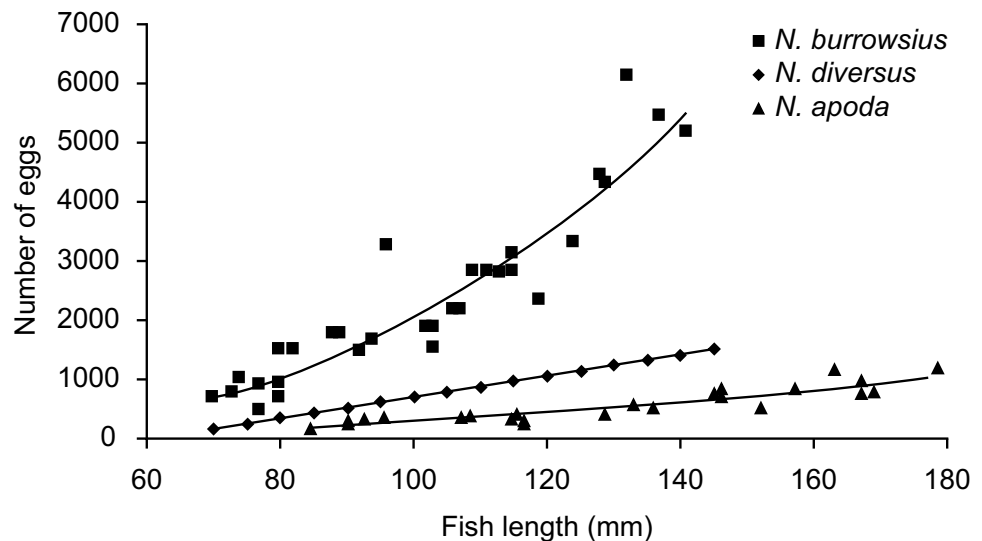


Figure 9. Number of eggs produced by female *N. burrowsius*, *N. diversus*, and *N. apoda* related to body length. Data from Eldon (1979c)—*N. burrowsius*, Eldon (1978b)—*N. apoda*, and generated from an equation from McLea (1986) as given by Hicks & Barrier (1996)—*N. diversus*. Equations being *N. burrowsius*: $y = 0.0027x^{2.9}$, *N. apoda*: $y = 0.012x^{2.2}$, *N. diversus*: $y = -1080 + 17.8x$.



at different stages of development, and may differ from the size of spawned eggs after they have expanded. Although these factors introduce variation, a fairly clear sequence in egg size is nonetheless present within the genus (Table 4). The less specialised species—*N. cleaveri* and *N. burrowsius*—have smaller, and more numerous eggs, compared with *N. diversus* and *N. apoda*. Thus, fecundity and egg size also support species placement in the transformation series (see Fig. 3).

Fecundity and egg size have implications for population dynamics and the ability to respond to disturbance and environmental change. There have been several suggestions as to the underlying reasons for the considerable differences in fecundity of *Neochanna* species. Cadwallader (1975a: 26) thought that differences were likely due to 'a less precise fertilisation process' in *N. burrowsius* than in *N. apoda* and *N. diversus*. Barrier (1993) proposed that *Neochanna* species could be placed on a continuum of r-K selectivity. This latter framework generated conclusions based on habitat stability, with *N. apoda* in stable habitats and *N. burrowsius* in disturbed habitats where high fecundity would be important. McDowall (1970) noted that Galaxiidae as a whole demonstrate a trade-off of egg size and fecundity and that it is strongly correlated with alternative life history patterns. Migratory *Galaxias* species whose fry develop in the marine environment have numerous small eggs, whereas wholly freshwater species tend to have fewer, larger eggs (Benzie 1961, 1968; Hopkins 1971, 1979). Thus, small egg size and, consequently, high fecundity in *N. cleaveri* are likely to be linked to its diadromous life-history. *Neochanna burrowsius* may have retained this ancestral trait, which is also likely to confer an advantage in flowing waterways where eggs and fry may be swept downstream into unsuitable habitat. In contrast, *N. apoda* often spawns within tree root hollows (Eldon 1978b). In such confined situations, numerous small eggs may not be advantageous, whereas fewer larger eggs that contain abundant yolk reserves may improve fry survival by promoting rapid development. Irrespective of the mechanism, it appears that selective pressures imposed by wetland life may not only have influenced the morphology of *Neochanna* species as detailed in the transformation series, but also their reproductive strategies.

5.2 SPAWNING BEHAVIOUR

Relative capture rates using a variety of methods suggest that female *Neochanna* outnumber males in many populations. The ratio of males to females was 1:1, 1:1.3 and 1:1.8 for *N. apoda* at different sites in the Hinai Valley, Wairarapa (Eldon 1978b) and 1:1.1 for *N. burrowsius* at a South Canterbury site (Eldon 1979c). However, Eldon (1979c) found that male *N. burrowsius* were more readily captured in traps during the spawning period, which he suggested indicated increased activity. Spawning involves scattering eggs throughout the habitat, a behaviour that is likely to reduce their detection by predators, including other adult *Neochanna*. Spawning has seldom been observed, but appears to involve males actively chasing females (Perrie 2004; O'Brien 2005). Pairs of *N. burrowsius* also move in unison during spawning activity in a way that suggests coordinated mating behaviour (Gay 1999; O'Brien 2005). Perrie (2004) recounted spawning behaviour in the early afternoon in *N. diversus*: 'The female(s), followed by one or both males would swim into a plant thicket... The male(s) would swim alongside, nudging and pressing their bodies against the females' (Perrie 2004: 9). Prior to spawning, unusual behaviour occurred in *N. apoda*, with adults resting amongst vegetation very near the surface of the water. These congregated fish were very still and did not startle when disturbed (Eldon 1971). However, vigorous activity must also occur during spawning in *N. apoda*, as eggs have been found splashed high above the water level of aquaria (up to 24 cm; Eldon 1971). There has been debate in the literature regarding the possible occurrence of terrestrial spawning (Eldon 1971, 1978b, 1979c; Cadwallader 1975a) and spawning migrations (Eldon et al. 1978b; Eldon 1979c).

5.3 TIMING OF SPAWNING

Environmental cues, including changes in water temperature, increasing photoperiod, and dissolved oxygen levels, may determine when *Neochanna* species spawn (Eldon 1979c, 1993). Cadwallader (1973) also proposed that rainfall or an increase in water level was a necessary stimulus for spawning in *N. burrowsius*. Such a cue is also potentially important for species that spawn immediately after the onset of rain. However, an increase in water level could not be confirmed as an environmental cue in experimental manipulations with *N. burrowsius* (O'Brien 2005). Spawning generally occurs from late autumn (*N. apoda* and *N. diversus*) until early spring (*N. burrowsius* and *N. cleaveri*), when water levels and temperatures are optimal (McDowall 1970; Eldon 1978b, 1979c; Ling 2001). Few field surveys for *Neochanna* eggs have been conducted, and the occurrence of spawning is usually deduced from the examination of gonads of individual fish (Eldon 1978b, 1979c; Koehn & Raadik 1991), or the appearance of fry (Eldon 1978b, Thompson 1987). Females typically spawn only once per year. However, there is some suggestion that *N. diversus* may spawn in autumn and spring if conditions are suitable (N. Ling, University of Waikato, pers. comm.). Indeed, *N. apoda* fry have been observed nearly year round if water is available (Eldon 1978b). Nonetheless, it is not known whether a female

is capable of spawning twice in a season, or whether females spawn once only, in either autumn or spring. In contrast, males spend only part of their milt at a time and may fertilise the eggs of several females in a season (Eldon 1979c).

Many of the habitats in which *Neobanna* species occur dry up during summer and autumn, so there is often only a short period of time in which successful reproduction and larval growth can occur. Rainfall is unpredictable and, possibly because of this, *Neobanna* females appear to be capable of retaining eggs for many weeks in anticipation of the onset of favourable conditions (O'Brien 2005). The ability to hold eggs may be facilitated by the development of a closed ovisac, as found in *N. apoda* (Davidson 1949). *Neobanna burrowsius* were found to delay spawning when no aquatic vegetation was present, and if disturbed or in situations of poor water quality (O'Brien 2005). The ability to control spawning readiness may be most pronounced in *N. diversus* and *N. apoda*, as they occur predominantly in seasonally dry wetlands and must be ready to spawn at the onset of rain (Eldon 1978b; Thompson 1987). However, female *Neobanna* do not synchronise their spawning and, as a result, the spawning season at any particular locality can extend over several months (Eldon 1978b, 1979c; Thompson 1987; McDowall 1990; O'Brien 2005). Thus, habitat-dependent patterns of water availability mean that *Neobanna* species do not necessarily have clearly defined breeding periods.

5.4 SPAWNING HABITAT

Little is known about the specific habitat requirements for spawning of most *Neobanna* species. However, as their spawning involves scattering eggs, it is likely that relatively large areas of suitable spawning substrate are required, whether these be dense aquatic vegetation, forest floor debris, moss, or, in the case of *N. apoda*, hollows under trees. Observations by Perrie (2004) of captive fish suggest that *N. diversus* prefers denser areas of aquatic vegetation for spawning. In a field study, O'Brien (2005) found that *N. burrowsius* eggs were associated with the presence of particular macrophyte species. These species typically had a complex architecture at the water surface, provided by either root hairs, e.g. *Glyceria* spp., or their general leaf structure, e.g. *Myriophyllum* spp. (O'Brien 2005). In another field-based investigation, Eldon (1978b) placed large bottomless tins into the substratum of a forested *N. apoda* habitat to ascertain the location of spawning through the enclosure of newly hatched fry. He concluded that spawning occurred within hollows amongst the tree roots that adults used as refugia during dry periods. Eldon (1978b: 26) also described sieving *N. apoda* eggs 'from gritty mud in a water-filled hollow about 300 mm in diameter formed by cattle tracks'. Thus, *N. apoda* may spawn in small shallow 'pockets' of habitat (Eldon 1978b).

5.5 SUMMARY

- New Zealand *Neochanna* species complete their life-histories in freshwater; however, larvae of the Australian *N. cleaveri* may develop in the marine environment.
- The number and size of eggs produced by females, of a given size, differ among the species so far studied in a way that conforms to the transformation series (Fig. 3).
- Females may outnumber males at some sites and males may be more active during the spawning period.
- Spawning has seldom been observed, but appears to involve co-ordinated and vigorous activity.
- *Neochanna* species scatter their adhesive eggs widely, but there is some evidence that they prefer particular places for spawning.
- *Neochanna* species exhibit flexibility in the timing and duration of spawning. Such flexibility illustrates the responsiveness of *Neochanna* species to their environment and is likely to enhance survival of vulnerable early life history stages.

6. Population characteristics

The survival, growth and fitness of each individual in a population determines general population-level characteristics, such as patterns in the density and size of the population. A ‘sustainable’ population is often identified as one containing a wide size range of healthy individuals. However, such ideal populations are seldom observed in the wild because of the interplay of abiotic factors, such as drought, and biotic factors, such as interactions with other fish. An important objective in conservation management is to determine if a population is in decline or limited by adverse factors. Population characteristics often reflect long-term environmental conditions and may be useful in guiding conservation action. This chapter provides information on recruitment, growth rates, and methods to determine density, size, and structure of *Neochanna* populations.

6.1 RECRUITMENT

Persistence of a *Neochanna* population requires recruitment and survival of sufficient juveniles to at least replace older individuals that die or emigrate. For *Neochanna* species, recruitment rate depends on many habitat and population characteristics, such as hydrological disturbance, habitat quality and fish density. Thus, recruitment can vary between sites and years. Drought patterns can have an important influence on recruitment success, as fry need to develop sufficiently before habitat dries up (Eldon 1978b). High water flows and flooding may also reduce recruitment, as fry are pelagic and can easily drift, or be washed out of a site into unfavourable downstream habitat (Kerr & McGlynn 2001). Interactions with other fish species, especially *Gambusia affinis*, may also influence recruitment, via predation and competition (Barrier & Hicks 1994; Kerr & McGlynn 2001; Ling 2004). Furthermore, Francis (2000a), speculated that

changes in farming practices contributed to low recruitment into some *N. apoda* populations. O'Brien (2005) also found very low recruitment, compared with the size of the adult population, in perennially flowing habitat, while populations in intermittently flowing habitats had consistently high recruitment.

6.2 GROWTH RATES

Generally, the growth of *Neochanna* individuals is initially rapid, and occurs mainly in the first year of life. Growth slows after fish reach reproductive maturity, at which time energy resources are diverted to reproductive activities and gonad development. In an extensive study of *N. burrowsius* populations, Eldon (1979c) calculated that in the first 10 months of growth, fish had mean monthly increments in body length (MMI) of 7–9 mm, whereas individuals 94–110 mm long had a MMI of 1.4 mm (range 0–3.8 mm). There may also be characteristic differences in growth rate between *Neochanna* species. For example, 1+ *N. burrowsius* had slower growth rates than similarly aged *N. apoda* (Eldon 1979c). Furthermore, after sexual maturity, growth slowed to a greater extent in *N. burrowsius* than in *N. apoda* (Eldon 1979c). Similarly, Perrie (2004) found *N. beleios* had lower growth rates than *N. diversus*. Site-specific growth from mark-recapture studies are compared in Table 5. It should be noted that such studies rely on the chance capture of a previously tagged fish, thus the length of time since, and timing of, capture varies. In all species, long-term estimates of growth, calculated over more than 300 days for fish > 80 mm long, are lower than short-term estimates (Table 5). This suggests that seasonal patterns in growth occur, with fish growing more during winter and spring than in summer and autumn (Eldon 1978b; Perrie 2004).

6.3 POPULATION DENSITY AND SIZE

Neochanna species can occur at high densities in suitable habitat, and records of more than 10 fish per trap occur in the NZFFD. Various methods exist to estimate population size and make assumptions of population characteristics.

TABLE 5. GROWTH RATES, FOR ADULT (> 80 mm LONG) *Neochanna* SPECIES AT VARIOUS SITES. THE AVERAGE LONG-TERM (CALCULATED OVER MORE THAN 300 DAYS) AND MAXIMUM GROWTH RATES MEASURED ARE GIVEN.

SPECIES	SITE	MEAN MONTHLY INCREMENT (mm)		PERIOD OF MAXIMUM GROWTH	SOURCE
		LONG-TERM GROWTH	MAXIMUM GROWTH		
<i>N. burrowsius</i>	Buchanans Creek	0.9*	6.9	July–Oct	Eldon (1979c)
<i>N. burrowsius</i>	Clearwell		2.5	July–Dec	Eldon (1979c)
<i>N. burrowsius</i>	Oxford	0.4†	2.5	June–Mar	Eldon (1979c)
<i>N. diversus</i>	Tekearau	0.8	3.9	July–Nov	Perrie (2004)
<i>N. diversus</i>	Waiparera	0.3†	1.4	July–Mar	Perrie (2004)
<i>N. diversus</i>	Whangamarino	0.7†	2.7	June–Oct	Perrie (2004)
<i>N. beleios</i>	Ngawha	0.3	0.7	Nov–Sept	Perrie (2004)
<i>N. beleios</i>	Omapere	0.2†	0.9	Apr–Sept	Perrie (2004)
<i>N. apoda</i>	Hinau	1.0	11.1	July–Feb	Eldon (1978b)

* Mean value from five fish calculated over 237 and 424 days.

† Sample size >5.

While catch per unit effort (CPUE) from sampling data provides an indication, population sizes can only be truly estimated using mark-recapture techniques. In both cases, habitat area is an important factor that influences estimates of population size. Eldon et al. (1978), in a study that used multiple trapping occasions in an extensive habitat, estimated that a population of *N. burrowsius* at Clearwell (Hinds River catchment), was likely to exceed 3000 adults. Similarly, O'Brien (2005) estimated population sizes for four *N. burrowsius* sites to range from 1200 to 3100 individuals. In contrast, 98 *N. apoda* were estimated to be present in a small 30-m² pond (Eastwood & Butler 1999). Perrie (2004) obtained population size estimates for *N. diversus* that ranged up to 540 fish per site.

Perrie (2004) also found that recapture rates of tagged fish were higher on consecutive days post tagging than over longer intervals. This indicates slow mixing rates, i.e. slow dispersal of individuals out of the immediate area they were released into after capture. Eldon et al. (1978) suggested that low mixing rates could result in poor estimates of population size. A further problem is defining the extent of a population. *Neochanna* species often occur in fragments of a larger wetland or lake system and in isolated pockets of habitat which, nonetheless, are connected during high flow and flooding, and/or by actively dispersing individuals (Eldon 1978b; Eldon et al. 1978). It is important, when deciding which methods of population size estimation to use, to determine the scale at which populations are 'open' or 'closed', i.e. whether individuals are moving in and out of the area of study or not. Many studies using mark-recapture methods have found that *Neochanna* populations at study sites are open, i.e. some fish disperse widely (Eldon 1978b; Eldon et al. 1978). However, both Eldon et al. (1978) and Perrie (2004) reported that the difference between estimates, assuming either open or closed conditions, resulted in only small differences in estimated population size.

6.4 AGE STRUCTURE

Determination of age of *Neochanna* fish requires the extraction of sagittal otoliths and, therefore, sacrifice of individuals. Eldon (1978b, 1979c) estimated that *N. burrowsius* and *N. apoda* live for 4-5, and 7 years, respectively. However, he suggested that *N. apoda* might live even longer, as otoliths from the largest fish he recorded were not examined. *Neochanna diversus* may be quite long-lived, as Thompson (1987) kept several alive in captivity for 8 years. By counting otolith annuli, Perrie (2004) estimated that a 114-mm-long *N. diversus* from Whangamarino wetland was 11 years old. However, *Neochanna* species exhibit differential growth patterns, with most growth occurring in the first year of life. After maturity, growth slows and otolith rings become very closely spaced and poorly defined, so counts may not be accurate. Thus, Eldon (1978b) commented that the number of rings recorded in independent counts of a single *N. apoda* otolith did not always agree, and sometimes three counts were all different. A further complication when determining age from otolith rings and check marks is that the variable seasons experienced in New Zealand result in growth patterns that relate poorly to annual patterns. Eldon (1979c) found contradictory results from otoliths of marked *N. burrowsius* of known minimum age and concluded that counts of otolith rings could not be used to determine the age of this species. Furthermore, the length of individuals is only very weakly related to their age. Perrie (2004) counted five annuli in a 105-mm-long

N. diversus from Whangamarino wetland, whereas an apparently similar-aged fish from Ngunguru wetland was 149 mm long. Considering the occurrence of site-dependent and seasonally variable growth rates, as well as the low reliability of otolith annuli readings, it is not possible to estimate the age of fish with any degree of confidence.

6.5 SIZE STRUCTURE

Another method used to estimate the age structure of a fish population is to determine how many cohorts in length-frequency data can be observed (as a high frequency of fish at certain lengths). By convention, and especially in small fish such as *Neochanna*, 5-mm length classes are recommended (Anderson & Gutreuter 1983). Examination of length-frequency histograms for *Neochanna* species is hampered by the difficulty in distinguishing cohorts after the first year because year classes merge as growth slows and becomes more variable at the onset of sexual maturity (Eldon 1978b; 1979c). The predominance of a particular sex may also influence size structure of a population. Eldon (1979c) found that *N. burrowsius* females attained a larger size than males at two different sites. This difference may be mistaken for different cohorts. In the Wairarapa, however, Eldon (1978b) found *N. apoda* males were larger than females at one site, but at two other sites there were no significant differences in the sizes of the sexes. Overall, it appears that estimation of age structure from cohort analysis is not possible for *Neochanna* species.

Nevertheless, important differences in the overall length-frequency structure of different populations has been noted by several workers. e.g. Eldon (1978b, 1979c), Francis (2000a), and O'Brien (2005). Observed structures were skewed to either the left or right, i.e. populations either had high recruitment or were mainly large fish, respectively. At a site in South Canterbury, Eldon (1979c) found 830 young-of-the-year *N. burrowsius*, and 65 adults between November and May, whereas at another site he found only 136 young-of-the-year, compared with 238 adults. Eldon (1978b) noted differences in length-frequency distributions in different *N. apoda* habitats in the Wairarapa, with fish being larger in pasture habitat than in forested habitat, even when the two kinds of habitat were adjacent. Eldon (1978b: 31) also reported that 'the largest mudfish recorded among 24 from West Coast (South Island) forest habitats was 118 mm, and the largest of 23 from West Coast open habitats was 160 mm'. Similarly, Francis (2000a) found fish attained a greater size in a roadside drain than in forest floor habitat at Fensham Reserve, Wairarapa. Eldon (1978b) suggested that differences in size attained by *Neochanna* reflected a relationship between growth rate and availability of food resources. He considered that *N. apoda* were smaller (125 mm) when only smaller prey were available (even if the site did not dry up requiring fish to cease feeding), whereas fish grew considerably larger (160 mm) when earthworms were included in the diet. O'Brien (2005) found differences in the maximum length attained by fish in intermittently and perennially flowing sites. These findings may reflect suppressed growth and stunting of fish in intermittent and ephemeral sites. Stunting may be related to starvation during habitat drying and the general availability of food resources. Habitat drying and hypoxia might also lead to selection of smaller-sized fish with lower oxygen uptake requirements (Meredith 1985).

6.6 SUMMARY

- Recruitment success differs greatly among populations. Drought, high water flows, habitat quality, and biotic interactions have been suggested as influencing recruitment.
- Rapid growth occurs before sexual maturity is reached at 1–2 years, and then slows considerably.
- Growth is seasonal, with more growth occurring during winter and spring.
- *Neochanna* species may exhibit characteristic growth rates, with *N. apoda* having the highest recorded growth rates and *N. beleios* the lowest.
- Estimates of population size require mark-recapture methods and may be affected by both low mixing rates and high rates of dispersal out of sites.
- Counts of sagittal otolith annuli and analysis of length-frequency structure are not reliable means of determining age in *Neochanna* species.
- Distinct population structures occur in different habitats and populations appear stunted in some situations.

7. Behavioural adaptations

Neochanna species exhibit a range of behaviours, including a diverse repertoire of foraging techniques, as detailed in section 4. However, they are most renowned for their amphibious tendencies and ability to persist in habitats that are periodically stagnant or dry up. The propensity to move over land may be a common behaviour in Galaxiidae (Woods 1963; McDowall 1990) that is especially pronounced in *Neochanna* species, especially *N. apoda* (Eldon 1978b). Main (1989) speculated that amphibious behaviour could have played a role in the dispersal of *N. apoda* into catchments after glacial retreat. Eldon (1978b: 33) reported that K.F. Maynard had observed *N. apoda* ‘splashing about all over the road’ during heavy rain; while Eastwood & Butler (1999: 4) reported that on the West Coast, *N. apoda* is referred to locally as ‘the fish with legs’. Such amphibious behaviour likely allows *Neochanna* species to persist in small, seasonally disturbed habitats. This chapter describes the behavioural response of *Neochanna* species to hypoxia (low dissolved oxygen), emersion (being out of water), and their refuge-seeking behaviours when habitats dry up.

7.1 RESPONSE TO HYPOXIA

Air-breathing is generally regarded as an evolutionary adaptation to hypoxia, a scenario thought to apply in the case of *Neochanna* species (Eldon 1979a, c; Meredith 1981; Dean 1995). When placed in poorly oxygenated water, *Neochanna* may move to the surface, take a gulp of air, and hold a bubble in the buccal cavity (Eldon 1979c; Meredith 1981; McDowall 1999b). Air-breathing also occurs when individuals are emersed (held out of water), with fish exhibiting distinctly distended jaws and inflated opercular regions. This is a primitive form of air-breathing more aptly described as air-gulping, as the behaviour is similar to feeding and coughing reflexes (Meredith 1985). Nevertheless, inflating the

buccal cavity with air is an efficient method of oxygen uptake (Meredith 1981). *Neochanna* will readily gulp air and do so in preference to utilising oxygenated water at the water surface (Meredith 1985; Barrier et al. 1996). When exposed to progressive hypoxia, gill ventilation in *N. burrowsius* increased in both amplitude and frequency, until the ability to compensate for declining oxygen uptake reached a threshold and air-breathing began (Eldon 1979c; Meredith 1981). Dissolved oxygen levels at which air-breathing commenced were approximately 15–30% saturation for both *N. burrowsius* (Meredith 1981) and *N. diversus* (McPhail 1999). A variety of air-breathing behaviours have been observed, indicating different strategies or progressive stages in response to increasing hypoxia (Eldon 1979c; Meredith 1981, 1985; McPhail 1999; McDowall 1999b).

During experimental conditions of declining aquatic oxygen concentration, *N. burrowsius* rose to the water surface, took an air bubble into the buccal cavity and then returned to the bottom of the tank and performed ‘burrowing like’ activities in an attempt to remain in benthic refuge (Meredith 1981). McPhail (1999) described *N. diversus* sinking to the bottom of the tank and remaining quiescent before the air bubble was expelled and the behaviour was repeated. *Neochanna diversus* continued gill ventilation while retaining the air bubble and McPhail (1999) suggested that this enabled water to pass over the air bubble. This behaviour differs from that reported by Meredith (1981), who observed that ventilation in *N. burrowsius* ceased during submerged air-breathing.

McPhail (1999) also described two distinctive postures assumed by *N. diversus* while holding an air bubble in the buccal cavity. Fish either assumed a head-up, vertical position against the glass, or a ‘cobra’ position with the head up and the anterior half of the body arched, while the posterior half of the body remained horizontal on the substratum. *Neochanna diversus*, *N. burrowsius* and *N. beleios* have all been observed ‘hanging’ at the water surface, sometimes with their head regions exposed to air (Eldon 1979c; Meredith 1981; Barrier 1993; McDowall 1999b). These fish did not expend as much energy as those that attempted to remain benthic while retaining an air bubble. Eldon (1979c) suggested that hanging at the water surface was a response to more chronic hypoxia and noted that fish were reluctant to move when disturbed. Remaining at the water surface exposes fish to increased risk of predation by birds (Barrier et al. 1996). Schooling and shoaling behaviour is a common anti-predator response in fish and may be adopted by *N. burrowsius* when air breathing at the water surface (Phillipps 1926a; O’Brien 2005).

As air breathing in an aquatic situation can be energetically demanding and potentially hazardous, *Neochanna* may attempt to leave the water before conditions become too extreme (Eldon 1979c; Meredith 1985; McPhail 1999). This was demonstrated in an experiment involving very low levels of dissolved oxygen, where some *N. burrowsius* actively left the water without establishing a regular pattern of air breathing (Meredith 1981). Meredith (1981: 19) observed that ‘fish were reluctant to return to the water and re-emerged if pushed back in’. In a series of investigations, Eldon (1978b) found that *N. apoda* would leave the water even if they were not threatened by stranding. McPhail (1999) also held the view that *N. diversus* left the water and moved to terrestrial vegetative shelter well before their habitat dried up. Pre-emptive movement in response to falling water quality would also allow *Neochanna* species to avoid being trapped in unsuitable habitat during a drought.

7.2 RESPONSE TO EMERSION

Movement from an aquatic to a terrestrial situation requires both physiological and behavioural adaptations. Meredith (1985) found that, during emersion, 40% of fish at any one time would lie on their dorsal surfaces with their abdomen exposed. Eldon (1978b) also noted that during experimental drought conditions *N. apoda* would sometimes lie on their backs. This behaviour may facilitate rehydration of the skin and enhance gas exchange efficiency through the skin to vital organs. The physical act of rolling may also improve distribution and mixing of water within the opercular cavities, or enhance waste excretion across the skin by allowing diffusion from different body surfaces (Meredith 1985). *Neochanna burrowsius* were thus observed to be active during emersion, changing positions frequently (Meredith 1985). Conversely, *N. diversus* moved infrequently in some studies (fish moved only 5 times in 10 weeks; McPhail 1999) but regularly in others, changing orientation, posture and location (Dean 1995; Davidson 1999). In all cases, however, *N. diversus* were responsive when touched, often reacting with vigorous activity, suggestive of an anti-predator response. Maintaining awareness would also allow fish to avoid environmental stressors by moving into better positions and seeking water when it returns (Meredith 1985). Barrier et al. (1996) speculated that while *N. diversus* may leave poor-quality water during the day when temperatures are high, they might return to feed during the night. Indeed, emersed *Neochanna* have been found with stomach contents that show little sign of decomposition (Günther 1867; Eldon 1979b). This may also explain the anecdotal observation that *Neochanna* catch rates are higher on rainy nights during otherwise drought conditions (Barrier et al. 1996).

The ability to bend into a deep, narrow U shape is also likely to be important for survival out of water (McDowall 2003). This posture allows maintenance of a dorsal-ventral position; ensuring fish do not roll onto their sides, which would affect their ability to inflate their gills. Coiling or bending behaviour also reduces the amount of exposed skin and, thus, the effective evaporative surface during emersion (Meredith 1985). In laboratory investigations, emersed *N. diversus* that were initially placed apart from each other would often end up coiled together in clusters (Davidson 1999). Similar behaviour has been observed in *N. burrowsius* and *N. apoda* (Eldon 1978b; Meredith 1985). Behaviour such as congregating in favourable microhabitats is likely to reduce desiccation during drought. Eldon (1978b) recounted how a drainage contractor with an excavator found 'pockets' or 'pods' of *N. apoda* in a grass field, and suggested that fish congregated in a few suitable refuges. O'Brien (2007) found large numbers (> 100) of *N. burrowsius* congregated in a cavity 0.5 m below the bottom of a former pond. It appeared that this subterranean cavity had been used for many years, as a large number of skeletons were also scattered about. Other searches for emersed fish have only found a small proportion of the fish known to be present there (Eldon et al. 1978; McPhail 1999).

7.3 REFUGE-SEEKING BEHAVIOUR

The ability of *Neochanna* species to find refuge well below ground level has been widely reported. For example, Roberts (1872: 456) described how a *N. apoda* 'was found at least eighteen inches down ... in a little chamber somewhat larger

than its own carcass. ... The chamber was dry and completely shut in from above'. Other depths recorded are 4 feet (Günther 1867), 10 feet (Hector 1869), 0.25 m (Eldon 1979c), 0.3 m (Andrews 1991), and 0.5 m (O'Brien 2005). These recorded depths suggest that active burrowing behaviour occurs; yet *Neochanna* have also been found in a diverse range of drought refuges, including car tyres and under corrugated iron (Eldon 1968, 1978a, b, 1979a, c; McPhail 1999). Selection of microhabitat during drought may therefore be largely random and opportunistic, with many fish dying in unfavourable places (Eldon et al. 1978; Eldon 1978b, 1979a, 1979c). There is also evidence from behavioural experiments that some individuals show little desiccation avoidance behaviour, such as burrowing (Eldon 1978b, 1979c). Davidson (1999) reported that *N. diversus* did not attempt to burrow into damp moss during emersion experiments. It appears, therefore, that the ability to survive drought requires an appropriate behavioural response, which may not always occur.

Neochanna species have well-developed muscles and bone ossification in the head region, likely an adaptation to facilitate burrowing through vegetation and damp mud (Davidson 1949; McDowall 1997a). However, there is debate as to whether *Neochanna* species are able to construct burrows, or whether they are limited to enlarging existing fractures or weaknesses in the substrate (Davidson 1951; Eldon 1978b, 1979a, c; McPhail 1999). Of 71 *N. burrowsius* that Eldon (1979a) found emersed, only 12 were in situations that bore any resemblance to burrows or a constructed refuge. Nonetheless, *N. apoda* may utilise the burrows created by *P. planifrons* (koura), although one study found that the characteristics of the burrows occupied by the two species differed slightly, with burrows containing *N. apoda* being vertical, compared with angled burrows that contained *P. planifrons* (Eldon 1968). *Neochanna burrowsius* have also been found in smooth-walled burrows and holes (Phillips 1926a; Eldon 1979a, c; O'Brien 2005). Cadwallader (1975a) observed three captive *N. burrowsius* occupying separate, small, smooth-walled chambers with tunnel entrances in a full aquarium. These burrows were located at or just above the water level, implying that *N. burrowsius* may also use burrows during non-drought conditions. However, in laboratory experiments, *N. burrowsius* and *N. apoda* failed to construct burrows, although there was some evidence of attempts (Eldon 1978b, 1979c). Burrowing behaviour has been observed in *N. cleaveri*, which commenced burrowing when exposed to conditions of hypoxia and declining water levels in an aquarium (Koehn & Raadik 1991). The behaviour consisted of the fish initially forming a vertical shaft when surface water was still present. By the time surface water had disappeared, horizontal tunnels connected to the original shaft had been constructed, and these still contained water. The fish then rested in the horizontal tunnels with their heads protruding into the water retained in the shaft, and remained in the tunnels as the substratum totally dried out (Koehn & Raadik 1991). This study demonstrated that at least one *Neochanna* species exhibits well developed burrowing behaviour. Considering the importance of this behaviour during droughts, further study to determine the extent of and requirements for burrowing is required for all New Zealand *Neochanna* species.

7.4 SUMMARY

- *Neochanna* species exhibit a wide range of behavioural responses that promote survival when their habitat is disturbed by drought.
- A variety of air-breathing behaviours have been observed and fish will also actively leave hypoxic water.
- When emersed, fish remain responsive and regularly move, which may enhance distribution of fluids and excretion of wastes.
- Individuals sometimes congregate in suitable micro-habitats, which may reduce water loss by evaporation and improve survival during drought.
- *Neochanna* species have sometimes been found in 'burrows' and underground cavities in the wild. However, New Zealand species have not been observed creating burrows in controlled situations (as has been observed with the Australian *N. cleaveri*) and there is debate over their ability to do so.

8. Physical adaptations

The ability of *Neochanna* species to persist in habitats prone to drying up has received much comment in the literature, and much has been made of this ability, e.g. Young (1996). While it is undeniable that *Neochanna* species can survive considerable periods without surface water, how they achieve this feat is poorly understood. Most studies have concluded that *Neochanna* species have few specialised anatomical adaptations to ensure survival (Meredith 1985; Dean 1995). Furthermore, although there has been much emphasis on their survival ability, field and laboratory studies of emersed *Neochanna* indicate significant rates of mortality during emersion (30–40%; Eldon et al. 1978; Meredith 1985; O'Brien 2005). The high level of mortality found conflicts with the general view that *Neochanna* species are well equipped to survive long periods without water (Dean 1995; McPhail 1999; Ling 2001). Physiological comparisons indicate that the potential to survive emersion may not necessarily be unique to *Neochanna* species within Galaxiidae (Meredith 1985). Indeed, *Neochanna* species do not have adaptations often found in more specialised air-breathing fish, such as highly modified gills (Davidson 1949; Meredith 1985; Davidson 1999), or the conversion of the swim bladder to an accessory gas exchange organ (Davidson 1949; Meredith 1985). It will be important to understand the extent to which *Neochanna* species can tolerate the absence of water if droughts become more severe as a result of climate change. This chapter details studies on characteristics of the gills, skin, mucus, blood, circulatory system, and metabolic rate of *Neochanna* species.

8.1 GILLS

Gills are important sites of gas and ion exchange in animals that live in water, but in the terrestrial environment, without the support of water, they may collapse and their ability to function may be reduced. Adaptations that improve gill function in air by preventing gill collapse generally lead to a reduction in gill surface area. This is due to the secondary lamellae (plate-like structures through which capillaries run) becoming shorter, broader, and spaced further apart on the gill filament (Meredith 1985; Dean 1995). Thus, a trade-off exists in that adaptations reducing gill area, which may improve survival in the terrestrial environment, may result in reduced ability to absorb sufficient oxygen from hypoxic water. How *Neochanna* species resolve these physiological constraints may provide insights into their likely survival strategies during drought.

Three studies have investigated the morphology of *Neochanna* gills (Table 6): Meredith (1985; *N. burrowsius*), Dean (1995; *N. diversus*) and Davidson (1999; *N. diversus*). Direct comparisons between these studies are difficult because of differences in the measurements made and data presented. However, it appears that morphological differences in gill structure may occur between the species studied. All studies agree that the structural components of *Neochanna* gills are not greatly reduced or modified, and resemble the gills of most other fish species, especially those of *Anguilla* species (Meredith 1985; Dean 1995). However, Dean (1995) found significant differences between *N. diversus* and *G. maculatus* in the spacing of secondary lamellae, with the latter species having long, closely spaced lamellae, whereas those of *N. diversus* are widely spaced (Table 6). On average, the secondary lamellae of *N. diversus* also appear stouter (lower height to width ratio) than those of *N. burrowsius* (Table 6). Comparisons of the bilateral (total) area of secondary lamellae also suggest that *N. diversus* may have a smaller functional gill area than *N. burrowsius* (Table 6). Thus, although more rigorous and direct anatomical comparisons are needed, *N. burrowsius* may (by having a larger gill surface area to extract oxygen from water) tolerate hypoxia for longer than *N. diversus*. Conversely, *N. diversus* may tolerate emersion better by having short, widely spaced gills. Although these conclusions need further verification, they reflect the ecological situations in which the two species are commonly found and support their placement in the transformation series (see Fig. 3).

TABLE 6. MEASUREMENTS OF SECONDARY LAMELLAE ON THE SECOND GILL ARCH OF *Neochanna* AND *Galaxias* SPECIES. MEANS ARE GIVEN WHERE PROVIDED. A DASH (-) INDICATES INFORMATION IS UNAVAILABLE.

SPECIES	SPACING (μm)	LENGTH (μm)	HEIGHT/WIDTH RATIO	BILATERAL AREA (μm^2)	SOURCE
<i>N. burrowsius</i>	31*	125	0.92	8800 [†]	Meredith (1985) [§]
<i>N. diversus</i>	36	102	-	-	Dean (1995) [§]
<i>G. maculatus</i>	22	105	-	-	
<i>N. diversus</i>	28	-	0.81	8600	Davidson (1999) [‡]
<i>G. maculatus</i>	22	-	-	-	

* Measured from supplied scanning electron micrograph.

[†] Calculated using allometric equation relating fish size to bilateral area.

[§] Sample size > 10.

[‡] Sample size of one 88-mm-long fish.

8.2 SKIN

Scaleless, permeable skin is considered a precursor of the primitive adaptations that enabled fish and amphibians to leave the water permanently. The skin of *N. burrowsius* acts as an effective supplementary gas exchange surface (Meredith et al. 1982), as it also does in several *Galaxias* species (Meredith 1985). Although cutaneous respiration is a general characteristic of galaxiid fish, it appears highly developed in *Neochanna* species. *Neochanna burrowsius* were capable of absorbing a greater proportion of oxygen (43%) through the skin than were three species of *Galaxias* (16–19%) in water (Meredith 1985). In the first 4 hours they were out of water, 40% of oxygen uptake and 45% of carbon dioxide loss occurred through the skin of *N. burrowsius* (Meredith 1985). Thus, considerable respiration can still occur in *Neochanna* even if gills collapse and their ability to function is reduced.

Metabolic waste products are also excreted through permeable skin, although valuable water may be lost through this process. Overall, the skin of *N. burrowsius* and, possibly, other *Neochanna* species is permeable to gases (oxygen, carbon dioxide, and ammonia) and water, but is weakly permeable to ions (important electrolytes) and chemical metabolites (urea; Meredith et al. 1982; Meredith 1985). These characteristics have consequences for the mechanisms and strategies that enable *Neochanna* species to survive in both air and water (Meredith 1985). Importantly, *Neochanna* species are unable to regulate water loss and have not evolved any specialisations in this regard, as are found in amphibians. However, the lack of anti-desiccatory mechanisms in *Neochanna* species is likely to be compensated for by behavioural responses and an extensive mucous covering (Meredith 1985).

8.3 MUCUS

Although Dean (1995) did not observe mucous cells in the skin of *N. diversus*, they are most likely present, as McPhail (1999) mentioned the presence of a mucous layer on the skin of these fish. The skin of *N. apoda* contains gland cells capable of excreting mucus (Davidson 1949). Similarly, the skin of *N. burrowsius* is covered extensively with mucous cells, which exceed 50% of the skin area. Their distribution and relative density were high compared with most other fish (Meredith 1985). The proliferation of mucous cells appears central to the function of *Neochanna* skin and is considered one of the outstanding features that allows *Neochanna* to survive in air (Meredith 1985). Indeed, mucus production increases substantially in response to emersion (Meredith 1985; Dean 1995; McPhail 1999).

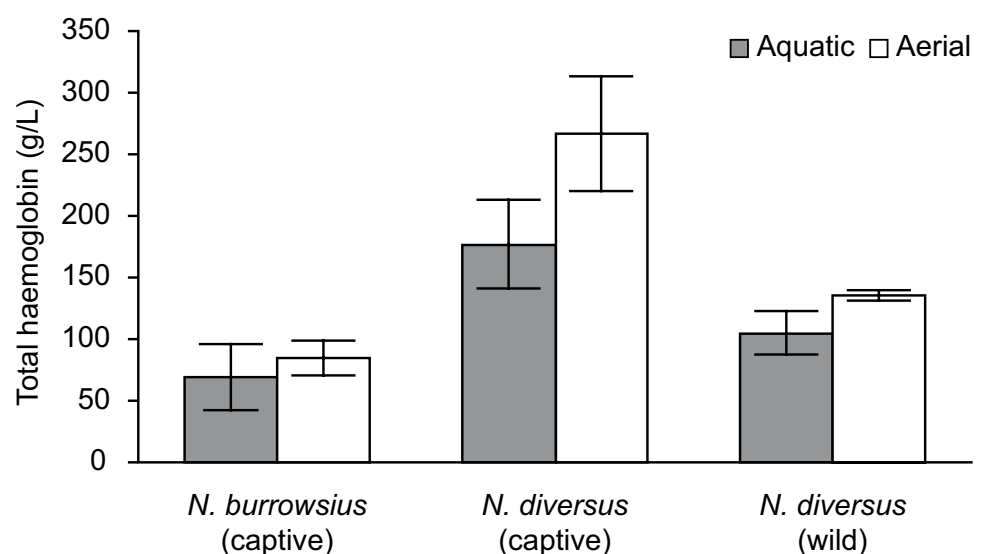
Mucus has many functions. For example, it has lubricating properties, performs protective and osmoregulatory roles, improves abrasion resistance, and is a barrier to pathogens (Meredith 1985). Importantly, mucus is hygroscopic and can store water, thus maintaining a moist layer around an emersed fish, potentially drawing and trapping water from the surrounding microenvironment (Meredith 1985). When *Neochanna* are emersed, they excrete copious quantities of mucus from their opercular apertures. This mucus may keep gill surfaces moist, thus preventing them from collapsing together, desiccating, and losing function (Meredith 1985; Dean 1999). Mucus may also aid in sealing the margins of the opercula, thus improving the retention of air bubbles in the buccal and opercular cavities when fish are air-breathing (Meredith 1985).

In some tropical aestivating fish and amphibian species, thick dried mucus can form a 'cocoon', sealing the emersed animal in a favourable microclimate. Although Hicks & Barrier (1996) speculated that *Neochanna* species formed such cocoons, there is no evidence that this occurs (Eldon 1978b; Meredith 1985; McDowall 2006). Furthermore, Meredith (1985) discussed whether a mucoid sheath, capable of resisting water diffusion, might explain the ability of *N. burrowsius* to survive for long periods without water. However, following laboratory studies, he concluded that it is unlikely to be a significant mechanism in emersed fish, as desiccation and death are likely to occur before a sufficiently protective cocoon had formed. The composition of mucus, however, may determine the effectiveness of its protective properties and osmoregulatory function, as certain types of mucus are related to amphibiousness in other fish. Several types of mucous cells are present in *N. burrowsius*, and their occurrence considered a general adaptation that has benefits for both aquatic and aerial survival (Meredith 1985).

8.4 BLOOD CHARACTERISTICS

Blood (and particularly the haemoglobin it contains) is a vital part of the physiology of most animals. It is the medium by which oxygen and nutrients are transported to cells, and carbon dioxide and other waste products are excreted. Fish living in low-oxygen environments typically have haemoglobin with a high affinity for oxygen. Interestingly, the haemoglobin of *N. diversus* has a high oxygen affinity (Wells & Ling unpubl. data, quoted in Dean 1995), whereas that of *N. burrowsius* does not (Wells et al. 1984). Thus, *N. diversus* may be capable of higher oxygen uptake in situations of limited oxygen availability than *N. burrowsius*, possibly compensating for its reduced gill surface area (Table 6). The concentration of haemoglobin in blood also reflects the blood's ability to transport oxygen and carbon dioxide within an animal (Davidson 1999). Davidson (1999) measured the blood characteristics of *N. diversus* in aquatic and emersed situations and found that total haemoglobin concentration was significantly higher in emersed fish (Fig. 10). However, this may not be the case in all *Neochanna* species, as emersed and aquatic *N. burrowsius* showed no differences in blood oxygen transport properties (Wells et al. 1984).

Figure 10. Total haemoglobin (g/L) \pm SEM in the blood of wild and captive *N. diversus* (Davidson 1999) and *N. burrowsius* (Wells et al. 1984), kept or found in aquatic and aerial situations.



The ionic concentration of the blood of *N. burrowsius* was more similar to that of euryhaline marine species of Salmonidae and Anguillidae, than most exclusively freshwater fish species (Meredith 1985). This may reflect a relatively recent marine ancestry for *N. burrowsius*, as demonstrated by the diadromous *N. cleaveri* (Fulton 1986). It would be interesting to see whether similar blood characteristics are present in other species of *Neochanna*. The blood of *N. burrowsius* also has a high buffering capacity (Wells et al. 1984), a feature that may moderate imbalances generated by the accumulation of waste products. Buffering or other protective mechanisms are necessary to mitigate ammonia poisoning. Certain amino acids may play a role in this, although the mechanism is still unknown (Meredith 1985).

8.5 CIRCULATORY SYSTEM

Although many aspects of the anatomy of *Neochanna* species are similar to those of other fish (Davidson 1949; Davidson 1999), *Neochanna* species differ from other fish in that they have especially efficient circulatory systems (Meredith 1985). In particular, some features may represent specialisations to enhance air-breathing and survival during hypoxia. For instance, *N. apoda* have an unusual vein that may be an accessory mechanism for eliminating carbon dioxide from the brain (Davidson 1949). The heart of *N. apoda* is also different from the usual teleost type, having an incipient septum subdividing the atrium, a feature found in the South American lungfish (*Lepidosiren* species). Davidson (1949) also described numerous capillaries on the buccal surfaces of *N. apoda*. Such extensive vascularisation would increase oxygen uptake, especially when fish retain an air-bubble in the buccal cavity. Increased vascularisation has not been observed in the buccopharyngeal cavity of *N. burrowsius* (Meredith 1985) or *N. diversus* (Davidson 1999). Thus, species-specific differences in vascular systems may occur that support the arrangement of *Neochanna* species in the transformation series (Fig. 3). On this basis, *N. apoda* exhibits the greatest adaptation to air-breathing of the *Neochanna* species.

An interesting question discussed in the literature is whether *Neochanna* species are able to alter their circulatory patterns in response to environmental conditions. Investigations into ammonia excretion, respiration and skin structure have generated debate over whether *Neochanna* can change skin permeability via the constriction and dilation of blood vessels (Wells et al. 1984; Meredith 1985; Dean 1999). It has been suggested (Meredith 1985) that blood could be directed to the skin when fish are emersed (to increase gas exchange), and away from the skin in hypoxic water (to reduce oxygen loss to the surrounding water). However, there have as yet been no definitive investigations, or a general consensus regarding this ability.

8.6 METABOLIC RATE

Metabolic rate is an important physiological variable (commonly measured indirectly by oxygen consumption) which reflects biochemical processes and provides an indication of energy requirements and metabolic waste generation.

The metabolic rate of a fish is dependent on many factors including size, temperature, activity, and stress. For fish in captivity, holding facilities and acclimatisation times are also important in determining standard or resting metabolic rates. Unfortunately, studies of *Neochanna* species carried out so far have used a variety of fish sizes (0.5–10 g), acclimation times (30 minutes – 5 days), experimental temperatures (15–17°C), and general procedures (Meredith 1981, 1985; Meredith et al. 1982; Dean 1995). This variation complicates comparisons; nevertheless, general patterns are evident and it is possible to come to some preliminary conclusions that can be used to inform further comparative research.

In some fish, such as the lungfishes (Sarcopterygii), rapid suppression of metabolic rate occurs in response to their removal from water. This is called aestivation, and is a state similar to hibernation. Such a response to removal from water has been demonstrated in *Galaxiella*, an Australian Galaxiidae fish species found in ephemeral wetland habitats. *Galaxiella* can halve their metabolic rate within hours of emersion (Thompson & Withers 1999). There have been several studies of resting oxygen consumption of Galaxiidae under aquatic (Table 7), and aerial (Table 8) conditions (Eldon 1978b; Meredith 1981, 1985; Dean 1995; Thompson & Withers 1999). The extent of metabolic suppression is determined from comparison of these separate aquatic and aerial measurements. These studies have generated debate as to whether, in response to their habitats drying up, *Neochanna* species exhibit true aestivation, which involves significant cardiorespiratory and metabolic changes as fish enter a state of torpor.

Eldon (1978b) discussed the use of the term aestivation and concluded that *N. apoda* does not truly aestivate. Similarly, Meredith (1981) showed that Galaxiidae in general were tolerant of emersion, and that aestivation was an inappropriate term to describe the response of *N. burrowsius* to survival out of water, because they do not enter a state of torpor. Indeed, it is widely recognised that *Neochanna* species do not become fully dormant or torpid when emersed, and that they respond to external stimuli (Cadwallader 1975a; Eldon 1978b,

TABLE 7. AQUATIC OXYGEN CONSUMPTION RATES FOR RESTING *Neochanna* AND *Galaxias* SPECIES, AND *Galaxiella nigrostriata*. SAMPLE SIZES AND THE MEAN, OR RANGE, OF FISH SIZES USED ARE GIVEN WHEN KNOWN.

SPECIES	SAMPLE SIZE	FISH WEIGHT (g)	O ₂ CONSUMPTION (μL O ₂ /g/h)	SOURCE
<i>N. burrowsius</i>	8		47	Meredith (1981)
<i>N. burrowsius</i>	11	4–10	37	Meredith et al. (1982)
<i>N. burrowsius</i>	15	6	54	Meredith (1985)
<i>G. maculatus</i>	6	6	141	
<i>G. brevipinnis</i>	4	5	102	
<i>G. vulgaris</i>	3	5	117	
<i>G. fasciatus</i>	6	5	94	
<i>N. diversus</i>	4	0.5–1	249*	Dean (1995)
	4	2–4	218*	
	7	4–10	116*	
<i>Gl. nigrostriata</i>		0.35	48	Thompson & Withers (1999)

* Means recalculated from extracted data and values converted using the equation:
1 Mol O₂ = 22.39 L O₂ STPD.

TABLE 8. EMERSED OXYGEN CONSUMPTION VALUES FOR RESTING *Neochanna* SPECIES AND *Galaxiella nigrostriata* HELD OUT OF WATER FOR VARIOUS LENGTHS OF TIME BEFORE MEASUREMENT. SAMPLE SIZES AND MEAN OR RANGE OF FISH SIZES USED ARE GIVEN WHEN KNOWN.

SPECIES	FISH WEIGHT (g)	SAMPLE SIZE	DAYS EMERSED	OXYGEN CONSUMPTION ($\mu\text{L O}_2/\text{g/h}$)	SOURCE
<i>N. burrowsius</i>		8	8	39	Meredith (1981)
<i>N. burrowsius</i>	4-10	11	<1	57	Meredith et al. (1982)
<i>N. burrowsius</i>	6	9	<1	48*	Meredith (1985)
	6	16	28	42*	
<i>N. diversus</i>	1-6	16	1	44 [†]	Dean (1995)
	1-6	16	7	37 [†]	
	1-6	16	28	41 [†]	
<i>Gl. nigrostriata</i>	0.4	6	<1	21	Thompson & Withers (1999)

* Extracted data.

[†] Means recalculated from extracted data and values converted using the equation:
 $1 \text{ Mol O}_2 = 22.39 \text{ L O}_2 \text{ STPD.}$

1979c; Eldon et al. 1978; Meredith 1981; Barrier & Hicks 1994; Dean 1995; Hicks & Barrier 1996; O'Brien 2005; McDowall 2006). Thus, *Neochanna* species are unlikely to undergo the same metabolic changes in response to drought as other truly aestivating fish and may better be described as 'emersion tolerant'.

It is important to determine the extent to which *Neochanna* species are capable of lowering their metabolism, as it is indicative of their ability to survive long periods of emersion. Meredith (1981) reported that *N. burrowsius* reduced their metabolic rate by 25% during 8 days emersed, although he later found (Meredith 1985) that on being removed from water, *N. burrowsius* initially increased their metabolic rate before it settled down, after 6 hours, to a level similar to the aquatic rate. The greatest reduction in metabolic rate has been reported by Dean (1995), who calculated that *N. diversus* reduced their metabolic rate by 70–85% when emersed. Dean's (1995) study involved comparing the results of two separate experiments using different methods and fish. Crucially, Dean (1995) reported aquatic oxygen consumption rates for *N. diversus* that were 2–3 times greater than those obtained in studies of *N. burrowsius* (Table 7; Meredith et al. 1982; Meredith 1985), and were more comparable to those of *Galaxias* species (Table 7). However, it is important to note that there were calculation errors in this study (acknowledged by T. Dean, DOC, pers. comm.). It is likely that the elevated aquatic oxygen consumption rate calculated for *N. diversus* led to the apparently large difference and reduction in metabolic rate when compared with results from fish in aerial conditions. This large reduction in metabolic rate in response to emersion suggested for *N. diversus* has been widely reported in the literature as evidence of the ability of *Neochanna* species to aestivate (Barrier & Hicks 1994; Hicks & Barrier 1996; Barrier et al. 1996; McPhail 1999; Ling 2001). Considering the importance of such a physiological adaptation in enabling fish to survive drought, further examination of these results and repetition of the study are needed.

In contrast to aquatic measurements, the oxygen consumption rates measured in studies of emersed *Neochanna* species were broadly similar (Table 8). Furthermore, the values obtained for emersed *N. diversus* were similar to those of *N. burrowsius* measured in both aerial and aquatic conditions (Tables 6 and 7). It is likely that *Neochanna* species have an inherently low resting or basal metabolic rate, even in aquatic situations. Meredith (1981) observed that aquatic respiratory patterns of undisturbed *N. burrowsius*, measured by counting opercular movements, were sometimes barely detectable, and often intermittent with apnoeic pauses of 5–60 seconds, indicating low oxygen consumption and metabolic requirements (Meredith 1985). Having low metabolic rates when resting is likely to enhance the survival of *Neochanna* species during periods of starvation, hypoxia and drought.

Although metabolic rate does not appear to lower immediately in response to emersion, or a state of torpor occur, oxygen consumption rates of *N. diversus* gradually fell over 20 days of emersion (Fig. 11). After 14–21 days of emersion, *N. diversus* exhibited an oxygen consumption rate half that measured after one day (Dean 1995), although it subsequently increased (Fig. 11). Meredith (1985) did not measure the oxygen consumption of *N. burrowsius* during this period and could have missed this minimum. A gradual reduction in metabolic rate may allow *Neochanna* species to be more responsive to changes in environmental conditions. However, there may be a limit to the ability to maintain a low metabolic rate, especially in smaller individuals. After 28 days emersion, oxygen consumption rates of the smaller *N. diversus* individuals (1–2 g) increased considerably, possibly as they became stressed (Fig. 12). In general, although aestivation does not occur, it is possible that the metabolic rate of *Neochanna* species slowly lowers if fish are emersed for moderately long periods, which would promote survive without water. However, further research is required on this topic, and direct comparisons of oxygen consumption rates are needed for all species under a variety of environmental conditions and activity levels.

Figure 11. Temporal patterns in oxygen consumption \pm SEM by emersed *N. burrowsius* (Meredith 1985) and *N. diversus* (Dean 1995).

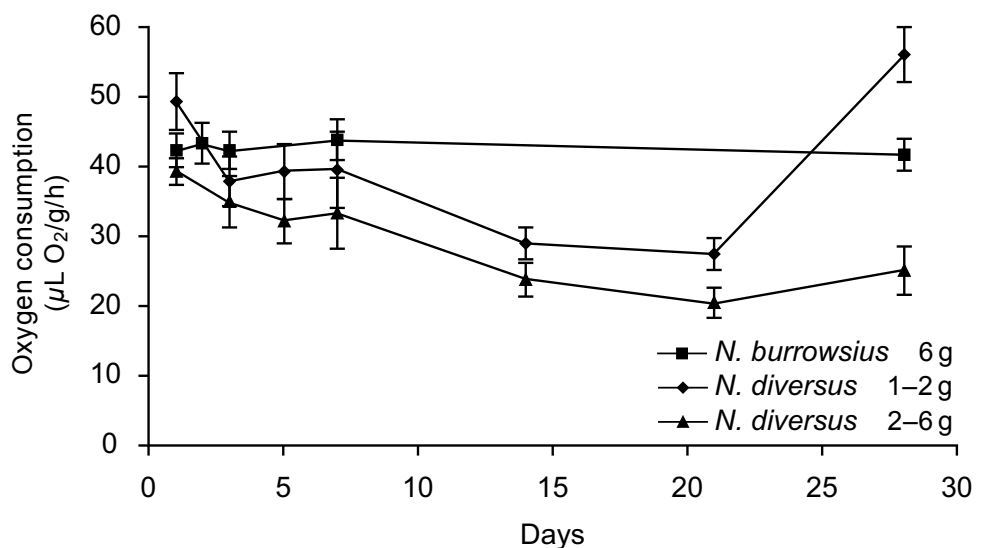
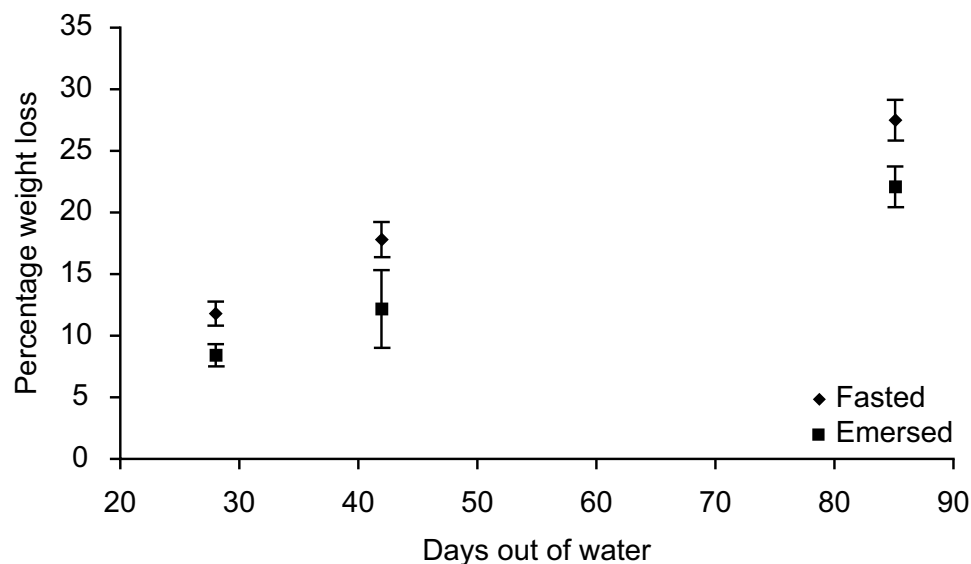


Figure 12. Results of two studies into weight loss in *N. burrowsius* and *N. diversus* held in aquatic conditions without food and emersed conditions (labelled fasted and emersed, respectively). Data from Ling (unpubl. data as cited in Dean 1995), and Meredith (1985).



8.7 SUMMARY

- The main characteristics of *Neochanna* species that allow survival of hypoxia and emersion are: generally small body size, inherently low metabolic rate, permeable mucus-covered skin with abundant blood vessels, an efficient circulatory system, and well-buffered blood.
- Preliminary comparisons indicate that there are differences in gill morphology, the circulatory system, and the oxygen affinity of blood, between the species studied.
- *Neochanna* species do not rapidly enter a state of torpor, indicating aestivation. Instead, their metabolic rate slowly drops when they are kept out of water and they remain responsive to external stimuli.

9. Survival out of water

When their habitats dry out, fish must cope with a progressive series of challenges as the quality of water deteriorates and its quantity diminishes. Survival without water poses numerous problems, including inhibited movement without the support of water, sensory perception in a different medium, passive loss of body fluids, reduced respiratory ability as gills collapse, toxic waste accumulation and food deprivation (Meredith 1985; McPhail 1999). In the terrestrial environment, *Neochanna* must tolerate abruptly changing and widely varying temperatures, desiccation, and substantial change in physiological processes such as increasing blood and tissue levels of carbon dioxide, ammonia, urea, osmolarity, as well as acid-base disruption (Meredith 1985).

Neochanna species exhibit the ability to tolerate severe internal disruption within the first 24 hours of emersion and this ability may be central to their survival (Meredith 1985). However, it is important to understand how *Neochanna* species prevent or control losses of essential molecules, such as water, and avoid the accumulation of toxic waste products, while maintaining respiration.

Determining the relative abilities to overcome these problems would indicate the vulnerability of *Neochanna* species to increasing drought severity. The response to drought may also be size-specific, as studies indicate that larger, adult fish may seek refuge before water recedes, and stranding may be more common in juvenile fish (Eldon et al. 1978; Eldon 1979c; McPhail 1999; O'Brien 2005). This section discusses the implications of water loss and the build up of metabolic waste during emersion and mechanisms to reduce this, as well as survival time and how this relates to fish size.

9.1 WATER LOSS

Neochanna species have a scaleless skin that is permeable to water, thus fish may suffer considerable loss of body water upon emersion. Meredith (1985) found that *N. burrowsius* were not able to restrict desiccatory water loss, as seen in amphibians. However, the mean rate of water turnover—and thus skin permeability—was not considered especially high. Nevertheless, the rate of water loss for emersed *N. burrowsius* was 2.4 times greater at 48% relative humidity than it was at 75% (Meredith 1985). At these two humidities, fish lost 10% of their initial body weight due to water loss in approximately 8 hours and 20 hours, respectively. Thus, without a mitigating mechanism, emersed *N. burrowsius* could be expected to dehydrate with lethal consequences within 48 hours (Meredith 1985). However, this estimated survival time is substantially less than that observed in the wild, so *Neochanna* species must rely on a combination of behavioural responses and habitat characteristics to avoid desiccation.

The large difference in water loss rates at different humidities highlights the importance of favourable microclimates/microhabitats in determining survival. This includes suitable conditions of ground moisture, a humid atmosphere, lack of strong air currents, cool temperature, and shielding from direct sunlight. A lack of any of these could lead to death through desiccation (Meredith 1985). However, terrestrial environments constantly fluctuate in their degree of dryness. Dehydrated *N. burrowsius* were able to regain 70% of lost body water through the skin from a damp surface within 6 hours (Meredith 1985). Thus, *Neochanna* species may survive brief periods of desiccation if regularly rehydrated from rain, dew or condensation. Further, subterranean refuges may be inundated periodically by ground water, which can fluctuate in level diurnally through expansion and contraction as temperature changes.

9.2 BUILD UP OF METABOLIC WASTE

Ammonia is the major nitrogenous excretory product in fish and is a highly toxic substance in alkaline conditions. However, fish can easily excrete ammonia into surrounding water, and it is only in the absence of free water that ammonia is likely to reach toxic levels in the blood. Meredith's (1985) studies of *N. burrowsius* showed that the accumulation of ammonia stabilised within 24 hours of emersion. Furthermore, levels of ammonia in the blood were low and relatively stable over 28 days of emersion. Meredith (1985) also found that in water, 80% of the nitrogen excreted by *N. burrowsius* was ammonia. However, the ammonia excretion rate measured when fish were emersed never achieved a level higher than 15% of the ammonia excretion rate in water. Thus, the remaining

85% was either excreted undetected, stored, or was not produced. If stored, the accumulated ammonia would likely kill a fish within one week if not suppressed or detoxified (Meredith 1985). Even if excreted, large accumulations of ammonia in the vicinity of a fish could be harmful to its skin surfaces unless they were protected by special mucoid compounds. However, the pH of *N. burrowsius* skin was close to neutral during emersion (Meredith 1985), which may indicate that its mucus has a buffering capacity.

Accumulated ammonia affects the acid-base status of blood and, hence, oxygen delivery. However, *Neochanna* may mitigate such acid-base disruptions through their high blood buffering capacity (Wells et al. 1984). In addition to ammonia, carbon dioxide (a by-product of respiration) levels rose in the blood of emersed *N. burrowsius*, further affecting the acid-base balance (Meredith 1985). Carbon dioxide accumulation leads to acidosis, whereas the accumulation of ammonia leads to alkalosis. Importantly, the time courses for build-up of carbon dioxide and ammonia were similar, thus their dual accumulation might have a neutralising effect, thereby reducing acid-base disruption (Meredith 1985).

9.3 MECHANISMS TO REDUCE TOXIC WASTE PRODUCTS

Although *Neochanna* species may be able to compensate for acid-base disruption, waste accumulation can still be detrimental. Thus, on re-immersion, *N. burrowsius* and *N. diversus* initially absorbed water, and then underwent a period of active waste excretion (Meredith 1985; McPhail 1999). As a mechanism for storing nitrogenous waste, McPhail (1999) suggested that *Neochanna* species convert ammonia into urea. Although Meredith (1985) found that 15% of nitrogenous waste in water was in the form of urea, he also found that after 8 hours of emersion, urea was not excreted by *N. burrowsius*, and after 48 hours urea had not accumulated. These results suggest that upon emersion, urea production by *N. burrowsius* was at least halved. Other methods *Neochanna* species may use for storing or detoxifying nitrogenous waste, such as amino acids, require further study.

Fish use protein as their primary source of energy for metabolism, rather than carbohydrate or lipid (as in mammals). Despite this, increases in metabolic rate can be supported by lipid metabolism. Ammonia is largely a product of protein-based metabolism; so, if fish can switch from protein to lipid or carbohydrate metabolism, the production of potentially toxic nitrogenous wastes could be suppressed. There is indirect evidence for such a change in the metabolic mechanism upon emersion. In immersed *N. burrowsius*, 72% of energy utilised for metabolism was estimated to come from protein. In emersed fish, however, only 9% was attributed to this source (Meredith 1985). Furthermore, fasting did not stimulate such a change in nitrogen excretion, which suggests that *N. burrowsius* switch their primary metabolic substrate only in response to emersion (Meredith 1985). *Neochanna burrowsius* is able to store appreciable amounts of lipids in body tissues and gonads, as shown by Eldon (1979c), who found that fat levels rose in spring and early summer. Although Meredith (1985) found no significant difference in the total lipid content of immersed, starved, and emersed *N. burrowsius*, Davidson (1999) found that triglyceride (a form of lipid) levels in the plasma of *N. diversus* were considerably lower in fish held out of water than in immersed captive and wild control fish,

suggesting the use of lipids for metabolism. Davidson (1999) also suggested that glycogen (carbohydrate) is sometimes utilised, as it is the primary energy source available to fish for metabolism when oxygen is limited. In *N. diversus*, glycogen was more concentrated in the liver than in the muscles, though emersion led to a reduction in glycogen concentration in both these tissues (Davidson 1999). Both carbohydrate and lipid metabolism could be viable solutions for limiting the production and accumulation of toxic levels of waste products during emersion.

9.4 SURVIVAL TIME

In drought situations, leaving the water may be advantageous, at least in the short term. Emerged fish have lower weight loss rates than fasted (starved) fish that remain in water (Fig. 12). This difference suggests that fish that remain in water likely expend energy in unsuccessful foraging, whereas emerged fish conserve energy, due to a low metabolic rate when resting. However, Meredith (1985) found that over an 85-day period, 40% of emerged fish died at irregular intervals between 28 and 85 days, yet there was no mortality of fasted immersed *N. burrowsius*. There are accounts of *Neochanna* being able to live without water for up to six months (Eldon 1979c; Close 1996). However, it is often difficult to determine whether fish survived in situ or recolonised habitat from an adjacent area that dried up to a lesser degree (Eldon et al. 1978). Laboratory studies have indicated that some *N. burrowsius* individuals can survive emersion for 85 days (Meredith 1985), *N. diversus* for 80 days (Davidson 1999), and *N. apoda* for 63 days in controlled conditions (Eldon 1978b).

Meredith (1985) suggested that *N. burrowsius* can tolerate weight losses exceeding 50% of initial body weight. Combining available data from three studies indicates that if fish are capable of tolerating a 50% reduction in weight, some may survive up to five months (Fig. 13). Most of the variation shown in Fig. 13 is due to the initial size of fish. However, this is an extrapolation and there is little evidence that any *Neochanna* species can survive more than 3 months of emersion. Over extended periods of emersion, fish would need to metabolise much of their body tissue and lipid stores (Eldon 1979c; Meredith 1985). Gonads appear to be significant lipid storage sites in *N. burrowsius* (Meredith 1985), but there have been no studies into the effects of emersion on subsequent reproductive output. This issue is especially important for *N. apoda* and *N. diversus*, which often spawn immediately after water returns in autumn. Not only do these species need to survive, they also need to maintain sufficient spawning condition and gonadal development. Thus, there may be further adverse consequences for individual fitness when fish are forced to endure drought for many months, even if they can survive (McDowall 1999b).

9.5 SURVIVAL AND SIZE

Neochanna may be required to utilise different survival strategies dependent on their size and the severity of drought conditions. Size influences energy reserves because there is a negative relationship between body size and metabolic rate. Smaller fish have higher metabolic rates, yet less body tissue to metabolise, which

will influence survival during periods of starvation (Meredith 1985). Furthermore, the surface area to volume ratio of fish controls efficiency of oxygen absorption through the skin, and the rate of desiccation. Thus, we would expect smaller fish to be more tolerant of immersed hypoxic conditions, whereas larger fish would be more likely to survive longer periods of emersion. Consequently, if juvenile fish have not grown sufficiently before summer drought commences, premature emersion may lead to desiccation or starvation (Eldon 1978b; McDowall 1999b). Hence, Meredith (1985) argued for the existence of an upper size limit for *Neochanna* species exposed to poorly oxygenated water, and a lower size limit for survival success during droughts.

These theoretically based conclusions have been verified by laboratory and field investigations. The combination of available data from three studies on *Neochanna* species shows a clear pattern between fish size and percentage weight lost per day (Fig. 14). Thus, fish size may be influential in determining maximum survival times during emersion. Additionally, smaller fish exhibit the greatest reduction in hepatic glycogen, an important energy source during starvation and emersion (Davidson 1999). McPhail (1999) also found significant differences in percentage weight loss between emersed adult (94–114 mm) and

Figure 13. Percentage weight loss over time for emersed fish. Combined data for various sizes of *N. burrowsius* (Eldon 1979c; Meredith 1985), *N. diversus* (Dean 1995; McPhail 1999), and *N. apoda* (Eldon 1978b). The regression line (\pm 95% CI) is based on all data.

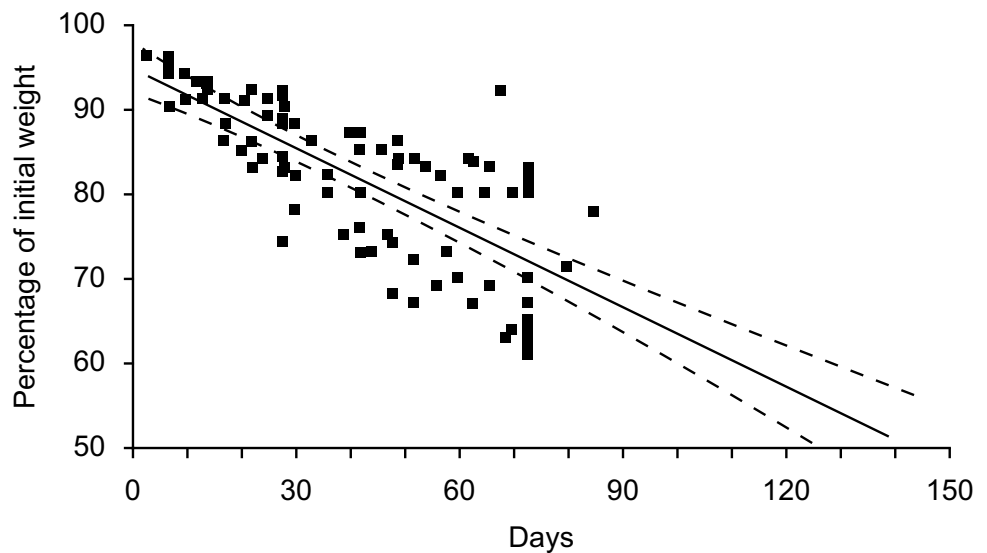
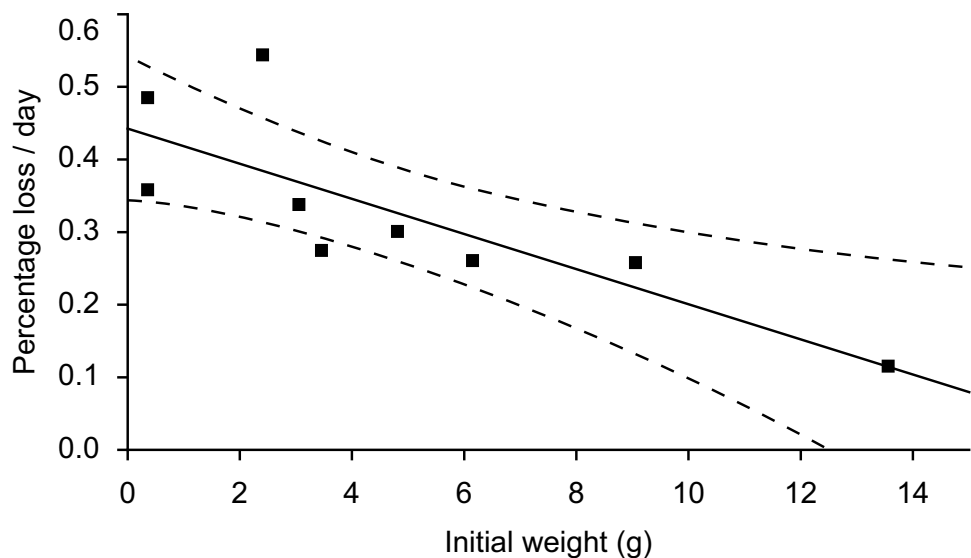


Figure 14. Relationship between fish size and weight loss during emersion. Combined data from *N. burrowsius* (Meredith 1985), *N. diversus* (Dean 1995), and *N. apoda* (Eldon 1978b). The regression line (\pm 95% CI) is based on all data.



juvenile (40–60 mm) *N. diversus*. Significantly, the two juvenile fish that died during McPhail's (1999) experiment were the smallest fish (40 and 45 mm) and had lost 40–45% of their initial body weight over 73 days.

From field investigations, McPhail (1999) suggested that a difference in the average size of young-of-the-year *N. diversus* before and after a drought was due to the smallest individuals suffering the greatest mortality. There is also evidence that larger *Neochanna* may leave hypoxic water before smaller juveniles, which remain immersed. For example, Eldon et al. (1978) did not find any emersed juvenile *N. burrowsius* during a drought, despite their previous abundance. However, large numbers of juvenile fish were observed in the remaining standing water. Such size-dependent strategies in response to a drought event were quantified for *N. burrowsius* by O'Brien (2007). *Neochanna burrowsius* found emersed were significantly longer than fish found swimming freely in an adjacent remnant pool. Furthermore, individuals that had left the water but had perished were significantly shorter than surviving fish. Thus, there is an upper limit to the size of fish remaining immersed, and a lower size limit related to survival of emersion. Generally, *N. burrowsius* > 50 mm long successfully adopted a strategy of emersion, whereas *N. burrowsius* < 50 mm long remained immersed (O'Brien 2007). Consequently, the severity of summer disturbance can influence the size structure of *Neochanna* populations.

9.6 SUMMARY

- *Neochanna* species cannot control water loss through their skin and thus require damp refuges to avoid desiccation.
- Initial studies have suggested that *Neochanna* species may be able to switch from protein to lipid or carbohydrate metabolism, thus using different metabolic pathways to avoid nitrogenous waste accumulation during emersion.
- Studies have indicated that some *Neochanna* individuals are capable of surviving three months of emersion in controlled conditions and extrapolation of weight loss measurements indicates that it would generally take them 5 months to lose 50% of their body weight.
- Fish size and the severity of drought conditions determine successful strategies for survival. Thus, there is an upper limit to the size of fish capable of remaining immersed in hypoxic waters, and a lower size limit related to the ability to survive long periods of emersion.

10. Threats

Two opposing constraints are generally considered to be the most important influences on *Neochanna* populations. These are environmental extremes, drought in particular, and negative inter-specific interactions (McDowall 1990; McDowall 2006). Both constraints can be related to the hydrological regimes of *Neochanna* habitats. In hydrologically stable and benign conditions, other fish species are more likely to be present, which may result in adverse interactions. Conversely, periods of summer habitat desiccation, which may initially eliminate other fish species, may become too severe, so that *Neochanna* also suffers mortality. While these two factors are likely to pose the most common threats to *Neochanna* species, analysis of 50 items of literature indicates that a multitude of threats have been identified by authors (Table 9). Despite the recognition of threats, few studies have quantified how they influence *Neochanna* species, the extent of the threats, potential for mitigation, and the relevant importance of each threat. Such studies are needed to clarify the implications of threats, and to ensure that appropriate actions are taken to protect remaining populations and habitat. This chapter reviews threats mentioned in the literature, with sections on habitat, hydrological modification, agricultural activities, competition and predation, and factors affecting fish health.

10.1 HABITAT

Wetland habitats of New Zealand mainland *Neochanna* species have been reduced, fragmented, and simplified by agricultural development and land modification. Much of this habitat change occurred in the late 1800s, and has been linked to the decline in *Neochanna* populations (McDowall 1980a, 1998a; McDowall & Eldon 1996). In some areas, changes in land use continue to threaten remaining populations. The threats identified range in severity from stock access, where impacts may be temporary (but which can degrade habitat over a longer period), to the complete removal of habitat (by land and water development, for example). Some habitat changes are irreversible, and processes such as fragmentation are likely to have long-lasting effects. Activities such as peat mining may pose a substantial threat through the removal of habitat (McDowall 1980a), while proposed hydroelectric power and irrigation developments have also been identified as having the potential to affect *Neochanna* habitats (Mitchell 1995 and Tipler et al. 2002, respectively). Fire is a potential threat to some *Neochanna* habitat (Eldon 1992; Grainger 2000), but may also lead to the development of new habitat, as in the rapid formation of peat lakes after fire on Chatham Island (Mitchell 1995). At present, the main threats to *Neochanna* habitat are hydrological modification and agricultural activities.

10.1.1 Hydrological modification

Activities on land surrounding wetlands, such as water abstraction for irrigation and drainage improvements that lower the water table, can have adverse effects on the hydrology of wetlands and springs (McDowall 1984). Moreover, most hydrological changes have long-term consequences that are largely irreversible,

TABLE 9. SUMMARY OF THREATS IDENTIFIED IN THE LITERATURE (50 SOURCES) AS BEING IMPORTANT IN INFLUENCING THE PERSISTENCE OF *Neobanna* SPECIES. VALUES INDICATE THE NUMBER OF TIMES THE PARTICULAR THREAT IS MENTIONED IN THE LITERATURE REVIEWED.

THREAT CATEGORY AND CLASS	<i>Neobanna</i> (IN GENERAL)	<i>N. rekobua</i>	<i>N. burrowsius</i>	<i>N. diversus</i>	<i>N. beletos</i>	<i>N. apoda</i>	TOTAL
Hydrology							
Continual wetland drainage	3		10	9	2	10	34
Severe drought	1		11	2		3	17
Hydrological change	1		8	3	2	2	16
Irrigation	2		11	1	1		15
Channel/flow modification			11	3		1	15
Flooding			3			1	4
Groundwater level fluctuations		1	1		1	3	
Piping of irrigation water		3				3	
Proposed hydro-power scheme		1					1
Sea level rise				1			1
Farming and land use							
Land intensification	1		11	5	3	3	23
Drain maintenance	1		6	1		8	16
Stock access			5	1	1	8	15
Deforestation		1	3			6	10
Agricultural sprays			2	3	1	2	8
Dairy farming	1		2	1	1	1	6
Landform modification		1	1	2		2	6
Fire/fire fighting	1	1		2	1		5
Cropping			2	1	1		4
Forestry				1	1	1	3
Domestic rubbish dumping					1		1
Peat mining				1			1
Subdivision of land				1			1
Population threats							
Fragmentation	2		10	5	3	8	28
Small population size	2		2	2	2	3	11
Restricted range	2	1		2	1		6
Genetic diversity	1		1	2		1	5
Lack of recruitment				2	1	1	4
Disease			2				2
Community influences							
Interactions with other fish	1	1	11	7		3	23
Excessive waterfowl			2			1	3
Terrestrial predators			1	1	1		3
Habitat quality							
Invasive plant species			6	1	1	2	10
Lack of aestivation habitat			2			4	6
Lack of food resources						1	1
Lack of spawning substrate			1				1
Physico-chemical properties of water							
Nutrient increases			2	1	1	2	6
Reduced water quality			1	1	1	1	4
Extreme temperatures			1		1		2
Low dissolved oxygen levels		2				2	
Acidification			1				1
Scientific knowledge							
Insufficient knowledge	2				1		3
Scientific collection	1		1		1		3
Total	22	6	135	63	29	77	332

e.g. the transformation of the Canterbury Plains from extensive wetlands to heavily irrigated farmland (McDowall 1998a). Hydrological change can have significant consequences for *Neochanna* species. For example, drainage and increased channelisation may lead to the loss of seasonally flooded wetlands and increased water velocity, which may limit suitable habitat and displace *Neochanna* into potentially unfavourable habitat (Kerr & McGlynn 2001; O'Brien 2005). Hydrological modification may also increase the frequency and intensity of droughts. In some situations, periodic drought may act to protect *Neochanna* populations (Ling 2001), because of the higher tolerance levels of *Neochanna* species compared with other fish species (Meredith 1985; Glova & Hulley 1998). However, drought is still a stressful situation for individuals and is likely to have consequences for growth, reproduction and condition. Thus, in situations where other fish species are neither present in *Neochanna* habitat, nor likely to invade it, modifications that increase drought events are likely to threaten the persistence of populations.

10.1.2 Agricultural activities

The main agricultural pressures on *Neochanna* species are water quality deterioration, damage of habitat by stock, and removal of habitat by drain maintenance. Despite agricultural activity being widely recognised as affecting *Neochanna* species, few studies have investigated the responses of *Neochanna* to such disturbances. However, it has been suggested (Eldon 1978b; McDowall 1990; Eldon 1993; Hicks & Barrier 1996; Young 1996) that *Neochanna* require relatively good water quality. Increases in nutrient levels through fertiliser and stock effluent run-off influence water quality and result in changes in the structure of aquatic communities. Further, drenches and biocides intended to kill internal parasites in farm livestock do not necessarily break down in the animals, and may pass through them into receiving environments, killing non-target microbes and changing ecosystem functioning (Tremblay & Wratten 2002).

The type of livestock with access to *Neochanna* habitat is also important, as smaller animals such as sheep are usually not heavy enough to seriously pug soils, except in extreme cases (Finlayson et al. 2002). Beef cattle and dairy cows represent the most serious threats, not only because of their weight and hoof morphology, but also because of their tendency to enter small wetlands and graze on aquatic plants (Johnson & Rodgers 2003; Reeves & Champion 2004). It has been suggested (Eldon 1993; Reeves & Champion 2004) that trampling by stock in drying wetlands during summer could 'rupture' burrows and refuges, leading to desiccation of emersed fish.

Neochanna species require a fairly complex or cluttered habitat. This complexity is usually provided by vegetation. Unfortunately, the presence of aquatic vegetation is not always compatible with current practices of drain management. Hudson & Harding (2004) reviewed current practices, and the development of *Neochanna*-friendly drain maintenance methods was identified by DOC (2003) as a priority. Drain maintenance can have a large and long-term impact on *Neochanna* populations, as indicated by Eldon (1968) who reported that a large *N. apoda* population took at least four years to recover after the initial clearance of aquatic plants. A population of *N. diversus* also took approximately three years to re-establish in the Holland's Road Drain (Hamilton) after severe mechanical clearance, despite it once having a remarkably high density (Barrier 1993; Hicks & Barrier 1996; Barrier et al. 1996).