

Effect of salinity on growth of juvenile Yarra pygmy perch (*Nannoperca obscura*: Percichthyidae)

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Abstract The threatened Yarra pygmy perch (*Nannoperca obscura*) is an obligate freshwater fish endemic to the south-eastern coast of mainland Australia, where a majority of river systems have been heavily modified since European settlement. The purpose of this garden study was to explore any relationship between the growth rate of juvenile *N. obscura* with respect to salinity. Trials were conducted encompassing perceived and potential ranges within the species' environment, through five salinity treatments and recorded growth after 8 weeks. Two brood-groups were trialed simultaneously to observe any intrapopulation variance in phenotypic response potentially linked to genetic variance. The salinity trials found a clear model indicating increasing growth for moderate salinities (i.e. 2.5–7.5 ppt), therefore identifying the ideal salinity range for *N. obscura* juveniles. Phenotypic response between brood-groups was varied and inconsistent, potentially reflecting low genetic variability. With a limited genetic variance restricting adaptability, tolerance to a changing environment would be reduced. Therefore increased salinization and subsequent flushing events within habitats may be key threats to the species' survival.

Keywords *Nannoperca obscura* · Pygmy perch · Salinity · Growth · Freshwater fish

Introduction

The Murray-Darling Basin (MDB)

Australia's Murray-Darling Basin (MDB) is a dryland river system in an arid/semi-arid temperate climate, exhibiting the characteristic highly variable [natural] and sometimes unpredictable flow regime (Unmack 2001; Morrongiello et al. 2011). The basin includes Australia's longest river system (south-central Queensland to South Australia), and drains roughly 1.073 million km². Forty-six known native fish species reside within the basin, with high endemism, and many species under threat (Lintermans 2007). A majority of the impact to native MDB ichthyofauna has been attributed to hydrological and land-use changes due to an increasing agricultural demand; influencing connectivity through weirs/levees, dams, alteration of floodplains and channelization (Walker et al. 1995; Balcombe et al. 2006).

The severely disrupted MDB has been subject to intense water abstraction for irrigation and civil use since European settlement (Arthington and Pusey 2003). The installation of tidal barrages and weirs near the mouth of the system (ca. 1940-50s) to prevent incursion of marine water resulting from upstream hydrological abstraction, modified the hydrology and ecology of extensive freshwater lakes known as the Lower Lakes (LL) and the Coorong estuary (Fluin et al. 2007;

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Wedderburn et al. 2012). Ever growing demands of water abstraction and continued river regulation significantly reduced freshwater inflow to the LL region of the system (Wedderburn et al. 2012). Hydrologic changes combined with extreme drought conditions during the ‘Millennium Drought’ (2001–2009), especially harsh from 2003 to 2009, accentuated these effects and saw many streams and water bodies cease their flow or completely dry out (Morrongiello et al. 2011; Wedderburn et al. 2012). The diminished water levels (reaching well below sea-level) also contributed to reduced water quality and disconnection of the Coorong from the LL when barrage flows were at zero for several years (Webster 2010; Kingsford et al. 2011). Thus a once predominantly freshwater system experienced salinization and cease-to-flow conditions beyond natural seasonal variation, leaving the MDB in critical condition especially in the LL region when stressors are cumulative. Conditions such as salinization, acidification, low dissolved oxygen levels or complete evaporation of habitat have generated an environment, which impairs the health, or directly kills, various native species (e.g. Hart et al. 1991). This is particularly damaging to species with small and/or isolated populations and habitat specialization, such as the Yarra pygmy perch (*Nannoperca obscura*, Klunzinger 1872).

Yarra pygmy perch (*Nannoperca obscura*)

The long tectonic isolation of Australia combined with a predominantly arid environment has provided a unique freshwater ichthyofauna with multiple endemic species across the southern regions (Unmack 2001). These highly isolated and varied habitats provide wide dispersion in discontinuous settings (biological hotspots) (Box et al. 2008) and emphasize how native ichthyofauna have adapted to localized environmental conditions.

The Yarra pygmy perch is a small (<80 mm) obligate freshwater percichthyid endemic to Australia’s temperate southeast including in the MDB (Hammer et al. 2010). This species is known to inhabit slow flowing and sheltered lowland streams, lakes and rivers; particularly in well-vegetated off-channel sites (Kuitert et al. 1996; Wedderburn and Hammer 2003; Wedderburn et al. 2012). Salinity at recorded sites is highly variable, but is typically low (<1.57 g L⁻¹) [<1.3 ppt] and sometimes moderate (≤7.0 ppt), especially during seasons or prolonged periods with low river flow (e.g. Zampatti

2001; Hammer 2002; Wedderburn and Hammer 2003). Yarra pygmy perch also has a low dispersal rate and relies on specific microhabitats; making it especially susceptible to the effects of reduced system health through the associated changes in water quality (e.g. salinization) and habitat connectivity (Hammer et al. 2010). The species can thus be considered an ‘ecological specialist’ according to Grinnellian specialization (reviewed by Devictor et al. 2010). *N. obscura* has been listed as ‘Vulnerable’ under both IUCN criteria and Australian federal legislation (EPBC Act 1999); with a fragmented distribution across the species’ remaining range (Saddler 1993). It faces extinction in several localities, being lost long ago from its type locality the Yarra River, and having recently undergone major declines and extirpation in the MDB (Hammer et al. 2010; Wedderburn et al. 2012; Saddler et al. 2013).

In response to the decline of Yarra pygmy perch in the wild, captive breeding programs have been implemented with the intention of restoring/restocking populations throughout the MDB (Hammer et al. 2013). The vulnerable status of the remaining populations is possibly accentuated by reduced genetic variance for the species (Carvalho et al. 2011; Brauer et al. 2013). Whilst contrasts in historic and contemporary levels of genetic variation are unknown, we do know that current levels of genetic variation found in *N. obscura* are amongst the lowest reported for a fish species (Brauer et al. 2013), so optimizing remaining genetic variation and potential to adapt to current and future environmental change is critical. This raises the difficulty of maintaining genetic variation in addition to increasing population numbers, and subsequently supporting the genetic integrity and continued viability of the species. Given current and future threats to flow regimes, how *N. obscura* responds to salinity needs to be addressed as this is potentially a primary environmental factor influencing the status of the species, and may be hereditary.

Influences on growth

When a species experiences a reduction in its genetic variance, the potential to adapt is reduced, restricting the range of tolerance (ability to survive) to environmental changes. Environmental variation can often lead to differential selection pressures, contributing

to the maintenance of intrapopulation variation (Robertson et al. 2011). Loss of this environmental variability (e.g. through river regulation or prolonged drought) can lead to increasing selection pressure towards a particular genotype, which is able to withstand the current environmental condition. However since no individual genotype can be superior to all conditions, the remaining population is threatened when the environment either exceeds the species' capacity to survive or changes considerably. Phenotypically this can be observed through reduced growth rate, low recruitment, or overall poor health (e.g. underweight, stunted growth). For many species an 'optimal' range can be identified, indicating what conditions the species thrives in beyond simply surviving. In fish (as in other species) this is typically expressed with strong growth rates (reviewed by Bœuf and Payan 2001). A majority of growth studies have focused on groups of commercial importance (e.g. salmon, trout, tilapia, carp and large perch). However for many species, which remain inconspicuous, there is little or no information of this nature available. If *N. obscura* restoration efforts are to be successful it is important to know their environmental requirements, particularly their 'optimal' range, to ensure survival and recruitment once released back into their natural environment.

Salinity has a major impact on the growth of the majority of freshwater species so far studied (reviewed by Bœuf and Payan 2001). A fish from the same river system, silver perch (*Bidyanus bidyanus*), demonstrates this, with growth rates highest at 4 ppt and decreasing with distance from this salinity (Kibria et al. 1999). Additionally, as the effect of salinity on growth may overlap with the effect of other environmental factors (e.g. temperature) and vice versa, it is important to clearly distinguish between those effects and that of salinity alone (Bœuf and Payan 2001).

The purpose of this study was to explore the relationship between the growth rate of juvenile *N. obscura* and salinity, and identify the optimal range to maximize growth. During this study, juveniles from two brood-groups were trialed simultaneously in an attempt to quantify intrapopulation variation in phenotypic response to salinity. The information provided by this study aims to increase the success rate of breeding programs and survival of *N. obscura* to re-establish wild populations and support remaining populations.

Materials and methods

Fish selection

The fish used in this study were captive reared first generation (F₁) offspring from *N. obscura* rescued from two different locations within the drying LL (Hammer et al. 2010). Brood-group founders (rescued individuals) were set-up to breed in groups with two males and three females, genetically characterized following Brauer et al. (2013) to maintain as much remaining variability as possible (and avoid inbreeding). Pairwise F_{st} comparisons were used to select the two brood-groups as the most divergent option available, albeit with a low F_{st} value (F_{st}=0.19438, $P < 0.001$) due to a limited natural gene pool and contemporary gene flow between locations within the LL (Brauer et al. 2013).

Specimens selected for use in the salinity trials were juveniles of 20 to 34 mm (standard length). These were big enough to handle without the risk of permanent damage, yet small enough that their growth rates would remain in the fast growing stage of a juvenile. A total of 300 individuals were selected; 150 from each of two brood-groups. Each brood-group was then separated into the five treatments (30 fish each), and each treatment was divided into three replicate tanks housing 10 individuals. This yielded a total of six replicates per treatment, divided between the two brood-groups with three replicates each.

All brood-groups were bred and raised within the same closed-circuit system, housed in separate but identical tanks prior to experimental treatment.

Experimental design

The salinity experiment trialed multiple low-level salinities based on suggested tolerance from field observations (Hammer 2002; Wedderburn and Hammer 2003; Bice and Ye 2006) and pilot observations. The tested salinity treatments were 0.3, 2.5, 5.0, 7.5 and 10.0 parts per thousand (ppt) running at 21 °C as suggested by the results of a pilot temperature trial based on environmental exposure. These conditions were maintained for each brood-group system for the duration of the 8-week trial.

System design

Twin systems were used to trial each variable for each brood group simultaneously, each with their own sump

for filtration and identical tank size and plumbing. Each system's total volume was 283 L; composed of a 49 L sump and three 78 L tanks each containing an inflow spout, an outflow box, an air-stone and an artificial plant mass in the center. Even flow ($\sim 4.3 \text{ L}\cdot\text{min}^{-1}$) through all replicate tanks provided adequate filtration and prevented the deterioration of water quality. The outflow box allowed water to leave the tank through a gravity feed and return to the sump, while a mesh covering prevented fish from being carried out. A pump located in the sump forced water through filter media (Kaldness K1) in the sump, up the inflow pipes and back into the tanks, circulating approximately $258 \text{ L}\cdot\text{h}^{-1}$.

The systems also received a series of regular, small volume water changes to assist in maintaining water quality through the removal of excess nutrients and the replacement of trace elements. This was carried out primarily through the sump, by allowing water to mix in the sump first and flow into the tank via the inflow with minimal direct impact to the fish. A built-in overflow in the sump allowed for excess water to drain away easily.

Water quality

During the course of the trial, daily water parameter measurements were conducted on one tank per system, rotating between tanks and focusing on salinity (ppt), temperature ($^{\circ}\text{C}$), pH, and dissolved oxygen (DO; % saturation). This was supplemented with weekly nutrient tests monitoring the total ammonia ($\text{NH}_3/\text{NH}_4^+$) and nitrite (NO_2^-) to ensure that levels remained negligible to fish health. A handheld field meter (YSI 63) was used for the daily temperature, pH and salinity measurements, while a portable DO meter (OxyGuard[®] Handy Polaris 2) measured the amount of DO in the water. Nutrient testing was carried out with AquaspeX[®] reagents (liquid and powder), and measured with a WinLab[®] Photometer LF2400.

Fish maintenance

Fish feeding was conducted on a daily basis, providing a combination of frozen and live foods. Frozen food was thawed in a container of water and a 1-mL pipette used to distribute multiple small portions to each tank, allowing time for consumption between servings. This was done to reduce waste and avoid fouling the water by providing little more than the fish could consume within a few minutes. The frozen food was often fed in a mix containing

any combination of bloodworms (Chironomidae), daphnia, brine shrimp (*Artemia* sp.) and freeze-dried cyclops (Cyclopidae). The bloodworms made up the highest ratio of the frozen feed, followed by the freeze-dried cyclops, which was added to the thawing mixture and allowed to soak in the contents. In addition to daily feedings, live mixed zooplankton, mostly daphnia, were provided weekly and were observed being preyed upon by each group of fish. Blackworms (*Lumbriculus variegatus*) were constantly present along the bottom of each tank, and regularly supplemented with fresh individuals to keep a continuous food supply available for foraging.

Daily visual inspections of fish health were used in conjunction with the aforementioned water testing to ensure that no signs of stress or disease were present. Care was taken to avoid external stressors from loud sounds, sudden movements within the room or unnecessary physical contact with the tanks. After weighing and measuring sessions, a mild dose ($5 \text{ mL } 283 \text{ L}^{-1}$) of a general antibacterial (Melafix[®]) was added to the system as a preventative measure. The room's lighting system was set to represent an average early summer day/night cycle of 12 h/12 h.

Weight and length measurements

All specimens of *N. obscura* were weighed and measured at both the start and finish of the trial. Tank groupings were removed categorically to maintain separation between replicates during measuring sessions. Fish length was measured to the nearest mm with a ruler using the standard length (SL), from tip of the jaw to the posterior of the hypural bone (Anderson and Gutreuter 1983). Weight was measured using a balance top-pan scale (Ohaus AdventurerTM) at 0.01 g increments. A small container of water was placed on the scale surface and tared. Each fish was added and weighed individually with the balance re-tared between specimens.

Calculations and statistical analysis

The initial set of individual measurements from each replicate group were averaged per tank, as individuals could not be matched up to their starting measurements, thus growth was calculated as a mean for each replicate tank. This resulted in three replicate means for each brood-group per treatment.

Absolute Growth (AG) was calculated as the difference between the initial and final measurements (Eq. 1)

using the initial mean (m_i) and final mean (m_f). Specific Growth Rate (SGR) (Eq. 2) was calculated to determine daily growth rate and assessed weight and length separately. Fulton's Condition Factor (FCF) was calculated (Eq. 3) to measure the overall health [bulk] of the fish (Cone 1989; Springer and Murphy 1990).

A two-way ANOVA was run for results analysis (IBM® SPSS® Statistics 21.0). When assumptions of ANOVA were not met (normality and Levene's test), non-parametric testing (Kruskal-Wallis test for independent samples) was used to determine significance of survival data. Homogeneity was tested for using Tukey in a post hoc test when applicable. An α level of 0.05 was used for all analyses.

A second order regression was run using all individual measurements from each salinity treatment and plotted with the replicate means of each treatment. The effective concentrations (EC) were calculated by determining the value of 'x' from the highest 'y' value. The EC90 and EC80 ranges represent where 90 and 80 % of maximum modelled growth has occurred respectively, or alternatively where growth has declined by 10 and 20 % from the maximum modeled values.

Absolute Growth (AG) for determining the total amount of growth for weight and length of each replicate group

$$AG = mf - mi \tag{1}$$

Specific Growth Rate (SGR) for comparing weight and length growth differences respectively between replicate groups

$$\left(\frac{\text{Log}N(mf) - \text{Log}N(mi)}{\text{days}} \right) \times 100 \tag{2}$$

Fulton's Condition Factor (FCF) for determining overall fish condition, taking into account the ratio of weight and length

$$K = 100 \left(\frac{W}{L^3} \right) \tag{3}$$

Results

Starting and finishing size

The *N. obscura* juveniles at the start of treatment ranged in replicate mean weight from 0.46 to 0.53 g, with a overall mean initial weight of 0.48 ± 0.15 g (mean \pm SD,

$n=300$). The initial replicate mean length was between 26.5 and 27.3 mm, with an overall mean initial length of 27 ± 2.9 mm (mean \pm SD, $n=300$). A significant difference was present between brood-groups for initial weight ($P=0.004$) and initial length ($P=0.034$), with brood-group B being on average both heavier and longer than brood-group A (Table 1).

The final replicate mean weight, after 8 weeks of exposure for each salinity treatment ranged from 0.77 to 1.05 g, with an overall mean final weight of 0.89 ± 0.024 g (mean \pm SD, $n=284$). The final replicate mean length ranged from 31 to 35 mm, with an overall mean final length of 33 ± 2.9 mm (mean \pm SD, $n=284$). A significant response was seen between brood-groups in both weight ($P=0.020$) and length ($P=0.001$), with brood-group B remaining heavier on average (Table 1). A significant response was also seen between salinities for both weight ($P<0.001$) and length ($P<0.001$).

Survival

There was no significant effect on survival, as the distribution across salinity treatments was the same across brood-groups ($P=0.545$), with a mean survival rate of 95 %.

Absolute growth

All *N. obscura* juveniles demonstrated positive AG for both weight (Fig. 1) and length (Fig. 2) after 8 weeks of treatment. Mean overall AGW was 0.41 ± 0.019 g and AGL was 5.9 ± 0.24 mm (mean \pm SE, $n=30$). No significant difference was seen in the response of brood-group for weight ($P=0.683$) or length ($P=0.102$), so data for brood-group was pooled, however a strong significant effect was detected for salinity ($P<0.001$). No interactive effect was detected between brood-group and salin-

Table 1 Mean initial ($n=300$) and final ($n=284$) sizes and of *Nannoperca obscura* in response to salinity

	Weight (g)		Length (mm)		Survival (%)
	Initial	Final	Initial	Final	
Brood-Group A	0.48 ^a	0.90 ^b	26.8 ^c	32.8 ^d	94.1
Brood-Group B	0.49 ^a	0.91 ^b	26.9 ^c	33.1 ^d	95.2

Means and survival are provided for each brood-group, with a significant difference between brood-groups for each corresponding set of measurements

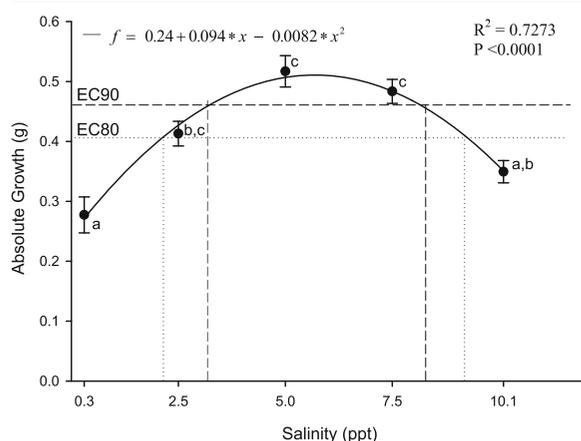


Fig. 1 Absolute Growth weight (AGW) of *Nannoperca obscura* in response to salinity. Means (\pm SE) ($n=6$) for AGW of all five salinity treatments after 8 weeks of exposure overlay a curve from secondary regression (\pm SE) ($n=30$). EC90 and 80 represent modeled declines of 10 and 20 % from the peak modeled AGW at 5.3 ppt. Significant difference between treatments is displayed in superscript at each point

ity for either AGW ($P=0.611$) or AGL ($P=0.810$).

Significant differences in AG were observed for both weight ($P<0.001$) and length ($P<0.001$) in response to salinity. The AGW showed a significant difference between the lowest salinity (0.3 ppt) and the three mid-range salinities of 2.5 ppt ($P=0.008$), 5.0 ppt ($P<0.001$), and 7.5 ppt ($P<0.001$); but not for the highest salinity of 10.1 ppt ($P=0.289$). Absolute growth for length also showed a significant difference between the lowest

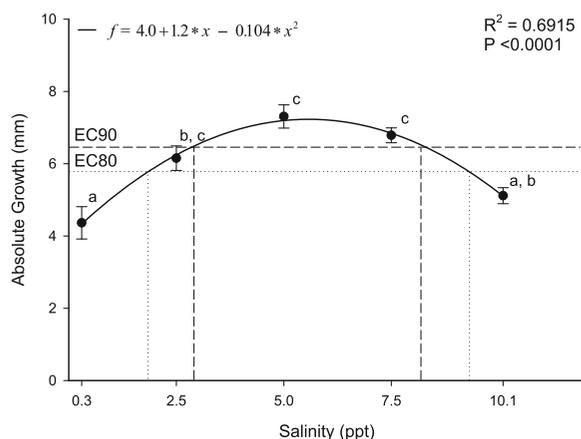


Fig. 2 Absolute Growth length (AGL) of *Nannoperca obscura* in response to salinity. Means (\pm SE) ($n=6$) for AGL of all five salinity treatments after 8 weeks of exposure overlay a curve from secondary regression (\pm SE) ($n=30$). EC90 and 80 represent modeled declines of 10 and 20 % from the peak modeled AGL at 5.6 ppt. Significant difference between treatments is displayed in superscript at each point

salinity (0.3 ppt) and the three mid-range salinities 2.5 ppt ($P=0.007$), 5.0 ppt ($P<0.001$), and 7.5 ppt ($P<0.001$); but not for the highest salinity 10.1 ppt ($P=0.484$). A significant difference was also present between the highest salinity (10.1 ppt) and two of the mid-range salinities; 5.0 ppt ($P=0.001$) and 7.5 ppt ($P=0.008$) for AGW, and 5.0 ppt ($P=0.001$) and 7.5 ppt ($P=0.012$) for AGL. There was no significant difference within the three mid-range salinities for either AGW or AGL.

Maximum modeled AGW occurred at a salinity of 5.3 ppt, with an EC90 range between 3.2 and 8.2 ppt, and EC80 between 2.2 and 9.3 ppt. Maximum modeled AGL occurred at 5.6 ppt, with an EC90 range between 2.9 and 8.2 ppt, and EC80 between 1.8 and 9.3 ppt.

Specific growth rate

N. obscura juveniles demonstrated positive SGRs for both weight (Fig. 3) and length (Fig. 4) at all salinity treatments. Mean overall SGRW was 1.1 ± 0.04 % daily and SGRL was 0.36 ± 0.014 % daily (mean \pm SE, $n=30$). No significant difference was observed in the response of brood-group for either weight ($P=0.171$) or length ($P=0.383$) so data for brood-group were pooled. A strongly significant effect was detected for salinity for weight ($P<0.001$) and length ($P<0.001$). No interactive effect was detected between brood-group and salinity for either SGRW ($P=0.267$) or SGRL ($P=0.771$).

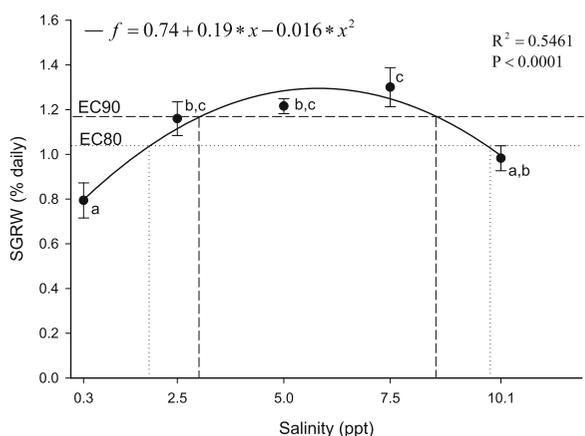


Fig. 3 Specific Growth Rate weight (SGRW) for *Nannoperca obscura* in response to salinity. Means (\pm SE) ($n=6$) for SGRW at all five salinity treatments after 8 weeks of exposure overlay a curve from secondary regression ($n=30$). EC90 and 80 represent modeled declines of 10 and 20 % from the peak modeled SGRW at 5.9 ppt. Significant difference between treatments is displayed in superscript at each point

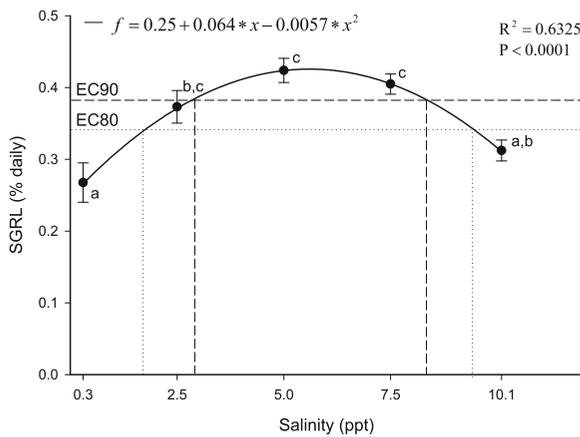


Fig. 4 Specific Growth Rate length (SGRL) for *Nannoperca obscura* in response to salinity. Means (\pm SE) ($n=6$) for SGRL at all five salinity treatments after 8 weeks of exposure overlay a curve from secondary regression ($n=30$). EC90 and 80 represent modeled declines of 10 and 20 % from the peak modeled SGRL at 5.7 ppt. Significant difference between treatments is displayed in superscript at each point

Length and weight showed a significant difference (respectively) between the lowest salinity (0.3 ppt) and the three mid-range salinities 2.5 ppt ($P=0.007$; $P=0.008$), 5.0 ppt ($P<0.001$; $P=0.002$) and 7.5 ppt ($P<0.001$; $P<0.001$). A significant difference was also present between the highest salinity (10.1 ppt) and two of the mid-range salinities 5.0 ppt ($P=0.005$) and 7.5 ppt ($P=0.022$) for length, and for weight with the salinity 7.5 ppt ($P=0.024$). There was no significant difference within the three mid-range salinities for either SGRW or SGRL.

Maximum modeled SGRW occurred at a salinity of 5.9 ppt, with an EC90 range between 3.0 and 8.6 ppt, and EC80 between 1.8 and 9.8 ppt (Fig. 3). Maximum modeled SGRL occurred at a salinity of 5.7 ppt, with an EC90 range between 2.9 and 8.3 ppt, and EC80 between 1.7 and 9.4 ppt (Fig. 4).

Fulton’s condition factor

Juvenile *N. obscura* final FCF values for all salinity treatments yielded a significant difference in response between brood-groups ($P=0.001$); however not for salinity ($P=0.672$). No interactive effect was detected between brood-group and salinity ($P=0.276$).

Initial mean FCF for brood-group A was 2.39 ± 0.385 and final mean was 2.53 ± 0.267 (mean \pm SD, $n_i=150$, $n_f=144$). Initial mean FCF for brood-group B was 2.48 ± 0.292 and final mean was 2.43 ± 0.203 (mean \pm SD, $n_i=$

150, $n_f=140$). Brood-group A initially had a lower condition value, however it experienced a +4.8 % change in final condition. While brood-group B initially had a greater condition value, it experienced a -2.5 % change in final condition.

Discussion

Response of *N. obscura* to salinity

The positive SGRs for weight and length exhibited by *N. obscura* juveniles in response to the salinity treatments were significant, with a distinct pattern evident. The optimal range for highest SGRW was modeled at EC90 3.0–8.6 ppt, and EC80 1.8–9.8 ppt. The optimal range for highest SGRL was modeled at EC90 2.8–8.3 ppt, and EC80 1.7–9.4 ppt. These results indicate that the Yarra pygmy perch is adapted to living in coastal freshwater environments with some degree of salinity, and that neither very fresh nor high salinity water is preferable for this species. This is not unusual as many obligate freshwater species such as silver perch (*B. bidyanus*) (Kibria et al. 1999), channel catfish (*Ictalurus punctatus*) (Burnside et al. 1975), silver carp (*Hypophthalmichthys molitrix*) (v. Oertzen 1985) and common carp (*Cyprinus carpio*) (Lam and Sharma 1985) have shown superior growth performances within a similar salinity range. The threat from river regulation is therefore still real for this and other MDB species, as this often leads to periods of increasing salinity and habitat disconnection/loss, followed by surges of freshwater which are often induced for agricultural purposes.

Effect of brood-group

No significant difference was seen between brood-groups of *N. obscura* in the positive SGRs for weight and length, in response to the salinity treatments. Although this work was conducted on fish as genetically differentiated as possible from within the LL study system by carefully selecting combinations from two different locations, these sites were in the recent past part of a broader panmictic population with the ultimate genetic divergence between the two brood groups unavoidably low (Brauer et al. 2013). Furthermore, population genetic variance of the species was well below average for a freshwater fish (DeWoody and Avise 2000; Brauer et al. 2013). Both genetic factors could

help explain the nearly identical response from the brood-groups, and lack of observable interaction between genotype and phenotypic response to the environment (or at least a lack of genetic variation relating to salinity tolerance).

Nevertheless, FCF showed significant differences between brood-groups. The degree of difference was on the order of 4 %, so it showed that despite the alterations in growth rate caused by exposure to different salinities, phenotypic differences in the two groups were evident. This may have implications for fitness in an ecological context, for when food is more scarce, as those groups prone to higher FCF should be more resilient (e.g. Altinok and Grizzle 2001). It has been observed in some species that distinct populations display opposing growth responses to salinity, which are attributed to genetic differences (e.g., Morgan and Iwama 1991; Overton et al. 2008). This should be explored further and considered in more detail when considering the suitability of species, and their sub-groups, for reintroduction programs.

Finding some difference, or at least some variability, between animals of different brood-groups, in terms of salinity, indicates that this species has sufficient variation to withstand a variety of environmental conditions. Future work should include specimens originating from multiple localities/lineages within the species' range (Brauer et al. 2013), noting that the species is now presumed extinct in the wild in the MDB.

Previous studies

Previous studies appear to focus on economically important species, with little or no interest in other, non-economically important groups such as small-bodied freshwater fishes (e.g. Bailey and Loudenslager 1986; Vøllestad and Quinn 2003; Robertsen et al. 2011). Investigations into whether differing genetic lineages [brood-groups] *within* a population display different growth responses to selected *environmental* factors have been generally lacking as far as is known. It has been generally accepted nevertheless, that growth rate in fish is highly variable with a large genetic component (Gjedrem 1983; Gjerde 1986; Altinok and Grizzle 2001; Vøllestad and Quinn 2003). Robertsen et al. (2011) implied that environmental conditions may influence relative growth performance among/between family

lines, however genetic traits beneficial in one particular environment may be less beneficial or possibly cost-incurring in another [per lineage]. Further investigation across a broader geographic scale is required to confirm this, especially considering the presence of four deep genetic lineages or evolutionarily significant units in discrete parts of the species' geographic range (Hammer et al. 2010; Brauer et al. 2013).

Practical applications and implications for the species

By determining what environmental factors are most suitable for raising healthy and fast growing Yarra pygmy perch juveniles into adults, the success of captive breeding programs can be enhanced. Salinity is an important factor contributing to *N. obscura*'s growth, and is easily manipulated in captivity. Additionally, it is important to know the ideal conditions for the species if the intent is to restore populations in the wild. If the juveniles are released into unsuitable water conditions, then few will survive and genetic variability will be further reduced, decreasing the chances of recruitment and restoring populations to be self-sufficient.

The history and current condition of the MDB has left *N. obscura* vulnerable through habitat loss and resource competition from generalist and/or alien species (Wager and Jackson 1993; Hammer et al. 2010, 2013; Unmack et al. 2011; Leblanc et al. 2012). Fragmented populations are believed to be confined to a narrow band inland along the coast in a separate drainage division (Saddler et al. 2013). Having a relatively low genetic variability (Hammer et al. 2010; Brauer et al. 2013), the species is at added risk from changing environmental conditions and anthropogenic influences. The low levels of genetic variation found in this species increases the potential for inbreeding and may result in decreased fitness of the population (Schwartz and May 2008). This could lead to the extinction of wild populations of the species, with fragmented populations suffering from habitat loss/disconnection and competition from more 'generalist' species.

Conclusion

The purpose of this study was to explore the relationship between the growth rate of juvenile *N. obscura* and

salinity. The experiment produced positive SGRs for weight and length for the juveniles. A clear model successfully demonstrated the effect of salinity on growth rates, identifying an optimal range for maximum growth. This highlights the subsequent threat the species faces from land-use change and altered hydrology that may result in elevated salinity levels, and indeed that have been witnessed in the Lower Murray in recent times (Wedderburn et al. 2012). Simultaneously, the present study has provided important information regarding what salinities are suitable for the species, which will aid captive breeding efforts to increase the success rate of re-establishing wild *N. obscura* populations in the wild. A larger experimental gene pool is required to further determine ecological exchangeability across major lineages (ESUs) and if any phenotypic response or linked genetic variance is present within the species, particularly between populations.

Further work is required in order to ensure that *N. obscura* populations are able to survive and are restored to self-sustaining levels. Of particular importance is a greater understanding of the species' population dynamics and geographic distribution, as well as phenotypic response(s) to various environmental factors with respect to genetic diversity. However restoring the MDB's environments is also essential, as efforts aimed at restoring native species such as the Yarra pygmy perch may be of little impact if environmental needs are not met. Without intervention, the continued survival of wild Yarra pygmy perch populations will be severely threatened.

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