Captive management of mudfish *Neochanna* (Teleostei: Galaxiidae) spp.

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CONTENTS

Abstract 5

1. Introduction 6

   1.1 Captive management considerations 7

   1.2 Characteristics of reproductive biology 7

2. Past captive breeding attempts 8

   2.1 *Neochanna burrowsius* 9

      2.1.1 Successful captive spawning 9

      2.1.2 Unsuccessful captive spawning 10

   2.2 *Neochanna diversus* 11

      2.2.1 Successful captive spawning 11

      2.2.2 Unsuccessful captive spawning 11

   2.3 *Neochanna apoda* 11

      2.3.1 Successful captive spawning 11

      2.3.2 Unsuccessful captive spawning 12

3. Guidelines for captive management 12

   3.1 Facility design 12

      3.1.1 Cost 14

   3.2 Collection and maintenance of stock 14

      3.2.1 Source population 14

      3.2.2 Collection 16

      3.2.3 Examining fish 16

   3.3 Feeding requirements 17

   3.4 Aquatic vegetation and spawning substratum 19

   3.5 Improving recruitment success 20

   3.6 Translocation 22

      3.6.1 Site assessment 22

      3.6.2 Transfer and release 23

      3.6.3 Monitoring and documentation 23

4. General breeding procedure 24

5. Acknowledgements 26

6. References 26

Appendix 1

Details of captive management facility equipment used at University of Canterbury 29

Captive management of mudfish *Neochanna* (Teleostei: Galaxiidae) spp.

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A B S T R A C T

This report provides guidelines for the captive management of mudfish (*Neochanna*) species in New Zealand. This includes the requirements for captive rearing, breeding, stock maintenance, and the establishment of new *Neochanna* populations. The reproductive and developmental biology of *Neochanna* species has been an infrequently studied topic, with the exception of Canterbury mudfish (*N. burrowsius*). Further, spawning in captivity has been documented only once in the cases of black (*N. diversus*) and brown (*N. apoda*) mudfish, and never for the Chatham (*N. rekohua*) or Northland (*N. heleios*) mudfishes. As each species has differing characteristics that are likely to influence its requirements, and much is still to be learnt, the aim of this report is to collate information and provide preliminary guidelines. Improving the survival rate of eggs and fry through successful captive breeding and rearing may provide a pool of recruits for the establishment of further populations. Translocation is an important method of safeguarding against species extinction by spreading the risk among a greater number of populations. However, captive management and translocation are not without risk, and should be contemplated thoroughly. *Neochanna* species have proved a challenge to breed in captivity, and many translocation attempts have failed to establish populations. These disappointments illustrate that a greater understanding is required to direct future conservation efforts. Thus, this report provides practical guidance to encourage further research into the biology of *Neochanna* species based on observations from both successful and unsuccessful experiences of breeding *Neochanna* species in captivity.

Keywords: *Neochanna*, *N. rekohua*, *N. burrowsius*, *N. diversus*, *N. heleios*, *N. apoda*, mudfish, captive breeding, reproduction, spawning, New Zealand.

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1. Introduction

Captive management has successfully brought many species back from the brink of extinction. The potential of captive breeding to enhance the conservation of threatened New Zealand mudfish (*Neochanna*) species has long been recognised and advocated (Eldon 1969, 1993; Swales 1991). Indeed, an objective of the Department of Conservation's (DOC's) mudfish recovery plan (DOC 2003) is to maintain and increase populations of all *Neochanna* species, with captive rearing and translocation being identified as actions towards achieving this goal. The aim of this report is to collate relevant information on *Neochanna* species in New Zealand and provide preliminary guidelines for their captive management.

A successful captive management programme is likely to include aspects such as collection, rearing, breeding, stock maintence, and the establishment of new populations. Moreover, because it is recommended that only fry be collected from wild populations (Eldon 1993), successful captive rearing (i.e. the collection of fry from wild populations to rear in a managed facility) can be considered the first step towards captive breeding (i.e. successful spawning and subsequent rearing of early life stages in a managed facility). As with many conservation measures, ensuring a captive management programme is successful requires an understanding of a species' behaviour and reproductive biology (Caro 1999; Shumway 1999; Knight 2001). Additionally, captive breeding programmes can enhance understanding of a species' requirements throughout its life cycle (Rakes et al. 1999). Such knowledge, especially of requirements for vulnerable life stages, may result in an improved awareness of appropriate actions to take when creating habitat or conserving existing wild populations. Thus, this report also aims to encourage further research into the reproductive biology of *Neochanna* species.

Neochanna species in New Zealand are non-migratory and often occur in small pond or sluggish stream habitats, meaning they are suitable candidates for breeding in tanks. However, endeavours to breed *Neochanna* species in captivity have not always been successful, nor attempted for some species, and much is still to be learnt. Furthermore, species-specific factors are likely to govern the conditions which promote reproduction. Although most *Neochanna* species have been described as occurring in predominantly lentic (i.e. standing water) habitats, the characteristics of the wetlands they inhabit can be quite different. *Neochanna rekohua* occurs in lakes within areas of deep peat bog and swampy scrubland (Mitchell 1995; McDowall 2004), whereas Eldon (1979a) described true *N. burrowsius* habitat as still or very slowly flowing, meandering, swampy streams with deep pools. *Neochanna diversus* is described as an obligate occupier of seasonally dry marginal areas of infertile peat bog wetlands (Barrier 1993; Dean 1995; Kerr & McGlynn 2001). Kerr & McGlynn (2001) concluded that the habitat requirements of *N. heleios* appear to be similar to those of *N. diversus*. *Neochanna apoda*, however, often occurs in shallow, debris-littered pools under forest canopy (Eldon 1978; Butler 1999). These diverse habitat requirements may, therefore, preclude identification of a standard methodology for captive management of all *Neochanna* species.

Nonetheless, studies of the reproductive biology of *Neochanna* species, in particular *N. burrowsius*, have provided observations and experimental information that present a starting point in the development of general protocols.

1.1 CAPTIVE MANAGEMENT CONSIDERATIONS

At the outset, the objectives of any captive management programme must be thoroughly considered. Moreover, before such a programme gets underway, a proposal should be prepared that addresses all issues relevant to the programme. Intentions for such programmes could range from rearing wild individuals past vulnerable life stages, breeding to supplement populations and / or establish new populations, through to conducting research into reproductive requirements. In all cases, careful consideration is required, including size, productivity and appropriateness of source populations, protection of genetic distinctiveness, maintenance of genetic diversity, long-term facility upkeep, long-term care of the captive population, quality of potential translocation sites, adequate monitoring of translocated populations, and the programme duration. Furthermore, the transfer of live aquatic life from or into water bodies during the course of the breeding programme must conform to The Freshwater Fisheries Regulations 1983, Section 26ZM of the Conservation Act 1987, and The Biosecurity Act 1993. Consultation with regulatory bodies, such as DOC, is necessary, and such consultations can also provide guidance on the relevant legislation and any approvals required before any translocation occurs.

1.2 CHARACTERISTICS OF REPRODUCTIVE BIOLOGY

Neochanna burrowsius appears to spawn more readily in captivity than other *Neochanna* species. As a result, its reproductive biology is well studied. Studies have been more sporadic for *N. diversus* and *N. apoda*, while little is known about reproduction in *N. heleios* and *N. rekohua*. However, all *Neochanna* species are likely to have broadly similar reproductive traits.

Many of the habitats in which *Neochanna* species occur dry out during summer and autumn. Thus, these fish often have only a small window of opportunity for successful reproduction. Spawning generally occurs from late autumn (*N. apoda* and *N. diversus*) through to early spring (*N. burrowsius*; McDowall 1970; Eldon 1978, 1979b; Thompson 1987; Ling 2001). Males partially spend their milt and may spawn with several females throughout the season, whereas females typically spawn only once per year. However, there is some suggestion that *N. diversus* and *N. apoda* may opportunistically spawn twice, or throughout the year, if water levels and temperatures are favorable (Eldon 1978; N. Ling, University of Waikato, pers. comm.). Additionally, females do not synchronise their spawning, which can lead to an extended spawning season, often over several months (Eldon 1978, 1979b; Thompson 1987; O'Brien 2005). Furthermore, *N. burrowsius* delay spawning when aquatic vegetation is not present, if they are disturbed, or when water quality is poor (O'Brien 2005). This reproductive plasticity may enhance *Neochanna* survival in hydrologically-fluctuating wetlands; however, it means that anticipating spawning can often be difficult.

The act of spawning has seldom been observed; however, it appears to involve males chasing a female and some co-ordinated breeding behaviour (Gay 1999; Perrie 2004; L. O'Brien, pers. obs.). Vigorous activity must occur during spawning in *N. apoda*, as eggs have been found splashed high above the water line (up to 24 cm, Eldon 1971). In all species, it is likely that eggs are scattered, and there is no indication of nest building or paternal care. Scattering of eggs amongst vegetation or, in the case of *N. apoda*, above the water line, will reduce their detection by predators, including other adult *Neochanna*. Indeed, considerable cannibalism can occur where complex spawning substratum is absent (O'Brien 2005). Additionally, Eldon (1979b) observed that clumped eggs suffered higher mortality than those scattered singly, suggesting further benefits beyond camouflage. Thus, recruitment success requires a large area of suitable spawning substratum, whether this is aquatic vegetation, wood debris, overhanging vegetation or accessible habitat above the water line.

Neochanna eggs are approximately 1.5–3 mm in diameter and extremely sticky. However, although they will initially adhere to almost any surface, they can easily be dislodged by disturbance (Eldon 1979b). Embryos take several weeks to develop, depending on water temperature and oxygen availability (Eldon 1978, 1979b). Newly hatched fry retain yolk sacs, although they are also able to consume food immediately (Eldon 1978). *Neochanna* species undergo an ontological shift in habitat, with fry being pelagic, feeding in open water, and at the surface until approximately 30–50 mm in length. In general, fry gradually become cryptic, benthic and nocturnal, moving into the adult habitat in late summer. *Neochanna* then reach sexually maturity in their first year, or later in the case of *N. apoda*.

2. Past captive breeding attempts

Neochanna species are readily kept in captivity and many authors have supplied useful information, especially for *N. burrowsius* (e.g. Cadwallader 1973, 1975; Eldon 1979b; Gay 1999), *N. diversus* (e.g. Eldon 1969; Thompson 1987; Gay 1999; Perrie 2004), and *N. apoda* (e.g. Davidson 1951; Eldon 1969). This section details the information available from previous studies to highlight situations in which spawning did or did not occur. Generally, these observations indicate that greatest success is achieved when *Neochanna* are kept in large tanks in a quiet location with few disturbances. The presence of aquatic vegetation is also important, particularly floating or dense mats of vegetation at the water surface. Situating tanks outdoors may also be beneficial, probably because they are subject to lower, diurnally fluctuating temperatures. Environmental cues may also be important for spawning initiation. However, there are still many unresolved aspects of *Neochanna* breeding, as spawning is

Figure 1. Modified plastic cattle troughs used to breed *N. burrowsius* at the University of Canterbury. *Photo by L. O'Brien.*

often unsuccessful, even when conditions appear completely suitable. The inhibition of spawning in captive adults may be the result of stress, low resource levels, lack of suitable spawning locations or partners, or poor water quality. However, spawning may occur without being noticed, and it is the mortality of early life stages resulting from developmental problems, predation, few suitable prey, or poor water quality which suppresses recruitment. It may be difficult to determine the underlying reasons why recruitment fails in a particular situation. Thus, detailed and regular observation, note taking, experimentation, and the methodical elimination of possible factors are necessary in situations where success has been sporadic. The development of captive management protocols that consistently increase initial survival rates could bolster numbers, safeguard species and improve understanding of *Neochanna* species' requirements.

2.1 *Neochanna burrowsius*

2.1.1 Successful captive spawning

Cadwallader (1975), Eldon (1979b), Gay (1999), and O'Brien (2005) all documented captive spawning of *N. burrowsius*. Furthermore, there are two examples of long-term captive management projects. *Neochanna burrowsius* have been maintained in a large (> 1000 L) tank, and in Lake Eldon (a small boggy pool on the NIWA Christchurch campus) since the mid 1980s (Eldon 1989, 1993). The University of Canterbury also has an outdoor facility in which *N. burrowsius* have successfully reproduced since 1999 (Fig. 1; O'Brien 2005).

Cadwallader (1975) suggested that *N. burrowsius* might spawn more readily in outdoor facilities because of the influence of environmental factors. Evidence for this was provided by *N. burrowsius* failing to spawn in indoor aquaria while, at the same time, spawning in a separate outdoor aquarium after a period of heavy rain. The outdoor aquarium contained willow (*Salix* spp.) roots, and it was on these that eggs were found (Cadwallader 1975).

Figure 2. Close-up of emptied cattle trough showing alcoves frequently favoured by *N. burrowsius* during spawning. *Photo by L. O'Brien.*

Multiple incidences of spawning were observed by Eldon (1979b) who used outdoor ponds and glass tanks containing tassels of nylon knitting material, mop heads and aquatic macrophytes as spawning substratum. Interestingly, although fish utilised artificial substratum, they showed a preference for natural vegetation (Eldon 1979b).

Gay (1999) found *N. burrowsius* spawned in a large indoor aquaria (900 × 400 × 400 mm, 144 L) filled with rainwater and maintained at 10–12°C. The tank was lit with natural light and a 15-W lamp. Water quality was periodically maintained by a biological filter and external power filter. The tank was lined with fine gravel and contained aquatic vegetation including free-floating species—water fern (*Azolla* spp.) and duckweed (*Lemna minor*), and submerged species—*Pratia* spp. and *Myriophyllum aquaticum*.

O'Brien (2005) found *N. burrowsius* readily spawned outdoors in 750-L circular cattle troughs (Fig. 1). These tanks had several characteristics considered advantageous to captive breeding, such as the ability to manipulate water level and flow. Water exchange was controlled using a ball-cock-restricted inflow, and a trickle gravity outflow. This maintained a water depth of approximately 40 cm. Water flow (from an aquifer bore) through each tank was approximately 1 L/minute. A feature of these tanks was the presence of alcoves and ledges in the moulded plastic (Fig. 2), which were often used by the fish for refuge and spawning. The ledge around the tank top prevented fish escape without the need of a cover, and sometimes provided a damp refuge above the water line. Dense aquatic vegetation was usually present, both submerged and free-floating species (see Section 4). Large cobbles and sand were placed on the bottom of the tanks. These provided refuges for the fish and a rooting medium for aquatic plants.

2.1.2 Unsuccessful captive spawning

Cadwallader (1975) found that *N. burrowsius* failed to spawn in indoor aquaria. These fish were kept under normal day–night lighting conditions, at 16–21°C, and a constant water level. It was suggested that spawning failure was due to a lack of environmental stimulus.

Spawning did not occur when attempts were made to film heavily gravid individuals (L. O'Brien, pers. obs.). The fish were placed in a small indoor aquarium in a temperature-controled room set at 15°C. Only sparse aquatic vegetation was supplied to avoid obstructing the view. It was thought that the 'unnatural' conditions, including a lack of aquatic vegetation (see Section 3.4), and noise and vibrations from the room's circulation fan, contributed to the lack of spawning.

2.2 *Neochanna diversus*

2.2.1 Successful captive spawning

Gay (1999) successfully spawned *N. diversus* in a large indoor aquaria (900 × 400 × 400 mm, 144 L) filled with rainwater (pH 6.2) and maintained at 13–16°C. The tank was lit with natural light and a 15-W lamp. Water quality was maintained by periodical filtration. The tank was lined with fine gravel (2–5 mm) and contained various pieces of bogwood to supply tannin. Aquatic vegetation included free-floating species—*Azolla* spp. and *L. minor*, and submerged species—*Pratia* spp. and eelgrass (*Vallisneria gigantea*). *Neochanna diversus* eggs were discovered on the tank bottom amongst plant debris, and submerged plants. Perrie (2004) observed spawning seventeen months after ten fish were captured and placed in a 120-L tank. This tank contained lengths of pipe, flowerpots and thickets of real and artificial plants. Spawning occurred after several months of stable conditions at 16°C with a 16-h light to 8-h dark cycle and pH of 7.45. However, no fry resulted from this spawning event.

2.2.2 Unsuccessful captive spawning

Eldon (1969) kept *N. diversus* in a tank with a water depth of 5 cm for three and a half years without any evidence of spawning occurring, despite both sexes being present. Thompson (1987) kept a small number of *N. diversus* for eight years without successful spawning observed. These fish were held in an *in situ* 'swamp cage', 50-L capacity, half filled with aquatic vegetation, with a relatively constant water level. More recently, Perrie found that females can become very gravid (to the point of apparent distress), which may lead to egg reabsorption and even death. However, in one case he successfully stripped fish and reared artificially-fertilised eggs to juvenile stage.

2.3 *Neochanna apoda*

2.3.1 Successful captive spawning

Eldon (1969, 1971) described *N. apoda* spawning in an indoor, covered aquarium housed in a poorly lit basement. *N. apoda* were found to scatter their eggs above the water in the aquarium, to a height of at least 24 cm. While the fish had not appeared to be gravid, approximately 4–6 weeks before the appearance of fry, adults had been observed resting amongst weed growth very near the surface of the water. These fish were found to be very still and did not startle when disturbed; an unusual behaviour potentially related to spawning.

Figure 3. Artificial ponds used in trials of rearing and breeding *N. apoda*. *Photo by D. Caskey.*

2.3.2 Unsuccessful captive spawning

After the above success, Eldon (1978) noted that several unsuccessful attempts were made to induce *N. apoda* spawning under controlled conditions. In an outdoor trial, Caskey (2002) placed eight *N. apoda* in one of two outdoor ponds (4.5 × 1.5 m) situated under forest canopy, protected by shade cloth (Fig. 3). Although fish were observed to be 'obviously gravid' in August 2001, successful recruitment has never been observed. Water level in the ponds remained constant and they contained a reasonable amount of leaf litter and surface cover of *L. minor*. Fern fronds draped into the water and the pond was well shaded. Despite no successful spawning occurring, these ponds were highly successful in raising juveniles (taken from the wild) to adulthood.

3. Guidelines for captive management

3.1 FACILITY DESIGN

It is important to consider the type, size and design of any facility intended to hold captive *Neochanna*. Experience suggests that captive rearing and breeding is best conducted in outdoor, semi-self-maintaining facilities (Eldon 1993). Thus, if the public has access to the area, fencing should be considered for the safety of young children. Artificial ponds can be used; however, if these are large, their construction may require resource consent. In comparison, artificial tanks are relatively easy to move and appropriately place, and allow greater control of water and habitat quality, as water levels can be manipulated. Furthermore, large tanks (> 500 L) allow the development of a 'self-maintaining ecosystem', which can help maintain water quality and reduce feeding and cleaning requirements.

At least two large tanks or ponds should be used, with adults maintained in one (adult / breeding tank), while the second is used to raise eggs and fry until they become benthic juveniles (rearing tank). A further tank, or old bathtub, used to culture supplementary live prey species, is also recommended. A greater number of tanks would provide increased operational flexibility, lower fish densities, and allow experimentation into optimal conditions for breeding.

Finding a suitable location to place tanks is also an important step in facility design. The design of the final set-up will depend on the numbers of fish intended to be held; availability of level ground; purity and proximity of water source (e.g. an aquifer bore, or a facility to collect rainwater or age municipal water supply); fall / head to provide gravity outflow (this can be enhanced by raising tanks on wooden palettes or a platform); and proximity of a suitable area for outflows. Water quality can be maintained either by regular partial replacement of water or, preferably, by a trickle-through system if a continuous source of good quality water is available. With a trickle system, control of water inputs, trickle outflow, and overflow from rainwater should all be considered. Water inputs can be controlled by taps or ball cocks, with the simplest method of controlling outflow and overflow being small (approx. 1 cm) holes. However, various aquarium and aquaculture solutions can be used, depending on the circumstances.

Tanks should be placed such that they receive adequate light to promote the productivity of zooplankton, and other small prey for fry. However, over-heating of tanks in summer should be avoided (this will depend on tank size, to a certain extent). Although *Neochanna* species are tolerant of warm water temperatures, including room temperature, elevated temperatures will increase metabolic demands and food requirements, causing stress. Warm water temperatures will also reduce dissolved oxygen levels. However, tanks should not be vigorously aerated, especially during the spawning period, as the turbulence and disturbance generated by this action appeared to delay spawning (in *N. burrowsius*, O'Brien 2005). Adequate dissolved oxygen levels can be ensured by placing tanks in an area which receives enough wind turbulence to circulate water and / or has overhanging vegetation that 'stirs' water during windy periods. It should be ensured, however, that tanks do not receive excessive inputs of debris such as leaves. Excess dead plant material needs to be removed, as it tends to cause rapid deterioration of water quality and promotes bacterial and fungal outbreaks. If tanks are placed under deciduous trees, covers made from shade cloth or the like may be required during autumn.

Tank placement may take some time to finalise and it is advised that tanks be seeded with appropriate pond-life well in advance of adding *Neochanna*. Monitoring the progress and success of tanks in developing and maintaining abundant 'pond-life' will also allow for the suitability of the facility for *Neochanna* species to be assessed. A further note of caution when considering facility design is that *Neochanna* will try and wriggle into any hole or crevice available, a behaviour which can have dire consequences (Eldon 1979b). Thus, any inlets or outlets need to be covered with mesh. Furthermore, care should be taken when using plastic-cased minimum-maximum thermometers, as fish may wedge themselves within them, becoming stuck (L. O'Brien, pers. obs.).

3.1.1 Cost

The capital outlay for a captive management facility was estimated by Eldon (1993) at no more than $20 000 dollars. However, expenses can be minimised by careful planning and use of recycled materials—e.g. by using old bathtubs to nurture prey species. The large University of Canterbury facility (Fig. 1; Appendix 1) cost approximately $3000. However, a well-equipped two- or three-tank outdoor facility could be constructed for less than $1000.

3.2 COLLECTION AND MAINTENANCE OF STOCK

3.2.1 Source population

The identification of appropriate populations from which to source *Neochanna* is an essential initial step. Ideally, these would be large, secure and stable populations, which have reached the capacity of the habitat to sustain further population growth. Additional candidates would include populations in situations where large numbers of fry are lost via downstream dispersal into inhospitable habitat, or which experience heavy predation pressure. A further consideration is the hydrological characteristics of the source and receiving habitat. For example, if a potential translocation site is known to dry out, success may be increased by sourcing *Neochanna* from a habitat that also dries out, as local adaptation to such disturbance is likely to occur.

Indeed, to fully conserve *Neochanna* species, consideration of the genetic attributes of populations is required. Local adaptations expressed in the genetic heritage of a population are important for ensuring persistence and need to be preserved (Ling et al. 2001). Recognition of the unique genetic characteristics of populations and their importance for conservation has lead to the development of the concept of evolutionary significant units (ESUs). An ESU is a reproductively isolated group of populations displaying unique evolutionary characteristics (Ling et al. 2001). The ESUs of all New Zealand *Neochanna* species have been determined (Fig. 4). It is important to source individuals only from within the same ESU in any captive management programme. However, individuals from several areas or populations could be collected, providing they are within the same ESU, as this would result in a diverse foundation of genetic characteristics in the captive population. This is likely to impart a benefit if the intention is translocation into a novel habitat; or if the species has especially low inherent genetic diversity (as is the case for *N. burrowsius*, Davey et al. 2003). The Department of Conservation could be consulted about selection of appropriate source populations.

Captive breeding programmes overseas have found that genetic diversity can be slowly lost in captive populations of fish. Maintaining genetic diversity is important, as the ability of individuals and populations to respond to a changing environment is related to the diversity of underlying genetic traits. It is not known if gradual loss of genetic diversity will be a problem in captive populations of *Neochanna* species. Blood samples from captive fish could be taken to assess diversity loss if a small population (e.g. < 50 individuals) has been reproducing in isolation for many years. However, the captive population of *N. burrowsius* in Lake Eldon which has reproduced in isolation for at least

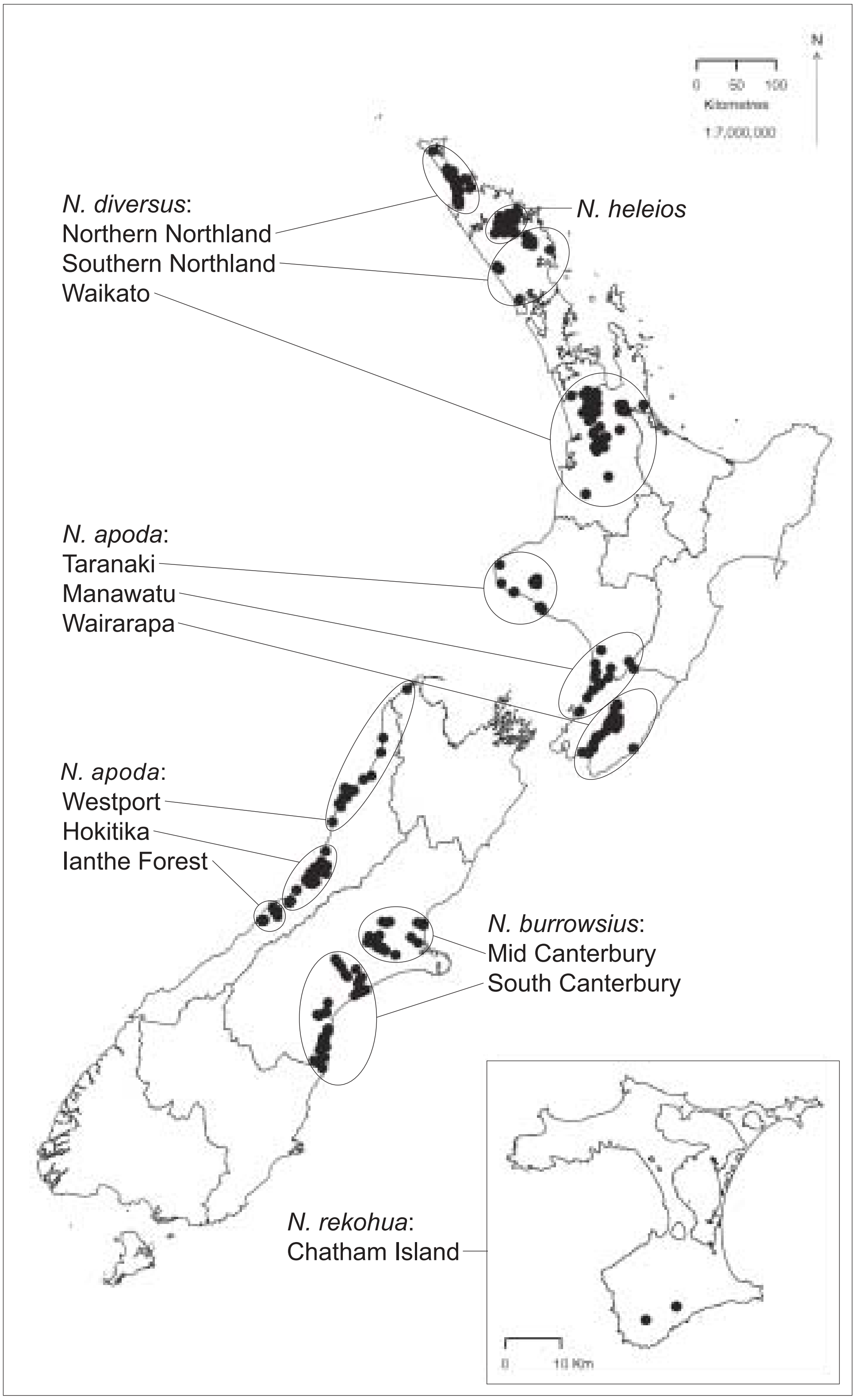

Figure 4. Distribution (black dots) of *Neochanna* species on the two main islands of New Zealand and on Chatham Island (inset), with evolutionary significant units (ESUs) for each species and DOC conservancy boundaries also shown. ESUs are based on mitochondrial DNA in the D-loop region. Distribution data is from the New Zealand Freshwater Fish Database. ESU designations are from Gleeson et al. (1997, 1999); Gleeson (2000); Davey et al. (2003); and Gleeson & Ling (unpubl. data).

20 years showed no loss of genetic diversity (Davey et al. 2003). One practical approach of maintaining genetic diversity, and similarity with wild populations, is to ensure some gene flow occurs. Thus, the genetic diversity of captive populations could be supplemented by regular addition of fry collected from appropriate wild populations.

3.2.2 Collection

Only fry and pelagic juveniles should be collected from the wild for captive management purposes (Eldon 1993). This is because they are easy to capture, and the sexes are randomly selected at this stage. Moreover, removal of fry has a minimal impact on a population, as fry production is normally far greater than the habitat's capacity to support all fry into adulthood. Removal of very small fry will have the least impact, and although they are more difficult to rear, they represent the greatest opportunity to improve recruitment.

Fry can be collected as soon as they appear, which is usually during autumn for *N. diversus*, *N. apoda* and *N. heleios*, whereas fry of *N. burrowsius* and, possibly, *N. rekohua* appear in spring (N. Ling, R.F.G. Barrier, L.K. O'Brien, R. Miller: Methodology to survey and monitor NZ mudfish species. In prep.). The number of fry required will be dependent on availability, project aims, and the size of captive facilities, but it is likely to approximate one hundred, especially for small fry. However, it is recommended that this number be collected in a series of small batches to spread risk. This way, collection and transportation methods can be tested, and initial fry monitored to ensure the facility is suitable before large numbers are introduced. Collection of fish and their transfer to captive management facilities may require approval from regulatory bodies such as DOC.

3.2.3 Examining fish

Best practice includes regularly checking fish for disease or poor condition. This can be easily done after trapping fish with Gee-minnow traps (further details will be published in N. Ling, R.F.G. Barrier, L.K. O'Brien, R. Miller: Methodology to survey and monitor NZ mudfish species. In prep.). Fish can usually be examined without anaesthetic, but should always be handled with wet hands. Anaesthetic should, however, be used for extensive handling of fish; including when measuring, sexing, or tagging, as it renders the fish easy to handle, thus reducing stress and injury. A fish biologist should be consulted for advice on the use of anaesthetising substances.

Neochanna species lack protective scales and the removal of mucus through handling or injury can increase an individual's susceptibility to infection (Meredith 1985; Dean 1995). Further, stress due to poor water quality may increase vulnerability to disease. Moreover, if disease occurs often, or condition is generally poor, it is likely that food availability is low and / or fish density high.

Individuals found with visible infections should be removed and treated. *Neochanna* species will respond to curative treatments commonly used on freshwater fish (Meredith 1985; L. O'Brien pers. obs.). Advice can be obtained from many sources, including most pet stores. Moreover, treatment to prevent disease (in a bucket after handling) is recommended and may reduce the risk of disease developing or becoming widespread. Post-handling treatment could include the addition of products such as Aqua Plus® (Rolf C. Hagen (USA) Corporation); this is a water conditioning treatment which reduces physiological stress in fish and coats the skin with a mucus-like protective coat. Further, a general antifungal and antibacterial agent could be added to the water. These two products are readily available from pet stores.

It may also be prudent to determine the sex of captive individuals to ensure both sexes are present. Anaesthetised fish can be sexed using a hand-held magnifying glass; and during the spawning season, sexes can be readily distinguished (Fig. 5). Males have a pointed papilla, whereas the genital area in females is more bulbous. Further, just prior to spawning, females may look gravid, with eggs visible through the thin abdominal skin, while males may have a distinctly white abdomen due to the presence of milt. Additional descriptions and illustrations of differences between sexes in Galaxiidae can be found in Cadwallader (1973), and McDowall & Waters (2003).

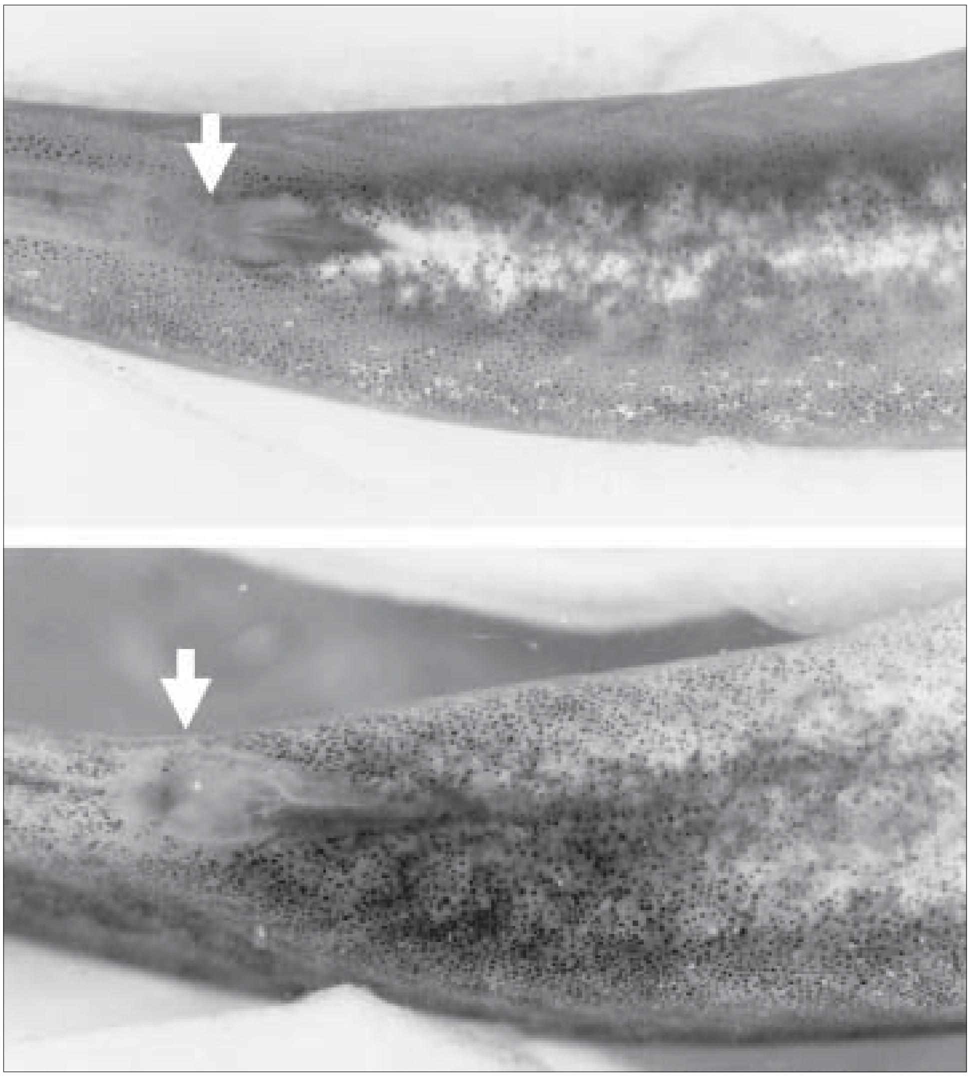

Figure 5. Genital region (indicated by arrows) of a near-ripe male (top) and gravid female (bottom) *N. burrowsius*. *Photos by L. O'Brien.*

3.3 FEEDING REQUIREMENTS

Neochanna are opportunistic generalist feeders, consuming predominantly small, slow-moving prey, such as planktonic micro-crustaceans, e.g. Cladocera, Amphipoda, Ostracoda, Copepoda (Fig. 6) and Chironomidae (midge) larvae. Fortunately, these are readily established and maintained in outdoor tanks and ponds. Prey species can be sourced in large numbers from fishless ponds, wetlands, or from *Neochanna* sites by running a very fine mesh net amongst aquatic plants, and collecting surface sediment. Once seeded with sufficient

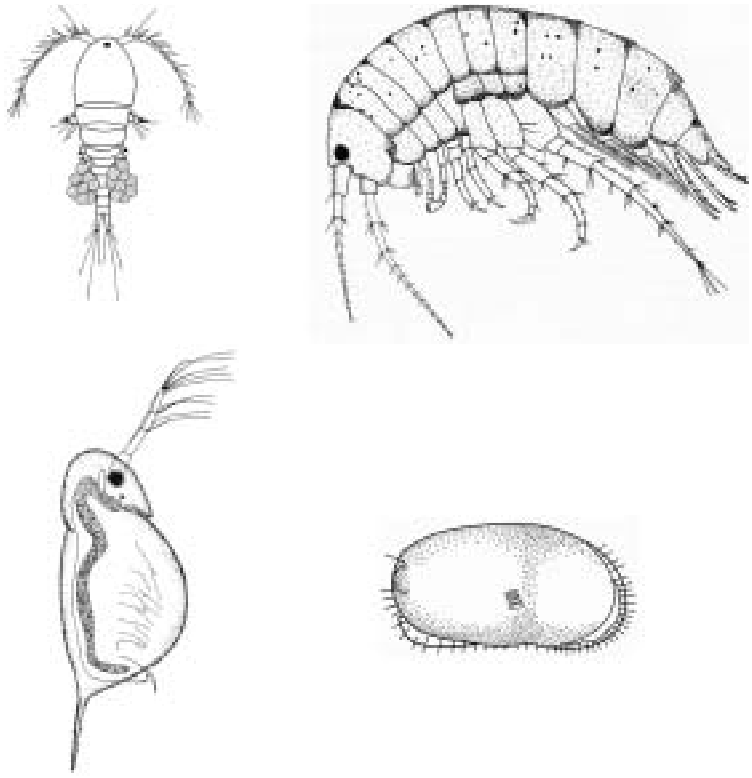

Figure 6. Representative micro-crustacean prey of *Neochanna* species. Clockwise from top left: Copepoda, Amphipoda, Ostracoda and Cladocera (not to scale). Drawings from Chapman & Lewis (1976).

live material, tanks require several weeks for a self-sustaining community to establish. Tanks must receive some sunlight to promote algal productivity, and some leaves or other detritus for invertebrates to feed on.

Neochanna should be fed regularly during the lead-up to spawning so that adults attain good pre-spawning condition. During this period, it is especially important to have established a thriving live prey community. The fishes' diet can also be supplemented with commercial or cultured food items. A diverse range of foods has been successfully fed to adult *Neochanna* (Davidson 1949; Eldon 1969; Town 1981; Meredith 1985; Thompson 1987; Dean 1995; Davidson 1999; Gay 1999; Perrie 2004). This includes live tubifex worms (Tubificidae), whiteworms (*Enchytraeus albidus*), freshly hatched brine shrimp (*Artemia* spp.) larvae, as well as scalded and chopped earthworms (*Lumbricus terrestris*). Terrestrial insects including houseflies (*Musca domestica*), spiders (Araneae), and aphids (Aphididae) will also be consumed, provided they are not too large (Eldon 1969). Commercially sourced flaked and moist pellet preparations, and frozen chironomid larvae (bloodworms) have also been used.

Newly hatched fry (because of their small size and inexperience) suffer high mortality rates, both in the wild and in captivity, and are more selective of their prey items. In captivity, many fry appear to die before first feeding (Eldon 1969; A. Perrie, University of Waikato, pers. comm.; L. O'Brien, pers. obs.). This may be due to developmental or behavioural inadequacies; however, it is important to maintain high water quality and provide open areas of water with abundant planktonic prey. Fry have been successfully fed commercial products, such as infusoria preparations (microscopic and near-microscopic aquatic life e.g. protozoa, rotifers, and paramecia), and can consume *Artemia* spp. once they get to about 10 days old. However, fry showed rapid growth when fed on micro-crustaceans (Gay 1999). Thus, supplying fry with very small live prey, either by transferring water from a live prey tank, or collecting prey with a very fine net, is recommended.

3.4 AQUATIC VEGETATION AND SPAWNING SUBSTRATUM

The presence of suitable spawning substratum may be a key factor in determining breeding success. Artificial spawning substratum such as mop heads, and tassels of nylon knitting material have been utilised by *N. burrowsius*; however, it was found that females preferred to spawn on real aquatic vegetation (Eldon 1979b). There may also be a preference for certain species of aquatic vegetation, particularly those with a complex growth form near the water's surface (O'Brien 2005).

It is considered preferable to use indigenous aquatic plant species and care should be taken not to use exotic species designated as pest or noxious species. Regulatory bodies, such as DOC and regional or district councils, may be able to supply identification brochures, if you are in doubt. In addition, any collected aquatic plants should be inspected carefully so that no predatory invertebrates or 'pest' fish eggs are transferred and, again, approval from regulatory bodies may need to be sought before collection.

Aquatic plants can either be rooted into substratum and / or left to float at the water surface. Suggested species are the water milfoils (*Myriophyllum* spp.) and the floating plants *L. minor* and Pacific azolla or water fern (*A. filiculoides*). If native aquatic plants are difficult to source, naturalised species such as watercress (*Rorippa* spp.) and starwort (*Callitriche stagnalis*) could be used, especially as floating mats.

Providing sufficiently complex vegetation at the water surface can be a challenge. A successful method used by O'Brien (2005) was the collection of emergent sections of *Rorippa* spp.; long sections were then broken into shorter (approx. 10 cm) segments, each with a growing end and rootlets. The tank's surface was then covered with a layer of these *Rorippa* spp. sections, which formed a mat of rootlets, being particularly favoured as spawning locations by *N. burrowsius* (Fig. 7). These mats of detached floating vegetation can be readily moved into a rearing tank once strewn with *N. burrowsius* eggs, then discarded once eggs hatch.

Sphagnum mosses (*Sphagnum* spp.) could also be trialled as spawning substratum, especially for *N. diversus*, which have been found in habitats described as heavily overgrown with these species (McDowall 1990). These mosses would provide a complex substratum due to their growth form, and a moist environment, if positioned terrestrially.

In the wild, *N. apoda* spawn within the holes around tree roots and buttresses, in which adults find refuge during habitat drying (Eldon 1978). *Neochanna apoda* scatter their eggs terrestrially within these holes, most likely to reduce mortality from cannibalism (Eldon 1971). However, survival requires a moist environment, as Eldon (1971) found terrestrially spawned eggs were often dehydrated and dead. Thus, *N. apoda* appear to have the most specialised requirements for spawning substratum of the New Zealand *Neochanna* species. The provision of spawning substratum for *N. apoda* may be achieved by providing wooden boxes, imitating the holes around tree roots, where spawning is thought to occur. A suggested construction would be a five-sided box made from untreated timber, placed half submerged into tanks, large

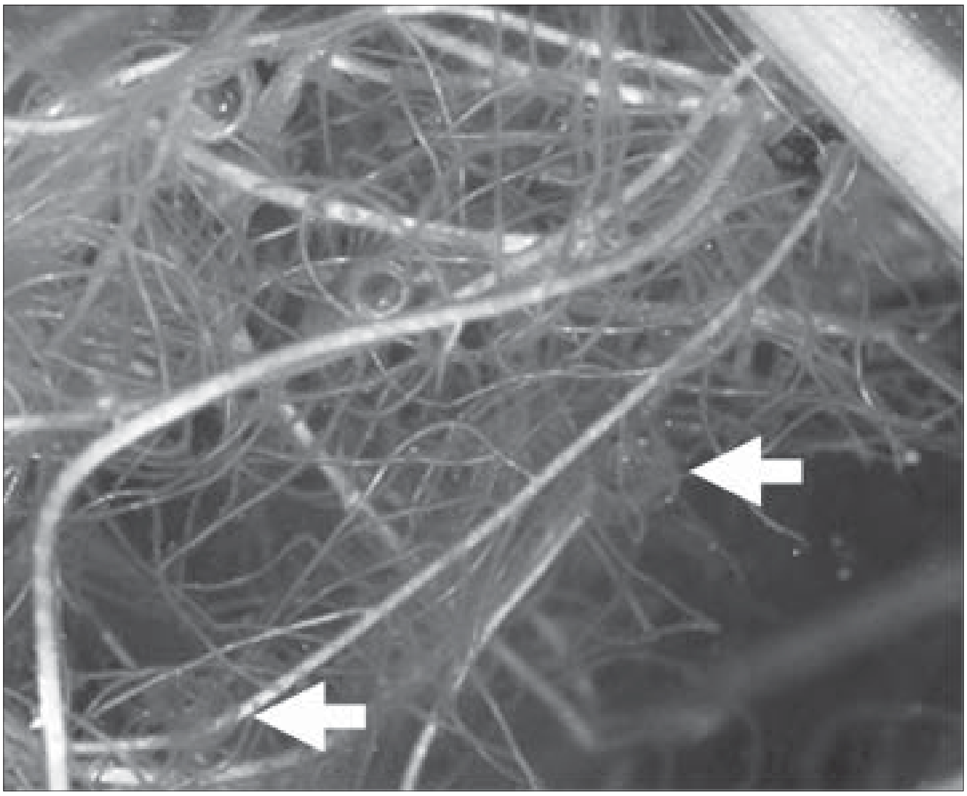

Figure 7. Aquatic vegetation camouflages eggs. *N. burrowsius* eggs attached to exposed root hairs of floating watercress (indicated by arrows). Eggs are often difficult to differentiate from gas bubbles emitted by plants (top left). *Photo by A. McIntosh.*

enough for several adults to swim freely into from below. The advantage of providing such spawning substratum is that the wooden surface facilitates checking for terrestrially spawned eggs. Once spawning occurs, the box can be removed and submerged in a rearing tank, thus allowing eggs to develop without the risk of predation.

Little is known about the spawning requirements of *N. rekohua* and *N. heleios*. However, both species often occur in peat lakes, and it is likely that eggs are scattered amongst margin vegetation in areas with little wind or wave disturbance. Recreating such habitat in captivity may be relatively straightforward.

3.5 IMPROVING RECRUITMENT SUCCESS

The most vulnerable *Neochanna* life stages, i.e. eggs, larvae, and small fry, experience the greatest mortality in the wild. The development of methods which increase the survival of these early life stages could secure the existence of threatened species by bolstering numbers. To ensure high recruitment in captive breeding facilities, eggs and fry should be removed from adult tanks and reared separately, as adults are likely to out-compete and predate their young. Cannibalism can be a significant cause of mortality of eggs and fry and is a common behaviour of *Neochanna* species, both in the wild and captivity (Cadwallader 1975; Eldon 1978, 1979a; Perrie 2004; O'Brien 2005).

Breeding and rearing tanks can be maintained in different ways to promote survival of early life stages. Tanks intended for housing adults should contain complex habitat, such as dense patches of submerged and floating aquatic vegetation, which acts to camouflage scattered eggs (Fig. 8). Rearing tanks

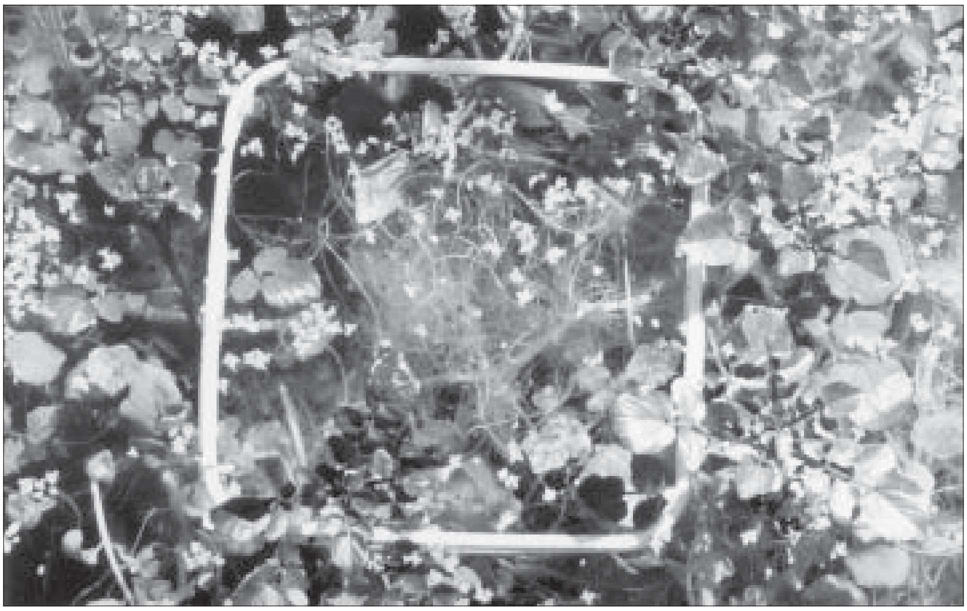

Figure 8. Location of a large concentration of *N. burrowsius* eggs amongst watercress roots at the water surface in outdoor tanks. Quadrate cut from a standard 1-L ice-cream container lid. *Photo by L. O'Brien.*

should contain little vegetation once fry have hatched. Generally, fry appear to prefer congregating in open water, where zooplankton is abundant, and they can have difficulty manoeuvring in dense vegetation. However, newly hatched fry of *N. apoda* preferred to take refuge in darkened areas, which is likely to be predator avoidance behaviour (Eldon 1978). Thus, wooden framing or the like could be used to provide shade and shelter. Moreover, to ensure fry survival in rearing tanks, predatory invertebrates should be removed and / or discouraged. Outdoor tanks are likely to be colonised by the flying adults of various aquatic insects. Although colonising chironomid larvae would provide considerable food resources, other invaders can be voracious predators of small fry, e.g. backswimmers (*Anisops* spp.), damselflies (Odonata), aquatic beetles (Coleoptera), and some caddisflies (Trichoptera), for example *Triplectides* spp. (L. O'Brien, pers. obs.). Damselflies are the most effective colonisers of tanks, but can be controlled somewhat by reducing the amount of aquatic vegetation present, as adults lay their eggs on or within the stems of aquatic plants. Moreover, the predatory aquatic larvae are ambush predators, and need some structure to launch an attack from, being reliant on fry swimming close by (L. O'Brien, pers. obs.). Thus, rearing tanks need to be inspected regularly and insect predators removed. Furthermore, care should be taken not to introduce any predaceous species into tanks when adding food and vegetation.

A key step in greatly improving cohort survival is the removal of recruits at the earliest stages of life and rearing them in isolation. Most importantly, this requires the ability to anticipate when spawning may occur, or early detection of spawning. This can be difficult, as individuals may spawn despite not appearing gravid (Eldon 1969), and obviously gravid fish may delay spawning for weeks if conditions are not suitable (Perrie 2004; O'Brien 2005). The ability of *Neochanna* species to retain eggs until conditions are suitable would be beneficial in the unpredictable and fluctuating habitats that they often occupy. This behaviour may be most pronounced in *N. diversus* and *N. apoda*, as they tend to spawn immediately after rains return in autumn and habitat is re-inundated after summer drying (Thompson 1987; Eldon 1978). Thus, various environmental cues have been suggested as being important in initiating spawning in *Neochanna* species. These include rainfall events with subsequent increases in water level, changes in water temperature and photoperiod (Cadwallader 1975). A link to lunar cycles has also been suggested, with spawning occurring most often during new moon and first quarter phases when luminosity is low (O'Brien 2005). Such behaviours could be utilised to synchronise spawning, through manipulation of water levels and imposing short dry periods at appropriate times. Such trials could possibly lead to better prediction and detection of spawning events in captivity. However, what conditions trigger spawning in *Neochanna* species are still largely uncertain, and further research is required.

3.6 TRANSLOCATION

Translocation is viewed as an important way of safeguarding against species extinction by spreading the risk among a greater number of populations (McHalick 1998). Translocation involves removing a relatively small number of individuals from an existing population and establishing them elsewhere, either directly or, more commonly for fish, after rearing and / or breeding them in captivity. Translocation is not without risk, and needs thorough contemplation, as many translocation attempts have failed to establish persistent populations (Eldon 1989, 1993; Caskey 1999). However, every new population established is likely to improve the odds of long-term species existence. Approval from regulatory bodies must also be obtained before any translocation occurs (see Section 1.1). This process may take several months.

3.6.1 Site assessment

Choosing translocation sites is a key step that may take many months or even years. It requires an understanding of the species in question as well as long-term knowledge of potential translocation sites. During the initial scoping phase, only those locations occurring within the same ESU as the individuals intended to be released should be considered. Translocating *Neochanna* species into sites beyond each species' natural distributional extent is not encouraged, especially if there is potential for interaction and / or hybridisation between species. Although they were developed specifically for *N. burrowsius,* Eldon (1993) outlined general features of optimum habitat that may be broadly applicable to all *Neochanna* species. These guidelines require consideration of habitat conditions during summer and the spawning period, vegetation, productivity of invertebrate community, and the presence of other fish species.

During summer, if a site dries, there must be accessible habitat or suitable refuge that remains moist; otherwise, mortality will be high. Thus, it is important to understand the hydrological characteristics of potential translocation sites. Aspects such as the usual source of water, degree of fluctuations in water level, and extent of the summer dry periods will influence survival and interactions of *Neochanna* with other species. These hydrological characteristics will influence water quality of a site. Although adults are tolerant of short periods of poor water quality, especially low dissolved oxygen levels, such conditions may inhibit reproduction and recruitment. Thus, sites with good water quality, at least during the spawning period, are preferable.

A complex and cluttered habitat is also required, including plenty of suitable vegetation, both riparian and in-stream, providing cover, refuge, and spawning substrate. Moreover, a site must contain an abundant aquatic invertebrate community. The presence and relative abundance of appropriate food resources, e.g. micro-crustaceans (Fig. 6) can be assessed by sweep netting the habitat. Another indication of a highly productive invertebrate prey community is the presence of large invertebrate predators such as Coleoptera, Odonata, and *Anisops* spp., which also require abundant prey to persist. These species, however, can prey on *Neochanna* fry, thus consideration of potential biotic interactions is important. Furthermore, *Neochanna* are most abundant in situations where few other fish species are present. *Neochanna* species are small, sluggish, and not usually aggressive, making them vulnerable to adverse interactions with other fish species. Thus, other fish species should be absent, or in low densities if present in the vicinity. If other species are present, water level fluctuations and summer drought may give the tolerant *Neochanna* species an advantage, and allow coexistence. In addition to these factors, there are likely to be species-specific preferences that require evaluation. Further information on the general habitat of *Neochanna* species can be found in McDowall (1990), Ling (2001), and will be published in L.K. O'Brien & N.R. Dunn: Mudfish: a review of literature regarding *Neochanna* (Teleostei: Galaxiidae) (in prep.).

3.6.2 Transfer and release

Before release, fish should be examined and preventive treatment for disease administered (see Section 3.2.3), with only healthy individuals being transferred. The number of fish introduced does not appear to determine whether a population will persist or not, as Eldon (1993) found around 100 fish have been used both in successful and unsuccessful attempts. However, it is recommended that as many individuals as possible be used to establish new populations. Further, if an initial transfer is successful, subsequent augmentation with further individuals could be undertaken, as this not only increases numbers but also ensures high genetic diversity in the founding population.

Eldon (1993) recommended the translocation of adults just prior to spawning, as this would ensure fish are concentrated in one area and have little time to disperse before spawning. Furthermore, translocation of pre-spawning adults allows the rapid evaluation of reproductive success in the new habitat. This method has also been successful in establishing populations of threatened fish overseas (Poly 2003). Another method Eldon (1993) described was the capture and rearing of fry to the benthic juvenile stage before subsequent release. However, the translocation of small individuals has not been successful in some cases. Further research into translocation methods, such as timing of release, is required, and further information on past translocations will be published in L.K. O'Brien & N.R. Dunn: Mudfish: a review of literature regarding *Neochanna* (Teleostei: Galaxiidae) (in prep.).

3.6.3 Monitoring and documentation

Insufficient monitoring subsequent to release is a severe impediment to the understanding of whether and why translocations are successful or not (Eldon 1993). Currently, a lack of monitoring in translocation projects has often meant that when populations fail to establish, the cause can only be speculated. Thus, the need for the documentation of captive management activities cannot be over emphasised. Understanding gained from sharing information on the establishment of new populations is essential to guide further conservation projects.

Records should be kept of the size, number and, if possible, sex of fish released. To assist in monitoring, it is recommended that released fish be individually identified using visual implant tags (VIT), implanted several weeks prior to translocation to ensure tag retention. Release dates should also be recorded so that, if multiple releases occur, success of fish released at different times can be assessed to determine the best times for releasing fish in that particular area. If good records have been kept and fish tagged, then growth, survival and dispersal can be evaluated. Monitoring during the spawning period, including measurement of water quality and hydrological characteristics, is especially important, as successful recruitment is essential for population growth. Further monitoring guidelines for *Neochanna* species will be published in N. Ling, R.F.G. Barrier, L.K. O'Brien, R. Miller: Methodology to survey and monitor NZ mudfish species (in prep.).

4. General breeding procedure

As little is known about the reproduction of many *Neochanna* species, it is difficult to outline standard procedures which are guaranteed to promote breeding in all species. Research is still required into spawning behaviour, recruitment limitations, and methods to replicate suitable conditions in a captive setting. However, the following procedure was found to be successful for breeding and rearing *N. burrowsius* at the University of Canterbury (O'Brien 2005), and could be used as a starting point in the captive management of other species. This procedure (Fig. 9) is relatively straightforward and brings together many guidelines discussed above.

Large (approx. 750 L) outdoor tanks supplied with aquifer-sourced water were used (see Section 2.1.1; Fig. 1; Appendix 1). Prior to the expected spawning period (early spring for *N. burrowsius*), tanks containing adult *N. burrowsius* were covered with a dense floating mat of aquatic plants. This consisted of detached, short (approx. 10 cm) pieces of *Rorippa* spp. and the water milfoil *M. propinquum*, plus the floating plants *L. minor* and *A. filiculoides* (see Section 3.4). Tanks were checked daily and floating vegetation with eggs attached was carefully moved into a secondary tank containing a thriving zooplankton community. Eggs were concentrated in certain places, such as on dense patches of floating roots (Fig. 7). The most effective method of moving eggs, which are easily dislodged, was lifting the floating macrophytes on a large square of 1 × 1 mm plastic mesh. A pool scoop may also be useful. Not all eggs were found attached to vegetation, and these were later collected as fry. Newly hatched larvae can be caught using a small mesh net or scooped out with an ice-cream container or similar. These early life stages are very vulnerable to injury, so particular care needs to be taken when handling them.

Aquatic vegetation in the rearing tank was kept to a minimum (see Section 3.5); thus, as eggs hatched, vegetation on which eggs were scattered was removed. The presence of live micro-crustaceans in the rearing tank was regularly

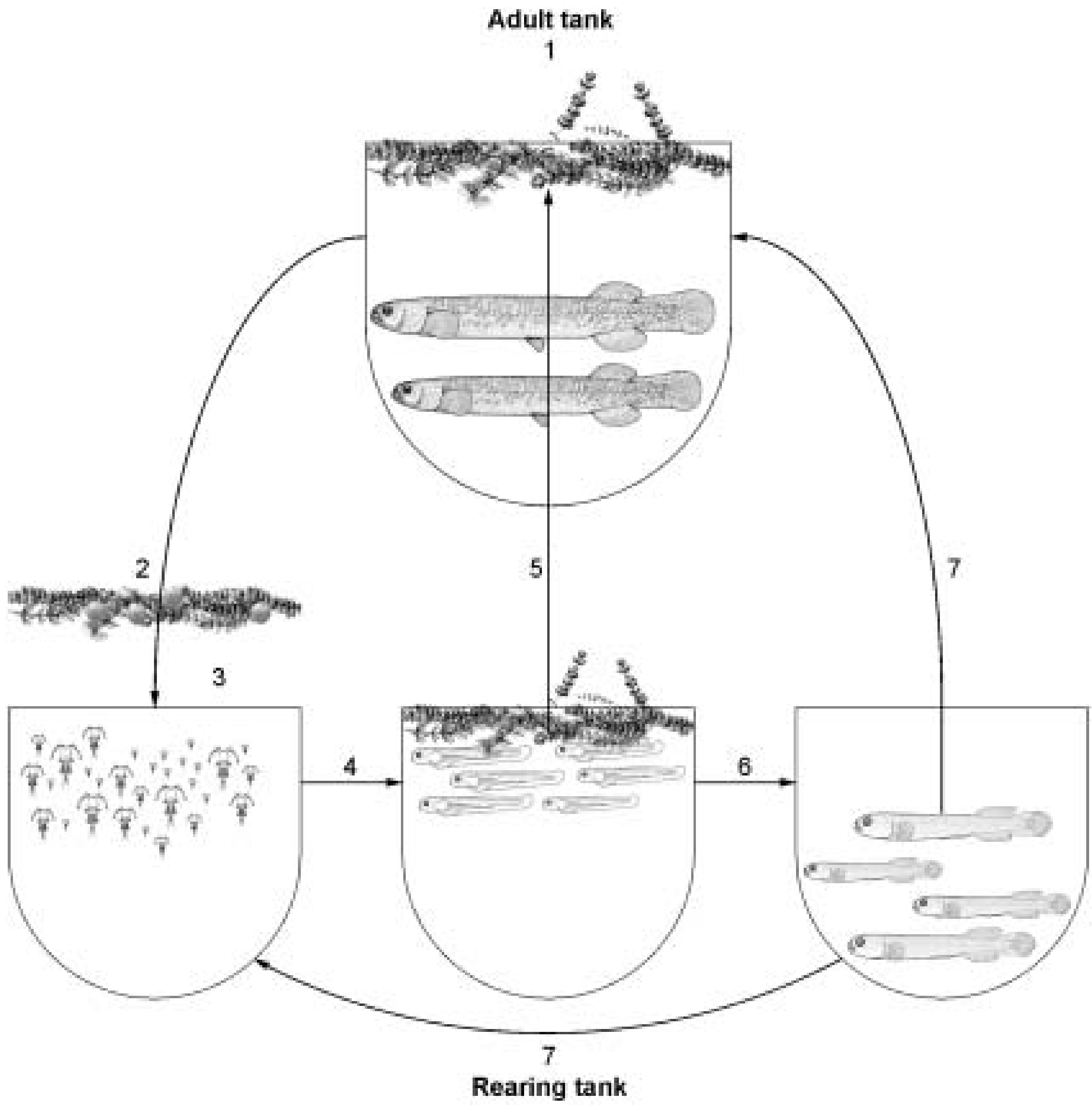

Figure 9. Diagrammatic representation of the technique found to be successful for *N. burrowsius* breeding. Further details can be found in the text. Drawings of *Neochanna* by R.M. McDowall and aquatic plants modified from Johnson & Brooke (1998).
Key to procedure steps:
1. Prior to spawning, place dense mats of floating aquatic plants in adult tank.
2. After spawning, carefully move vegetation with eggs into rearing tank.
3. Rearing tank should contain abundant micro-crustaceans, and no adults or invertebrate predators.
4. Allow eggs to develop and hatch.
5. Carefully remove vegetation and place in adult tank or discard.
6. Allow fry to develop to benthic juveniles (approx. 6 months).
7. Before next spawning season, move all juveniles to adult tank to allow prey communities to re-develop in rearing tank.

checked and the tank restocked, if required. As fry grew, their diet was supplemented with frozen bloodworms. It was found that fry did not all grow at the same rate, possibly due to intra-specific competition. Thus, Gee-minnow traps were regularly used to trap larger individuals (approx. 40 mm), which were then transferred into adult tanks, to allow smaller fry to develop. Eventually, before the next spawning season, all juveniles were moved to the adult tanks. Following this procedure, the captive population of *N. burrowsius* was at least doubled in one season.

5. Acknowledgements

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Appendix 1

Details of captive management facility equipment used at University of Canterbury

Tanks: Twelve 750-L plastic cattle troughs with ball-cock inflow, and outflow either as a trickle hole or a standpipe. Similar troughs can be sourced from farm supply companies. Additional prey culture tanks consisting of old bathtubs were also used.

Pipe: 19-mm diameter black alkathene pipe is recommended. Length required will be dependent on number of tanks and proximity of water source and outfall.

Pipe connectors: T-junctions, elbows, adapters etc. can be sourced from farm supply companies, specialised plumbing stores such as Water Dynamics or hardware supply stores such as Mitre 10.

Pallets: these can be used to ensure a flat platform for tanks and to raise them, increasing fall from the outflow of tanks.

Sand: a 5-cm layer per tank of clean *river* sand (avoid coastal beach sand due to its high salt content) is required as a substratum for the growth of aquatic plants and as refuge for burrowing invertebrates. This can be sourced from building supply companies such as Placemakers.

Miscellaneous: items such as very fine meshed aquarium nets, water quality testing kits, disease treatment solutions, frozen bloodworms, live *Daphnia* spp. and other supplementary food, as well as basic aquarium advice, can be sourced from pet supply stores. Libraries are also a source of books on basic information about keeping fish in aquaria.