20°C. At hatch the larvae measure around 7 mm. The embryo remains attached at the spawn site with a large yoke sac still inside the chorion for around 19 days. The larvae then wriggle free and are negatively buoyant. At this stage larvae are approximately 13.5 mm total length. Jackson suggests yoke-sac is almost completely absorbed after 26 days and the eleutheroembryonic phase is complete. Around this stage, at approximately 15 mm, the juveniles begin to actively forage.

3 MATERIALS AND METHODS

3.1 Sourcing and general maintenance

Fish (total *n* = 9) were collected from Rodwell creek using box traps baited with crushed yabbie on 22 and 26 March 2008. Fish were transported in aerated buckets and allowed to gradually acclimate to the temperature and water quality in captive facilities. This took over 2 hours by the slow addition of water before being placed into a 2000L tank recirculating at 7500L/hour through a canister type biological filter. At SARDI Aquatic Sciences Centre (West Beach, South Australia) water temperature was maintained around 10-14°C during winter and 17-21°C during summer months. For 2 weeks during December 2008 the air-conditioning system failed and water temperature rose to 26-27°C within two days. Attempts were made to alleviate heat stress via additional aeration and ice. Fish appeared stressed, but survived this event. It was noted that fish became more aggressive than normal during warm conditions where water temperatures were above 20°C. In June 2009, broodstock fish were transferred to three 500L fibreglass tanks as these allowed for easier observation. On 6 October 2009 fish were moved to a controlled environment room (CER) and held in pairs in 300L aquaria.

3.2 Spawning inducement

Fish were held in 500L fibreglass tanks initially and later moved into 300L aquaria in a controlled environment room. When moved to the 300L aquaria fish were paired where possible, though at this stage is was apparent of the nine fish in total, only 2 fish were male. Photoperiod in the Aquarium room facility was 12:12 and water temperature varied whilst temperature in the CER room facility was steady (table 1).

Table 1. Water quality parameters in broodstock tanks

Location	Temperature (°C)	Dissolved oxygen (ppm)	TDS (ppm)	pH
500L tanks (March 08 -Oct 09)	10-17.9	9.3 ± 2.2	0.57	7.62
300L tanks in CER (0ct 09 -Dec)	17 ± 1.1	9.46 ± 1.9	0.46	7.49

Photoperiod was increased over three week periods from a 12:12 light dark ratio to 13:11 and then 14:10. No hormone manipulation was used in the current trial. To condition fish for spawning they were fed daily on a varied diet including live *Daphnia* sp., live earthworms and chopped prawn. Two species of Cladoceran, *Daphnia magna* and *Daphniopsis australis*, were cultured in outdoor ponds (figure 1) using methods similar to that described by Hoff and Snell (1989).

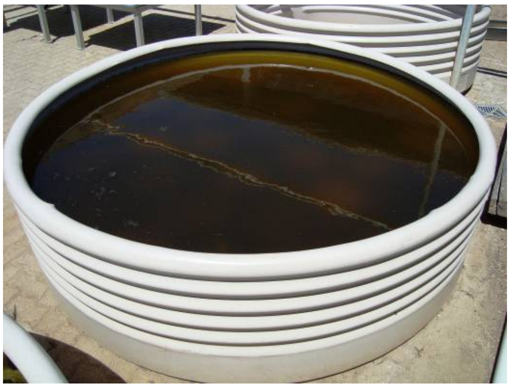

Figure 1. Tank used to culture *Daphnia sp*.

A backlighting technique was trialed for determining gender on 20 September 2009 and 9 October 2009 when moving fish from the 500L tanks into the 300L aquaria and also provided an indication of gonad development. This involved placing fish in a small aquarium, with a fluorescent light shining from behind. The light penetrating through the fish made it possible to see the orange colour and outline of eggs within the gonads in female fish. Males appeared darker with less light being able to pass through the gonad.

3.3 Egg development and husbandry

Eggs were removed from broodstock as soon as spawning was complete and incubated in 50L aquaria. Eggs were held in hatching trays made with a fibreglass fly wire base. Gentle airlifts supplied water flow near the trays which were kept horizontal to prevent eggs from clumping together. Daily water changes were made to ensure adequate water quality. The addition of methylene blue at a rate of less than 100 ppm (Ramnarine, 2001) was used to prevent fungal infection for the first spawn of eggs. Concentrations of 1500 uL/L of formalin for 45 minutes every second day have also been suggested for the treatment of fish eggs (Rach, 1997). For Murray cod Ingram (1999) suggested that during the incubation period and until commencement of hatching, eggs are given a daily prophylactic treatment of 1.0 mL/L formalin for 30 min to prevent fungal infection. This method was used for the second spawn of eggs. After spawning, dead (opaque) and infected eggs were carefully removed with tweezers to prevent further spread. Tweezers were dipped in alcohol between batches to prevent spread of infectious pathogens.

3.4 Larval and juvenile development and rearing

Digital images of egg and larval development were recorded using a microscope and camera linked to a desk-top PC using Image-pro version 7.0. An image was captured daily for the first 5 days and then every second day until day 25 post hatch, with final image taken on day 30 at completion of yoke sac.. Post hatch lengths (TL in mm) were measured from a random sample of fish (*n* = 3 or 4) on the same day that images were taken using a calibrated digital measurement function in Image-pro. Fish were offered *Artemia* sp. nauplii from day 20, prior to this endogenous energy is supplied completely from the yoke sac. live *Daphnia* sp. and frozen Bloodworm after day 30.

3.5 Statistical analysis

Linear regression analyses were performed to determine the relationship between larval length and age.

4 RESULTS

4.1 Spawning inducement

Adult river blackfish were successfully induced to spawn in captivity using 'natural' stimuli. Fish responded well to tank conditions and live feeds offered. Fish gained condition quickly through july and august with gonad development obvious after live *Daphnia* sp. and earth worm were regularly consumed (figure 2). Gonad development was apparent in all female fish when the backlighting technique (figure 2) was used. This backlighting technique was useful even for the largest fish (290 mm TL, total length). This technique showed that out of the nine fish only two were male, this limited production severely and lead to attempts to re-spawn from these male fish in the same season.

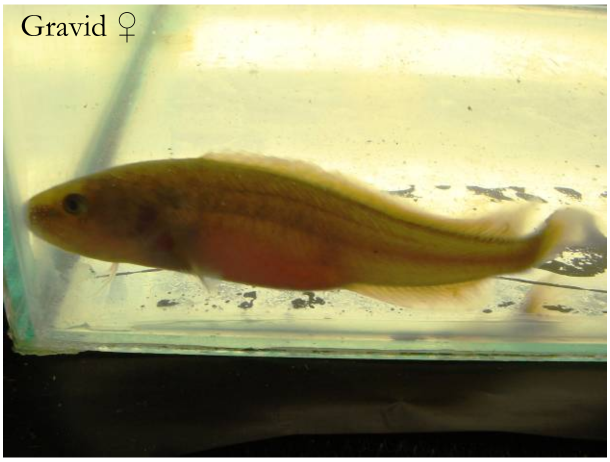

Figure 2. Gravid female river blackfish (6wks before spawn). Gonad development is obvious when backlighting is applied.

The first spawning event occurred prior to planned photoperiod and temperature manipulations when temperature was at 14°C with a light dark ratio of 12:12. There was a trend of increasing temperature over the prior three months (figure 3). This trend occurred due to tank exposure to increasing ambient temperature prior to fish being transferred to tanks within the CER room. The trend of increasing temperature appears to correlate with observations of gonad development in the female fish (figure 3). A the first spawning occurred earlier than expected in conditioning tanks and not in spawning tanks that allowed better observation, a number of eggs were dislodged from the spawn site when inspecting PVC tubes.

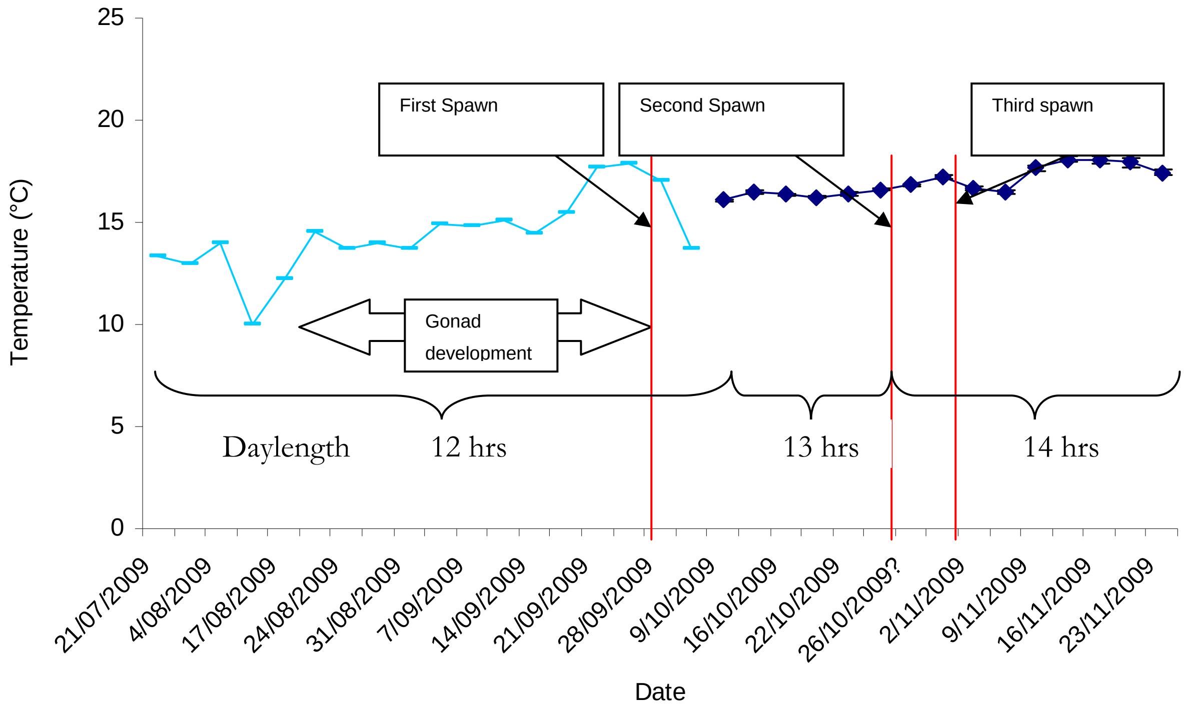

Figure 3. Temperature proceeding spawning, showing where gonad development is believed to have occurred subsequent to slight trend of increasing temperature, with subsequent spawning events.

The second spawning event this time from a different pair of broodfish occurred 13 days after day-length was increased to 13 hours and 18 days after temperature rose to a steady 16°C. A third spawning occurred 7 days after day length was increased to 14 hours, however these eggs were immediately eaten by the male. It should be noted this third spawning event was the second spawning involving this male fish, having spawned only 4 weeks earlier, and had in previous weeks behaved aggressively towards advances by the second female. Due to the earlier mentioned lack of male fish, further attempts to pair females with males that had spawned in previous weeks were not successful.

During unsuccessful spawning female fish were observed to approach and chase males with males showing no interest or in some instances displaying aggression towards the female. During successful spawning female fish were observed to present near to males, which showed interest by displaying and nudging the female who then followed the male fish into the PVC tube spawning site Eggs from successful spawning were discovered in tubes less than 15 hrs post lay in both successful spawning events (figure 4a). Eggs were removed from the male's care and incubated artificially (figure 4b).

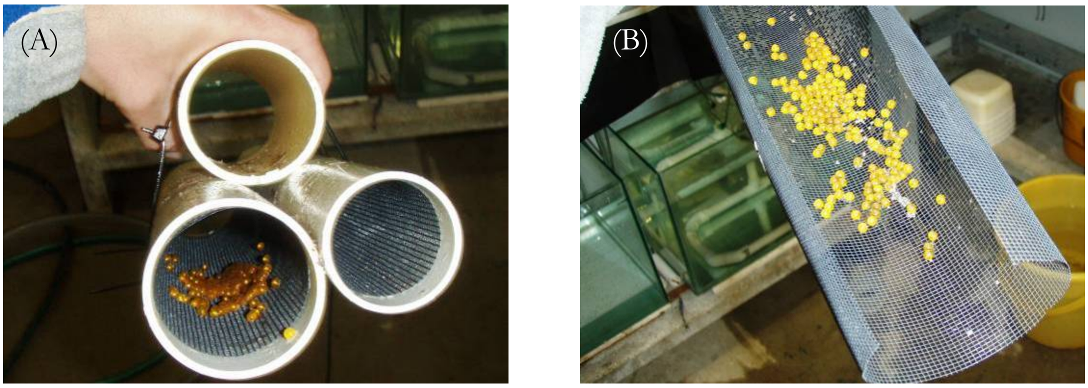

Figure 4. (A) River blackfish eggs within spawning tube; (B) carefully removing eggs attached to a fly-wire insert.

Observed fecundity is presented in Table 2, however the number of eggs spawned in the third spawning event could not be determined as eggs were consumed by the male.

Table 2. Observed river blackfish fecundity and fish length.

	♀ Broodfish TL (mm)	Fecundity (no.eggs)
Spawn 1	Bf7 - 224	147 + unknown lost
Spawn 2	Bf3 - 150	168
Spawn 3	Bf2 - 142	unknown

4.2 Egg development and husbandry

Eggs removed from the male fish's parental care were incubated artificially in glass aquaria supplied with aeration (figure 4b). These received a 50% daily water exchange and outflow from airlifts were directed towards eggs. Mean egg diameter (width) was 4.15 mm ± 0.01 s.e. (n = 72), and mean egg height was 3.78 mm ± 0.04 s.e. (n = 30). Embryonic axis was first observed on day two (figure 5). A heart beat was first observed on day six, located anterior to the embryos head. On day nine circulating pigmented red blood was noticeable (figure ?). Eggs hatched mostly on day 15-16 with a few hatching as late as day 21 (figure 6) post-lay at 16°C.

The first spawn was discovered before fish were moved to the new facilities, as such observations were limited to the discovery of eggs while lifting a PVC tube from the tank. As a result many eggs were dislodged from the spawning tube. These eggs were gathered and placed in a hatching tray but suffered from fungal infections and a subsequently high mortality rate of 90% was recorded. A number of eggs from the second spawn also suffered fungal infection resulting in a mortality rate of approximately 80% during the mid-late stages of egg development. Prophylactic treatment with methylene blue at a rate of less than 100 ppm (Ramnarine, 2001) for spawn 1, and formalin at 1500 uL/L for 45 minutes every second day (Rach, 1997) for spawn 2 combined with careful removal of dead (opaque) and infected eggs with tweezers reduced infection rates, though the infection continued to persist.

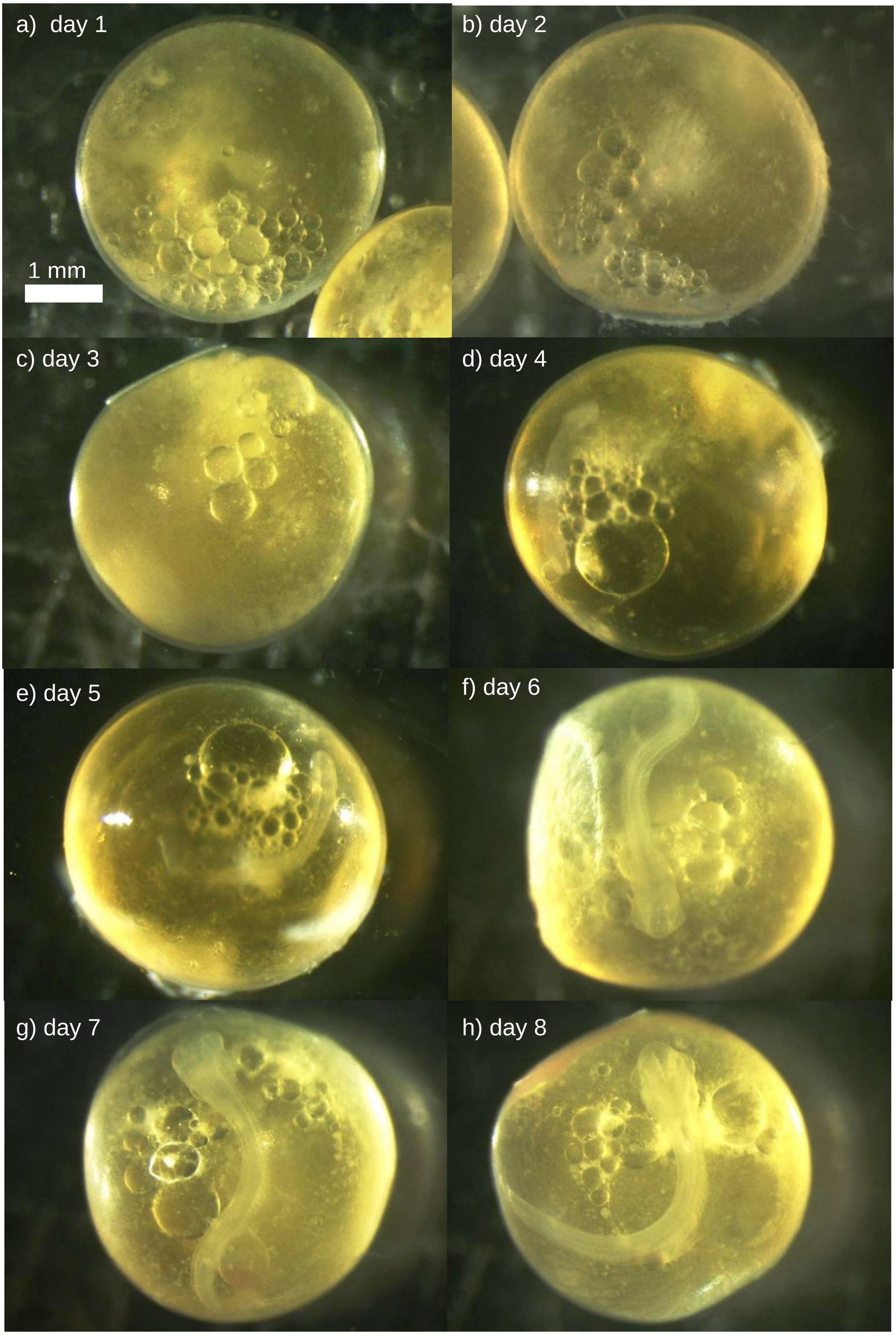

Figure 5. (a-h), River blackfish egg development day 1 through to day 8; images taken every 24hrs. Day 1 photograph is less than 15hrs post lay.

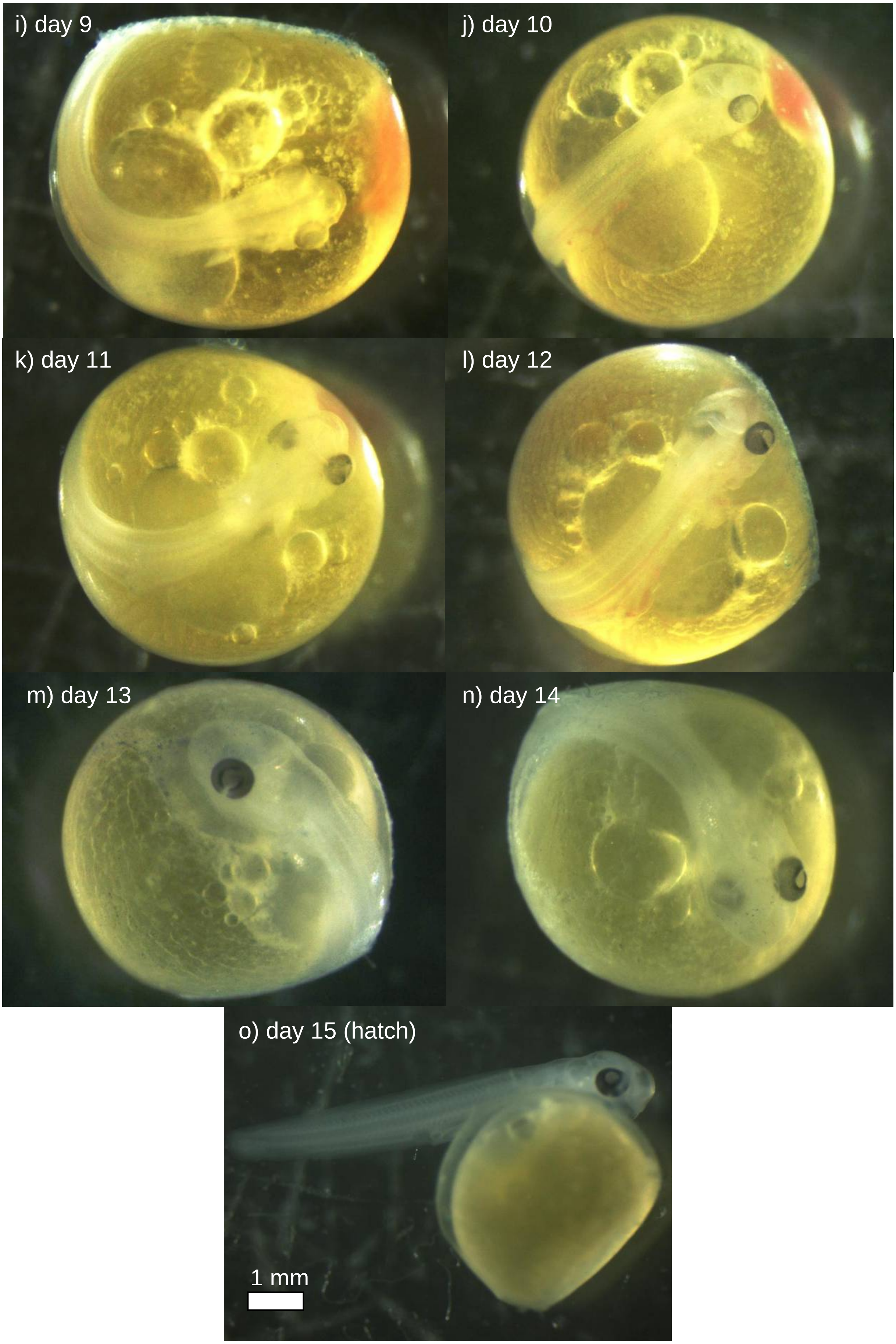

Figure 6. (i-o), River blackfish egg development day 9 through to hatch at day 15.

4.3 Larval and juvenile development and rearing

Mean total length of larvae at hatch was 7.78 mm ± s.e. 0.20 (*n* = 10). Larvae carried yolk for at least 30 days post hatch (figure 8 and 9). Although Larvae were offered *Artemia* sp. nauplii at day 20 they began feeding on day 22-25. Linear regression analysis was performed on total length at age until completion of yoke sac absorption (day 30?) (figure 7). Larval total length showed a close relationship with age (R^2 = 0.9154) with length increasing with age in a linear fashion.

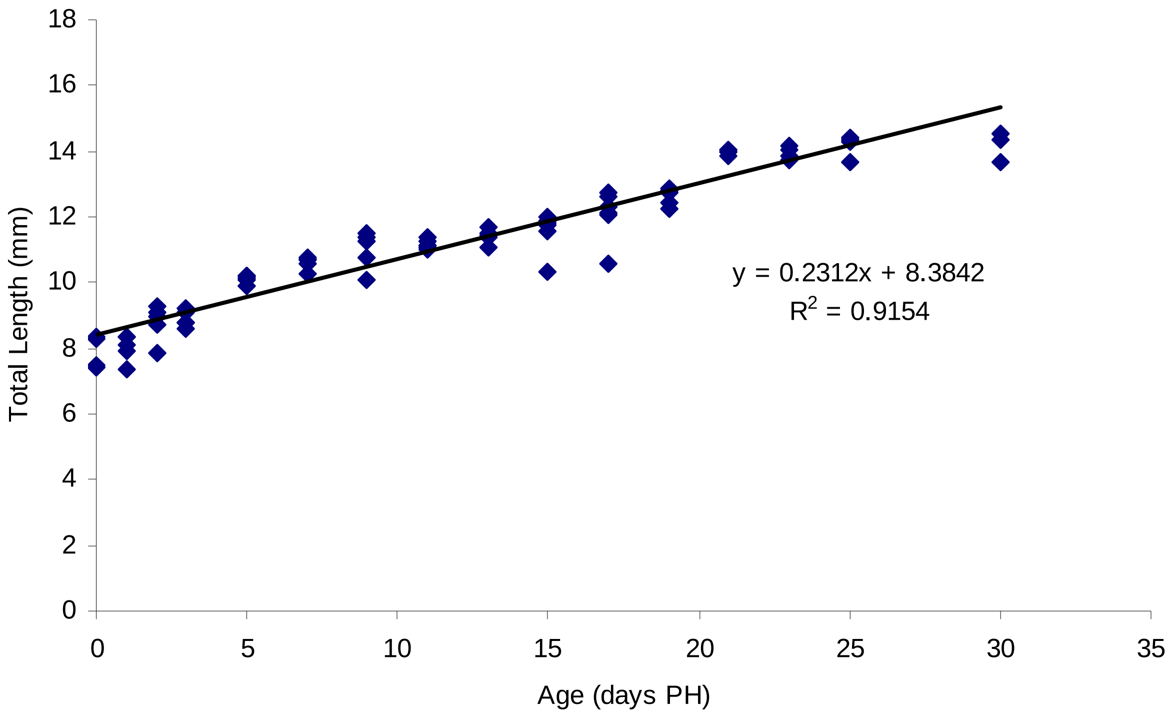

Figure 7. River blackfish larval length with age (days post hatch).

While handling larval fish for image collection was performed with the greatest of care, a number of larvae were lost during larval and early juvenile stages, probably due to post handling stress complications (i.e. secondary infections). In total 6 fish made it through to a juvenile stage and a length of 30-40 mm TL at 4 months of age.

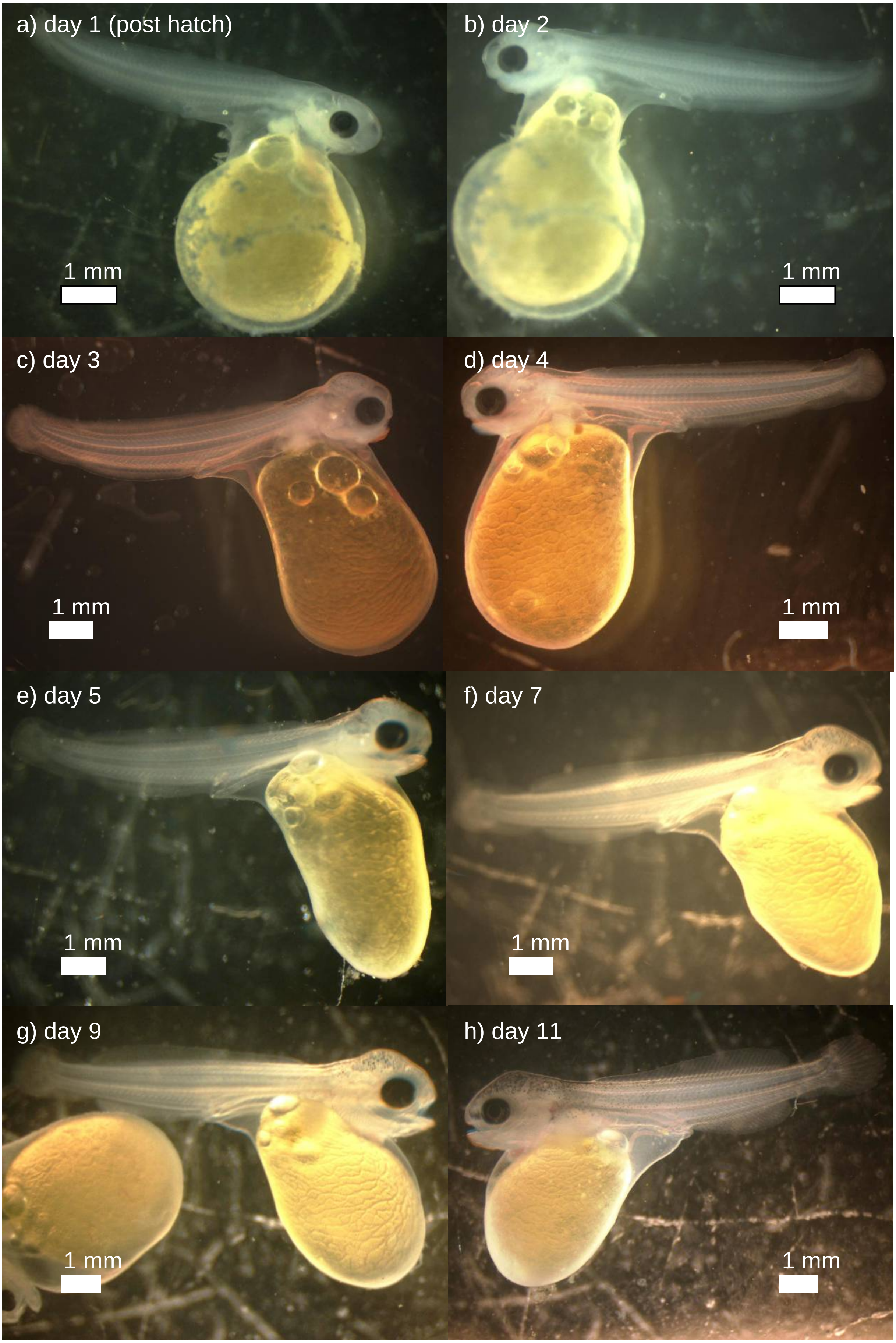

Figure 8. (a-h), River blackfish larval development day 1-11 post hatch.

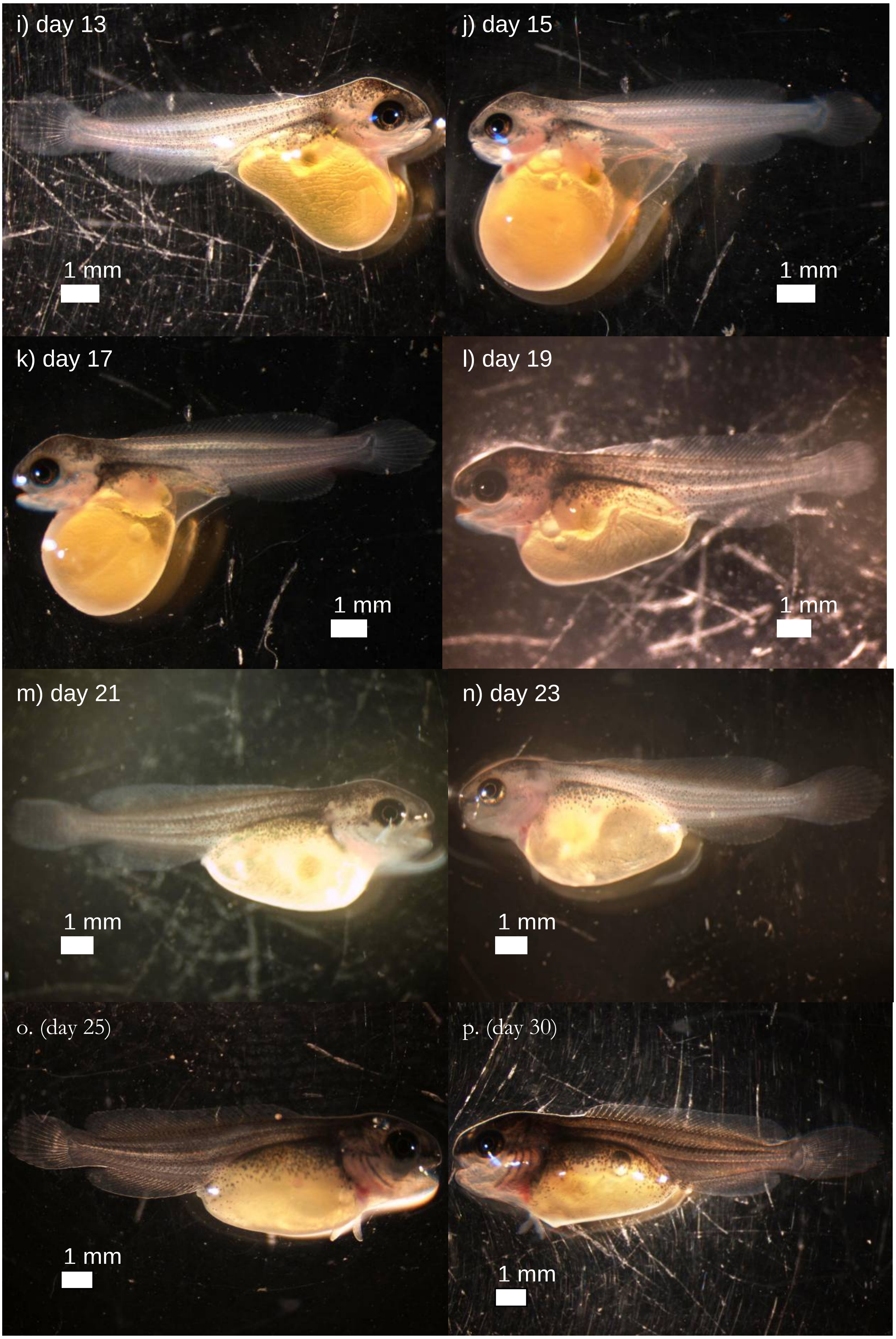

Figure 9. (i-p), River blackfish larval and juvenile development day 13-30 post hatch.

5 DISCUSSION

5.1 Spawning inducement

Previously attempts to spawn river blackfish in captivity have been made (NSW-DPI, 2006) without any reported success. This report describes the first account of captive spawning of river blackfish and was achieved using 'natural' spawning cues without the use of hormones.

Fish responded well to tank conditions and were observed to gain condition rapidly. The use of live cultured *Daphnia* sp. appeared crucial to conditioning for this trial, as no other foods offered were accepted and consumed with such vigour and in such volume. As it is difficult to determine river blackfish gender from external characters, the ratio of male: female fish was not known until fish began to condition and become ripe. By chance, the majority of captive held fish were female, 7 females and 2 males. All female fish showed gonad development and successful spawning was undertaken by all male fish. Attempts to pair ripe females with males that had spawned in weeks prior, highlighted an unwillingness by the male fish to spawn a second time. This suggests that male river blackfish may only spawn once in a season. To make full use of captive specimens in the future it is suggested that paired fish be acquired to maximise efficiency and outcomes.

The temperature regime preceding spawning is believed to have played a crucial role in inducing gonad development in the current trial as has been suggested for other species such as Murray cod (*Maccullochella peelii*) and golden perch (*Macquaria ambigua*) (Lake, 1967a; Humphries *et al* 1999). Gonad development is believed to have occurred subsequent to a slight trend of increasing temperature. This hypothesis for river blackfish is supported by observations of gut shape during this period. Indeed during the later course of this period, ova were seen inside the fish when backlighting was applied. Future captive spawning of the species may be improved with enhanced temperature manipulation.

The fecundity of *Gadopsis* species is low with that observed here comparing well to that reported by Jackson (1978), therefore efficient production of juveniles for re-stocking would require a larger number of paired fish. Nonetheless, the species responds well to tank conditions and thus such a program could be feasible. When handled appropriately, the adult river blackfish (Rach, 1997) show few symptoms of stress in the captive environment which is desirable given that stress is an important factor that can effect egg or sperm quality (Campbell, *et al* 1992; Rurangwaa. *et al* 2004.

Indeed the successful egg fertilisation rate and observations of what appeared amble yoke supplies providing energy well for advanced development in the current study suggests fish did not likely suffer considerably from stress. Though it cannot be completely discounted that egg or sperm quality may have influenced egg survival rates it is though that because diet was varied and fish behaviour gave no indication of stress these factors are unlikely to have influenced any mortalities.

5.2 Egg development, larval and juvenile rearing

Fungal infections during the later stages of egg development, was the main limitation on hatch rate success. Preventative daily baths of both methylene blue (>100 ppm) and formalin (1.0 ml/L) failed to completely control fungal infection In the future, it is suggested pre-treating the incubation tank with sodium hypo-chlorite, then neutralising with sodium thio-sulphate and treating eggs with formalin (1.0 ml/L) before moving eggs into incubation tanks may prevent infectious pathogens further for future trials. Daily prophylactic treatment of 1.0 mL/L formalin for 30 min and careful removal of any infected or dead eggs may offer the best prevention of fungal infection. This is based on those methods used for Murray cod (Rach, 1997). Egg and larval growth rates from the current study compared closely with those reported by (Jackson 1978).

As a result of the larvae being large and well developed at completion of yoke sac absorption, providing *Artemia* sp. nauplii as a first food was very successful. The long endogenous yoke sac absorption period and subsequent large larval size, negates labour intensive larval feed production which benefits larval or juvenile survival and limits costs. Majority of larval mortalities with the current study are likely linked to handling, handling was performed to collect images and assess health, this will not likely be required for future rearing as such larval survival rates should be much improved. With experience from this current trial future attempts are likely to be much more productive.

5.3 Implications for conservation management

This report illustrates that river blackfish will respond favourably to tank conditions and spawn successfully in captivity. Thus, captive spawning for later reintroduction is a feasible tool for the conservation of this species. Nonetheless, several improvements may be made to the methods used in the current study to increase the number of larvae/juveniles produced.

The current CER facilities would not reduce temperatures below 15°C, thereby not reaching winter temperatures commonly seen as low as 8-10°C in there natural habitats (Hammer 2006). Making it difficult to ensure replicating spring conditions where-by temperature and daylength gradually increase to stimulate spawning. Future captive spawning of the species would be better assisted with improved ability to manipulate temperature; heater-chillers may provide greater ability to condition fish in preparation of spawning production runs.

In addition, it is suggested that for future production, a greater number of male brood-fish are collected to maximise the number of paired fish, given that it appears male fish are likely to spawn only once in a season. It is apparent that better methods for egg husbandry need to be developed to ensure adequate production of juveniles for any restocking efforts. Methods suggested are based largely on those used for Murray cod (Rach, 1997) as was trialed here, but in the future should be trialed with some extra precautions as suggested in the above discussion of egg development.

Immediate priorities for further research should include replicating the breeding trial using the knowledge and recommendations from the current trial. This would involve;

- Collection of an additional three or more male fish from Rodwell creek to achieve even paired numbers of broodfish in captivity and to acquire more breeding pairs if possible to promote a wider gene pool for restocking.
- Purchase of heater-chiller units to achieve improved manipulation capabilities for temperature.
- Testing the viability of spawning and rearing larger numbers of juvenile river blackfish in captivity.

The methodology developed is likely to be useful for other at risk populations of river blackfish in southeastern Australia.

The genetic relationship between SAMDB river blackfish and those elsewhere is as yet unknown (Miller *et al* 2004), and should also be investigated. This could identify genetic units of conservation significance informing management of priorities for conservation. A reintroduction plan for river blackfish should be developed to accommodate future successes in spawning and rearing river blackfish in captivity. This could provide protocols for determining when, where and how to reintroduce river blackfish in the EMLR and SE to secure their future.

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