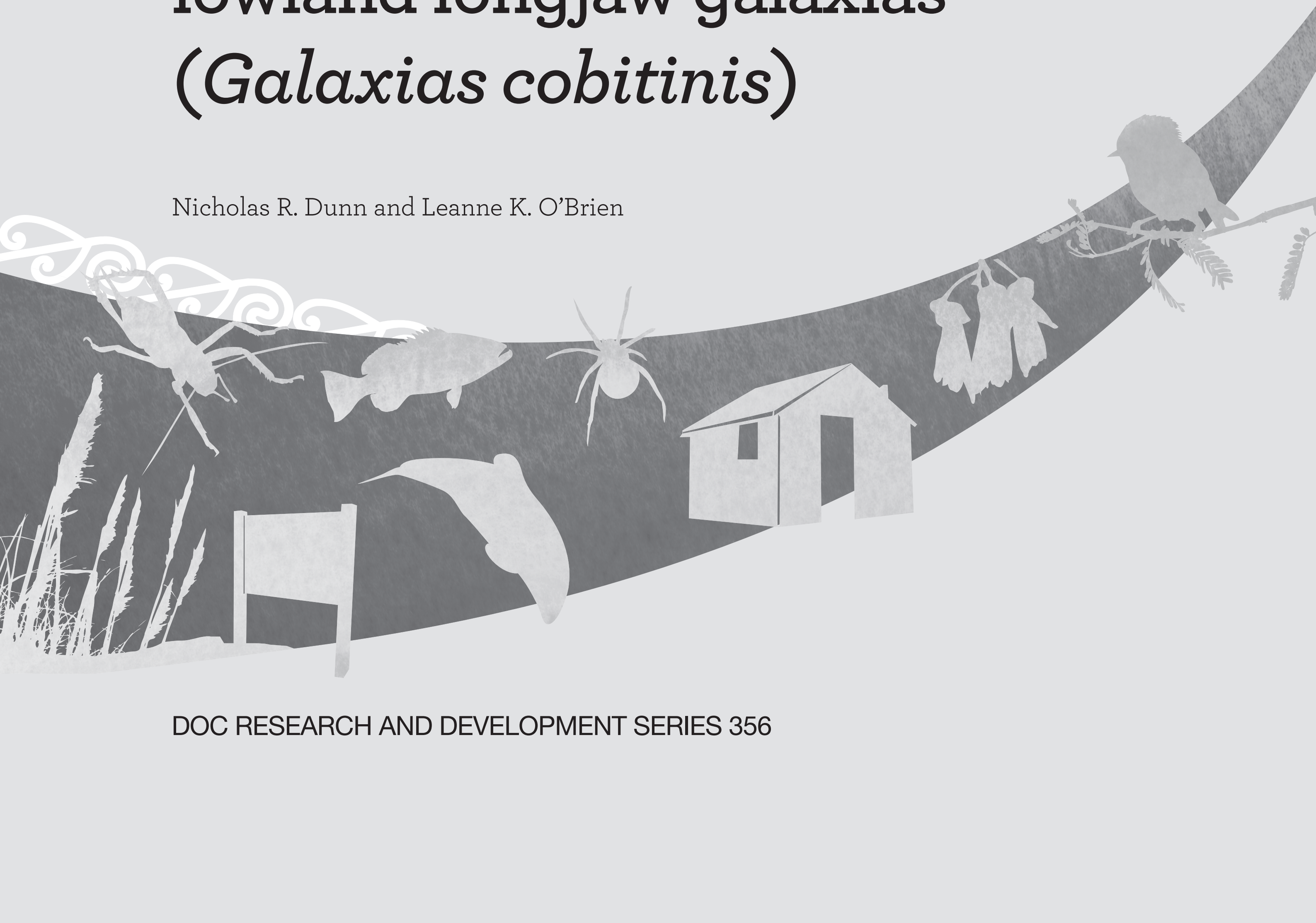
Considerations in the transport and captive management of lowland longjaw galaxias (*Galaxias cobitinis*)

Nicholas R. Dunn and Leanne K. O'Brien



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CONTENTS

Appendix 1

Considerations in the transport and captive management of lowland longjaw galaxias (*Galaxias cobitinis*)

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Abstract

Lowland longjaw galaxias (*Galaxias cobitinis*; Threatened: Nationally Critical) only occur in the Kakanui River catchment in North Otago, New Zealand, which can experiance reduced surface flow conditions during summer and early autumn. To guide conservation options during future low flow events, studies of the optimum conditions for holding *G. cobitinis* in captivity for periods of up to 3 months were undertaken. Methods were also developed to transport *G. cobitinis*, hold fish in temporary facilities, feeding, and the treatment of parasitic, fungal and bacterial infections. Investigations focussed on growth rates, and relative condition of *G. cobitinis* held at (i) different densities and (ii) in differing substratum treatments, with or without additional water current. We found that *G. cobitinis* can be successfully held in captive conditions for several months with low mortality, even at densities of > 50 fish/m^2. *Galaxias cobitinis* readily adapted to a diet of frozen bloodworms and any incidences of disease were readily treated with standard off-the-shelf preparations. Our studies indicated that characteristics of the holding tanks were important. *Galaxias cobitinis* held in tanks with angled substratum, imitating riffle habitat, had higher growth and relative condition, and less ectoparasites, than those in tanks with a flat layer of substratum. These results were possibly influenced by the differing amounts of cover, potentially affecting stress levels and susceptibility to disease; and that deeper water required greater energy expenditure in the flat substratum treatment. Furthermore, supplying additional water current with submersible pumps reduced fish condition, likely due to additional energy demands. Optimal conditions for holding *G. cobitinis* likely involve shallow water levels in tanks with abundant cover, and water current supplied by aeration only. These findings may also be useful for the captive management of other, small, range restricted, non-migratory fish species that are threatened by drought disturbance or water abstraction.

Keywords: *Galaxias cobitinis*, lowland longjaw galaxias, Galaxiidae, collection, captive management, fish transport, captive diet, fish growth in captivity, fish salvage, disease

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1. Introduction

Reduced river flows associated with drought disturbance or water abstraction have been identified as a major risk to the future security of range restricted, non-migratory *Galaxias* populations (Allibone 2000; Baker et al. 2003; Dunn 2003; DOC 2004). A number of these populations occur in gravel-bed streams and rivers on the east coast of the South Island of New Zealand (McDowall 2000), where it is considered that the frequency and severity of drought disturbance will increase in the future (Mullan et al. 2005).

Galaxias cobitinis McDowall & Waters 2002, is only found in the Kakanui River catchment, North Otago, and has the conservation status of Nationally Critical $_{\text{CD, EF, OL}}$ (Dunn et al. 2018). The Kauru River, a Kakanui River tributary, can support significant numbers of *G. cobitinis*. However, large population size fluctuations can occur, due in part to the prevailing hydrologic conditions (Dungey 2003), such as the loss of surface water flow during summer and early autumn; yet, subsurface flow can remain in some areas (P. Ravenscroft, formerly Department of Conservation, Dunedin; personal communication). For example, during severe drought conditions in the summer of 2001–02, the known *G. cobitinis* population was estimated at only 250 individuals (Allibone et al. 2003). Consequently, several short-term conservation management options have been considered in the event of drought, including the use of excavated refuge pits as a means of retaining water within the riverbed, and fish salvage with subsequent holding in captive facilities.

The keeping of Galaxiidae in aquaria has been widely reported on previously (e.g. Davidson 1949; Benzie 1961; Eldon 1969; Cadwallader 1973; Meredith 1981, 1985; Dean 1995; Davidson 1999; Gay 1999; Perrie 2004; O’Brien 2005; O’Brien & Dunn 2005; McQueen 2010; Dunn 2011). However, careful consideration is required when designing holding facilities for rare species such as *G. cobitinis* which presents a particular challenge, as its small size (usually less than 70 mm Total Length (TL)), allows individuals to enter the smallest of spaces (Dunn & O’Brien 2006).

The present study aimed to establish the feasibility of, and optimum conditions for, holding *G. cobitinis* in captivity over a period of 3 months, as may be required during a fish salvage operation in the event of a severe drought, fulfilling Action 7.3 of the non-migratory galaxiid fishes recovery plan (DOC 2004). This study also developed methods for the transporting and holding of *G. cobitinis* in temporary facilities, their feeding, and preventative treatment of common infections.

This report is structured in two parts. The first section describes the general methodology we developed for the transport, facility design, feeding regime, and maintenance of water quality and fish health, during the study. The second section summarises the results of two experiments that investigated optimal *G. cobitinis* density in tanks, and the degree to which tank conditions need to replicate those *G. cobitinis* experience in the wild.

2. Methodologies and observations in fish transport, facility design, and fish health

2.1 Transport

In the event of a drought or other immediate threat to their natural habitat, fish are likely to already be experiencing stressful conditions as surface water flow ceases. Thus, it is important that steps be taken to minimise any additional stresses during a salvage operation. Under any transport situation, fish will be subjected to changes in water temperature or quality, and external stimuli such as light, engine vibration or exposure to air during handling (Swanson et al. 1996). These stresses are difficult to avoid, but can be minimised by ensuring that the design of transport containers allow some degree of temperature control, provide sufficient aeration and minimise 'slosh' to reduce the risk of injury to fish, particularly during transport over gravel-bed rivers. During transport, water temperatures should be monitored, and maintained as low as possible, to enhance oxygen saturation and reduce stress to the fish. This is especially important if fish salvage is conducted during the height of summer.

We have progressively developed containers for the transport of large numbers of galaxiids. Most successful have been Willow® Quick Serve 44-L chilly bins, modified to hold plywood baffles, airlines and air diffusers. Chilly bins were found to better maintain low water temperatures than single-walled buckets. The design of this particular chilly bin allowed the water temperature to be kept lower than normal, and the bins and fish to be checked without having to fully remove the lid (Fig. 1A & B). Closed cell foam was positioned vertically on the inside of the bins to secure the baffles in place while still allowing their quick removal to aid fish capture following transport (Fig. 1C).

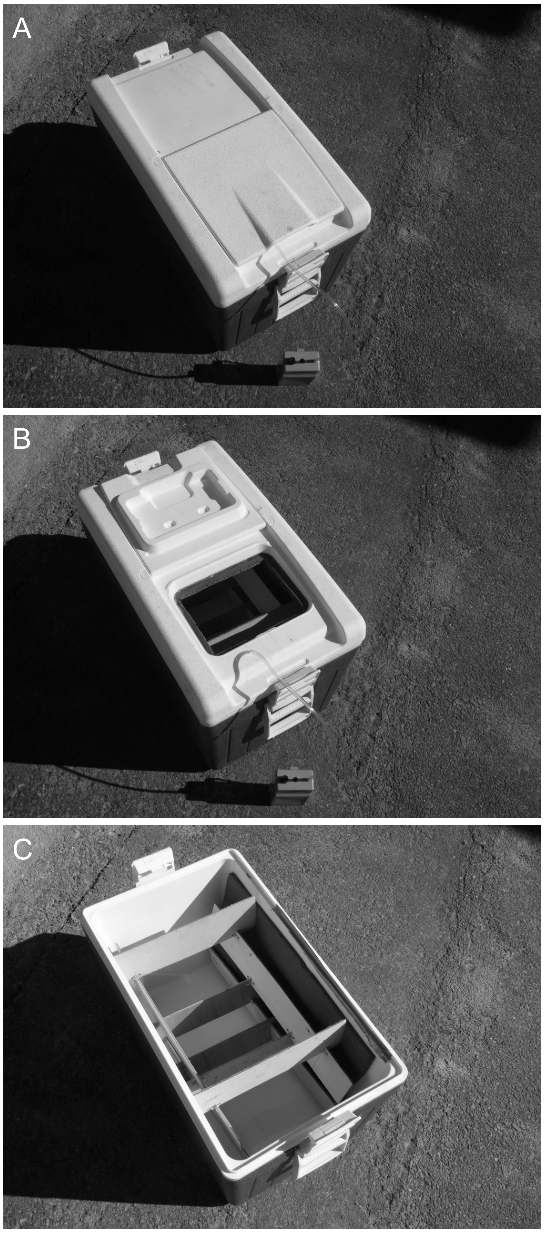

Figure 1. Willow Quick Serve 44-L chilly bin and portable 1.5-V battery-powered bubbler used to transport *Galaxias cobitinis* , showing the Quick Serve lid A. closed; B. open; and C. completely removed to show the plywood baffles and position of the foam.

We transported 130 *G. cobitinis* (divided evenly between two bins) on a 4-hour journey from the Kauru River to Christchurch, on 10 February 2005, a very hot (> 25°C) summer day. Low water temperatures were maintained by adding an ice cube (non-chlorinated water) to each bin at approximately 10-minute intervals. Aeration was supplied by either a portable 1.5-V battery-operated pump or a 240-V electric pump powered through an inverter from a 12-V battery. No *G. cobitinis* mortalities occurred during transport.

During transport, several 'off-the-shelf' standard treatments were also added periodically to the water at recommended concentrations to reduce stress and prevent infection. Treatments included transport salts (e.g. Brooklands tonic salt, Brooklands Aquarium Ltd; non-iodised rock salt can also be used), as small amounts of salt have previously been shown to reduce stress during the transport and initial holding of freshwater fish (e.g. Hattingh et al. 1975) by reducing osmoregulatory dysfunction and plasma cortisol levels (Swanson et al. 1996). Salt also reduces the susceptibility of fish to infections (Benzie 1961; Hattingh et al. 1975). The stress-reducing formulations Aqua Plus (Rolf C. Hagen (U.S.A.) Corporation) and Stress Coat (Aquarium Pharmaceuticals Ltd) were also added at recommended dosages. These products contain stress-reducing plant extracts and polymers, and coat fish in a protective artificial mucous, remove chlorine and neutralise heavy metals. A similar product, NovAqua, was found by Swanson et al. (1996) to be effective in increasing the survivorship of delta smelt (*Hypomesus transpacificus*) during and after transport.

2.2 Temporary holding facility design and operation

The transport containers described in section 2.1 and shown in Fig. 1 were also useful for the temporary holding of *G. cobitinis*, with the addition of half-round sections of plastic pipe for cover. However, although this facility was convenient, allowing quick setup, it was more time consuming to maintain than the long-term reticulated flow-through facility outlined in section 2.3. Partial (c. 50%) water changes, and removal of uneaten food and faecal matter were required on a daily basis to maintain water quality. And it was also likely to have been more stressful, as it was difficult to change the water without startling the *G. cobitinis*. We initially held 130 *G. cobitinis* in transport containers for 16 days, during which time only three fish died (2.5% of fish held). One *G. cobitinis* mortality showed obvious signs of an existing pathogenic infection and a probable tumour. The cause of death for the remaining *G. cobitinis* was not obvious, but was likely related to the stresses involved in transport and acclimation to captivity. This level of mortality was lower than that reported for previous captive holding experiments (e.g. Hattingh et al. 1975; Swanson et al. 1996).

2.3 Long-term holding facility design and operation

2.3.1 Facility design

The long-term *G. cobitinis* holding facility consisted of thirteen 120-L lidded storage bins ('tanks'; 75 cm long × 50 cm wide × 45 cm high) with a trickle-through flow system (Fig. 2). This facility was situated outdoors in a semi-shaded position and had a total footprint of 15 m^2. Twelve of these tanks were used in the experimental investigations outlined in section 3, while the thirteenth held extra *G. cobitinis*. Assembly of such a facility can be time consuming, and a trade-off exists between time and money. The materials used in this facility were chosen for their widespread availability, compatibility and low cost (see Appendix 1). However, many other options would have been suitable as a holding facility, and time could be saved by using ready-made components, such as cattle troughs already fitted with ball cocks.

Figure 2. The long-term *Galaxias cobitinis* holding facility with lids removed and placed to the sides of tanks. The thirteenth tank is in the foreground with the mesh insert on the lid visible.

2.3.2 Facility operation

Tank water was sourced directly from the Christchurch municipal water supply. At the time of this study, this water supply was not treated in any form and had a high Ministry of Health grading (Ministry of Health 2004). Water was supplied to each tank via a system based on 19-mm (c. ¾ inch) lateral polyethylene pipe. The average depth of water in each tank was 28.5 ± 0.46 cm (± 1 SE). Water flow through each tank was controlled by a ball cock regulated inflow and a stand pipe outflow. The inclusion of a ball cock prevented the tanks from overfilling and the stand pipe prevented them from completely emptying if water inflow stopped. The rate of flow resulted in the complete exchange of water in each tank over a c. 24-hour period. The small size and rheotactic behaviour of *G. cobitinis* required that all inlets and outlets be covered with 1 × 1 mm mesh. This is essential, even for small gaps, as *G. cobitinis* can enter the 3-mm-wide inlet slots of small submersible water pumps (Dunn & O'Brien 2006).

Tanks were situated on wooden pallets to ensure a flat foundation. Pallets also provided height to allow for an effective fall for a gravity outflow to a garden drip line or into the municipal sewerage system. Aeration in tanks was supplied by an 80-W aquarium air compressor (Resun; pressure 0.030 MPa, output 0.088 m^3/min), which was distributed via 5-mm-diameter tubing and control valves to 15-cm-long air diffusers in each tank. This air compressor provided sufficient aeration to serve all 13 tanks.

Tanks had secure lids to reduce the entry of wind-borne debris, prevent *G. cobitinis* from escaping, and avoid accidental drowning. Sections were cut from the lids and replaced with fine nylon mesh to allow atmospheric exchange with the water (as shown in the front tank in Fig. 2). Tanks were also strengthened against buckling under the weight of the water by tensioning cord across the top of them.

Tanks were conditioned for 1 week before *G. cobitinis* were introduced, to allow biofilms and algae to develop on the plastic surfaces. This process was accelerated by the addition of specific, commercially available bacterial enzymes (Appendix 1). Once *G. cobitinis* were introduced, tanks were checked daily, with water flow and aeration being adjusted as necessary. During warmer periods, algal growth was prolific, which could potentially lead to the blocking of outlets. Thus, excess loose strands of algae were removed daily from tanks using a turkey baster. The retention of a thin algal layer was considered beneficial, however, as this promoted biological balance and nitrogenous waste uptake in tanks. Faecal matter and any uneaten food items were also removed daily using a turkey baster. Such tank maintenance is essential to maintain good water quality, and prevent prolific fungal and bacterial growths. Basic water chemistry tests (e.g. Aquarium Pharmaceuticals Ltd liquid test kits; Appendix 1) were performed weekly to check water quality. Water was never found to contain constituents at levels that were likely to have a negative effect on fish health.

Water temperature in the facility was monitored using both a data logger (HOBO; Onset Computer Corporation) and aquarium thermometers, to facilitate a quick estimation of current water temperatures. Situating the facility outdoors allowed natural variation, with minimum, mean (± 1 SE), and maximum daily water temperatures of 5.2, 12.6 ± 0.33 and 20.0°C, respectively, being experienced over the 3-month period (Fig. 3). The sudden drops in temperature recorded on 25 April and 9 May 2005 were due to a severe hail storm and a hard frost, respectively.

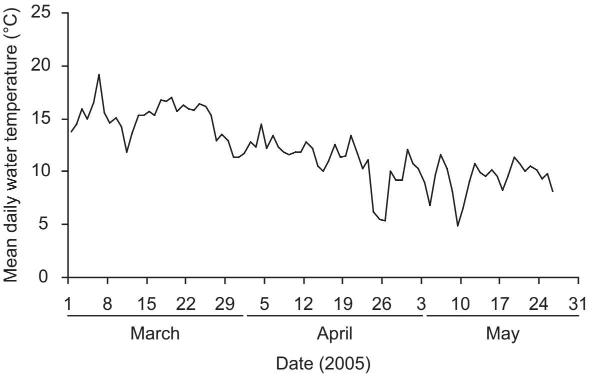

Figure 3. Mean daily water temperature (°C) recorded in *Galaxias cobitinis* holding tanks over the period 1 March – 26 May 2005.

2.4 Foraging behaviour and feeding regime

2.4.1 Foraging behaviour

Bonnett et al. (1989: 457) considered that the protruding jaw of *Galaxias prognathus* (upland longjaw galaxias) allowed it to obtain 'food from the underside of gravels and cobbles'. However, the observed behaviour associated with feeding in captive *G. cobitinis* in this study was initially typified by extremely active, apparently random, searches in the water column for extended periods, rather than searching on the tank bottom. This behaviour appeared to be extremely energy inefficient as, although *G. cobitinis* sometimes orientated themselves in the flow as if drift-feeding, they did not maintain station, i.e. stay in the same feeding position. This initial behaviour, which lasted for c. 2 weeks, may reflect attempts to escape, or may be a successful foraging behaviour in the wild. Feeding in captivity was conducted at discrete intervals, rather than as a continuous supply of prey, as would occur in the wild. Following the initial 2 weeks in captivity, *G. cobitinis*' foraging activity reduced to predominately those periods when food was given or the tanks were disturbed. Within 1 month, *G. cobitinis* had associated the disturbance of tank maintenance with the appearance of food, whereupon they would most often feed by darting to and from the underside of rocks to capture food in the water column and on the water surface.

2.4.2 Feeding regime

During the first month of the study, both live prey and commercially available foods were trialled as a suitable diet for the captive fish. Initially, invertebrates were collected from Cust Main Drain, North Canterbury, by kick netting. However, this approach was discontinued due to concerns over the likelihood of regularly introducing common pathogens and parasites to the tanks, coupled with transport costs. Moreover, many *Deleatidium* spp. mayflies appeared to be too quick to be captured by *G. cobitinis*; and cased caddisflies did not appear to be consumed. Stream-sourced samples also needed to be screened to remove predatory species such as *Archichauliodes diversus* (toe biter) which, although unlikely to consume *G. cobitinis*, may inflict injury, and thus increase the incidence of fungal and bacterial infections in the fish.

Culturing was found to be a convenient method of supplying live prey to *G. cobitinis*, and theroretically, the presence of live prey species in the tanks may help to process fish waste. Microcrustaceans, consisting predominantly of 'pond' species, were cultured in a mesocosm tank system. However, *G. cobitinis* tended to pursue larger, more visible prey, with smaller prey items, such as copepods, going largely unnoticed and persisting in tanks. This may have been due to *G. cobitinis* having small eyes (McDowall & Waters 2002) and, being a pencil galaxias, possibly lacking an accessory lateral line (McDowall 1997).

Various commercially available fish foods were also trialled. We found that dry, flaked fish foods were not readily taken; dry tubifex worms were apparently too small; and frozen brine shrimp were expensive and dissipated quickly into small particles, which floated and blocked stand pipe outlets, and were not consumed. However, *G. cobitinis* readily consumed frozen bloodworms, which became the major food type provided. During the experimental investigations (see section 3), *G. cobitinis* were fed approximately 0.15 g of frozen bloodworms per fish every second day. This equated to ¼ cube for 6 *G. cobitinis*, ½ cube for 10 fish, ¾ cube for 12 fish and 1 cube for 20 fish. The frozen cubes floated whilst thawing, slowly releasing bloodworms into the water column, which were large enough for *G. cobitinis* to easily see and capture. In summary, the final feeding regime adopted in our investigation was to provide small amounts of frozen bloodworms at regular intervals, whilst ensuring that live prey species were also present.

2.5 Fish health

During our investigations, we conducted regular observations and inspections of *G. cobitinis* to allow the early detection of disease outbreaks, so that we could intervene before the infection or prevalence of the pathogen intensified. Incidences of ubiquitous pathogens, most commonly the protozoan *Ichthyophthirius multifiliis* (ich or white spot) and the fungus *Saprolegnia*, were detected during the captive holding of *G. cobitinis*.

Ichthyophthirius multifiliis is a protozoan ectoparasite that is often carried at low levels in wild fish populations. However, when fish are confined and stressed, as occurs in captive conditions, fatal outbreaks can occur (McDowall 1990). The life cycle of the protozoan usually involves the detachment of a mature parasitic cyst from within the upper epidermis of the fish's skin; this then sinks to the substrate forming an encapsulated cyst (tomont), before bursting and releasing mobile, ciliated swarmers (theronts); these swamers must then find a host fish within 1–2 days (van Duijn 1973; Schubert 1987; Wurtsbaugh & Tapia 1988). This life cycle takes approximately 2 weeks, meaning that treatment of *I. multifiliis* needs to continue for at least 2 weeks following the appearance of cysts, which cannot be treated as they are protected by the fish's skin. The swarmers are vulnerable, however, and can be killed by chemical treatment or physical removal through regular complete water exchanges and wiping down the tank surfaces.

Fungal infections are also commonly encountered in freshwater fish. The spores of filamentous *Saprolegnia* species cause saprolegniasis. This is an opportunistic secondary fungal infection as it requires some pre-existing mechanical injury to be present, such as the lesions caused by *I. multifiliis* (van Duijn 1973; Schubert 1987).

Mortality occurred in four *G. cobitinis* individuals that had been weakened due to a combination of *I. multifiliis* and subsequent secondary fungal and bacterial infections; thus, a combined treatment regime was deemed necessary. Few guidelines have been published concerning the treatment of disease in captive Galaxiidae. However, Benzie (1961), Meredith (1985), Mitchell (1989), and Dean (1995) reported on their own experiences in treating disease in *Galaxias* and *Neochanna*.

In our experience, we found that using and following the directions given on standard off-the-shelf aquarium products obtained from a pet supply store (see Appendix 1) were sufficient to treat and eliminate *I. multifiliis*, bacterial, and fungal infections. *Galaxias cobitinis* were found to be tolerant of all preparations used, including malachite green, which is usually avoided when treating scaleless tropical fish. We commonly used malachite green, non-iodised salt, methylene blue, acriflavine and 1% melaleuca or cajeput oil – an extract similar to tea tree oil (Melafix; Aquarium Pharmaceuticals Ltd) to treat fungal and bacterial infections; and 2-phenoxyethanol was also beneficial, as it has anti-fungal properties (van Duijn 1973; Appendix 1). We also regularly used the off-the-shelf formulation Wunder Tonic (1.5% methylene blue, 0.1% malachite green, 0.05% acriflavine, 0.04% quinine; Brooklands Aquarium Ltd) to treat fungal infections. To treat parasitic infections, we used 5% formalin and the proprietary formulation MasterPet Cure Ex (0.1% negavon, 0.2% trichlorphon, 0.01% formaldehyde). Non-iodised salt and malachite green were also effective in eliminating *I. multifiliis* as well as secondary infections.

Our strategy when using chemicals to treat pathogenic infection was to use a combination of full strength treatment on fish held in temporary holding facilities, higher concentration treatment of tanks while fish were removed, with subsequent regular low-dose treatment of full tanks containing fish. The rational for this was that temporary holding facilities, including buckets, had smaller volumes of water, thus dosages were easier to administer, whereas, regular full-strength treatment of larger tanks required large volumes of chemicals, and was thus uneconomical. Intensive treatment of tanks in the absence of fish allowed tank surfaces and substratum to be thoroughly treated. This involved initially scrubbing substratum and tank surfaces; tanks were then partially filled, sufficient to just cover the substratum and treated with salt, formalin and malachite green at concentrations that would dilute to recommended levels once tanks were fully filled. The substratum was soaked in the concentrated treatment for at least 24 hours before tanks were filled and fish returned.

3. Optimal conditions for holding *G. cobitinis*

Two experiments were conducted simultaneously to determine whether, (i) the density of fish stocked, and (ii) substratum and water current conditions within holding tanks, affected individual *G. cobitinis* growth and condition. Concurrent to these experiments, investigations into batch and individual marking of *G. cobitinis* were also conducted O'Brien & Dunn (2018).

3.1 *Galaxias cobitinis* housing and handling during holding conditions experiments

Over the course of the experimental investigations, *G. cobitinis* were removed from the long-term holding facility at monthly intervals, and housed in a temporary facility that consisted of 12 aerated 25-L plastic bins (Fig. 4). This also allowed tanks to be emptied of both water and substratum, and cleaned.

Figure 4. Part of the temporary facility used to hold *Galaxias cobitinis* during monthly handling periods. Each 25-L bin has an air diffuser with air supplied through the tubing, and a half-round plastic pipe 'fish house'.

Prior to handling, fish were anaesthetised with 2-phenoxyethanol (0.6 ml/L), then measured to the nearest 0.5 mm TL, and weighed to the nearest 0.1 g using a Scout Pro balance. Initially, mean (± 1 SE) *G. cobitinis* length and weight were 51.9 ± 0.2 (range 34 – 61) mm TL and 0.56 ± 0.01 (range 0.05 – 1.0) g respectively, in the density and holding conditions experiments combined. The number of ectoparasitic cysts per *G. cobitinis* were also recorded during the April and May handling periods. Once handled, *G. cobitinis* were returned to their respective tanks and treated with stress-reducing preparations and preventative treatments that are effective against fungal and bacterial infections (as detailed in section 2.5).

3.2 Statistical analysis

Identification of individual *G. cobitinis* (see O'Brien & Dunn 2018), either by mark or dissimilar initial lengths of unmarked fish within a tank, allowed individual growth in length (mm/month), and relative condition (*Kn*) to be calculated between successive months, and over the entire experimental period. Relative condition was calculated using the allometric equation of the form $Kn = W/a{\cdot}L^b$, where W is an individual's weight (g), L is its total length (mm), a is the y-intercept and b the slope (Le Cren 1951; Anderson & Gutreuter 1983). Values of a and b were calculated from combined data for all individuals within each comparison. For the density investigation, one-way ANOVA was used to examine differences in growth in length and relative condition in relation to the numbers of *G. cobitinis* in tanks. Whereas for the holding conditions investigation, factorial ANOVA was used to examine differences in growth in length and relative condition in relation to water current and substratum treatments. All analyses were carried out using Statistica 6.0 (Statsoft Inc. 2001). Where mortality had occurred in a tank, *G. cobitinis* from the thirteenth tank (see section 2.3) were used to restock the experimental tanks to the required density; however, these replacement fish were not included in the analyses.

3.3 Investigation 1 – effect of *Galaxias cobitinis* density on growth and condition

3.3.1 Methods

The growth and relative condition of individually marked *G. cobitinis* held at four different densities were compared to determine whether detrimental intraspecific interactions were occurring. Each of the four tanks contained a 5-cm layer of substratum and was aerated, but did not contain a pump providing supplementary water current. Tanks were stocked with 6, 10, 12, or 20 *G. cobitinis*, equating to densities of 16, 26.7, 32, and 53.3 fish/m^2, respectively, for the four treatments.

3.3.2 Results and discussion

There were no significant differences in the growth in length or relative condition of *G. cobitinis* held at each of the four densities, as determined by ANOVA. Further, there was also no trend indicating that individuals held at higher densities had reduced growth or relative condition, on average (Fig. 5). This finding is supported by a lack of observed negative intraspecific interactions, indicating little territoriality, dominance hierarchies, or other aggressive interactions between *G. cobitinis*. This is despite all four of the densities tested being substantially greater than that observed in the field, for example, Dungey (2003) recorded a maximum estimated density of 0.81 *G. cobitinis*/m^2 in a section of the Kauru River in June 2003. Thus, provided that sufficient food is given and high water quality is maintained, *G. cobitinis* can be held at high densities (50/m^2) for 3 months without significant adverse consequences.

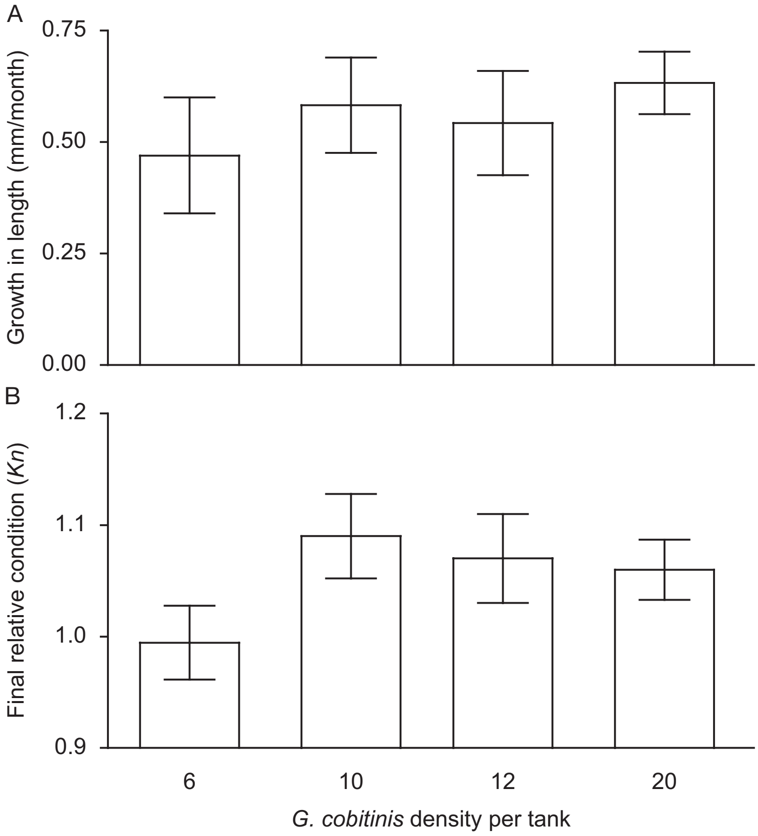

Figure 5. Mean (± 1 SE) A. growth in length and B. relative condition for *Galaxias cobitinis* held at densities of 6, 10, 12 and 20 fish per tank in the density investigation.

3.4 Investigation 2 – effect of substratum and water current on *Galaxias cobitinis* growth and condition

Adult *G. cobitinis* occur mainly in gravel/cobble-dominated riffles with shallow water depths (predominantly less than 15 cm; Baker et al. 2003). Such habitat is likely to be well oxygenated and have fast-flowing water. The aim of this investigation was to determine the extent to which mimicking these 'natural' conditions in a facility represented optimum conditions, or whether a less expensive/intensive tank setup is equally suitable for holding *G. cobitinis*.

3.4.1 Methods

This investigation was conducted using a 2 × 2 factorial design, whereby experimental treatments were randomly assigned across eight tanks, in which substratum characteristics and the presence or absence of water current were manipulated (Fig. 6). The effect of treatments on the growth and relative condition of six randomly assigned, individually identifiable *G. cobitinis* in each tank were measured.

Substratum manipulation treatments involved tanks having either cobbles placed as a single flat layer approximately 5 cm deep across the floor (referred to as flat; Fig. 7A); or as a pile of cobbles, angled from near the water surface at one end to depths similar to that of the flat substratum at the other (referred to as angled; Fig. 7B). This latter treatment was intended to imitate a steep riffle habitat and provided refuges for *G. cobitinis*, both deep within the substratum and near the water surface at the shallow end (Figs 6 & 7B). Water current was manipulated by the presence or absence of 6 W submersible pumps (Aqua One; 400 L/hr) to create surface flow (Figs 6 and 7B).

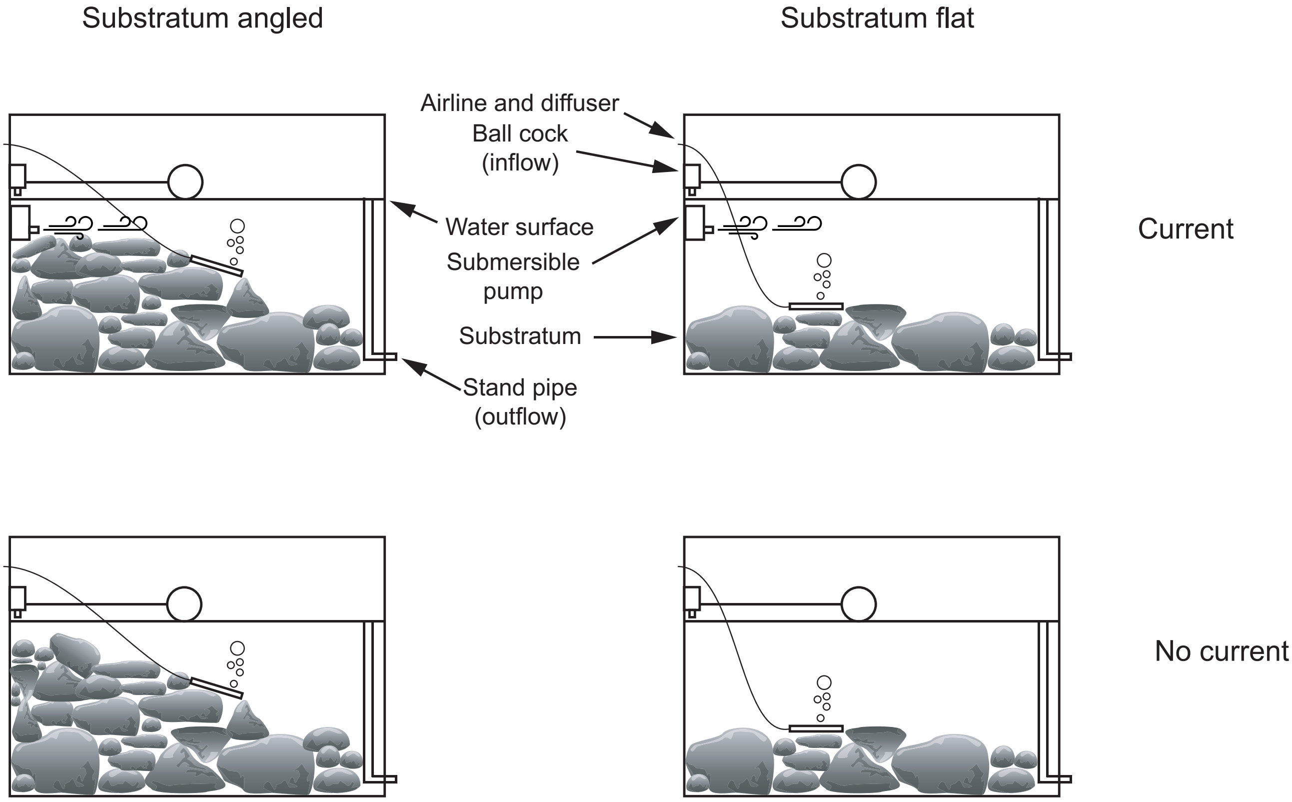

Figure 6. Schematic of the 2 × 2 factorial design of the substratum and water current treatments used in the holding conditions investigation.

Figure 7. Examples of treatments in the holding conditions: A. an angled substratum, water current treatment tank, showing extra substratum; and B. a flat substratum, no water current treatment tank. Submersible pump, air diffuser, ball cock, stand pipe and tensioning cords common to both.

3.4.2 Results and discussion

Survivorship over the three months of investigation was highest in tanks containing angled substratum without extra water current supplied, and lowest in tanks with a flat substratum and water current (Table 1). In total, ten mortalities occurred in *G. cobitinis* initially stocked in the experiment – one occurred during tank cleaning, four during an outbreak of the parasite *Ichthyophthirius multifiliis* (ich, white spot), and five when they squeezed themselves into submersed pumps (three within the first 4 days of the investigation). A further two *G. cobitinis* died 2 months later, when they managed to enter a small (c. 2 mm) opening when one of the suction feet holding a submersible pump to the tank wall became dislodged from its housing. Interestingly, the three *G. cobitinis* that initially entered pumps were in tanks containing flat substratum; by contrast, fish in tanks containing angled substratum, while having the same opportunity to enter pumps, did not. Therefore, the amount of substratum cover provided may have influenced this behaviour, which could be interpreted as being attempts by *G. cobitinis* to escape the tank.

The arrangement and quantity of cobble substratum in the tanks also had a significant effect on the growth of *G. cobitinis* (Table 2). Of the *G. cobitinis* that survived 3 months in captivity, those in the angled substratum treatment had significantly greater growth in length than conspecifics in the flat substratum treatment (Fig. 8A). The relative condition and health of *G. cobitinis* was significantly lower in tanks that had a water current provided by a submersible pump (Fig 8B & Table 2), and the mean number of ecoparasites per individual *G. cobitinis* (as a measure of health), was highest in tanks containing a flat substratum in April and May combined (Fig. 9 & Table 2). Thus, tank substratum and water flow characteristics significantly influenced *G. cobitinis* growth, relative condition and health.

Table 1. Percentage survivorship over 3 months for *Galaxias cobitinis* held in differing treatment conditions. Initially, 12 *Galaxias cobitinis* were assigned to each treatment.

	ANGLED SUBSTRATUM	FLAT SUBSTRATUM
No water current	100%	92%
Water current	83%	42%

Table 2. Factorial ANOVA results examining the effect of substratum characteristics and water current on *Galaxias cobitinis* growth in length, relative condition and number of ecto-parasites per individual (as a measure of health) between treatments. Significance: * $0.01 < P < 0.05$; ** $P < 0.01$.

	d.f.	MS	*F*	*P*
Growth in length				
Substratum	1	13.2	6.6	0.015*
Water current	1	0.01	0.01	0.933
Interaction	1	0.02	0.01	0.919
Error	34	67.7		
Relative condition				
Substratum	1	0.03	1.9	0.174
Water current	1	0.07	4.8	0.036*
Interaction	1	0.02	1.3	0.261
Error	34	0.02		
Mean number of ectoparasites per individual				
Substratum	1	29.1	35.2	<0.001**
Water current	1	4.1	5.0	0.03*
Interaction	1	1.8	0.2	0.16
Error	34	0.8		

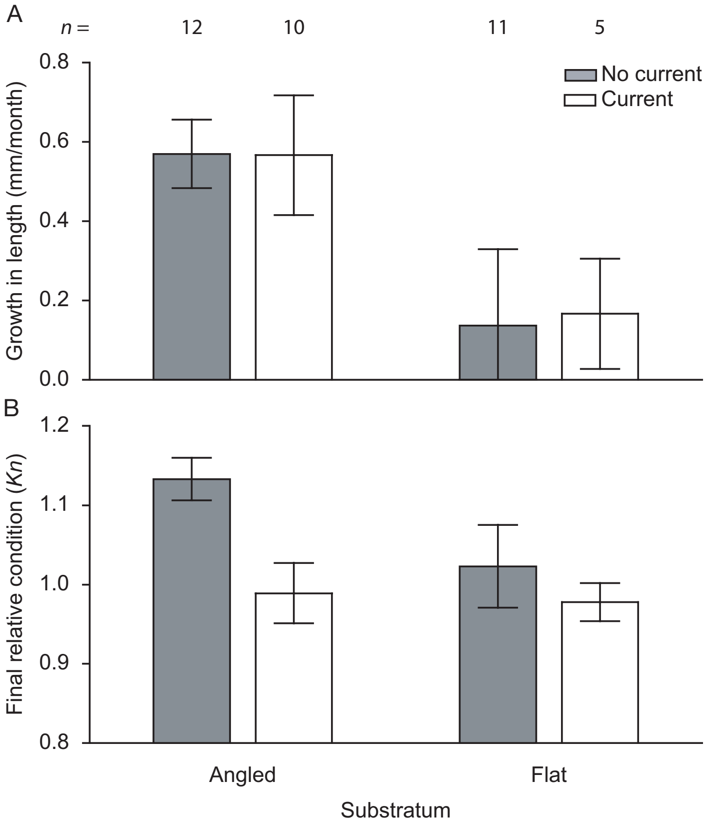

Figure 8. Mean (± 1 SE) A. growth in length and B. relative condition for *Galaxias cobitinis* held in different substratum and water current treatments in the holding conditions investigation. *n* values are the number of *G. cobitinis* surviving in each treatment at the conclusion of the investigation, from an initial 12 per treatment.

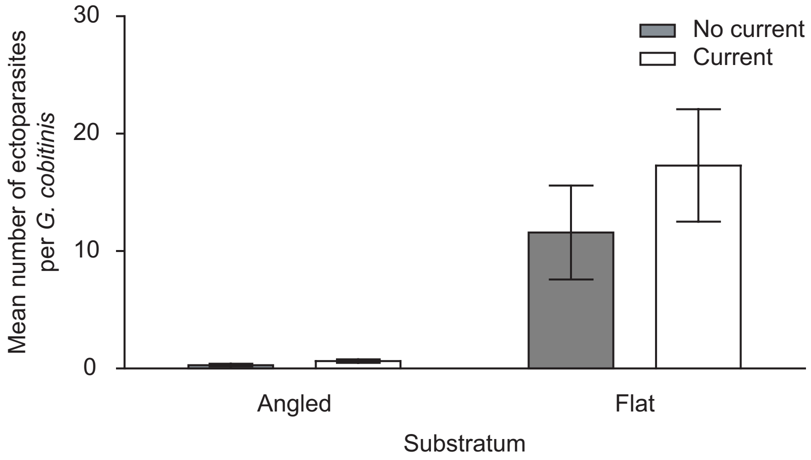

Figure 9. Mean (± 1 SE) number of ectoparasites per *Galaxias cobitinis* held in angled and flat substratum treatments in the holding conditions investigation for the April and May handling periods combined.

4. Discussion of optimal holding conditions

This study demonstrated that *G. cobitinis* can be successfully held in captivity for several months in the event of drought or other adverse conditions in the wild, and so fulfils Action 7.3 of the non-migratory galaxiid fishes recovery plan (DOC 2004). Investigations indicated that characteristics of the substratum and water current within tanks can affect the growth and relative condition of *G. cobitinis* in captivity. The provision of hidden resting areas, both near the water surface and within interstitial spaces, as occurred in the angled substratum treatments, resulted in the highest growth and final condition of captive *G. cobitinis*. Underlying this result is differences in energy expenditure required during swimming, with less vigorous swimming required when feeding in tanks with no water current, and with refuges near the water surface. A lack of suitable refuges, and thus possibly increased stress levels may have also resulted in the observed differences in susceptibility to *I. multifiliis* infection.

Small organisms such as *G. cobitinis* usually have an intrinsically high metabolic rate relative to similar, larger organisms, resulting in high basic energy requirements. Consequently, increased energy demands incurred from swimming, either in the presence of the supplied water current and/or a greater distance from refuge to the water surface where food items were most often taken, as in flat substratum treatments, may have been sufficient to result in significant differences in growth and relative condition. Although water current is a characteristic of the natural habitat of *G. cobitinis*, it is likely to be unnecessary in a captive setting and imposes greater energy demands. Furthermore, considering the mortality risk posed by submersed pumps, we would not recommend the use of pumps in a captive holding facility for *G. cobitinis* or any similar small galaxiid.

The provision of many large cobbles may benefit *G. cobitinis* by providing more cover resulting in less stress. However, the greater the amount of substratum particles in tanks, the more time consuming and problematic routine tank maintenance is, and the more likely and more quickly water quality will deteriorate. A compromise to this problem could be the provision of a dense, single layer of cobbles to provide adequate cover, combined with a shallow maximum water depth of c. 15 cm in the tank, to reduce energy expenditure during feeding.

We acknowledge that further experiments are needed to confirm that the ecological mechanisms we have suggested actually explain the observed results. However, the information provided by these investigations is sufficient to provide guidance on holding *G. cobitinis* in captivity, and is considered applicable to other non-migratory *Galaxias* and *Neochanna* species.

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We would like to thank Marcus Simons (Coastal Otago District Office, Department of Conservation (DOC)), Murray Neilson (formerly Otago Conservancy, DOC) and Pete Ravenscroft (formerly Coastal Otago District Office, DOC) for the opportunity to conduct these investigations, and for useful discussions on the conservation and management of *G. cobitinis*. Simon Madill (formerly Coastal Otago Area Office, DOC) helped in the collection of fish. Patrik Stenroth (formerly Lunds universitet, Sweden) assisted in setting up the tank facility. Tony Rippen (South Canterbury Museum, Timaru) provided useful discussion on the treatment of disease in captive fish.

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Appendix 1

Materials used for transport, temporary, and long-term holding facilities.

An X indicates that this item was used during transport or in a particular facility.

CATEGORY	ITEM	TRANSPORT	HOLDING	
			TEMPORARY	LONG TERM
Tanks and bins				
Plastic goods	Willow 44-L ‘Quickserve’ chilly bin	X		
Hardware	3-mm plywood for baffles	X		
Plastic goods	20-mm closed cell foam	X		
Plastic goods	25-L hobby bin		X	
Hardware/plastic goods	Get Organised 120-L bin with lid			X
Hardware	1 × 1 mm plastic insect mesh			X
Piping and fittings				
Irrigation	RX Plastics 19-mm lateral polyethylene tube			X
Irrigation	RX Plastics trough valve			X
Irrigation	Apex 115-mm ball float			X
Irrigation	Philmac 19-mm tee junction			X
Irrigation	Philmac 19-mm elbow			X
Irrigation	Philmac quick action valve 19-mm tails			X
Irrigation	Philmac elbow 19-mm tail to ½ inch BSP female			X
Irrigation	Philmac director 19-mm tail to ½ inch BSP			X
Irrigation	Philmac elbow 19 mm tail to ½ inch BSP male			X
Irrigation	Hansen reducing socket 20 × 15 mm			X
Irrigation	RX Plastics 15-mm tank fitting			X
Irrigation	Philmac 19-mm joiner			X
Irrigation	Philmac 19-mm tee × 15-mm BSP			X
Irrigation	Philmac Cray clips			X
Air and water current				
Pet supply	Resun electromagnetic air pump	X	X	X
Pet supply	Petz 4-mm-diameter airline tubing	X	X	X
Pet supply	Hagen Elite air control valve	X	X	X
Pet supply	Hagen Elite aqua fizz 6-inch air diffusers	X	X	X
Pet supply	Aqua One 101 Maxi Power Head	X	X	X
Hardware	PDL RCD electrical safety switch adaptor		X	X
Food				
Pet supply	Aqua One frozen bloodworms		X	X
Treatments and test kits				
Pet supply	Aquarium thermometer	X	X	X
Instrument supplier	Onset Computer Corporation HOBO data logger	X	X	X
Pet supply	Brooklands tonic salt		X	X
Supermarket	Non-iodised salt		X	X
Pet supply	Wunder tonic		X	X
Pet supply	Wunder formalin		X	X
Pet supply	Blue Circle malachite green		X	X
Pet supply	MasterPet Antiseptic		X	X
Pet supply	MasterPet Cure Ex		X	X
Pet supply	Hagen Aqua Plus	X	X	X

Continued on next page.

Table A1.1 continued

CATEGORY	ITEM	TRANSPORT	HOLDING	
			TEMPORARY	LONG TERM
Pet supply	Aquarium Pharmaceuticals Stress Coat	X	X	X
Pet supply	Tetra bactozym (bacterial enzymes)			X
Pet supply	Aquarium Pharmaceuticals pH test kit		X	X
Pet supply	Aquarium Pharmaceuticals ammonia test kit		X	X
Pet supply	Aquarium Pharmaceuticals nitrate test kit		X	X
Chemical supplier	Anaesthetic	X	X	X