Stocky Galaxias – captive breeding strategy, Snowy 2.0

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December 2022

Arthur Rylah Institute for Environmental Research
Published Client Report

Arthur Rylah Institute for environmental research

VICTORIA State Government
Environment, Land, Water and Planning

Acknowledgment

We acknowledge and respect Victorian Traditional Owners as the original custodians of Victoria's land and waters, their unique ability to care for Country and deep spiritual connection to it. We honour Elders past and present whose knowledge and wisdom has ensured the continuation of culture and traditional practices.

We are committed to genuinely partner, and meaningfully engage, with Victoria's Traditional Owners and Aboriginal communities to support the protection of Country, the maintenance of spiritual and cultural practices and their broader aspirations in the 21st century and beyond.

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Citation: Stoessel, D.J. and Raadik, T.A. (2022). Stocky Galaxias – captive breeding strategy, Snowy 2.0. Published client report for Snowy Hydro Ltd, Cooma. Arthur Rylah Institute for Environmental Research, Department of Environment, Land, Water and Planning, Heidelberg, Victoria.

Front cover photo: (clockwise from top) Murrumbidgee River at junction with Tantangara Creek; Macquarie Perch; alpine plain in snow; Stocky Galaxias (Images: Tarmo A. Raadik).

Edited by David Meagher, Zymurgy SPS.

ISBN 978-1-76136-195-1 **(pdf/online/MS word))**

Accessibility

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Stocky Galaxias – captive breeding strategy, Snowy 2.0

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Caveat: This report was completed in October 2021 and consequently does not contain more recent information which may have become available.

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**Published Client Report for Snowy Hydro Ltd, Cooma,
Department of Environment, Land, Water and Planning**

Acknowledgements

We thank Kyle Weatherman for providing information regarding aquaculture facilities at Charles Sturt University (CSU, Albury), Matthew McLellan (NSW DPI) for information on the Gaden and Narrandera facilities, and Neil Hyatt (Victorian Fisheries Authority) for information on the Snobs Creek Hatchery facility. We also thank Phil Littlejohn (Aquarium Industries, Melbourne) for additional advice on captive fish quarantine and health. Mark Lintermans, Jarod Lyon, Lizzie Pope (Snowy Hydro) and Lachlan Barnes (SLR) are thanked for providing comments on various drafts of this report. Lindy Lumsden (ARI) provided invaluable additional comments on the final draft of this document.

Contents

Tables

Figures

1 Introduction

Snowy Hydro Limited received approval in 2020 to construct a new large-scale pumped hydro-electric storage and generation scheme (Snowy 2.0), to increase hydro-electric capacity within the existing Snowy Mountains Hydro-electric Scheme. This will involve the connection of the existing Talbingo and Tantangara reservoirs via a series of underground pipes and an underground power generation station. Water will be transferred in both directions between the reservoirs, which are in separate river catchments.

The Arthur Rylah Institute for Environmental Research has been engaged by Snowy Hydro to provide specialist advice that can inform the selection of options and preparation of various aquatic Management Plans required as part of the New South Wales (NSW) and Commonwealth approvals for the Snowy 2.0 project. This report details a captive breeding strategy for Stocky Galaxias (*Galaxias tantangara*). It outlines the known requirements and identifies the key knowledge gaps required to establish a captive population and breeding program for the species should this be attempted in the future. Given the long-term nature of such an endeavour, the value and relevance of this strategy will extend beyond the Snowy 2.0 Management Plans.

Threatened species that have undergone severe decline in range and abundance and persist as a few isolated, small populations are at high risk of extinction due to deteriorating genetic condition and impacts from stochastic environmental conditions (Furlan et al. 2016; Pavlova et al. 2017; Brown et al. 2022). The management of these species often involves ex situ conservation techniques (Robert 2009), such as captive breeding which supports reintroduction programs (release of captive-bred individuals) into the wild to either maintain or establish new populations (Philippart 1995; Margan et al. 1998; Williams and Hoffman 2009; Armstrong et al. 2015; Dolman et al. 2015). Captive breeding, therefore, falls within a broader framework of species recovery planning or ecological restoration, driven by maintaining or re-establishing viable populations and wild habitats (Collares-Pereira and Cowx 2004; Shute et al. 2005).

Breeding individuals in captivity, under expert care and sound management, provides an insurance against potential extinction, and/or a stock for reintroduction or reinforcement efforts (Leus 2011). A critical aspect of captive breeding is the maintenance of genetic diversity and fitness of individuals (Leus 2011). Where these aspects have been ignored, captive breeding programs have an inherent risk of failure (Snyder et al. 1996; Frankham 2008; Williams and Hoffman 2009). Therefore, genetics must underpin breeding for conservation purposes to ensure offspring have the necessary genetic diversity and fitness to persist and adapt to environmental change (Philippart 1995; Montgomery et al. 1998; Robert 2009; Gómez-Romano et al. 2016; Pavlova et al. 2017).

Currently, Stocky Galaxias exists in a single, small population in a short, narrow, shallow section in the headwaters of Tantangara Creek, in the upper Murrumbidgee River system (Raadik and Lintermans 2022a). Because there is one small, global population remaining in the wild, it is at high risk of extinction from stochastic events such as predator invasion, impacts from fire and drought, as well as genetic deterioration leading to loss of evolutionary potential and adaptability (Lintermans and Allan 2019; Raadik and Lintermans 2022a; Raadik and Stoessel 2022). Conservation recovery of the species is therefore, by necessity, currently focussed on the protection of the last remaining population and establishment of additional populations by translocation to the wild, to spread the extinction risk.

However, the high risk of extinction to the remaining small population most likely prevents its use as a source for individuals for wild-to-wild translocations, although such a decision is dependent on a genetic assessment of the population. To minimise risk, an appropriate ‘insurance’ strategy is to establish a captive population in suitable facilities and to undertake captive breeding to produce offspring to commence reintroductions into the wild, and reinforcement of the Tantangara Creek population (Raadik and Stoessel 2022).

1.1 Relevance to priority conservation actions

Whilst captive breeding does not specifically align with any of the recommended management/research actions for Stocky Galaxias (NSW DPI 2017; NSW FSC 2019; TSSC 2021 – summarised in Raadik and Lintermans 2022a), it is a critical component to the success of the following recommended action:

- Formulation of a detailed translocation plan and undertake translocations to establish additional, viable populations to spread extinction risk (NSW FSC 2019; TSSC 2021).

Further, developing techniques for captive management also contributes to the following recommended high priority action (NSW DPI 2017):

- Undertake emergency rescue of Stocky Galaxias in response to drought, oil spills/ pollution, detection of biosecurity threats (e.g. disease or pests), or to avoid other detrimental impacts.

1.2 Aims and objectives

The aim of captive breeding for Stocky Galaxias is to:

- Improve the resilience of the species by increasing the number of individuals and populations through the captive production of viable offspring with evolutionary potential.

The specific objectives of this captive breeding strategy are to identify:

1. Existing knowledge and knowledge gaps in relation to the captive maintenance and breeding of Stocky Galaxias.
2. The requirements for captive breeding of Stocky Galaxias.

Given that Stocky galaxias currently exist as a single, small population, it is at high risk of extinction. A key task to decrease the risk of extinction is to increase the size of the population if numbers are reduced in the future and establishment of additional wild populations (assuming that no further populations are detected during a catchment survey).

Given the estimated small size of the Tantangara Creek population, it is unlikely that there are sufficient individuals available for wild-to-wild translocations to be a suitable approach to establish a new population initially (Raadik and Stoessel 2022). Therefore, captive breeding is considered the preferred approach to produce enough viable offspring for translocations, at least initially. An additional value of a captive breeding program is developing a process to successfully maintain fish in captivity. Establishing a viable captive population is a key activity that would serve to mitigate the risk of extinction in the wild prior to the establishment (or detection) of other wild populations (Raadik and Stoessel 2022). Establishing techniques for captive management would also prove valuable if emergency extractions are required due to an identified imminent threat to the population (Raadik and Lintermans 2022b).

2 Captive breeding knowledge

The purpose of this section is to summarise the current knowledge of Stocky Galaxias captive maintenance and breeding requirements and identify key knowledge gaps.

2.1 Current knowledge

Stocky Galaxias have not been bred in captivity, and only recently have adults and juveniles begun to be maintained in captivity at facilities at the Gaden Trout Hatchery in Jindabyne and at the Charles Sturt University campus in Albury (Raadik and Lintermans 2022a). Whilst the species general ecological requirements for captive survival have been somewhat established (though not published), the potential requirements for reproduction and egg and larval survival can only be inferred from observations in the wild (Allan et al. 2021; Raadik and Lintermans 2022a), and from captive breeding of similar species in the Mountain galaxias complex (Stoessel et al. 2015; Stoessel et al. 2020a).

Briefly, Stocky Galaxias is a high elevation, cold-water species (< 2–18 °C) that lives and spawns in clear, flowing and well oxygenated, soft, freshwater. Spawning occurs when photoperiod is increasing from late October to mid-November. Fecundity increases with fish length from 200 to < 900 oocytes. Eggs are relatively large, sticky, and demersal, hatching in 35–40 days, with free-swimming, newly hatched larvae about 10.1 mm in length (Allen et al. 2021).

2.2 Key knowledge gaps

Specific key knowledge gaps which need to be resolved and may influence the success of captive breeding for Stocky Galaxias are:

1. Population genetic information
    - Degree of population level genetic diversity, inbreeding, effective population size.
2. Requirements for successfully maintaining broodstock in captivity.
    - Captive maintenance, annual reproductive development, post-spawning recovery.
    - Determining the optimal water quality parameters and fish densities, feeding/nutritional requirements, disease management, etc.
3. Undertaking captive breeding.
    - Timing of breeding, substrate for eggs, etc.
    - Breeding method (natural spawning – breeding pairs or groups, artificial fertilisation – hormones, hand stripping, etc.).
    - Time required and optimal water parameters required to initiate reproductive conditioning, trigger spawning, incubate and hatch eggs, and disease prevention and treatment.
4. Care and growth of larvae.
    - Time until free-swimming and exogenously feeding, appropriate larval density, optimal water parameters, food, and nutritional requirements.
5. Care and growth of juveniles before release.
    - Juvenile diet and feeding requirements, requirements to maximise growth and survival.

Guidance on appropriate techniques and methods to trial are detailed in Appendix 1 which is largely based on work by Stoessel et al. (2015, 2020a).

2.3 Prerequisites

As noted above, the establishment of a functional captive population, for the purposes of species protection, and full-scale captive breeding program for Stocky galaxias is hindered by the current lack of knowledge regarding the genetics of the existing population and proven techniques for captive breeding. As such, the following key activities are required prior to the establishment of a captive population:

1. Undertake genetic assessment of the current population (as part of monitoring program)
2. Commence a captive breeding trial using existing captive fish

If insufficient captive fish are available for a breeding trial/s, consideration should be given to procuring additional fish from the wild if the genetic analysis indicates that it is safe to do so i.e., if removing fish from the wild population would not significantly affect the viability of the population.

The commencement of a full-scale captive breeding program is also dependent on the identification of suitable translocation sites via a catchment survey where new populations could be established and/or an identified need to increase the population of fish in Tantangara Creek. A catchment survey would also potentially lead to the discovery of other Stocky galaxias populations which may have implications for the need for and/or scale and nature of a captive breeding program.

As such, captive breeding activities can be separated into 2 phases, an investigative phase (Phase 1) and full-scale breeding (Phase 2). These are described in more detail in Section 4.

3 Captive breeding

The captive breeding strategy for Stocky Galaxias follows four key steps partnered with several key activities following collection from the wild (Figure 1). Methods to be used for fish collection have been previously described in Raadik and Lintermans (2022b). The type of hatchery and captive breeding facilities considered appropriate for Stocky Galaxias are discussed below and in further detail in Appendix 2.

The following has been informed by the only known captive breeding which has been undertaken on freshwater-resident, non-migratory galaxiids (Stoessel et al. 2015; Stoessel et al. 2020a; Stoessel in review), and for conservation of other small-bodied native species (Hammer 2007, 2008; Hammer and Wedderburn 2008; Stoessel et al. 2020b). It is also informed by the available biological information of Stocky Galaxias (Raadik 2014; NSW FSC 2016; Allan and Lintermans 2019; Lintermans and Allan 2019; Raadik and Lintermans 2022a), including reproductive biology (Allan et al. 2021). Further details on each of these steps are provided in Appendix 1. As knowledge of the specific captive breeding requirements of Stocky Galaxias accumulate, revision/s may be required.

The major key steps for captive breeding of Stocky galaxias are:

1. Quarantining fish entering the facility to ensure fish health may be monitored closely and maintained, and any parasites or disease isolated and treated.
2. Captive maintenance and promotion of reproductive progression in broodstock fish.
3. Breeding to produce offspring.
4. On-growing and maintaining offspring until release.

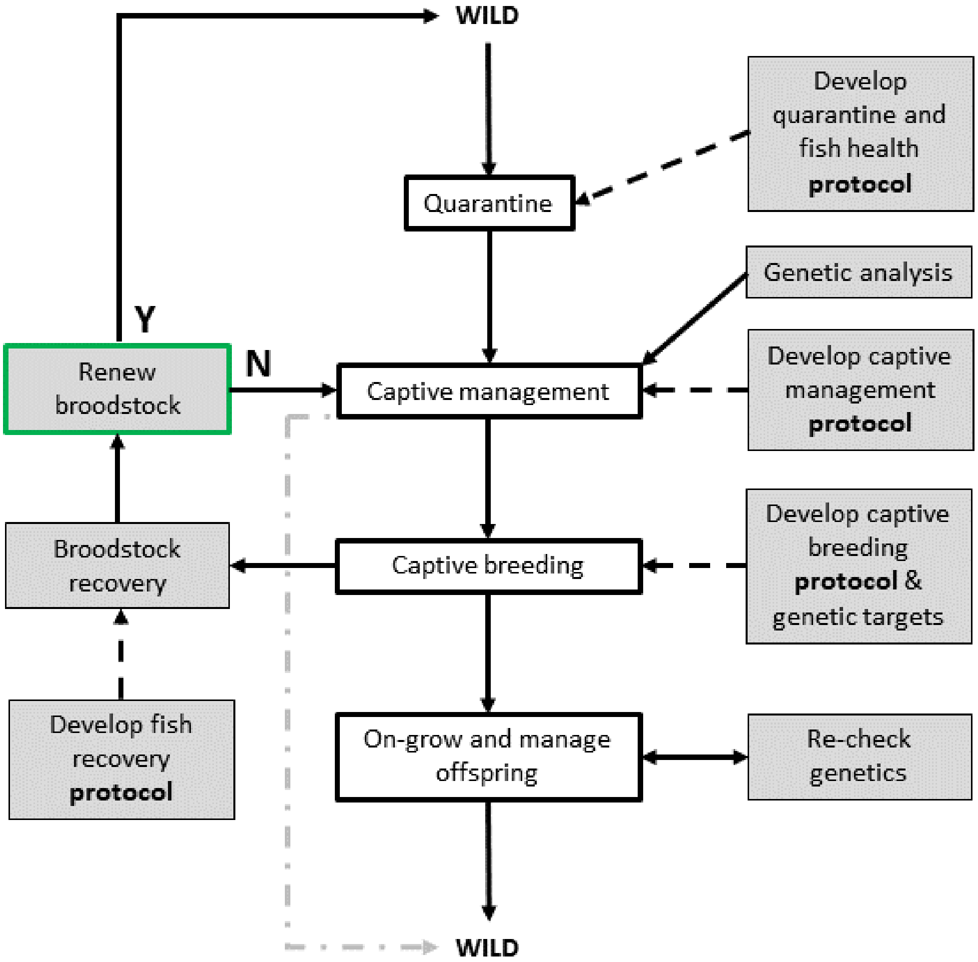

Figure 1. Flow chart of captive breeding program steps (unshaded), major activities (shaded) and decision point (green box).
Dashed black lines indicate an activity undertaken once; grey line represents the pathway if only captive management is required (e.g. emergency extraction).

Major activities for each step follow, and key knowledge gaps are provided in 2.2, above:

1. Quarantine:
    - Development of quarantine protocol specific to hatchery facility, including fish health protocol.
    - Isolation and observation of batches of fish entering the facility, and treatment for parasites or disease, to ensure no transferal of disease or parasites, and that only healthy fish are maintained.
2. Captive maintenance:
    - Development of a captive maintenance protocol to maximise fish health and vigour, to promote reproductive progression, and to minimise mortality.
    - Undertake captive maintenance of fish.
    - Genetic analysis of individuals to provide data to the breeding program (i.e. selection of broodstock or brood-pairs) to meet genetic targets of the captive breeding plan (see further down).
3. Captive breeding:
    - Development of a captive breeding protocol that is guided by genetics and that ensures reproductive success (egg fertilisation and development, hatching, and larval growth and survival) and genetic targets for offspring are met.
    - Development of post spawning recovery guidelines to maximise broodstock recovery, and, if necessary, the replacement of all or part of the broodstock if needed before captive management continues.
    - Undertake captive breeding (spawning, fertilisation, egg development and hatch, larval survival and development).
4. On-growing offspring:
    - Develop larval rearing protocol.
    - On-grow offspring and maintain until release
    - Assessment of offspring genetics to monitor compliance with the genetic target of captive breeding (if required).

3.1 Aquaculture facility

To achieve captive maintenance and breeding of Stocky Galaxias, a suitable aquaculture facility will be required. Consideration of a facility will require an evaluation of several key factors, including:

- Facility requirements (regardless of aquaculture system used).
- Type of aquaculture system required for captive management and breeding.
- Location of the facility with respect to the location of source fish and potential translocation sites.
- Scale of breeding required.
- Cost implications of building a new facility or modifying an existing facility.
- Suitability of available facilities.

It should be recognised in this decision that Stocky Galaxias are much smaller than other large-bodied species commonly produced (e.g. Murray Cod (*Maccullochella peelii*), Golden Perch (*Macquarie ambigua*), or the threatened Macquarie Perch (*Macquaria australasica*)). Further, existing data suggests Stocky Galaxias is far less fecund, and consequently produce far less offspring per breeding event, and so hatching and on-growing facilities may also be smaller than those used to produce large-bodied natives.

As an example, the facility used to produce captive bred stock from two galaxiids in Victoria (Stoessel et al. 2015, Stoessel et al. 2020a) was approximately 160 m^2 in area. Therefore, besides the option of building a facility, there is the potential to utilise a portion of an existing, larger aquaculture facility, and modifying it if necessary.

3.1.1 Facility requirements

Regardless of the type of facility chosen for captive breeding of Stocky Galaxias, common to all are requirements of water quality and security of supply, aquatic life support systems, and construction considerations. An outline of requirements is provided below, and details are provided in Appendix 2.

Water quality/supply

Water quality and its security are the most important factors in maintaining the well-being of aquatic animals. Water security (= enough when required) is generally not an issue where a facility is located next to a permanent stream, or in a rural or urban area with a consistent supply of potable water. However, water security may be problematic at times where seasonal or supra seasonal conditions occur such as drought, particularly at locations where water supply capacity is small (e.g. small rural town).

Adequate water quality can be difficult to maintain for many reasons. Storm/flood, or low flow events, can temporarily reduce the quality of water sourced from a stream, while municipal water supplies contain chlorine, which is highly toxic to aquatic animals. Therefore, frequent monitoring of the quality of water entering a facility is required, and appropriate treatment undertaken where required.

Aquatic life support systems

Adequate aeration of water is required for aquatic animals. In indoor aquaria, this is generally achieved by air being delivered along a hose, off which feeder lines terminate into air stones placed into the bottom of aquaria, and which is connected to one or more electric air-compressors. The facility should also allow for increasing or decreasing water temperature using heaters or chillers, provide water movement where necessary using pumps, coarse particulate filtration using a sponge and/or sand filter, processing animal waste products using a biofilter and/or zeolite filtration, and for some systems, the ability to sterilise aquarium water for viruses and bacteria (e.g. ultraviolet light, ozone sterilisation, etc.). To ensure continuity of service of these to avoid fish mortality, the equipment should be replicated to provide a back-up system in case of component failure, particularly the air compressor. An automated alarm system to notify of failure of larger equipment, or power loss should also be installed, along with a back-up generator in case of longer-term electrical supply outages.

Construction considerations

The facility should have good airflow, particularly if animals are kept indoors, to ensure excess humidity, which can cause build-up of bacteria and fungi, to escape. Further, the facility should have adequate climate control, enabling rooms to be heated or cooled, thereby assisting with maintaining conditions and reducing the reliance on heaters and chillers within the aquaria themselves to maintain appropriate water temperature. Consequently, structural material for floor, walls and roof should be capable of insulating from external temperatures, usually 10–15 °C from ambient air temperature.

For water flow within aquaria, tanks, ponds and/or raceways, should be designed to remove suspended solids and wastes to maintain fish condition and help with maintaining water quality parameters. Water in isolated aquaria, tanks, ponds, or raceways should also be independent of the next to limit the possibility of transferring pathogens, fungus and disease. Where water is recirculated, it should not be able to, be introduced from one aquarium to the next without appropriate treatment.

Material used in the construction of the aquaculture facility should also not contain products toxic to aquatic animals, such as various metals (copper, nickel, cadmium, or brass). Floors should be non-slip and sealed, so they are waterproof and easily cleaned. Similarly, benches and racks for aquaria should be made of non-corrosive material (i.e. stainless steel), and, along with the floor, designed and built to withstand well-above the maximum carrying capacity of the number of filled aquaria/tubs required.

Lighting in the facility should be able to be timed to gradually, and automatically, increase or decrease in intensity, to avoid startling fish and to mimic natural photoperiods. To help maintain quarantine security of animals, access to facility should be secure, and able to be restricted to personnel required for maintenance or animal care.

3.1.2 Type of aquaculture system

Three types of aquaculture systems, used for conservation or commercial breeding, are considered here for use in captive breeding of Stocky Galaxias. A summary of each system is provided below, with detailed information in Appendix 3.

Flow-through system

An open-ended system which requires a waterway for source water which flows through the facility and back out to the waterway.

Advantages: system is relatively easily maintained (e.g. biological waste, aeration, equipment), requires less skilled staff, the water in the facility will reflect seasonal changes in physico-chemical parameters of the source water, not prone to sudden changes in quality unless this occurs in the source stream, and the biological carrying capacity (volume of animals) can be high.

Disadvantages: requires a large volume of water and needs to be located near a waterway, the physico-chemical parameters (e.g. temperature, hardness, etc.) of source water are not easily adjusted, aquatic fauna may escape the facility, and animals in the facility can be exposed to pathogens in inflowing water.

Recirculation system

A semi-closed system where incoming water can be treated and stored before use in the facility, with a portion of water in the facility being recycled and reused.

Advantages: uses a municipal water supply, can maintain high water quality for long durations, uses less water than a flow-through system, water temperatures and other water quality parameters can be adjusted to the requirements of the species being held, carrying capacity can be medium to high, groups of aquaria, tanks, ponds, etc. can be run separately from others, pathogens can be treated before water reaches the facility, and animals cannot escape to the wild.

Disadvantages: the system is more complex than a flow-through system and therefore requires specialised maintenance and operational staff

Closed freshwater system

A closed system where incoming water can be treated and stored before use in the facility, then supplied to individual and isolated aquaria, tanks or ponds. Water exits to waste from each isolated unit during regular scheduled water exchanges, being replenished from the facility’s stored water supply.

Advantages: can use a municipal water supply, can maintain high water quality for long durations, uses less water than a flow-through system, water temperatures and other water quality parameters can be adjusted to the requirements of the species being held in each isolated unit, aquatic fauna cannot escape, animals are quarantined from pathogens in the incoming water and between units, and the system can be dismantled and stored when not required (reduced facility operation costs).

Disadvantages: system is more complex to manage and maintain than a recirculation system, biological waste is more difficult to manage, separate units are prone to rapid changes in water quality, carrying capacity is usually low, requires time to set up and establish system has been dismantled, requires a high level of specialised maintenance, and experienced staff are required for its operation.

Further considerations

The choice of system to be used for captive maintenance and breeding will largely depend on the programs budget, scale and frequency of breeding required, water availability and reliability, and ability of the facility to manipulate temperature and photoperiod to assist captive breeding. The suitability for each aquaculture system is summarised in Table 1, with specific considerations, important for breeding Stocky Galaxias outlined below.

Water source – the need for a flow-through system to be situated near a waterway restricts where such a facility may be located. Alternatively, recirculation and closed freshwater systems can use municipal supply, however, the water needs to be treated to remove chlorine before use in a facility.

Removal of contaminants – more difficult in a flow though system as entire supply would need to be treated. Where municipal water is used, contaminants can be removed before water enters the facility, and can be further treated once on site (i.e. in a recirculation system).

Removal of waste products – essential for artificial aquatic systems, and therefore needs to be easily achieved. Can be undertaken in all systems, however most complicated in a closed-freshwater system.

Ability to adjust water physico-chem – essential to maintain or manipulate water quality parameters throughout the year in captive aquaculture systems to promote reproductive maturation, spawning and grow-out of larvae and juveniles.

Manipulation of photoperiod – essential to promote reproductive maturation. Possible in all system types, but needs ability to be adjusted (e.g. indoors, no outside light, maintained by ‘natural daylight’ LED room or aquarium lighting with a programmable timing facility for day/night on-off and photoperiod increase/decrease).

Biological carrying capacity – important to match to fish production requirements. As Stocky Galaxias are small-bodied the holding and breeding/hatching/on-growing space requirements are less than for large-bodied species.

Inducing repeat spawning in broodstock – If repeat spawning events can be promoted in Stocky Galaxias, it will allow larger numbers of offspring to be produced, despite the species low fecundity (Raadik and Lintermans 2022a) and will be of particular benefit where broodstock is limited. Such a strategy relies on the provision of appropriate nutrition and conditions (photoperiod, water temperature) to broodstock post-spawning to stimulate fast recovery and a resumption of reproductive maturation, thereby enabling more than one spawning event to occur during or outside of the natural spawning period (e.g. Stoessel et al. 2020b).

Table 1. Comparison of suitability (green shading) of three different aquaculture system types: Flow through, Recirculation (Recirc) and Closed Freshwater (Closed). Considerations important for captive breeding of Stocky Galaxias in bold.

H – high; L – low; M – medium; N – no; P – partially; Y – yes.

System Consideration	Flow-Through	Recirc	Closed
Needs to be located near a stream	Y	N	N
Capable of removing contaminants from inflowing water	N	Y	Y[1]
Waste products relatively easily removed	Y	Y	N
- Less problematic with nitrogenous waste	Y	N	N
Prone to rapid changes in water quality	N	N	Y
Physico-chemical parameters of water easily adjusted/maintained regardless of time of year	N	Y	Y
- Individual aquaria maintained at appropriate temperatures	N	N	Y
- Groups of aquaria maintained at appropriate temperatures	N	Y	Y
Photoperiod easily manipulated	P	Y	Y
Biological carrying capacity (number of individuals)	M-H	L-H	L
Potential to induce broodstock into multiple spawning/stripping per year	N?	Y	Y
Animals quarantined from pathogens	N	Y	Y
Aquatic fauna may escape	Y	N	N
Alarms, fails safes, back-up systems	Y	Y	Y
Regular specialised attention and maintenance	L-M	M	H
Requires highly trained staff	P[2]	Y	Y
Difficulty to operate and maintain (cost)	L-M	M	H
Can be dismantled and stored when required	N	N	Y
Time consuming to dismantle or set-up	N/A	N/A	Y

[1] – for closed systems this is undertaken before water reaches the facility's holding tanks, as it cannot be undertaken in the aquaria themselves.

[2] – oversight required by experienced staff member.

Animal quarantine / biosecurity – control of pathogens, and prevention of animal escape from a facility, are a standard biosecurity requirement for operations in aquaculture (Perera et al. 2008; SCAAH 2016). Animals should be quarantined from pathogens to ensure the health of broodstock, and offspring are maintained, and to avoid introduction of pathogens to novel environments during translocation of offspring or return of broodstock. Control of pathogens is also important within the facility, particularly between groups or within individual aquaria.

Maintenance and staff expertise – the amount and complexity of maintenance/operation of a facility, and the level of skills required by staff increases from a flow-through to closed-freshwater system, though this needs to be balanced against the complexity of the operation of a suitable facility required to meet project objectives, particularly with highly threatened species with little existing aquaculture knowledge; a high level of skill and effort (maintenance, operation) as well as sufficient capacity to ensure constant care is required for threatened species to minimise risk of loss in captivity.

Whilst all aquaculture system types could be used for Stocky Galaxias captive management and breeding (Appendix 3), on balance the recirculation system is more suited (Table 1). Properly established, this type of facility, whilst more complex than a flow-through system, is more flexible with respect to system location based on the water supply it can use, allows manipulation of required water quality parameters important for breeding of Stocky Galaxias, and has a higher level of biosecurity. It is also less complex, less time-intensive and has less risk than maintaining a closed freshwater system, while also having a greater carrying capacity to house broodstock and their offspring.

3.1.3 Location of facility

The location of an aquaculture facility with respect to sites of source populations for broodstock collection and emergency extraction of fish or return of extracted fish and release of captive-bred offspring, also needs consideration. This is important as the location of the facility may have implications to risk to Stocky Galaxias and therefore success of the program, e.g. loss or injury to fish during transportation, or at a facility located outside of the species normal tolerance of environmental conditions.

Fish injury or loss during transportation (and in the aquaculture facility) can be minimised by following robust protocols (see Raadik and Stoessel 2022), Where possible, transport distance/time, and changes to natural environmental conditions should be minimised. Therefore, a facility closer to the source and release sites would be preferential to one further away, that may have different physiography (e.g. water quality, change in elevation (air pressure), maximum air temperature, etc.). This will also reduce transport costs.

Regardless, two options for a suitable captive maintenance and breeding facility for Stocky Galaxias, exist. The choice of which will have implications for travel times and potential risk. These are:

- An established facility that may require modification.
- A constructed new facility, located at a suitable nearby site, that has required utilities and nearby staff facilities.

Overall facility cost (capital and operating) will also influence facility location (e.g. use established facility or build new, purchase outright or rent, if an existing facility is used does it require modification, etc.). For example, modification of an established facility potentially has lower cost than construction and fit-out of a new facility, but less flexibility with respect to distance from source/release sites.

3.1.4 Suitability of available facilities

Several existing aquaculture facilities, potentially suitable for captive management and breeding of Stocky Galaxias, were evaluated for their suitability (Table 2). They ranged in distance (as hrs of driving time) from the Stocky Galaxias population in Tantangara Creek (currently only known source population), and varied in several important characteristics, such as aquaculture system type, whether an upgrade was required, water supply suitability, and specific characteristics of the aquaculture system (also see 2.3.2 to 2.3.4, above).

The existing facilities were:

NSW

- Gaden Trout Hatchery, Jindabyne (NSW Department of Primary Industries).
- Narrandera Fisheries Centre, Narrandera (NSW Department of Primary Industries).
- Institute for Land, Water and Society, Charles Sturt University, Albury-Wodonga Campus, Albury.

VICTORIA

- Snobs Creek Hatchery, Snobs Creek (Victorian Fisheries Authority).

- Arthur Rylah Institute for Environmental Research, Heidelberg, Melbourne (Department of Environment, Land Water and Planning).

Three additional areas, all reasonably close to the Stocky Galaxias catchment and source and potential release sites, were included, where a new hatchery facility could potentially be constructed. These were at:

- Cabramurra (NSW)
- Cooma (NSW)
- Canberra (e.g. University of Canberra) (ACT)

Follows comments on considerations outlined in Table 2:

Existing facility capacity for galaxiids – relates to the availability of a suitable aquaculture system for maintaining galaxiids.

Facility upgrade – based on information provided from the facilities, whether an upgrade is required to improve facility needs for galaxiids, particularly for the full-scale production breeding.

Driving time – a surrogate for distance the facility is from the Stocky Galaxias location in the upper Tantangara Creek. An assumption is the greater the travel time, the more risk there may be to fish health, though this can be reduced by other means (see 2.3.3, above). Further, this does not consider the ability to transport offspring from a given facility by air to release locations: all facilities have airport facilities nearby, except Snobs Creek.

Elevational change – elevational change from the source location to hatchery facility was calculated, and relates to change in air pressure, and to maximum air temperatures, etc. Whilst the influence of a pressure increase on Stocky Galaxias health is unknown, and may be minimal with adequate acclimation, a change in elevation to some facilities exceeds 1 km in height and is included in consideration as a precaution. Again, influence of increased external temperature may be able to be controlled by hatchery construction material. This seems to not be as important as other factors, considering that Stocky Galaxias have successfully been housed at CSU since last year, which is 1290 m lower than their source stream.

Water supply – a water supply which can be modified or controlled for physico-chemical parameters and contaminates is considered more suitable (see 2.3.2, above).

For remaining conditions, see 2.3.2, above.

Table 2. Comparison of suitability of existing aquaculture facilities for breeding Stocky Galaxias, including options for new facilities (green – suitable; yellow – partially suitable; red – unsuitable)

C – closed system; FT – flow through; L – large; M - medium ; part – partial; Rec – recirculation; S – small, Temp - temperature.

Possible facilities	Existing facility / capacity for galaxiids (captive manage-ment)	Facility upgrade needed for captive breeding 1 minor 2 medium 3 new	Driving time (hrs)	Elevation change (m)	Suitable water supply	Water temp control (year-round)	Maintain water temp at 2 °C	Photoperiod control	Independent water to each group of aquaria, tanks, ponds, etc.	Fail safe systems (back-up power supply, alarms, air compressors, chillers, etc.)
EXISTING Gaden Hatchery	🟩 Yes, FT / S-M	🟨 2–3	🟩 2.0	🟨 - 535	🟨 Thredbo R	🟥 No	🟨 Part (in winter)	🟥 No	🟥 No	🟨 Part
Narrandera Fisheries Centre	🟩 Yes, Rec / M-L	🟨 2	🟨 4.0	🟥 - 1310	🟨 Murrumbidgee R	🟩 Yes	🟥 No	🟩 Yes	🟩 Yes	🟨 Part
Charles Sturt University	🟩 Yes, Rec / M-L	🟩 1	🟨 3.7	🟥 - 1290	🟩 Large country centre	🟩 Yes	🟩 Yes	🟩 Yes	🟩 Yes	🟩 Yes
Snobs Creek Hatchery	🟩 Yes, Rec & FT / M	🟨 2–3 (Rec) 2 - FT	🟥 6.3	🟥 - 1200	🟨 Snobs Creek	🟩 Yes	🟨 Part (in winter)	🟥 No	🟩 Yes	🟩 Yes
Arthur Rylah Institute	🟩 Yes, Rec & C / S-M	🟨 2–3 (Rec) 2 (C)	🟥 7.0	🟥 - 1340	🟩 Capital city	🟩 Yes	🟥 No	🟩 Yes	🟩 Yes	🟨 Part
NEW (nearby) Cabramurra	🟥 No	🟥 3	🟩 1.0	🟩 + 10	🟥 Small town supply, 60 ML reservoir	N/A	N/A	N/A	N/A	N/A
Cooma	🟥 No	🟥 3	🟩 1.5	🟨 - 660	🟨 Small country centre	N/A	N/A	N/A	N/A	N/A
Canberra (e.g. University of Canberra)	🟥 No	🟥 3	🟨 2.9	🟨 - 810	🟩 Capital city	N/A	N/A	N/A	N/A	N/A

Of the existing aquaculture facilities (Table 2), all have systems which are potentially suitable for Stocky Galaxias captive maintenance, however they all require some level of modification to improve their suitability, particularly for full-scale breeding production. The three NSW facilities are within four hours' drive of the upper Tantangara Creek, with the Gaden Trout Hatchery at Jindabyne the closest. Elevational change is ignored (see above), and one facility in NSW and one in VIC have suitable water supply, however other hatcheries in NSW have partially suitable supply. The only facility that currently has suitable controls for manipulation of water temperature, photoperiod, ability to run separate groups of aquaria/tanks or ponds and had suitable fail-safe back-up systems was the facility at Charles Sturt University, Albury.

Other advantages of the CSU facility over the others are that it also has a recirculation system, which has more advantages than a closed or flow-through system (section 3.2.3, above), is relatively close to the source population, and potential modifications to the recirculation facility to improve suitability are relatively minor.

All existing facilities could be rendered suitable following various modifications. The three options for constructing new facilities (Table 2), were all relatively similar, and could be built with the most suitable and flexible aquaculture system, however, the water supply at Cabramurra was considered less secure. All new facilities would have initial outfitting costs which are not applicable to existing facilities, however, all existing established facilities require modification, which may be similar, if not higher, than the cost of fit-out of a new facility.

3.2 Scale and intensity of captive breeding required

The feasibility and scale of breeding required can only be determined once the following key activities are undertaken and data collected:

- monitoring and genetic analysis of the existing population (to estimate the number of and genetic diversity of the population/s.
- a catchment survey (Raadik and Lintermans 2022c) which will determine whether other remnant populations exist and/or identify potential translocation sites.
- A proof-of-concept breeding trial to establish appropriate conditioning techniques, breeding triggers, and egg and larvae rearing techniques (Phase 1)

The scale of, and need for, captive breeding will likely change and potentially diminish over time relative to the degree of improvement in the conservation status of Stocky Galaxias, e.g. as more viable wild populations are established or discovered, wild-to-wild translocations may replace the need for captive-breeding to provide individuals for translocation or active intervention may cease. However, as captive breeding will have been developed, a capacity should be maintained, for when it may be required, such as if genetic decline, or poor recruitment, are not able to be rectified by wild-to-wild translocations.

Due to the lack of captive breeding knowledge on the species (see 2.2, above), several methods and protocols for successful breeding need to be established (Phase 1) (see 2.3 above). This phase will need to be experimental, trialling and modifying existing protocols established for captive breeding of other similar species in the Mountain galaxias complex (Stoessel et al. 2015; Stoessel et al. 2020a). These should address all knowledge gaps (2.2.2 above), methods and protocols required (2.3 above). Key components include fish health management and larval/juvenile/adult maintenance, to promote sexual maturation for natural spawning, or artificial stripping and fertilisation, and to assess whether this can be stimulated at other times of the year or repeated within a year to maximise hatchery production. Consequently, this phase will be intensive in time and effort, and potentially low in output of offspring (e.g. 100's of offspring).

Once genetic assessments are complete, captive breeding protocols are developed and assuming suitable habitat is available for translocation, full-scale captive breeding can commence (Phase 2), which will require more facility resources (equipment, space) and staff for production. This may lead to offspring output in the 1000's of individuals. The scale of captive breeding required at any given time will be influenced by the need for captive breeding to produce a given quantity of offspring for population recovery or translocation, which will be directed by outcomes of the catchment survey, population monitoring and any preceding translocation programs and associated monitoring. Scenarios requiring captive maintenance and/or captive breeding of fish, including translocation types, are shown in Table 3. Once additional, viable, populations of Stocky Galaxias are established, reliance on captive breeding will diminish, with any translocations required potentially only undertaken by wild-to-wild transfer (Table 3). However, captive management, and captive breeding to a lesser extent, may be required as part of emergency response (e.g. poor recruitment, drought, genetic decline, wildfire) in the future.

Table 3. Scenarios potentially requiring captive management and/or captive breeding of Stocky Galaxias, including translocation types.

Bold – high reliance, un bold – low level of reliance.

Scenarios	Captive insurance population	Captive breeding	Captive-wild reinforcement	Captive-wild reintroduction	Wild-wild translocation
Single population and location (current)	**Y**	**Y**	**Y**		
Single population and translocations sites (transition)	**Y**	**Y**	**Y**	**Y**	
More populations (transition)	Y	**Y**	**Y**	**Y**	Y
More viable populations established (future)					**Y**
Emergency (future)	**Y**	Y	Y	**Y**	

4 Captive breeding program (Phase 1 – Experimental trial)

To ensure success of captive breeding of Stocky Galaxias, many methods and protocols need to be developed (Phase 1, see 2.5, above), including investigation of knowledge gaps, before full-scale captive breeding can commence (Phase 2). These are detailed in sections 2.2 and 2.3 above and are not repeated here. Ideally, these should aim to promote “natural” spawning behaviour in Stocky Galaxias in captivity, thereby reducing the reliance on invitro propagation techniques for fertilisation. Doing so would reduce stress and potential harm related to handling of individuals during invitro procedures. However, as invitro propagation techniques have proved successful for two other species of galaxias (Stoessel et al. 2020a), it may be beneficial to trial a similar method for the Stocky Galaxias.

Many factors will influence the cost of undertaking the preliminary experimental phase of captive breeding, such as:

- The availability of a fit for purpose, functioning, facility or the need to construct a new facility(see 2.4, above).
- The level of prior expertise the facility staff have in the aquaculture of small freshwater fish species, and therefore the level of understanding of fish husbandry and complex water chemistry interactions to minimise the risk of failure.
- Methods and protocols can be developed within 12 months, and review and revision of these may need to be undertaken.
- Genetic analysis (collection, laboratory procedures and data interpretation) of broodstock individuals will have to be undertaken as it may differ to earlier, broader genetic analysis of populations.
- Whether techniques that have been successful for the captive breeding of other native small-bodied galaxias prove successful for the captive breeding of Stocky Galaxias.
- The amount of staff salary, facility running costs, consumables, and genetic input to the breeding protocol required, including a revision of the genetic breeding plan if additional populations of Stocky Galaxias are discovered and included in the captive breeding program.

Such a program could be undertaken by a suitably qualified aquaculture facility, however, as this is the initial developmental phase, a collaborative approach with other institutions with some captive breeding experience with smaller fish species, such as galaxiids, would be beneficial from a knowledge sharing perspective (e.g. CSU, NSW DPI and/or ARI). The cost of this phase may also be reduced if some knowledge gaps have been partially filled and protocols are in development. Therefore, aside from adequate aquaculture facilities, collaboration with institutes with prior or developing expertise and knowledge in Stocky Galaxias captive maintenance and/or breeding is logical.

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6 Appendices

APPENDIX 1. Captive maintenance and breeding requirements

The following section provides additional detail on considerations for a captive breeding program. Note that appropriate protocols for the collection and transfer of fish to, and translocation of offspring from, the breeding facility are dealt with in Raadik and Stoessel (2022), as are permit requirements.

A1.1 Introducing animals to the facility

To minimise fish stress, on arrival at the facility, the time fish are held in transport containers must be minimised. Therefore, holding facilities must be ready, and acclimatisation of fish to hatchery conditions (water temperature, salinity, and dissolved oxygen level) must commence immediately. The water quality parameters should be equalised slowly to ensure physiological shock does not occur, and where adjustment is required, water should be slowly transferred, over several hours, from the receiving aquarium to the transport container, with dissolved oxygen levels in each maintained as close to 100% as possible. Minimising handling and disturbance to fish during this time, and using low, indirect lighting, will also help reduce fish stress (DeTolla et al. 1995; Johnston and Jungalwalla 2005).

A1.1.1 Quarantine

On first entering the facility broodstock must undergo a period of quarantine to ensure fish health, where they are isolated from other parts of the aquaculture system and species already in the facility, treated for pathogens (e.g. DPI VIC 2005, 2008) and visible sign of injury, and visually monitored for odd behaviour. A quarantine period for fish with unknown pathogens is three to four weeks, and this period can also be used to settle the fish into captivity. During this time, fish can be treated using common prophylactics such as malachite green/formalin combination, oxytetracycline or praziquantel (see also A1.8, below).

For quarantine and general fish holding, if infections or mortality occur, microscope examination of fish should be undertaken to identify the cause and treatment required, or diseased/moribund individuals should be appropriately preserved and sent to an appropriately qualified Veterinarian for examination.

A1.1.2 Density of fish

Adult and juvenile Stocky Galaxias have displayed aggressive intraspecies behaviour in captivity at Gaden Trout Hatchery and at CSU, Albury (L. Baumgartner, pers. comm.). Consequently, if this behaviour continues, particularly in newly introduced broodstock, fish may need to be separated (individually, size groupings, lower density, etc.). into separate tanks or aquaria to minimise mortality. Each tank/aquarium must be labelled with a unique number, and appropriate details (waterbody, site and date of capture, date introduced into facility, number of individuals), including management regime (target water parameters, feeding regime and any treatment(s) required).

An advantage of housing each fish separately is that, following collection and analysis of genetic data, paired individuals or known genetic quality can be easily selected as brood pairs, guided by the genetic hatchery protocol, if required.

A1.1.3 General fish holding

Each aquarium should hold a minimum of 10 L of water, have fine mesh covers on all water inflow and outflow valves, and a well-fitting cover to avoid fish escape. To reduce fish stress, each aquarium should be covered on three sides with black material (e.g. plastic). Each aquarium should be filled with aged (chlorine-free), carbon-filtered water which should be trickle-fed, recirculated, filtered, and chilled. To minimise the risk of pathogen spread across broodstock, water in each aquarium should be independent of the next, and no water or equipment should be shared between aquaria (fully isolated tanks). This can be achieved, to a degree, in recirculation systems where a group of aquaria are joined, with the water treated using ultraviolet light, however, this will not kill parasites, which will need to be treated (see above).

A1.1.4 Feeding

Following transfer of animals to aquaria, fish should be gradually reintroduced to feeding. It is common for fish that have recently been transported to refuse food for a couple of days. If no feeding continues, alternative plans should be in place, including consultation with a feed manufacturer, a fish nutritionist, or a veterinarian (Batt et al. 2005). Broodstock fish should be fed a varied diet of predominantly live food (e.g.

Tubifex sp., Rotifers, *Artemia* nauplii) at least once daily. Feeding fish a varied diet aids in attaining appropriate nutritional requirements, and live food reinforces behaviour required for survival in the wild if broodstock are returned.

A1.2 Husbandry

Common to the husbandry of all aquatic species is a requirement to maintain an environment that is as least-hostile as possible. This requires a good understanding of water quality so that problems can be identified and rectified early, and that procedures and protocols are in place to guarantee a level of care and ultimately the health of animals. The following provides a basis to husbandry required in a captive breeding facility (i.e. recirculated or closed freshwater system).

A1.2.1 Water chemistry

The nitrogen cycle

When an aquarium is first set-up, "new tank syndrome" may occur prior to good bacteria being established in filters as part of the initial stages of the nitrogen cycle. Where the nitrogen cycle is not understood well, and nitrogenous compounds are not controlled, mass death of captive fish may occur. It is therefore vital that staff have a good understanding of water parameter requirements, and in particular the nitrogen cycle (Figures A1-1).

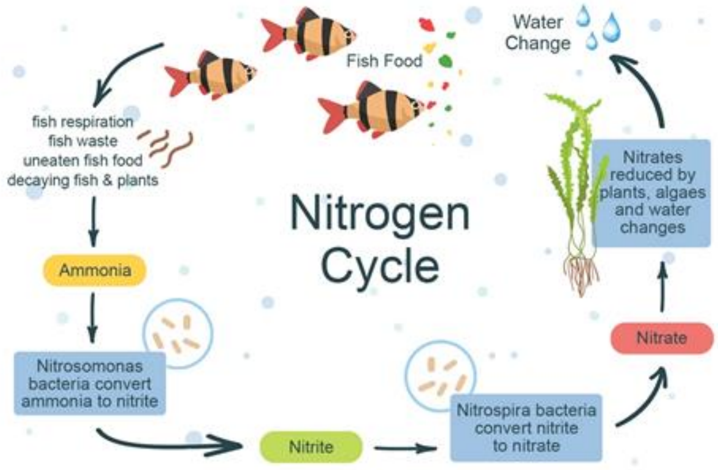

Figure A1-1. The nitrogen cycle

https://www.aquaticexperts.com/blogs/blog/nitrogen-cycle-freshwater, accessed 18th June 2021

When aquatic animals excrete waste, ammonium (NH^{4+}) and ammonia (NH^{3}) are formed. The latter is extremely toxic, causing damage to the delicate gill tissue at relatively low levels (Moe 1992, DeTolla et al. 1995; Johnston and Jungalwalla 2005; Linbo 2009). The toxicity of the compound also increases in waters that display higher pH (more alkaline waters), temperature and salinity (Johnston and Jungalwalla 2005, Nickum et al. 2004). These must be maintained within an appropriate range for Stocky Galaxias.

Ammonia may be reduced in aquaria in the short term by undertaking partial water changes, altering fish density, reducing temperature and salinity, or with the use of an ammonia-absorbent compound (such as Amguard^{TM}, Seachem). In the longer term, however, to ensure stability, a matrix of nitrifying bacteria (e.g. Nitrosomonas and Nitrobacter) need to be established in biofilters to convert ammonia to nitrite, and ultimately to nitrate (Batt et al. 2005). Providing a nutrient source is present in aquaria (e.g. fish waste), these bacteria will establish naturally in filters over time. The filter substrate can be media such as zeolite, crushed oyster shell, sand, dolomite, pea gravel, or synthetic substances (e.g. at ARI we most commonly use Bio Balls; Batt et al. 2005). In general, the larger the surface area to volume ratio of the filter, the more effective the filter, bearing in mind that such a filter needs to optimise the balance between water flow and surface area (Batt et al. 2005). Once nitrifying bacteria have established, such a system should not be considered ‘set and forget’, as nitrifying bacteria are sensitive to sudden changes in pH, generally not growing well outside the range of pH 7.2–8.5, and can be affected by chemicals used for disease treatment, as well as by sudden changes in temperature (Batt et al. 2005) and salinity. Colonies of nitrifying bacteria may therefore quickly collapse, and constant vigilance (and testing) is therefore required. Weekly (or more regular in the initial stage of the nitrogen cycle) monitoring of ammonia, nitrite, and nitrate is therefore critical.

To control the concentration of nitrate, the final compound in the nitrogen cycle, regular water changes need to be undertaken. As a guide, a 30% water change should be conducted on a weekly basis, however, greater amounts of water will need to be exchanged in instances where nitrate concentration is found to be excessive (i.e. > 40 ppm).

Establishing colonies of nitrifying bacteria in filters, which allow conversion of toxic ammonia into less toxic nitrite and more inert nitrate, can take between a few weeks to a couple of months (Figure A1-2). In high concentrations, nitrite causes methemoglobinemia and ultimately, hypoxia (Williams and Eddy 1986). The toxicity of the compound may be mitigated by chloride ions, thus having slightly elevated salinities (i.e. up to 3 ppm) in aquarium housing Stocky Galaxias may be beneficial during the establishment phase of nitrifying bacteria (i.e. where nitrite is a concern; Batt et al. 2005). Alternatively, Prime™ may be used in recommended doses at this initial stage to detoxify not only nitrite, but also ammonia and nitrate, while still allowing the nitrogen cycle to continue to completion. A combination of these methods (i.e. increasing salinity and the addition of Prime™ is therefore recommended in the initial stages of the nitrogen cycle. For chronic exposure in freshwater systems, 0.0 mg/l ammonia and ≤0.1 mg/l nitrite is suggested as safe (Batt et al. 2005).

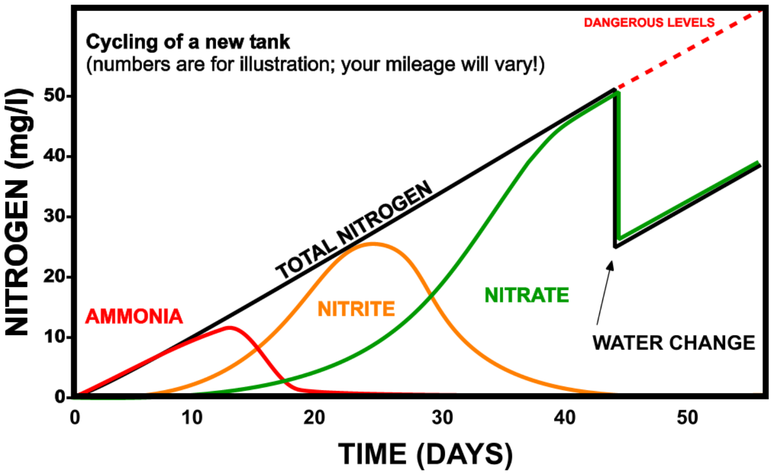

Figure A1-2. Progression of the nitrogen cycle

http://www.theaquariumwiki.com/wiki/File:Cycling_graph.png, accessed 18th June 2021

Establishing nitrifying bacteria in filters does not, and preferentially should not, occur when fish are first added to an aquarium for the reasons mentioned above. The nitrogen cycle may be started, and good bacteria established in fishless aquaria several weeks or even months before aquatic animals are placed into them by adding a source of nutrients (e.g. a small amount of pelletised fish food). Doing so limits the potential for problems to arise once aquatic animals are added. Where such a strategy is used, however, any remaining source of nutrient (e.g. fish food), in addition to any fungus (which is toxic to aquatic animals when ingested), should be removed from aquaria prior to animals being placed into them.

A1.2.2 General water parameters

In addition to nitrogen compounds mentioned above, the management of dissolved oxygen, salinity, temperature, pH, and water hardness will be critical to maintaining Stock Galaxias in captivity. These parameters should be monitored at an appropriate frequency (at least once a week, but more frequently in the weeks immediately following the placement of animals in aquaria) at a time reflecting greatest demand on the system (usually after feeding). Such a strategy allows predictive, rather than reactive, management of water quality (Wedemeyer 1996, Kreiberg 2000, Batt et al. 2005).

Dissolved oxygen

Dissolved oxygen is often the first limiting factor compromising aquatic species welfare (Johnston and Jungalwalla 2005). In general, the concentration of the parameter decreases with increasing water temperature, atmospheric pressure, and salinity (Johnston and Jungalwalla 2005; Batt et al. 2005), while demand by individuals increases with temperature and level of physiological activity (i.e. due to an increase in metabolic rate; Johnston and Jungalwalla 2005, Batt et al. 2005). Although aquatic animals vary widely in their requirements for oxygen, in general, cold-water species, such as the Stocky Galaxias, have lower tolerance for low oxygen levels than warm water species (Batt et al 2005), and few species are capable of good growth at concentrations below 50%, while concentrations of less than 35% should be considered critical from a welfare perspective (Johnston and Jungalwalla 2005). To maintain healthy aquatic animals in captive maintenance systems, dissolved oxygen should therefore be as near 100% saturation as possible (DeTolla et al. 1995).

Salinity

Some species can tolerate a wide range of salinity, while others only tolerate narrow ranges that may alter according to life stage (Batt et al. 2005). Where fish are held at salinities significantly outside their range of tolerance, osmoregulatory stress, impaired growth rates and reduced disease resistance may occur (Batt et al. 2005). At worst freshwater animals may have difficulty in maintaining fluid and electrolyte balance due to excessive water loss and dehydration, analogous to terrestrial animals deprived of access to drinking water (Johnston and Jungalwalla 2005). Alternatively, for marine animals maintained in freshwater, excess water retention (haemodilution) is likely to prove harmful, if not deadly (Johnston and Jungalwalla 2005). It is critical for these reasons to ensure Stocky Galaxias are kept at salinities within their range of tolerance (i.e. <3ppt). Any change in salinity, even if it is within a species known tolerance range, should be undertaken gradually (Johnston and Jungalwalla 2005).

Temperature

Many aquatic animals, including fish, crayfish and mussels are ectothermic, meaning that their body temperature and vital functions vary with that of the surrounding waters (Johnston and Jungalwalla 2005, Batt et al. 2005). The health, nutrient requirements, performance, reproduction and, indeed survival of aquatic animals are therefore dependent on the temperature of the water they are held (DeTolla et al. 1995). Temperature requirements vary among species as well as between estuarine and freshwater fish (Tomasso 1993). Stocky Galaxias should therefore be kept within a range considered appropriate for the species. Present data suggests Stocky Galaxias regularly tolerate water temperatures between approximately 2 °C to 18 °C in the wild (Allan et al. 2021; Raadik and Lintermans 2022a). Recommended maximum rates of change to water temperature are ≤ 2 C/hr or ≤ 10 C/day (Johnston and Jungalwalla 2005), however, should ideally be limited to about 1 C/hr (Tomasso 1993).

pH

pH effects dissolved gases, organic acids, phosphates, the ratio of non-ionized to ionized ammonia, solubility of metals, and oxygen uptake by aquatic animals (DeTolla et al. 1995). Most freshwater species in the wild live in waters with pH values ranging from 6 to 8 (Batt et al. 2005). A pH greater than 8.5 should be averted as ammonia toxicity is increased at higher pH, and colonies of nitrogenous bacteria in biological filters, that do not generally grow well outside the range of pH 7.2 to 8.5 (Batt et al. 2005), may collapse leading to catastrophic shifts in water quality (see section 5.7.1).

Aquaria should therefore be maintained at the lowest pH suitable for Stocky Galaxias being held, and within the range that nitrogenous bacteria grow well (i.e. pH of 7.2 to 8.5; Batt et al. 2005). Where pH levels approach 8.5, it will be necessary to undertake a partial water change (Johnston and Jungalwalla 2005). Alternatively, pH levels below 7 should be readjusted upwards (gradually) via the use of coral pieces (calcium carbonate). Where such changes are undertaken, however, they should be done gradually (Batt et al. 2005) and limited to less than 0.5 units over a 24-hour period (Johnston and Jungalwalla 2005). It is of utmost importance that any unplanned shift in pH, particularly those which are rapid, need to be investigated immediately and thoroughly, as they are a likely indicator of issues elsewhere in the system, most likely being the presence of waste products that in turn may detrimentally impact nitrogenous bacteria, water quality, and ultimately the health of the fish being held.

Water hardness

The critical component of total water hardness is the calcium concentration, or “calcium hardness”, and it is this component that should therefore be tested and monitored regularly (i.e. rather than general water hardness). The component is crucial as it maintains precise levels of internal salts in aquatic animals by influencing the permeability of gill membranes, preventing diffusive ionic loss to water (Flik and Verbost 1995), which is required for normal heart, muscle, and nerve function (Wurts 2002; Boyd et al. 2016). For most aquatic species, including the Stocky Galaxias, sufficient calcium will often be present in source water of captive breeding facilities and therefore it is unlikely required to be adjusted. In general, aquatic animals tolerate calcium hardness concentrations between 75 and 200 mg/L (Wurts 2002).

A1.2.3 Cleaning

Faecal material is rarely toxic, but its degradation causes deoxygenation of the water, production of nitrogenous compounds and development of toxic gasses. In addition, faecal material will contribute to organic suspended solids that can lead to gill diseases. No specific limits can be suggested but in general accumulation of faecal matter within an aquarium system should not be allowed (Johnston and Jungalwalla

2005). Debris should therefore be siphoned off on a regular basis to maintain water quality parameters within the predetermined range for species-specific requirements.

Aquaria also require cleaning when algae form on the front or sides and/or debris begins to gather on the bottom (Varga 2016). Cleaning is best undertaken using a magnetic aquarium glass cleaner and a siphon. To limit the possibility of transferal of disease between aquaria, cleaning equipment should not be transferred from one aquarium to the next without being sterilised (i.e. using a commercially net sanitiser). Ideally, each aquarium should have its own set of cleaning equipment.

Depending on biological load, biological filters should be rinsed, but not totally cleaned, in seasoned water taken from the aquarium they have been filtering. This preserves the bacterial community (Batt et al. 2005) and eliminates the risk of disease transferal from one aquarium to the next.

If equipment is to be stored, to minimise the risk of pathogens being present and to ensure aquaria are ready to be used when required, they should be drained, air dried, sprayed internally and externally with 70% ethanol, wiped down with clean paper towel, and stored with their lid in place. All piping, biofilters etc, should be flushed with a bleach solution (20 mL/L) and air dried. To reduce the spread of diseases among aquatic animals, countertops should be cleaned with 70% ethanol or isopropanol solution and then wiped down with a clean paper towel.

A1.3 Sexual maturation

The initial attempt to promote annual sexual maturation of individuals should be primarily via the manipulation of temperature and photoperiod (daylength) to mimic the onset of the spawning period (Stoessel et al. 2020a); late spring for Stocky Galaxias. Alterations to photoperiod and temperature have been shown in other aquatic species to cause faster final reproductive development in captive individuals (Pérez et al. 2011), while photoperiod alterations have produced correlative changes in the timing of spawning (Norberg et al. 2004). Manipulation of the annual photoperiod and temperature cycle is therefore a powerful hatchery technique in the maintenance of reproductive stocks (Norberg et al. 2004; Pérez et al. 2011). Fish should be considered reproductively ripe when ovaries are visually determined to fill approximately >90% of the body cavity.

It is likely that multiple spawning/stripping may be possible throughout a calendar year by repeatedly altering daylength and temperature to that of the winter solstice (~10 hours daylight, ~2 °C), and then into the late spring/early summer breeding period (i.e. ~14 hours daylight, ~11 °C). However, achieving water temperatures of ~2 °C, except in natural settings (i.e. waterbodies close to where the remaining population persists), is unlikely to be achieved for reasons outlined in Appendix 3. Such an extreme lowering of temperatures may not, however, be necessary, providing photoperiod is altered to mimic the winter solstice, and temperature in partnership is reduced as far as possible at the time, after which photoperiod and temperature are gradually increased to mimic that of the breeding season.

If this is done, a spawning cycle should be undertaken over several weeks (to a couple of months), to ensure there is sufficient time to allow broodstock to recover condition (i.e. following a spawning/stripping) and to reach sufficient sexual maturity prior to another round of spawning/stripping being undertaken. A note of caution, however, is that temperature is a key parameter in the denitrification process (Chen et al. 2018), and at low temperatures denitrifying bacteria will not be able to process metabolic waste efficiently (or at all). Therefore water parameters, particularly ammonia, nitrite, nitrate, and pH need to be monitored very closely at this time (i.e. daily), regular 30% water changes undertaken (daily), and treatments used such as Prime^{TM} in addition to slightly elevated salinity (i.e. < 3ppt) used where necessary to control water parameters. Failing to do so may be catastrophic, resulting in ill health, and potential death of individuals.

A1.4 Captive breeding

Once fish are determined as reproductively ripe, broodstock pairs can be maintained in individual aquaria under conditions conducive to natural spawning (e.g. flowing, shallow,, etc.) though a natural spawning protocol has yet to be developed. Alternatively, following Stoessel et al. (2015, 2020a), broodstock pairs can be hand-stripped without hormonal treatment or anaesthetic, ensuring the following:

- Operator hygiene - ensure hands are washed, and anything encountering the fish (including hands) are wet with water to minimise loss of protective mucus layer on the skin of broodstock.
- Pathogen control - to minimise the risk of disease transfer, all equipment is to be sterilised (using 250 g of salt per 5 L of water). Equipment is to be soaked in the solution for a minimum of 5 minutes.
- Egg stripping - the total oocytes produced by each female should then be stripped as a single layer onto the bottom of two or more plastic petri dishes that contain 5–10 mm of water (to aid in sperm motility)

from the aquaria into which they are to be placed, and that are lined with 1.5 mm diameter nylon mesh (e.g. to enable shed oocytes to be able to be easily transferred).

- Fertilisation - within 30 seconds of eggs being stripped, the genetically paired male/s should also be hand stripped directly into the water covering the oocytes. If required, the water can be gently moved over the oocytes using a fine, soft brush.
- Following fertilisation - within 30 seconds, the fertilised eggs should be placed into individually labelled incubators within 20 L aerated, glass aquaria (Figure A1-3, Figure A1-4; see Bacher and O'Brien 1989). Water within each aquarium should be aerated and recirculated.
- Egg holding temperature - aquaria containing eggs should be chilled to 11 °C to mimic that of the mean temperature of the breeding period (Allen et al. 2021).

Figure A1-3. Circular, poly-pipe egg incubator (left) and fertilised egg mass on nylon mesh inside an aerated egg incubator
(Images: Tarmo A. Raadik).

Figure A1-4. Circular egg incubators in a 20L glass aquarium (Imaged: Dan Stoessel)

A1.5 Recovery of broodfish

Spent adult fish should be treated in a saline solution (10 g/L) for 20 minutes, and then placed into a recovery aquarium, containing chilled water (i.e. 11 °C), for a further 30 minutes to monitor recovery, and treated with fungicide (Aquatopia® fungus eliminator) to prevent fungal infection. Following recovery, fish should be returned to the aquarium from which they were sourced, ensuring that it is at the same temperature as the recovery aquarium, and that it has had ProTech (Aquasonic Pty Ltd) added to counter loss of protective mucus on the skin of fish due to handling.

A1.6 Egg hatching

To maintain health of developing eggs, each incubator should be removed daily, placed in a shallow dish that contains water from the source aquarium, checked for fungus, and any fungus, dead (white) or non-fertile eggs, removed using a sterilised pipette. This method avoids eggs being damaged, which can allow a substrate for fungal growth. Egg incubators should then be placed in an aerated salt solution (10g/L) for 20 minutes for fungal treatment, ensuring the water is at the same temperature as the source aquarium, before being returned to the incubation aquarium. The time egg batches are out of water should be kept to a minimum (i.e. less than 30 seconds).

A1.7 Larval grow out

When larvae begin hatching, they should be transferred into static, aerated, 4 L aquaria and fed twice daily (starting with a liquid feed (Aquasonic Pty Ltd Complete Fry Starter), live rotifers, encapsulated food (JBL NovoBaby 01), and lastly *Artemia* nauplii). When free-swimming, larvae should be transferred into 10 L aquaria, and into 100 L aquaria once the yolk sac is absorbed ad a density of about 3 fish L^{-1}.

All water inflow and outflow valves on these aquaria should be wrapped in foam to avoid loss of individuals, and flow through of water should be minimised to avoid physiological stress due to water movement. As fish grow, they should be monitored closely, and if aggression is observed, density of animals adjusted appropriately (see A1.9).

Where possible, live food should be provided to the larvae, particularly prior to release to the wild, to limit food related aggression, and to promote natural feeding behaviour, which in turn maximises the likelihood of establishment of individuals in the wild. Human contact during larval growth should also be minimised, and feeding preferentially automated (where possible), to reduce altered behaviour of individuals that may increase predation of individuals in the wild. Where automated feeders are used to deliver pelletised food, they should be cleaned daily to halt the growth of mould and fungus, which, if ingested is extremely toxic to fish (Wedemeyer 1996).

A1.8 Health surveillance

Suspected health problems should be investigated promptly (Johnston and Jungalwalla 2005). The best time to check animal health is during feeding, as many species become active as food is placed into an aquarium. An unusually sluggish response to food may be a warning sign of a health issue (Huntley and Langton 1994), and other signs include unusual swimming behaviour, increased or laboured respiration, repeated rubbing or ‘flashing’ against hard surfaces, protruding scales, clamped fins, bulging or cloudy eyes, emaciated appearance, erosion at the fin extremities, long, stringy faeces, and blotches, spots, lumps, ulcers, or cotton-like growths on the body or fins (Huntley and Langton 1994).

In the case of an outbreak of ill-health in individuals housed in either broodstock, larval or grow-out aquaria, the following should be immediately investigated:

- Water parameters, including the functioning of aerators, chillers/heaters and filters.
- Possibility of poisoning, e.g. from iron, copper or fungal toxins, or contamination by detergents, soaps, perfumes, paints, glues, solvents, or insecticides (Huntley and Langton 1994).
- Possibility of physical damage because of antagonistic and territorially related aggression by other animals.
- Potential nutritional deficiencies, e.g. manifesting as spinal deformities, cloudy eyes, and skin haemorrhaging (Huntley and Langton 1994).
- Pathogens (parasites, bacteria, viruses, fungi) (e.g. DPI VIC 2005, 2008).

Whilst some health and welfare problems can be fixed by innocuous treatments (such as a temporary change in salinity) or by changing management practices (flow rate, diet, structural modifications, etc), pharmaceutical treatments for control or elimination of pathogens are also often required (Johnston and Jungalwalla 2005) (see A1.1.1, above). Key steps to maintaining fish health are constant surveillance leading to early detection, accurate identification of the cause and correct treatment, and often may involve a veterinarian with aquatic expertise.

A1.9 Density of animals

Stocking density in an aquatic system incorporates more than just the number of animals in a space (Beleau 1990). Aquatic fauna, particularly fish, inhabit a complex three-dimensional medium and are influenced by their environment to a much greater degree than terrestrial species (Williams et al. 2009). Quantifying the adequate space for each animal is therefore complex. The number of fish that can be carried in each volume of water is extremely variable and depends on the species, size of individuals, water temperature, pathogen load, dissolved oxygen level, metabolic rate of the fish, feeding rate, and how fast the water is being exchanged (Batt et al. 2005). Nevertheless, maintenance of tolerable levels of oxygen, metabolites, and suspended solids, integrated with changes in temperature, will be a good guide to the appropriateness of stocking density in the system. Any drift of conditions outside of desirable parameters will require remedial action; either improving water quality or reducing density (Johnston and Jungalwalla 2005). Where animals display territoriality and aggression, they should be graded periodically to ensure similar sizes (and sexes where necessary) within groups (Batt et al. 2005) and densities reduced if necessary.

A1.10 Feeding and nutrition

Fish brought in from the wild will often learn to feed effectively on pellets and show remarkable flexibility in their ability to ingest and digest formulated feeds (Goddard 1996). Where used, however, dry feeds should be stored at temperatures < 20°C and humidity < 75% (Goddard, 1996), as high temperatures destroy certain vitamins and degrade lipids, and high humidity increases susceptibility of the feed to mould (Goddard, 1996). Mouldy food is of most immediate concern as if ingested, it can be highly toxic; affected food should never be used (Wedemeyer 1996). Vitamins in artificial feeds can also be destroyed by oxygen, ultraviolet light, and lipid peroxidation (Goddard 1996). Certain feeds can be frozen to extend their shelf life, especially when relatively low amounts of feed are required, however, freezing and thawing may degrade micronutrients. Where such a strategy is employed, the use of additional supplements is as a result commonly required (Goddard 1996).

Live food, such as bloodworm, earth worms, etc. are also valuable sources of nutrients, but can be time-consuming to prepare or source. As a varied diet has more value, fish can be fed a range of formulated and live feeds.

Formulated feeds can be expected to provide the nutritional requirements of the species for which they are designed (Nickum et al. 2004). General commercial formulated feeds, however, are unfortunately designed to meet broad, non-species-specific nutrient requirements (i.e. protein, carbohydrates, and fats; Nickum et al. 2004). Captive fish of varied species for which a feed is not specifically designed, such as Stocky Galaxias, will frequently consume these feeds; however, their requirements may not be met over time (Nickum et al. 2004). For longer term health, essential amino acids, vitamins, and minerals must be in proper ratios (DeTolla et al. 1995).

As nutrition is integral to longevity, disease prevention, growth, and reproduction (Dierenfeld 1997), deficiency of nutrients can result in reduced growth rate and feed consumption and disease (NRC 1993; Conklin 2000). It is therefore important that a varied diet is offered to Stocky Galaxias broodstock and offspring. Overfeeding, or refusal by fish of artificial foods can also cause serious water quality problems via deoxygenation of the water, production of nitrogenous waste products and toxic gasses, while also promoting the growth of potentially harmful bacterial and fungus (Johnston and Jungalwalla 2005; Batt et al. 2005). Where these types of foods are used therefore, close monitoring, and regular siphoning of remnant food is required to limit problematic water quality and disease.

A1.11 Record keeping

A1.11.1 Care records

Each broodstock, egg development or larval rearing aquarium should be uniquely numbered to ensure they can be easily identified, and records attributed to them.

Detailed records should be kept of:

- The source, date of capture and number of Stock Galaxias entering the facility, and which aquaria they are placed into.
- The movement of animals between aquaria. For example for treatment in hospital aquaria, and any mortalities suffered, when they occurred, the likely reason for deaths (e.g. fungus, parasites etc.), and whether the carcass of the deceased animal was preserved (i.e. frozen or placed in ethanol or formalin) and where it is stored.
- Which individuals were used for breeding and which pairs were mated.
- The location of fertilised egg masses in incubator tanks.
- Location of larvae reared from individual fertilisation events, etc.
- Date, tally of individuals and destination when leaving the facility.

A1.11.2 Checklists

Checklists should be developed that ensure staff complete daily and weekly duties and that there is a record of all incidents and equipment service (i.e. by date; Shepherd and Bromage 1988; Batt et al. 2005). Doing so ensures a consistent level of care is provided to the animals while in captivity, the facilities and aquarium are maintained to a high standard, and that handover from one staff member to another is undertaken with ease. These records need to be sufficient to allow staff who care for the animals on entry to the aquarium to quickly identify what has been undertaken and when, what needs to be done, and what are potential issues of concern. As such the checklist should include checks on:

- Personnel hygiene – have personnel washed their hands prior to undertaking aquarium work? This is important to ensure chemical and bacterial contamination does not occur.
- Visual checks on animal health – are animals displaying unusual behaviour or signs of disease, and if so in which aquaria?
- Treatments – was medical treatment provided and if so for which individuals, what aquaria, and for what?
- Aquaria hygiene - do aquaria require cleaning and if so which aquarium and was it done?

- Facility hygiene practices - are floors and benches clean and clutter free? Was cleaning undertaken?
- Illness and mortality – were there any deaths and if so from which aquaria, and what was the likely cause?
- Feeding – were the animals fed and if so with what?
- Movement of animals – were animals moved from one aquarium to another and if so, was this recorded?
- Water quality - were water quality parameters (see section 5.7) recorded, was a 30% water change on any aquarium conducted, and were any treatments relative to water parameters (e.g. the addition of Amguard or Prime) and animal health (e.g. antibiotics or fungus treatments) undertaken? Are any water quality parameters of concern, and if so if which aquaria?
- Aquarium functionality - is the air on, water flowing, filters functioning, chillers/heaters on, any water leaks, lids are on aquarium etc.? Is there any equipment of concern that may not be functioning properly?
- Maintenance undertaken or required - is equipment functioning correctly, and if not, were repairs undertaken, repairs arranged, or the equipment replaced? Does anything need to be ordered, and if so what equipment, and has the order been completed?

A1.11.3 Standard operation procedures

Standard operation procedures (SOPs) should be developed to ensure consistent guidelines exist for the care of all animals being held, that sanitation of aquaria and equipment is guaranteed, and that maintenance of the facility is undertaken. A hard copy of SOPs should be kept within the facility, so they are easily accessible. These should also be regularly reviewed and updated as required.

A1.12 Considerations for offspring release to the wild

As a rule, larger fish are more likely to establish after release, and they also provide a more controlled reintroduction program (e.g. can be marked for monitoring) (Storck and Newman 1988; Hyvärinen and Vehanen 2004; Hammer et al. 2012). However, there are trade-offs between the size of fish released and the time in captivity (Hammer et al. 2012). For example, younger and smaller fish are likely to experience much higher mortality due to limited feeding ability and increased vulnerability to predators (Garrido et al. 2015), preferentially therefore, younger, and smaller individuals should not be released. However, this needs to be weighed against the economic cost of maintaining individuals in captivity, as the longer an individual is kept in captivity, the higher the economic cost.

Predation and competition at sites already established may also present a barrier to establishment from larvae as will the timing of when fish are released (Hammer et al. 2012), and this should form part of the consideration in the Translocation Plan. The time to translocate individuals from when hatched will therefore depend on the conditions at proposed translocation sites, whether Stocky Galaxias are already present at the site (and therefore what size they are and the extent of the cannibalism threat), the size of release individuals, and the time of year.

Where animals are being returned to the wild from captivity, in addition to treatments outlined in Raadik and Stoessel (2022), fish should also be fasted for 12 to 48 hours prior, to ensure an empty gut, thereby limiting nitrogenous waste production, oxygen demand and water pollution during transport (DeTolla et al. 1995; Weirich 1997; Batt et al. 2005).

APPENDIX 2. Hatchery facility requirements

A2.1 Water quality/supply

Water quality is the most important factor in maintaining the well-being of aquatic animals and in reducing stress and the risk of disease (Batt et al. 2005). Where water quality parameters fall chronically outside requirements, welfare, growth, and eventually survival of animals are compromised (Johnston and Jungalwalla 2005).

If a captive breeding facility is to be established, a comprehensive analysis of water quality parameters (ions, pH, metals, etc.) should be conducted of water entering the facility to ensure it is suitable (Batt et al. 2005). As chlorine is highly toxic to aquatic animals, where a municipal water supply is used the water must be either dechlorinated by aeration or by filtration through activated carbon or chlorine-precipitating compounds such as sodium thiosulfate (DeTolla et al. 1995; Nickum et al. 2004). Where contaminants are identified, additional measures such as reverse osmosis may also be required (Huguenin and Colt 2002).

A2.2 Construction materials

Construction materials should not contain copper, nickel, cadmium, or brass as these are toxic to aquatic fauna (DeTolla et al. 1995). The floor and benches of the facility should be sealed, smooth, easily cleaned and made of impervious material (Batt et al. 2005). Benches, shelves, and floors should also be designed to ensure catastrophic collapse of equipment, and potential injury to staff and animals does not occur. The use of timber (particularly treated) should be avoided.

A2.3 Additional considerations

Where life support components are used to treat, oxygenate, heat or chill water, they should be replicated, to provide a back-up system in case of component failure. An automated alarm, in addition to a back-up generator and air compressors should also be present to ensure the loss of electrical supply or malfunction of essential equipment (such as air compressors if used), do not result in the loss of animals. Where animals are kept within a building, airflow within the facility should ensure that excess humidity is removed to discourage structural damage to room fixtures and growth of pathogenic bacteria and fungi, both of which may be harmful to employees as well as aquatic fauna (Batt et al. 2005). The facility should also have good climate control, enabling rooms to be heated or cooled, thereby assisting with maintaining conditions and reducing the reliance on heaters and chillers within the aquaria themselves to maintain appropriate water temperature. Furthermore, water flow within aquaria, tanks, ponds and/or raceways should be sufficient to remove suspended solids and wastes, to ensure that water parameters are maintained within acceptable levels, and to promote fishes to swim correctly and to maintain normal behaviour.

To eliminate potential stress to aquatic fauna in the form of a startle response caused by sudden changes in light intensity (Williams et al. 2009), or if necessary to mimic natural photoperiods to promote annual reproductive progression (i.e. in addition to shifts in water temperature) lighting should be able to be timed to gradually and automatically increase or decrease in intensity, and ideally be restricted in its dispersion or placed at a lower level than the aquarium surface (Batt et al. 2005).To limit the potential for the introduction of disease, access to facilities should be secure, and restricted to personnel required for maintenance of the facility and the care of animals (Batt et al. 2005).

The water within each aquarium, tank, pond, or raceway should also be independent of the next to limit the possibility of transferring pathogens, fungus, and disease. Where water is recirculated, it should never, without appropriate treatment (ozone, active carbon and sand filtration, oxygenation) be introduced from one aquarium to the next. Nets, buckets, or any other equipment used within the facility should be sterilised after each use and should never be shared between aquaria to limit the possibility of transfer of disease, parasites, or spores.

APPENDIX 3. Types of aquaculture systems

Three types of aquaculture systems, flow-through, recirculation, or closed freshwater systems are considered here for use in captive breeding of Stocky Galaxias. The choice of system (flow-through, recirculation or closed freshwater aquaria) will largely depend on the programs budget, scale of breeding required, in addition to water availability and reliability. Each system, nevertheless, has benefits and potential challenges.

A3.1 Flow through system

Flow-through systems are commonly used in commercial aquaculture (Figure A3-1). This type of system uses water taken from a waterway, that then flows through a settling pond, and into a system of aquaria, tanks, ponds and/or raceways (Figure A3-1). Water is then directed back into the waterway from which it was removed. Such systems, unless already pre-existing, require a considerable investment and a large quantity of water that is of a consistent high quality (Batt et al. 2005), and therefore such a facility needs to be built close to a waterway. Water parameters including turbidity, dissolved oxygen, water temperature, pH and hardness are ultimately determined by that of the inflowing water. If used for the purpose of captive breeding of Stocky Galaxias, such facilities would therefore need to be located near waterways that display comparable annual water parameters to the reach of Tantangara Creek from which the fish are sourced (i.e. lows of about 2 °C and maximums of about 18 °C). If year-round breeding of the species is required, then temperature manipulation (in addition to photoperiod) is also required, something that would be difficult to achieve using such a system without appropriate facilities being constructed. It also remains unresolved whether Stocky Galaxias can be maintained as self-sustaining populations in tanks, ponds, or raceways. There is also a possibility using this system that where a facility is located away from waterways within which the species is native, that fish may be exposed to parasites or disease that they are unaccustomed (i.e. due to water coming straight from a waterway without being treated). If infected, these parasites and diseases may then be introduced into the wild on release which may prove devastating for the species. Furthermore, where fish are kept in ponds and raceways there is a possibility, although small, that fish may escape to local waterways, where they may establish. On the positive side, flow-through systems located near the last remaining population of Stocky Galaxias would be able to achieve annual temperatures as low as 2 °C (i.e. to mimic the winter solstice) that would be beneficial for promoting annual sexual maturation of broodstock.

Figure A3-1. Examples of flow through systems

(eurofishmagazine.com)

A3.2 Recirculation system

Comparatively, a recirculation system can be located away from waterways as they use less water than flow-through systems, with a portion of water that would normally be expelled being recycled and reused by passing it through filters to remove metabolic and other waste products (Fisher 2000; Figure A3-2, Figure A3-3). Such a system may use aquaria placed on shelves or benches (rack style), a series of tanks, or a combination of both (Figure A3-4). When well designed and operated, these systems can maintain water of adequate or even superior quality than a flow-through system (Nickum et al. 2004), while also being able to customise temperatures to the requirements of species being held (i.e. within groups of aquaria).

Despite the superiority of these systems in maintaining water parameters, and therefore desired outcomes, they are costly to build, operate, and maintain (DeTolla et al. 1995; Ngoc et al. 2016), and require regular attention by specialised maintenance personnel. At the extreme low temperatures likely required to promote sexual maturation in Stocky Galaxias (i.e. 2 to 11 °C), such a system, without additional engineering, may also be incapable of converting highly toxic ammonia and nitrite into less toxic nitrate, due to denitrifying bacteria failing to function, or even survive at such low temperatures. Such problems, nevertheless, can be overcome via the addition of chemicals to the water (e.g. Prime^{TM} Seachem), or with the use of zeolite filters. Depending on the size of the system in question, however, a considerable volume of chemicals may be required to neutralise ammonia and nitrite, or where zeolite filtration is used, the substrate requires regular replacement, both of which would add considerably to the cost of operating the system.

As a final note on the application of this system, it is unlikely that the extreme low water temperatures potentially required to promote final maturation (i.e. ~2 °C) could be achieved due to the volume of water needed to be consistently chilled, and limitations of engineering in doing so. At best, such a system may be able to achieve temperatures between 5 and 10 °C. Optimistically, however, such extreme water temperature may not be required to achieve sexual maturation, providing water temperature declines occur in partnership with reductions in photoperiod to mimic winter conditions. Regardless of the temperature limitations of this system, however, it is undoubtedly suitable for captive breeding where broodstock is captured from the wild at an advanced stage of sexual maturity (i.e. late winter to early spring). Its suitability in promoting sexual maturation of Stocky Galaxias following a spawning/stripping is, however, at present unknown, and therefore requires further work if such a system is used.

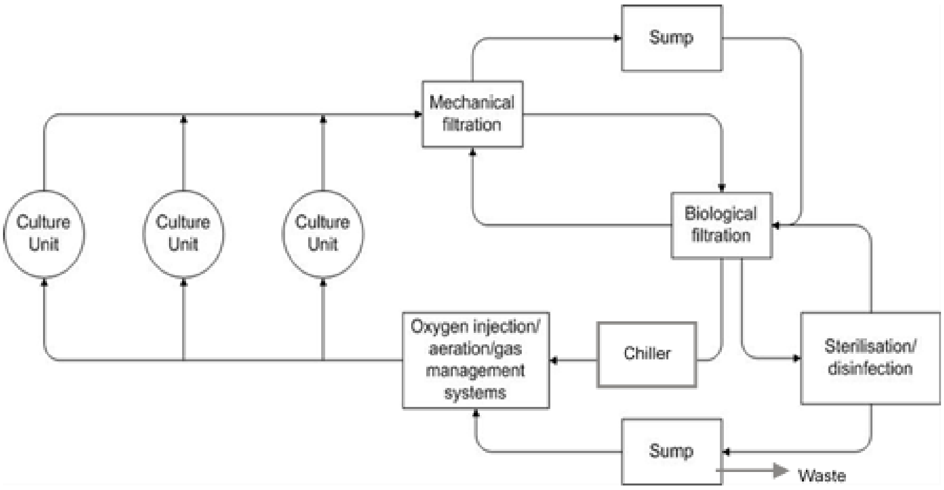

Figure A3-2. Conceptual drawing of a recirculation system

Figure A3-3. Example of recirculation system (Taiwantrade.com)

Figure A3-4. Examples of a rack style aquarium set-up at ARI (left) and Narrandera Fisheries Centre (right).

(Images, from left to right: Tarmo A. Raadik; NSW DPI)

A3.3 Closed freshwater system

A closed freshwater system is generally set-up in a rack style, with multiple aquaria arranged either along a bench, or on custom built shelves (Figure A3-4, Figure A3-5). The benefit of these systems is that they are comparatively cheap to set up, can be customised to accommodate a variety of species that have varied requirements, and that they can be broken down into their components which can then be stored when not in use. To their detriment, however, they are time consuming to set up and break down, challenging to maintain (and therefore require dedicated staff with an in-depth knowledge of the systems and the management of water parameters), have very low inherent biological carrying capacity, and are prone to rapid changes in water quality (Batt et al. 2005). Water management is therefore crucial (DeTolla et al. 1995) with such systems requiring considerable, regular attention. As a result, they are often difficult and comparatively labour intensive (and costly) to operate and maintain.

As with a recirculation system, it is also unlikely that the extreme low water temperatures potentially required to promote final maturation could be achieved using this system, however, it is undoubtedly suitable for captive breeding where broodstock is captured at an advanced stage of sexual maturity.

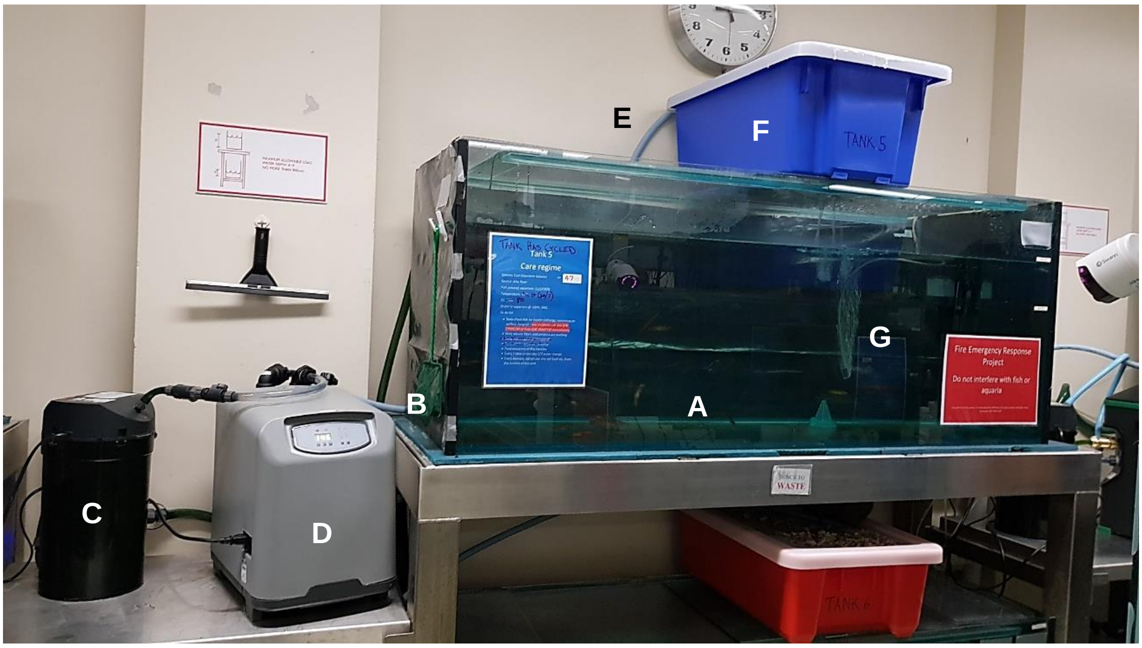

Figure A3-5. Example of a closed freshwater system at ARI

A – glass aquarium, B – location of outlet, C – Eheim Classic 2217 filter and pump, D – Teco TR20 chiller, E – biofilter inlet, F – biofilter, G – aeration. (Image: Tarmo A. Raadik).

www.delwp.vic.gov.au

www.ari.vic.gov.au