Production of sterile male brown bullhead catfish as a biocontrol method

Progress report and review

Prepared for Bay of Plenty Regional Council

March 2021

Climate, Freshwater & Ocean Science

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NIWA CLIENT REPORT No: 2021089HN
Report date: March 2021
NIWA Project: BOP21204

Quality Assurance Statement		
	Reviewed by:	S.Pether
	Formatting checked by:	Carole Evans
	Approved for release by:	Scott Stephens

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Contents

Figures

Executive summary

NIWA was engaged by the Bay of Plenty Regional Council to develop an effective method of producing reproductively sterile male brown bullhead catfish as a biocontrol method. Initial investigations focused on testing methods utilised for closely related species, namely inducing triploidy in IVF-produced embryos that will be masculinised (grow into males only) by treating the resulting larvae with androgens.

Overall, trials have indicated further research is required to produce sterile male catfish through triploidy and masculinisation. Presently, the investigation has shown that mature brown bullhead catfish are highly amenable to captive rearing and will breed in captivity. The key female used for breeding in 2020 produced two batches of eggs between December 2020 and January 2021. However, the established method of using human chorionic gonadotropin for spawning induction to synchronise gamete release among captive fish and, therefore, enable in-vitro fertilisation, was not effective in brown bullhead catfish. As such, IVF could not be performed, and triploidy induction techniques could not be tested.

Although triploidy and hence sterilisation of larvae could not be tested in 2020/21, egg incubation and larvae rearing techniques were successfully developed. Preventing proliferation of fungal growth through continual antifungal treatment is critical for successful incubation, along with sufficient but gentle water flow over the eggs. Once hatched and feeding, the larvae are robust, with c. 90% survival to the juvenile stage.

Masculinisation was trialled at day 37. We were unable to source trenbolone, the compound known to induce masculinisation in closely related channel catfish, as this compound is banned in New Zealand due to abuse as an anabolic steroid. As such, 17α-methyltestosterone (MT) was tested. MT is effective at masculinising salmonids but has the opposite effect in channel catfish and feminises (creates female) larvae. The fish were split into two rearing tanks with one tank given normal dry feed (control) and the other dry feed coated with MT (50 µg/g feed dose). These control and MT fed fish are presently being reared to a size where gender will be easily identifiable through histological examination. Initial histology will be undertaken in July 2021 to determine if MT can effectively masculinise brown bullhead catfish.

Further research is required to develop an effective method of producing reproductively sterile male brown bullhead catfish. Firstly, reliable induction of ovulation and spawning is necessary to enable IVF, which is crucial for effective triploidy induction. Once large-scale production of triploid larvae is possible, masculinisation is required to maximise the number of sterile male catfish produced. At the moment, there is no effective method of masculinising brown bullhead catfish. Trenbolone will need to be sourced from overseas for testing and MT requires further testing to determine if it feminises brown bullhead catfish. Alternatively, investigating the use of other masculinising compounds would be required. Therefore, given the project is much more complex than originally thought, we recommend collaborating with Bay of Plenty Regional Council on obtaining multi-year external funding that involves a Ph.D. or two M.Sc. students.

1 Background and rationale

Brown bullhead catfish (catfish, *Ameiurus nebulosus*) were first discovered in March 2016 in Te Weta Bay, Lake Rotoiti and December 2018 in Lake Rotorua. The population is now an estimated 186,000 in Lake Rotoiti as of March 2020, despite intensive efforts using fyke nets by contractors and volunteers over the previous four years. Catfish are a known predator of kōura and there has been a significant negative effect observed on kōura populations in Lake Rotoiti and Rotorua (Kusabs 2020). At low abundances, invasive fish can be controlled by use of biocides, physical removal, barriers and environmental modification, such as blocking access to spawning grounds. This window of opportunity has likely passed for controlling catfish in Lakes Rotoiti and Rotorua using the aforementioned means.

In late 2019, Bay of Plenty Regional Council (BoPRC) commissioned NIWA and SF Tech to undertake a feasibility study for two potential biocontrol methods that could supress to a high level or eradicate catfish as a long-term management tool. Modelling indicated that releasing sterile male catfish into the population could be an effective method of biocontrol to supress or eradicate catfish from the Rotorua lakes.

“Sterile Male” biocontrol has been widely applied in aquaculture and fisheries for over thirty years. The sterile male approach involves creating “triploid” catfish, which are unable to breed with other catfish. Triploid catfish contain three sets of chromosomes as opposed to the normal “diploid” two sets, which renders the fish sterile or produce the occasional unviable offspring. These fish are then hormone treated to bias development towards males (masculinisation). In order for BoPRC to release sterile male catfish as a biocontrol method, a reliable method of creating the sterile fish is needed, a task for which NIWA has been engaged.

To that end, NIWA has tested the effectiveness of triploid and masculinisation induction methods employed in closely related species for the brown bullhead catfish. This report will outline activities to date, outcomes and recommendations for the way forward.

2 Project methods and outcomes

2.1 Broodstock husbandry, hormonal treatment and spawning

BoPRC delivered 60 mature catfish of various sizes to NIWA Northland Marine Research Centre (NMRC) on 29 October 2020. The fish were placed in three 1,500 L circular tanks with flow-through water, and treated with Chloramine-T as a disinfection bath. Over the next weeks, the fish were provided with pelleted feed as they acclimate to the captive environment. Within two weeks, most if not all of the fish were eating the pellet feed. The temperature of the water was kept at 20°C as this was what has been reported as the spawning temperature of the species (Blumer 1985).

After this acclimation period, eight 200L tanks were set up that each housed a pair of catfish. Across the eight tanks a selection of putative spawning habitats were provided, namely smooth gravel, sand, PVC pipes, hollow cement bricks and artificial vegetation. In one 1,500 L tank, we placed one female and two males along with trays of gravel and sand. After two weeks, none of the pairs produced eggs. At this time, opportunistic necropsy (dissection) of two freshly dead females showed that oocytes (egg cells) in one of the females were uniformly at the late developmental stages (late vitellogenic), an indication that they are near spawning condition.

On 30/11/2020, we applied a spawning induction protocol effective in the closely related channel catfish (Goudie 1992, 1995). This method was also selected due to the ready availability of the compound in New Zealand, human chorionic gonadotropin (hCG). We injected 11 fish (4 pairs, and one three fish assemblage) with hCG at doses 100 µg/kg for females and 50 µg/kg for males, while the rest of the pairs were not injected. The hCG injections were repeated once after 48 h. Unlike the published method, we did not repeatedly inject the fish until spawning occurred to err on the side of caution as we have had experience in other species whereby such an aggressive approach caused high mortalities of the females from being egg-bound.

Soon after, one pair of injected fish in the 1,500 L tank (the three fish assemblage), displayed nesting behaviour over the gravel tray, and spawned eight days after the second injection. However, no other fish spawned or showed nesting behaviour which begged the question if this was the result of the induction or coincidental with natural spawning behaviour. The eight-day latency period is also much greater than that reported for channel catfish (Goudie 1992).

Therefore, the spawning induction was repeated in January. To confirm that the gonadal stages of the fish were appropriate for induction, we terminally sampled 2 males and 2 females on 7/1/2021. The oocyte stages were similar to what was found in late October, but the males had small testes and no motile sperm. On 8/1/2021, we injected 3 females and 8 males with hCG, and formed 2:1 M:F assemblages. The female that previously spawned had again showed nesting behaviour and distended belly, and was not injected, but was kept with 2 males and 1 female in the large tank. As before, injections were repeated at 48 h.

The female that previously produced eggs but was not injected this round, again spawned a similar number of eggs but no other fish spawned. This indicates that the hCG treatment as applied was ineffective at inducing spawning in this species. However, we can confirm that a female can lay two batches of eggs in quick succession.

2.2 Egg incubation and larvae rearing

2.2.1 Egg incubation

From each of the two egg batches spawned by the same female, (on 10 December 2020 and 16 January 2021) clumps of 'sticky eggs (c. 3mm diameter, Figure 2-1) were laid onto smooth gravel trays.

For the first batch, approximately 1/3 of the eggs were removed within the same day and placed in conical 8L incubators with upwelling flow and bubbling. The rest were left with the parents so we could observe their incubation behaviour as that may be useful for developing incubation methods.

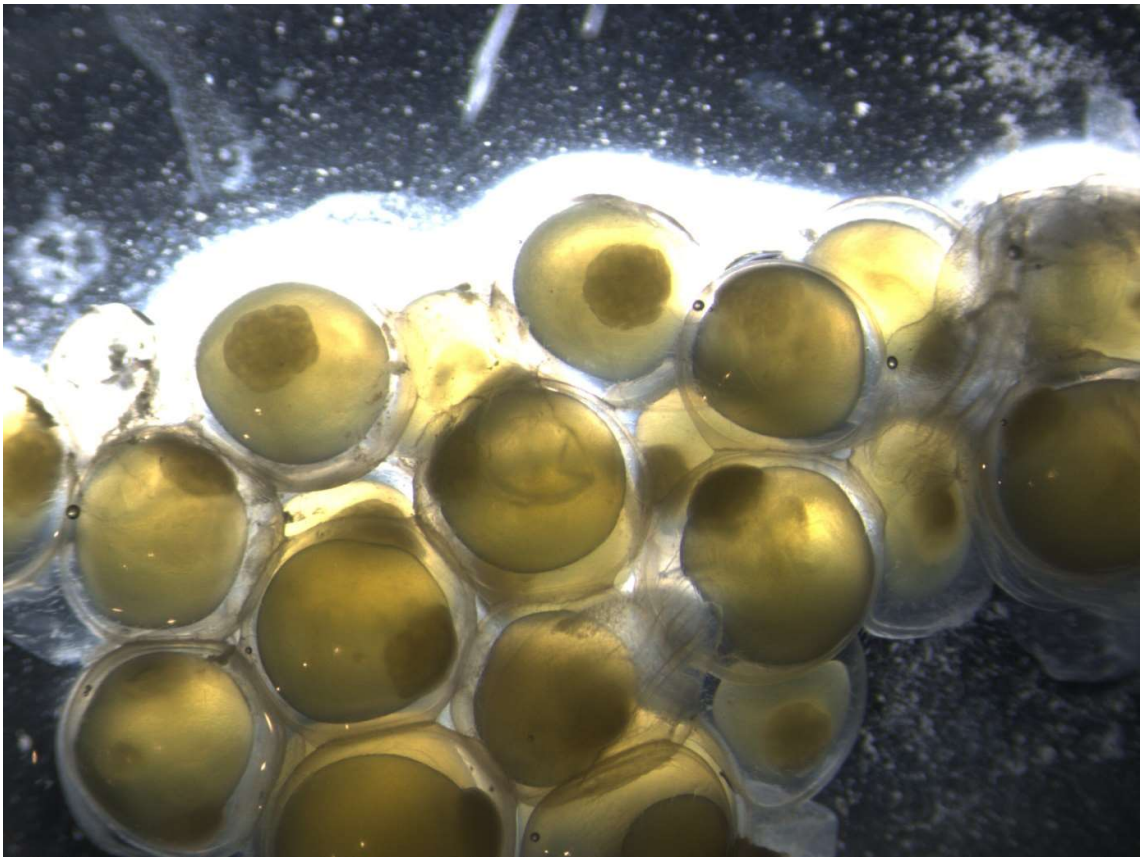

Figure 2-1: Spawned catfish eggs. The zygotes can be seen at the 64-cell stage.

By day 2, dead eggs and fungal growth were noticed in the incubators, and by day 4 the same issue was found in the tank (incubated by parents, Figure 2-2). At day 4, dead eggs were removed from the incubator. As we judged that there was insufficient water flow over them, the remaining eggs were split into three incubation methods:

1. A 13 L conical incubator with eggs sitting on screen midway up the incubator, and light bubbling.
2. A 25 L bin filled with pebbles, with water flowing over the top of the eggs with no bubbling.

3. Eggs were declumped using 1.5% sodium sulfite solution to breakdown the adhesive matrix. They were then placed in a 13 L incubator with vigorous upwelling and aeration to maintain the eggs in suspension.

However, by the next day (day 5) all incubated eggs had died, and by day 6, all eggs in the tank had also died.

For the second batch of eggs laid on 16/1/2021, we administered daily antifungal treatment (10 ppm FMC) from the day of spawning until 8 days post hatch (dph). While dead eggs were observed, fungal growth was arrested in incubators.

Figure 2-2: A clump of developing eggs. Yellow eggs are live, white dead, and fuzzy eggs are fungus-infected eggs. Two days after spawning, approximately half of the eggs were removed from the tank. A portion were treated to dissolve the adhesive matrix (separated eggs) and the rest left as clumps. The eggs were incubated in the following ways:

A. Clumped eggs in a 13 L conical incubator with 2.5L/min flow, eggs rested on a bed of smooth pebbles on a screen halfway up the incubator, with aeration (bubbling) from underneath.

B. Separated eggs in a 13 L conical incubator with 2.5L/min flow and vigorous bubbling to keep eggs in suspension.

C. Clumped and separated eggs in a square 25 L tub with 5L/min surface flow.

D. Separated eggs in a 2 L conical incubator, 1 L/min flow, eggs rested on a bed of smooth pebbles on a screen halfway up the incubator, with aeration (bubbling) from underneath.

Five days after spawning, we removed all the eggs from the spawning tank due to the increased proportion of dead eggs. The dead eggs were then graded out and the remaining live eggs were combined with the others in configurations a) to c). Then, all eggs from configurations a) to c) were placed in four 'tipper tub' incubators (Figure 2-3). In this configuration, a flow of water fills a tub at one end of the tank, which over time tips over creating an intermittent flow/wave. This more closely mimics the incubating behaviour of the parents.

Figure 2-3: Tipper-tub incubators.

The larvae hatched 9 days after laying, which is within the range reported for the species (6-13 days). Incubator configurations a) to c) did not produce viable larvae. Configuration d) only contained a small number of eggs but resulted in 60 hatched larvae. The tipper tub configuration seemed very promising as a large number of viable embryos were observed up to 8 days after laying (Figure 2-4). However, they perished due to unexpected and unavoidable staffing changes imposed by a local community COVID-19 case emergence.

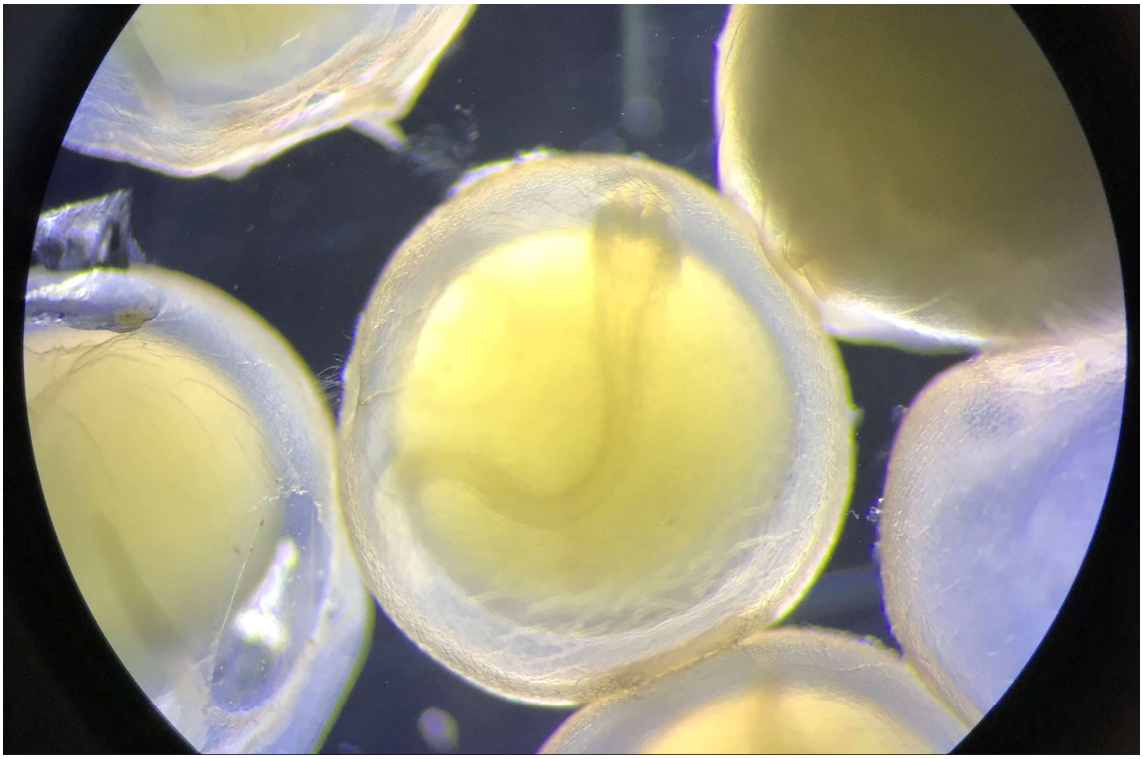

Figure 2-4: Early embryo development.

2.2.2 Larvae rearing

The larvae were moved to the tipper tub, but with the tipping mechanism disabled and reared in 20°C. They exclusively utilised their yolk reserves for at least 4 days, with some starting to feed on enriched artemia (nutritionally enhanced live feed) at day 5. By day 8, all fish were eating artemia. At day 15 all fish were eating dry feed. From hatching to present day (44 days), c. 90% of the fish has survived to the juvenile stage.

2.3 Methyltestosterone (masculinisation) treatment

Owing to the small number of larvae, our priority was to arrive at an effective larvae rearing protocol on normal fish. As such, we did not attempt induce masculinisation until much later than what had been reported in channel catfish.

Furthermore, we were unable to source trenbolone, the compound that had been reported to be effective at inducing masculinisation in channel catfish (but see next section). Trenbolone had been a commonly administered veterinary drug, including in New Zealand. However, we later learned that this substance is no longer available in New Zealand due to its increasing illegal use as a human growth steroid.

Therefore, we sourced 17α-methyltestosterone (MT), an androgen that is effective for inducing masculinisation in salmonids and other species. However, it should be noted that MT causes feminisation in channel catfish.

Nevertheless, it is possible that brown bullhead catfish respond differently to MT and thus, to maximise the value of the now juvenile fish, we tested the use of MT. On day 37 (4/3/2021), the fish were split into two rearing tanks with 28 and 27 fish in each. One group was given normal dry feed (control) and the other dry feed coated with MT (50 µg/g feed dose).

These control and MT-fed fish are presently being reared to a size where gender will likely be identifiable through histological examination. In July 2021, five individuals from each treatment (control and MT) will be sacrificed to determine if their gender can be discriminated. If so, the remaining fish will be examined to determine if the MT treatment was successful in biasing the sex ratio towards males. If the fish are still too young to determine gender, the remaining juveniles will continue to be reared under their existing treatment until January 2022, when further histology will be undertaken.

3 Recommendations

In order to create sterile male catfish through triploidy and masculinisation, the following research activities would be required. The first two elements below are likely to require significant research effort over multiple years.

1. *Control of spawning and ovulation to enable IVF.*
   This is necessary for an 'on-demand' production of sterile individuals.
   a. Investigate the use of more aggressive hCG induction, i.e., 48-hourly injections until spawning has occurred.
   b. Investigate the use of a gonadotropin-releasing hormone analogue (GnRHa) either alone or in combination with other compounds (e.g., hCG, dopamine inhibitor). This compound has also been used to reliably control reproduction in channel catfish.
   c. Investigate environmental priming of ovulation.

2. *Masculinisation/production of sterile males*
   At the moment, there is no effective method of masculinising brown bullhead catfish. Trenbolone will need to be sourced from overseas for testing, if it's possible to import the compound into New Zealand. The other commonly used compound, MT, induced feminisation of males in channel and yellow catfish. Thus, if MT also causes feminisation in brown bullhead catfish, it may be necessary to investigate the use of other masculinising compounds (e.g., aromatase inhibitor (Kitano et al. 2000, Shen et al. 2015), which would require dose response (amount and timing) testing.

3. *Triploid induction*
   Triploid induction can only be undertaken once an effective IVF technique is developed for brown bullhead catfish. This requires being able to reliably strip eggs and milt.

Therefore, given the complexity of the project, we recommend collaborating with BoPRC on obtaining multi-year external funding that involves a Ph.D. or two M.Sc. students. It is likely that this approach would allow for thorough and cost-effective investigations and also result in capacity building.

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