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Captive spawning trials of river blackfish (*Gadopsis marmoratus*) during 2010/11

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

Simon Westergaard and Qifeng Ye

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SARDI Aquatic Sciences
PO Box 120 Henley Beach SA 5022

November 2012

Report to Department of Environment, Water and Natural Resources

Government of South Australia

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Cover: Juvenile river blackfish from previous years breeding hiding in PVC tube.

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DISCLAIMER

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EXECUTIVE SUMMARY

The river blackfish, *Gadopsis marmoratus,* population in Rodwell Creek (Bremer River catchment, eastern Mount Lofty Ranges (EMLR)), was deemed critically threatened in March 2008 when one pool dried and the population became restricted to a single pool. Prior to habitats deteriorating and potentially drying, nine fish were rescued and transported to SARDI Aquatic Sciences in 2008/09, where they have been captively maintained. The primary goal was to achieve short-term population survival with a view to eventual restocking when environmental conditions improved.

In 2009/10, a project was established by SARDI Aquatic Sciences, at the request of the South Australian Department for Environment and Heritage (now Department of Environment, Water and Natural Resources), to trial captive spawning and rearing techniques for the first time and develop protocols for captive breeding of river blackfish with the nine fish rescued from Rodwell Creek (Westergaard and Ye 2010). The current work follows on from the 2009/10 breeding trials.

During the two trials conducted in 2009/10, captive spawning of river blackfish through 'natural inducement' was successful. This was achieved by gradually increasing day-length (photoperiod) and temperature over 6 month trial periods in 300 L aquaria tanks. Within these 300 L tanks paired male and female fish were held in an effort to mimic spring-early summer conditions in order to induce gonad development/conditioning and spawning. Photoperiod was increased from an initial 8:16 light and dark ratio to 10:14, 12:12 and 14:10, respectively. Water temperature was also increased from 10 °C to 17.5 °C over the 6 month period. Fish responded well to tank conditions with viable eggs and larvae produced. In this first attempt, offspring were successfully raised through to the juvenile stage. However, production was limited by a low number of brood-stock, a low male:female ratio of brood-stock (2 males vs 5 females) and fungal infections during egg incubation.

In the current trials (2010/11), the same methodology was applied as the previous year's trials with the exception of new water chillers being used to help facilitate better temperature control in brood-stock tanks. Again as in the previous year, two 6 month trials were conducted in 2010/11. Fish responded well to tank conditions, showing gonad conditioning/development and some mating behaviours, however, spawning was unsuccessful. Although it's unclear why the trials were successful in the previous year but not in 2010/11, it is speculated that spawning did not occur in the current trials due to a low total number of brood-stock and a low number of male brood-stock, hence limiting mate preference/compatibility. It is also possible that brood-stock may not spawn more than once in a season and that the relatively small size of holding tanks in which the two pairs of fish were held may have been contributing factors. However, further research will be needed to validate these possibilities and to refine the methodology for river blackfish captive breeding. Developing a reliable breeding method will provide a conservation measure for this threatened species in the future.

1 INTRODUCTION

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 2007b; Hammer 2009; Slater and Hammer 2009). The drought conditions have the potential to severely impact upon native fish populations, especially those that are already restricted and threatened with either local or state wide extinction (Hammer 2006; Hammer 2007a; Hammer 2007b; Hall *et al.* 2009).

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, nine fish were rescued and transported to SARDI Aquatic Sciences in 2008/09, where they have been captively maintained. The primary goal was to achieve short-term population survival with a view to the eventual restocking when environmental conditions improved.

In order for successful reintroductions or to preserve the currently held captive population, the development of techniques for propagating the species is crucial. In 2009/10, a project was established by SARDI Aquatic Sciences, at the request of the South Australia Department for Environment and Heritage (now Department of Environment, Water and Natural Resources), to trial captive spawning and rearing techniques for the first time and develop protocols for captive breeding of river blackfish (Westergaard and Ye 2010).

The 2009/10 report (Westergaard and Ye 2010) described details of the first account of captive spawning of river blackfish using 'natural inducement' methods, with viable eggs and larvae produced. In this first attempt, offspring were successfully raised to a juvenile stage, however, production was limited fore-most by low numbers of brood-stock fish, a low male:female ratio of brood-stock and fungal infections during egg incubation. Based on this work, Westergaard and Ye (2010) suggested a number of changes to the methodology and equipment improvements in order to increase production numbers. These include:

- A header supply system be used for supplying consistent flow-through water to the incubation system;

- Disinfecting the header supply system and the incubation tank with sodium hypo-chlorite (100 ppm) for greater than 20 minutes (Lilley and Inglis 1997), then later neutralising with sodium thio-sulphate;
- Pre-treating eggs with formalin (1.0 ml/L) before moving eggs into incubation tanks; and trial using;
    - Formalin as a daily prophylactic treatment of 1.0 mL/L for 30 minutes (Rach 1997) with careful removal of any infected or dead eggs; and/or
    - Malachite green at 0.5 ppm as suggested by Lilley and Inglis (1997) with careful removal of any infected or dead eggs.

Being the second year of trials, the current work follows on from the 2009/10 breeding trials with refinements via extra preparations made and precautions taken from the above suggestions. Nevertheless, the general approach remained similar to the previous year's (i.e. using 'natural inducement'). The objectives of this study were:

- To further trial and refine the method for captive spawning of river blackfish from the currently held stock of the threatened Rodwell Creek population.
- To rear any subsequent offspring and assess the techniques used.

These objectives were targeted to meet the recommendations from the Drought Action Plan for South Australian Freshwater Fishes (Hall *et al.* 2009). The current report documents the method applied in the captive breeding of river blackfish during 2010/11, and provides a discussion about the outcomes.

2 MATERIALS AND METHODS

2.1 General Maintenance

Nine fish were originally collected from Rodwell Creek in 2008 (Westergaard and Ye 2010). These fish have been maintained at SARDI's Aquatic sciences facility at West Beach, with some breeding success through the trials in 2009/10 (Westergaard and Ye 2010). Two mortalities had occurred after the trials with seven adults left. Fish were housed in pairs in 300 L aquaria tanks, in a controlled environment room (CER), where they remained for the duration of the 2010/11 breeding trials. The total number of brood-stock fish was seven (5 females and 2 males) (Figure 1.A). Due to the limited number of male fish, only two male:female pairs were possible at any one time.

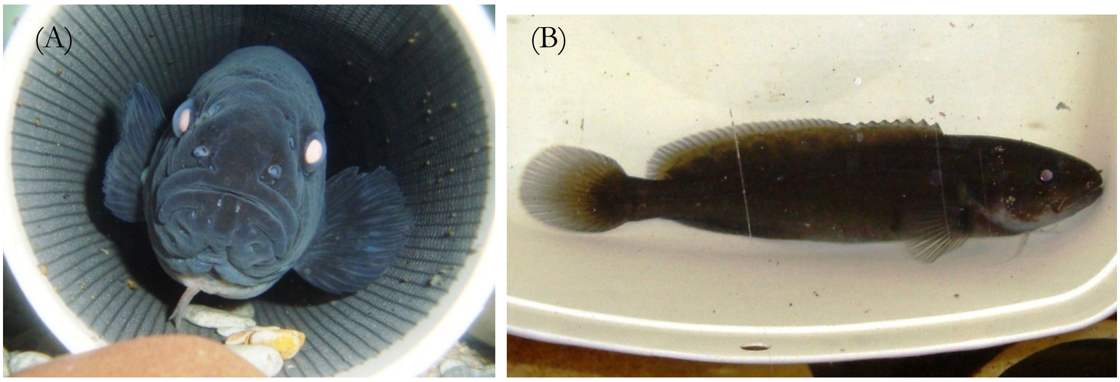

Figure 1. (A) A brood-stock river blackfish inside a breeding hollow. (B) 104 mm total length (TL) juvenile from the 2009/10 breeding trial

Activities of blackfish maintenance involved daily feeding (Monday-Friday, however, feeding also occurred on Saturday and Sunday when nearing the spawning periods), regular water quality checks and fortnightly water exchanges of 25% of the system volume. Holding tanks were used to house the extra female in the CER, where temperature was regulated by the room air-conditioning. An additional six juveniles produced from the 2009/10 trials were also housed in the CER (Figure 1.B).

2.2 Attempts to Induce Spawning

The general approach remained similar to the previous year's (i.e. using 'natural inducement'). However, there were extra preparations made and precautions planned, mainly to address complications during egg development (fungal infection and mortality). In addition, a chiller unit (TECO, TR-20®) was purchased, allowing greater ability to manipulate temperature in the breeding tanks. Two separate trials were conducted for a duration of 6 months each. Conditions for both trials were similar. Fish were paired where possible, though given there were only two male fish, only two paired fish were possible at any one time. Fish were held in 300 L breeding tanks in the CER. Water was pumped through these tanks then returned to a sump/bio-filter fitted with the chiller unit before being re-circulated to the tanks. The combination of CER and chiller unit allowed temperature control in the system to be manipulated between 10-30 °C. The breeding tanks were furnished with PVC pipes (i.e. to create hides and act as a spawning substrate) and gravel. Dividers were placed in each tank so male and female fish could be separated. The dividers were fitted with rudimentary opening and shutting gates; thus fish could be introduced to each other (Figure 2.B). This was in response to the aggression displayed by the fish when housed in close proximity. As water in the system recirculated through the bio-filter/sump-chiller system (Figure 3.B) and through to both tanks, water quality in these tanks would be identical. Therefore, water quality monitoring was done from the bio-filter/sump on a weekly basis with measurements of temperature (°C), dissolved oxygen (DO, ppm), salinity (psu), pH, ammonia (ppm), nitrite and nitrate (ppm) being recorded.

Photoperiod and temperature were manipulated to produce conditions of increasing day-length and temperature in an effort to mimic spring-early summer conditions in order to induce conditioning/gonad development and spawning for the blackfish. Photoperiod was increased over the 6 month trial from an initial 8:16 light and dark ratio to a ratio of 10:14, 12:12 and then 14:10 (Figures 5 and 7). No hormone manipulation was applied in the current trials. Water temperature was increased from 10 °C to 17.5 °C in the CER over the course of the trial (Figures 5 and 7). To condition fish for spawning they were fed daily on a varied diet including live *Daphnia sp.*, live earthworms (where possible) and chopped prawn. The Cladoceran, *Daphnia sp.* were cultured in outdoor ponds (Figure 2.A) using methods similar to that described by Hoff and Snell (1989).

Figure 2. (A) A tank used to culture *Daphnia sp.*. (B) Opening and shutting gates on tank dividers.

A backlighting technique was used to determine gender and provide an indication of gonad development (Westergaard and Ye 2010). 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 penetrate through the gonad. A header supply system was set up in July 2010 to disinfect water (Figure 3.A) for the egg incubation tank, although the system was not used as successful spawning did not occur.

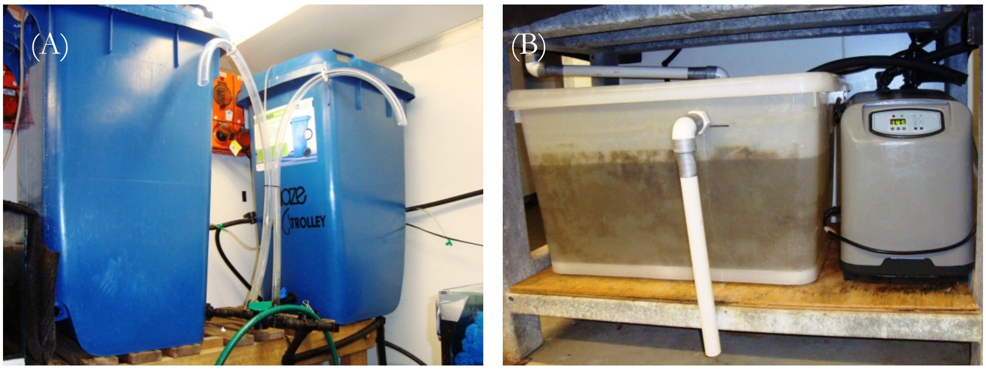

Figure 3. (A) Header supply system on egg incubation set-up for water disinfection. (B) Bio-filter/sump-chiller system on brood-stock tank.

2.3 Trial One

Trial One ran from 1st July 2010 to 27th of January 2011. Production from the *Daphnia* ponds was consistent during this period and the fish received a consistent and diverse range of foods (i.e. live *Daphnia sp*., live earthworms and chopped prawns). Photoperiod and water temperature during this trial period are presented in Figure 5. Pairings of brood-stock for this trial consisted of BF1 female and BF6 male, and BF5 female and BF4 male.

2.4 Trial Two

Trial Two ran from 1st February 2011 to 30th of June 2011. Production from the *Daphnia* ponds was consistent for most of this period except for a three week period in early June. However, during this period, the brood fish received a consistent supply of chopped prawn. Photoperiod and water temperature during this trial period are presented in Figure 7. Pairings of brood-stock for this trial consisted of BF7 female and BF6 male, and BF3 female and BF4 male.

3 RESULTS

3.1 Fish Maintenance in Holding Tanks

All fish fed well with no disease or mortalities during the 2010/11 trial. In the holding tanks, the mean water temperature was 16.7±1.8 °C; DO was 7.6±1.5 ppm; salinity was 0.97±0.10 psu and pH was 8.0±0.4. Temperature in the holding tanks generally remained steady throughout the trial period except during September when a spike was observed in the temperature from 15.5 to 27.0 °C for a period of three days due to a malfunction in the air conditioning system. The extra female fish in holding tanks that were not involved in the spawning trials were exposed to the same photoperiod conditions as the brood fish in the trial tanks (Figures 5 and 7). It should be noted that the extra female fish in these holding tanks displayed the same gonad development as the brood fish in the trial tanks.

3.2 Trial One

Fish responded well to tank conditions and live feeds offered. Fish gained condition quickly with distinct gonad development, particularly after live *Daphnia sp.*, earthworms and chopped prawn were consumed (Figure 4). In the brood-stock tanks, water temperature ranged between 10.5 and 18.3 °C; mean DO, salinity and pH were 7.2±1.1 ppm, 1.00±0.10 psu and 8.2±0.3, respectively. No detectable levels of ammonia or nitrite were observed. Photoperiod and water temperature were effectively controlled and a significant increasing trend in both parameters was observed in trial tanks over 6 months (Figure 5). Observations of gonad development in the female fish appeared to coincide with the trend of increasing temperature and photoperiod. Gonad development was apparent in all female fish when the backlighting technique (Figure 4) was applied, and development was most distinct after November. However, all river blackfish involved in this trial failed to spawn successfully.

Aggression was observed on a regular basis between male and female brood fish. On the 14th October, blackfish (BF1 female) and (BF6 male) were observed nudging and displaying to each other and inhabiting the same hide. Such mating behaviour lasted for several days, however it later progressed into aggression with scars and scratches evident on both fish. The male (BF6) being the smaller of the two was later observed displaying avoidance behaviour, whereby confining himself into the corner of the tank. Observations indicated that the male (BF6) fed little in the following days, possibly stressed by the aggression displayed by the female (BF1). Following consistent aggressive behaviour, BF1 and BF6 were separated for approximately one week. When later reintroduced for a period of a week, aggression was again evident, thus these fish were separated again. Three more attempts to introduce this pair were made in November, December and January

2010/11. Unfortunately this aggressive behaviour continued to persist. No evidence of courting or aggressive behaviours were observed in the second blackfish pair (BF5 female and BF4 male) during this trial.

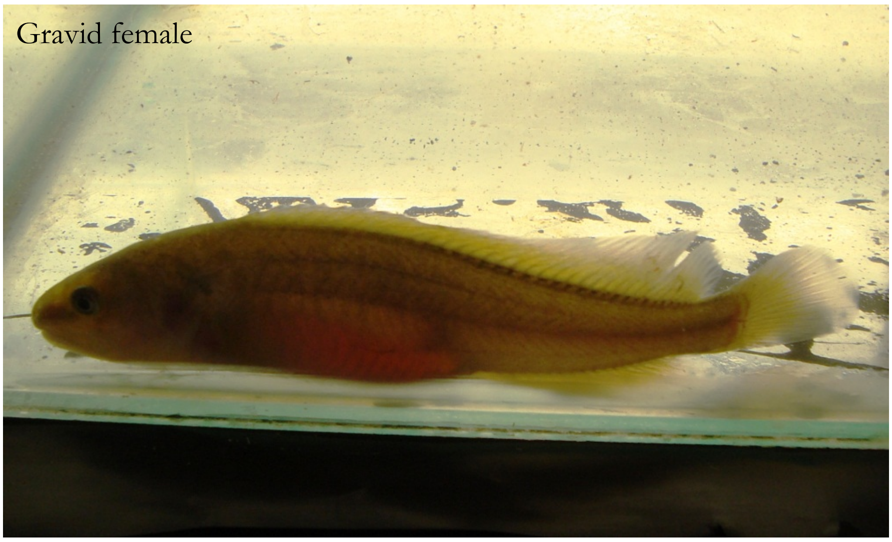

Figure 4. Gravid female river blackfish, gonad development obvious when backlighting was applied during Trial One.

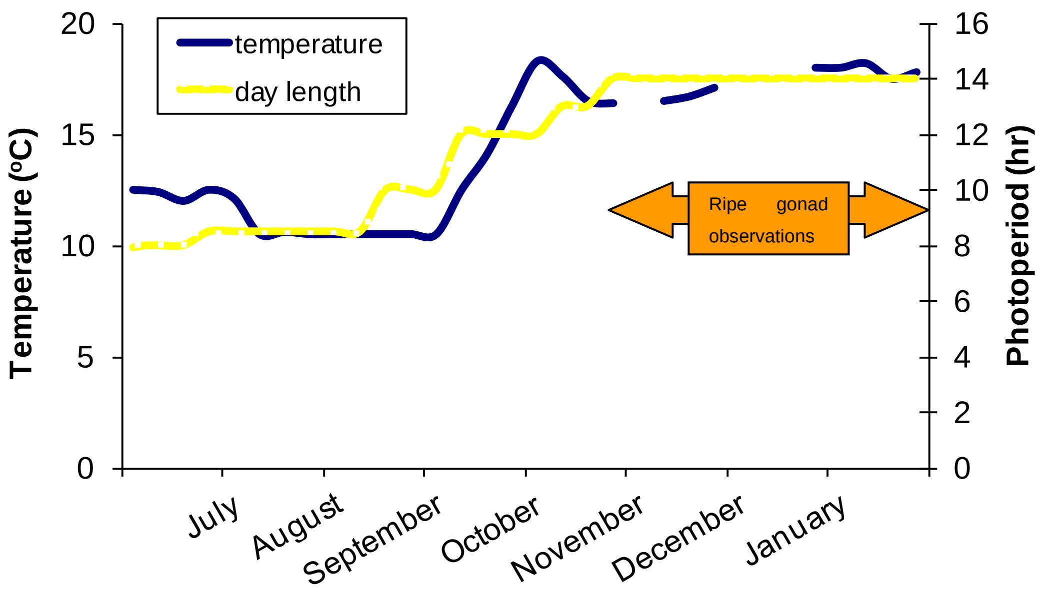

Figure 5. Photoperiod and temperature in trial tanks for Trial One (July 2010 to January 2011).

3.3 Trial Two

Similar to the first trial, fish responded well to tank conditions and live foods offered, gaining condition quickly with distinct gonad development. During a three week period in early June when *Daphnia* supplies had crashed, a consistent supply of chopped prawn was used as a substitute and appeared to maintain fish condition. In the brood-stock tanks, water temperature ranged between 10.0 and 16.8 °C; mean DO, salinity and pH were 9.6±0.1 ppm, 0.80±0.06 psu, and 7.5±0.1, respectively. There were no detectable levels of ammonia or nitrite. Photoperiod and water temperature were effectively controlled and a significant increasing trend in both parameters was observed in all trial tanks (Figure 7). However, the increase in water temperature was gradual during this trial over the 6 months (Figure 5). Gonad development was apparent in all female fish seen under backlighting, being most distinct in May and June (Figure 6). Similar to Trial One, observations of gonad development in the female fish appeared to coincide with increasing photoperiod and temperature. However, in this trial there was no evidence of courting behaviour or aggression between any of the paired fish (BF7/BF6 and BF3/BF4), and no spawning was observed.

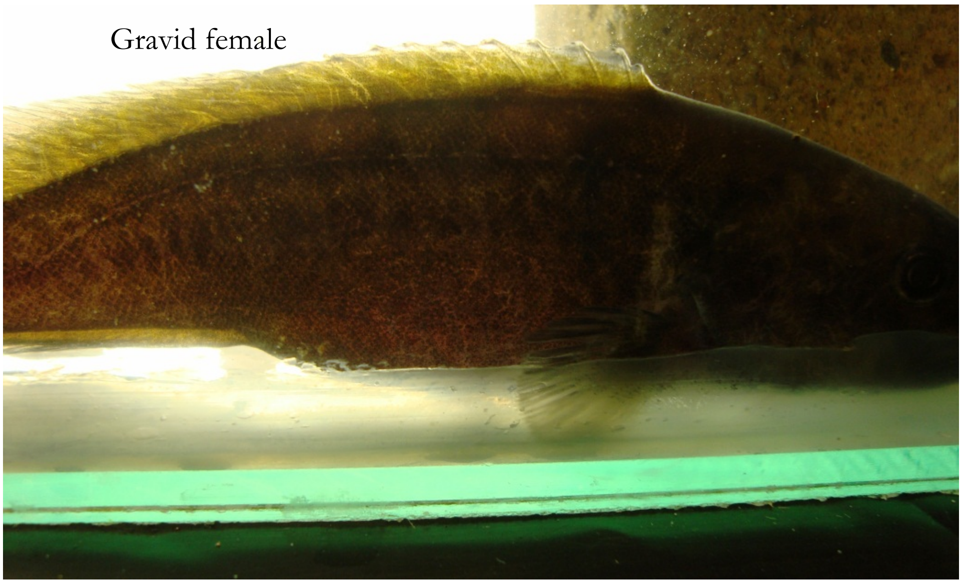

Figure 6. Gravid female river blackfish, gonad development obvious when backlighting was applied during Trial Two.

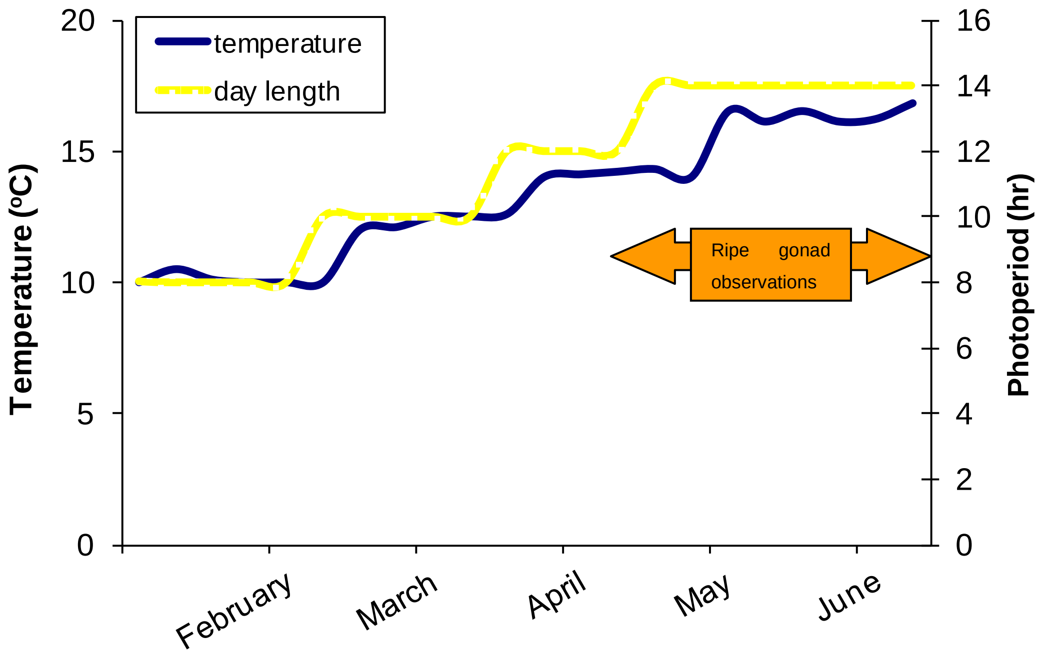

Figure 7. Photoperiod and water temperature in trial tanks for Trial Two (3rd February 2011 to 30th June 2011).

4 DISCUSSION

4.1 Lack of Spawning Success

In 2009/10, Westergaard and Ye (2010) gained success in the first captive spawning program of river blackfish by using 'natural' spawning cues, and without the application of inducing hormones. However, in the current trials (2010/2011), successful spawning was not achieved. Despite this, fish responded well to tank conditions, with rapid gonad development into spawning condition. Live cultured *Daphnia* sp. appeared crucial in aiding good fish conditioning, this was also evident during the previous year's successful trials (Westergaard and Ye 2010). However, as shown in the current trials it cannot be attributed to the eventual success in persuading the two pairs to spawn.

The limited number of males (n=2) in the current trials was likely the main factor in the unsuccessful attempt to entice blackfish pairs to spawn due to either a compatibility issue (mate choice) and/or chance effect (i.e. a potential mismatch in the timing of gonad development among limited number of individuals). There was an apparent unwillingness to spawn by the male fish and/or aggression by the females towards the males when attempting to pair ripe females with the only two males. The reasons for this are inconclusive, however, it has been shown in some species, such as guppies, the discrimination of female mate choice away from males to which previous matings have occurred (Eakley and Houde 2004); therefore, mate choice was possibly a contributing issue to the spawning failure during the 2010/11 trials given only two males available. In addition, the 2009/10 trials suggest that male river blackfish may only spawn once in a season (Westergaard and Ye 2010). Therefore such a 'narrow window' spawning opportunity for males may have further limited the chance of reproductive success during 2010/11. Gonad development observed during the 2010/11 trials was more likely associated with the increasing trend in photoperiod rather than temperature, as gonad development was also seen in female fish in the holding tanks where temperature generally remained constant. Observing gonad development in all female fish reassures that much of the husbandry practices for river blackfish in this study were optimal.

There were no significant stressors noted during any of the trials. However, pH and salinity levels were slightly higher during Trial One, with pH being 8.2±0.3 and salinity 1.0±0.1 psu. This was due to the higher levels found in the water supply. In a recent study, Bice *et al.* (2011) found eggs in the Marne River, South Australia at a salinity of 5000 $\mu S.cm^{-1}$ (2.4 psu), suggesting that this level may not be a limiting factor for the successful spawning of river blackfish. However, it was uncertain whether these eggs were fertile and developing. It was possible that female fish might have spawned but males might either be lacking interest or unable to produce viable sperm, hence fertilisation and egg/larval survival rates could have been minimal or non-existent. This was somewhat supported by the lack of river blackfish recruitment at the Marne River site with no new recruits found in the following year (Bice *et al.* 2011). However, in the Angus River, Bice *et al.* (2011) observed successful recruitment of river blackfish in salinities of approximately 2000 $uS.cm^{-1}$ (1.2 psu) during winter and early spring, suggesting salinity concentrations in the current trials were all within an acceptable range. The slightly higher pH levels in Trial One were unlikely to be problematic either, as Bice *et al.* (2011) reported similar levels in the Angus River where annual river blackfish recruitment had been observed.

Despite pH levels and salinities being lower in Trial Two, no mating/spawning behaviour was observed in any of the paired fish. Explaining this is somewhat difficult, due to knowledge gaps associated with the courting behaviours and limited breeding success of river blackfish in captivity. However, with such low numbers of brood-stock, individual fish may have been limited in their compatibility, therefore highlighting that male and/or female individuals are likely to be highly selective in choosing a mate. In addition, there may have been tank/captivity effects given fish were in a controlled environment that differed from spawning in the wild. For example, it is also possible that their courting behaviour may require more space. Therefore, future experiments could be conducted in larger outdoor tanks with perhaps small groups of fish rather than separate pairs as in the current trials. Recent experience in the production of pygmy perch (*Nannoperca spp.*) has shown some benefits of this method with greater spawning success observed (Westergarrd, unpublished data). Nonetheless, these potential influencing factors may warrant the need for further research

4.2 Implications for Conservation Management

This report illustrates that breeding river blackfish in indoor tank conditions may be unpredictable using the current techniques, particularly when the number of brood-stock fish is limited. Thus, captive spawning for later reintroduction requires improvements in methodology in order to develop a more reliable and feasible tool for conservation management.

While indoor captive breeding could continue to be trialled for improvements, recent experience in the production of pygmy perch has shown benefits by using a less intensive method of outdoor tank culture, whereby the off-spring are maintained in large (1,200-10,000 L) outdoor breeding tanks with the brood-stock fish until the juvenile stage (15-25 mm TL). This method is less intensive; thus labour costs are kept to a minimum. It is recommended that such a method be trialled for river blackfish breeding in the future. The relatively large larval size of blackfish should mean that they are likely to be well suited to this type of aquaculture, given numerously available *Daphnia* produced in outdoor tanks would provide the first suitable sized prey for larval river blackfish.

In addition, it is suggested that a greater number of male brood fish be collected for future trials so that fish pairs could be maximised and diversified. This is important given that male fish are likely to spawn only once a year (Westergaard and Ye 2010) and both female and male fish may be selective in choosing a mate (Eakley and Houde 2004). Immediate priorities for further research should include outdoor tank culture breeding trials based on the knowledge and recommendations from the 2009-2011 trials and techniques adapted from pygmy perch culture (Westergaard, unpublished data). This would involve:

- Collection of an additional three or more male fish from Rodwell Creek to achieve even paired numbers of brood fish in captivity and acquire more breeding pairs to promote successful spawning and a wider gene pool for restocking.
- Testing the viability of spawning and rearing larger numbers of juvenile river blackfish in captivity in less intensive outdoor tanks.

The genetic relationship between South Australian Murray-Darling Basin river blackfish and those elsewhere is yet to be fully understood, and should be investigated. This will allow identifying genetic units of conservation significance to inform and prioritise management. Recently river blackfish populations in the South Australian Murray-Darling Basin have been recognised as a genetic asset of conservation significance (Moore *et al.* 2010). Given the threatened status of river blackfish in many regions, developing a reliable method for captive breeding will provide a conservation measure in the future.

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