Structural habitat selection by Southern pygmy perch (*Nannoperca australis)*

Prepared by: Amina Price, Rick Stoffels, Kyle Weatherman, Rebekah O'Keefe, Warren Müller

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Final Report prepared for Murray Local Land Services by The Murray–Darling Freshwater Research Centre.

Murray Local Land Services
PO Box 797
Albury NSW 2640

Ph: (02) 6051 2200; Fax: (02) 6051 2222

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For further information contact:

Amina Price

The Murray–Darling Freshwater Research Centre
PO Box 821
Wodonga VIC 3689
Ph: (02) 6024 9650

Email: Amina.Price@latrobe.edu.au
Web: www.mdfrc.org.au
Enquiries: mdfrc@latrobe.edu.au

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Cover Image: Southern pygmy perch utilising artificial macrophyte habitat

Photographer: Kyle Weatherman

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The Murray–Darling Freshwater Research Centre offices are located on the land of the Latje Latje and Wiradjuri peoples. We undertake work throughout the Murray–Darling Basin and acknowledge the traditional owners of this land and water. We pay respect to Elders past, present and future.

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Executive summary

The **objectives** of the present work were to determine whether:

- Southern pygmy perch (*Nannoperca australis*) preferentially select structured habitats over open ones and if so, whether they show a preference for macrophyte habitats over woody habitats
- observed patterns of habitat selection vary when a predator (Redfin perch - *Perca fluviatilis*) is visible
- the activity levels/metabolic rate of Southern pygmy perch differs in the presence or absence of structure (artificial macrophytes)
- observed patterns in activity levels/metabolic rate among habitats vary when a predator (Redfin perch) is visible.

The **anticipated outcomes** of this project were to provide managers with an improved ability to:

- manage habitat in order to better support existing populations
- identify suitable translocation sites based on structural habitat availability.

Habitat selection:

- Southern pygmy perch demonstrated a statistically significant preference for structured habitats (macrophytes and wood) as compared to unstructured habitats.
- Macrophyte habitat patches were selected by a significantly greater number of Southern pygmy perch than woody habitat patches.
- Habitat selection was not influenced by the presence of Redfin perch.

Metabolic response:

- Metabolic rates were significantly higher when macrophytes were present than when they were absent.
- Metabolic rates were not influenced by the presence of Redfin perch.

Implications for management of existing Southern pygmy perch habitats and the selection of translocation sites:

- The results from this project strongly suggest that whilst Southern pygmy perch may use non-macrophyte habitats such as those containing wood, macrophytes represent their preferred habitat type. Consequently, management of existing Southern pygmy perch sites should focus on maintaining or creating significant areas of aquatic vegetation. Similarly, in the event that Southern pygmy perch are translocated to new sites, site selection should be strongly based on the presence of aquatic vegetation, as well as other habitat features such as low velocities and relatively shallow depths.
    - *Knowledge need*: There is a need to determine whether macrophyte type, architecture and density, location within the water body (e.g. fringing) and total area of macrophyte cover influence habitat suitability for Southern pygmy perch.
- Contrary to our predictions, the metabolic rates of Southern pygmy perch were higher when macrophytes were present than in open habitat. Given that the explanation for this is unlikely to be related to elevated stress levels, these results are indicative of reduced routine movement when macrophytes are absent. This suggests that processes essential for survival and growth (e.g. foraging movements) as well as reproduction may be negatively impacted if structured habitat is not available.
    - *Knowledge need*: Whilst we have established the importance of structured habitat, particularly macrophytes, as important habitat for Southern pygmy perch, it is not

    clear what the implications of little or no macrophytes are for the species. For example, how does the presence or absence of macrophytes affect foraging rates, reproductive output and success? What are the implications for growth and carrying capacity for this species?

- Southern pygmy perch are highly susceptible to predation by introduced species that also utilise macrophyte habitats (e.g. Redfin perch). This is based on the fact that habitat selection and metabolic rates did not vary in the presence or absence of Redfin perch. We suggest that these results are a consequence of:

1. the Southern pygmy perch utilised in this study, which were collected from a system where Redfin perch are absent, not recognising Redfin perch as a potential predation threat, and
2. the preference for macrophyte habitats being driven, at least in part by longer-term predatory pressures from native species which are more strongly associated with snags and woody debris than aquatic vegetation. This suggests that Thus, Southern pygmy perch may be highly vulnerable to predators such as Redfin perch, which utilise aquatic vegetation.

Consequently, management of existing Southern pygmy perch sites should aim to exclude predators that utilise macrophyte habitats, particularly introduced species, and translocation sites with these types of predators present should be avoided unless the predators can be eradicated prior to translocation.

    - *Knowledge need:* In order to determine whether Southern pygmy perch can co-exist with key predators such as Redfin perch, increased knowledge is needed regarding (1) the effectiveness of macrophytes as refuge habitat for Southern pygmy perch against predators which also utilise these habitats, and (2) the ability of Southern pygmy perch to quickly learn avoidance behaviours with respect to predators that they are not familiar with.

1 Introduction

1.1 Background

The Southern pygmy perch (*Nannoperca australis*) is a small-bodied fish species that historically was widely distributed throughout the Murray and lower Murrumbidgee systems, as well as coastal Victoria and northern Tasmania (Hammer 2002; Lintermans 2007). The species has experienced dramatic declines in range and abundance and is now listed as endangered in NSW and South Australia (Lintermans 2007). In NSW, there are only three known populations, one in the Lachlan Catchment and two in the Murray Catchment near Holbrook and Albury (Lintermans 2007). Currently, management of the two Murray populations is focused on maintaining the limited number of existing populations with a view to establishing new populations via translocations to suitable habitats in the future. Southern pygmy perch appear to have very limited dispersal (Cook *et al.* 2007; Dexter *et al.* 2014; Stoffels & Weatherman 2016), but locally, can breed up to good numbers quickly if good quality habitat is available (Hammer 2002). Consequently, management actions are most likely to be successful if they are focused on local-scale habitat maintenance/improvement and threat abatement. Therefore, an understanding of the critical habitat requirements of Southern pygmy perch is vital.

Southern pygmy perch are most commonly described as being associated with relatively shallow, low velocity habitats with dense cover (Hammer 2009; Humphries 1995; Kuiter *et al.* 1996). Whilst some authors have noted that Southern pygmy perch can occur in association with woody debris or rocks (Bond & Lake 2003; Hammer 2009), the majority of studies suggest that Southern pygmy perch occur almost exclusively in aquatic vegetation (Humphries 1995; Kuiter *et al.* 1996; Lintermans 2007; Woodward & Malone 2002). This may be submerged aquatic vegetation (Hammer 2009; Humphries 1995) or emergent and overhanging edge vegetation (Hammer 2009). Newly inundated edge vegetation has been proposed as being particularly important as larval and juvenile habitat (Hammer 2009; King *et al.* 2009) and as refuge habitat for all life-stages during high flows (Hammer 2002).

Aquatic and fringing vegetation is thought to function as shelter from high flows and also as spawning habitat for Southern pygmy perch (Hammer 2009; Tonkin *et al.* 2008). However, on a day-to-day basis, aquatic and fringing vegetation are thought to provide both a refuge from predation by piscivorous fish such as Redfin perch (*Perca fluviatilis*), and foraging habitat due to their increased invertebrate densities (Closs *et al.* 2005; Hammer 2002; Humphries 1995; Kuiter *et al.* 1996; Warfe & Barmuta 2004).

Whilst it is generally thought that aquatic macrophytes are a critical habitat feature for Southern pygmy perch (Hammer 2002; Humphries 1995; King *et al.* 2009), robust scientific evidence for this is lacking and there is anecdotal evidence of self-sustaining Southern pygmy perch populations occurring in habitats with little or no macrophytes. It is not currently known whether Southern pygmy perch are associated specifically with macrophytes, or whether other structural habitat types, such as snags, can fulfil the same functional requirements.

Other types of instream structure such as snags, or woody debris can also play an important role in providing structure and habitat complexity in freshwater systems (Crook & Robertson 1999; Matthews 1998). Snag cover influences the quality of food and habitat resources by contributing to overall habitat complexity, and like aquatic vegetation, it is believed to play an important role in providing refuge from high current velocities, as spawning sites for a number of species and as camouflage from predators or prey (Crook & Robertson 1999; Matthews 1998). For a number of species, snags also provide excellent foraging substrates, since high densities of macroinvertebrates and algae are often associated with snags (Crook & Robertson 1999). It is not known whether microinvertebrates also occur in higher densities around snags.

Consequently, it would seem that snag habitat may have the capacity to offer similar benefits to Southern pygmy perch as aquatic and fringing vegetation, however, there is little documented evidence of Southern pygmy perch being strongly associated with coarse or fine woody debris.

Thus, a major knowledge gap in our knowledge of the habitat requirements of Southern pygmy perch is a clear understanding of the circumstances under which macrophytes or other structural habitat types are used as habitat and whether this represents a true habitat requirement. Given that this is currently a key habitat factor which is likely to be considered in the management of current and future pygmy perch populations, having a clear understanding of the functional role of structural habitat is important.

1.2 Project objectives and outcomes

The objectives of the present project were to determine whether:

1. *Southern pygmy perch preferentially select structured habitats over open ones and if so, to assess whether they show a preference for macrophyte habitats over woody habitats.*
   Based on current knowledge of the species, we predicted that structured habitats would be preferentially selected over open habitats.
2. *Observed patterns of habitat selection vary when a predator (Redfin perch) is visible.*
   We predicted that Southern pygmy perch would show a stronger preference for structured habitats, in particular macrophyte habitats, which are likely to provide greater refuge from predators, when Redfin perch were present.
3. *The activity levels/metabolic rates of Southern pygmy perch differ when the fish are in structured versus open habitats.*
   The measurement of metabolic rates using respirometry techniques is a useful approach to determining how a fish is responding to environmental conditions in relation to its activity levels and degree of stress (Cech Jr. 1990). Respirometry is used to measure oxygen consumption rates of the fish and this, in turn, determines the metabolic rates. High metabolic rates are indicative of high levels of stress or activity (Cech Jr. 1990). We predicted that metabolic rates would be higher due to increased levels of stress when no structure was present.
4. *Observed patterns in activity levels/metabolic rate among habitats vary when a predator (Redfin perch) is visible.*
   We predicted that metabolic rates would be higher due to increased levels of stress when a Redfin perch was present.

The scientific knowledge delivered by this project will give managers an improved ability to:

- manage habitat in order to better support existing populations, and
- identify suitable translocation sites based on structural habitat availability.

2 Materials and methods

2.1 Species description

2.1.1 Southern pygmy perch

The Southern pygmy perch is a short-lived species that inhabits a range of still or slow-flowing waters in streams, lakes and wetlands (Kuiter *et al.* 1996; Lintermans 2007). Southern pygmy perch usually spawn between September and January, producing non-adhesive demersal eggs, which are scattered over aquatic vegetation or the benthos (Humphries 1995; Kuiter *et al.* 1996; Lintermans 2007). Early-stage larvae are poorly developed and are associated with the benthos (Morrongiello *et al.* 2012).

Southern pygmy perch consume epibenthic and epiphytic invertebrates that include small crustaceans such as amphipods, cladocerans, ostracods and copepods as well as aquatic insect larvae (e.g. chironomid, mayfly, hemipteran and mosquito larvae) (Humphries 1995; Kuiter *et al.* 1996; Woodward & Malone 2002).The diet of smaller individuals tends to be dominated by microcrustaceans, whereas larger individuals consume larger prey such as macroinvertebrates (Humphries 1995).

2.1.2 Redfin perch

Redfin perch are native to Europe and were first introduced to Australia in the 1860s (McDowall 1996). This species is now widely distributed throughout temperate areas within the Murray–Darling Basin (Lintermans 2007). Redfin perch grow to a maximum length of ca. 60 cm, and utilise a variety of habitats, showing a preference for still and slow-flowing waters with instream structure such as abundant aquatic vegetation or snags (Lintermans 2007; McDowall 1996). These habitats are particularly important during the spawning period (late winter and spring), during which time thousands to tens of thousands of eggs are laid in a gelatinous ribbon amongst aquatic vegetation (Lintermans 2007; McDowall 1996).

Redfin perch are described as a voracious predator (Lintermans 2007), consuming a wide variety of prey ranging from small invertebrates to fish. Within the Murray–Darling Basin, they consume small native species such as Southern pygmy perch, rainbowfish and gudgeons as well as the early-life stages of larger-bodied species such as Silver perch (*Bidyanus bidyanus*), Golden perch (*Macquaria ambigua ambigua*) and Murray cod (*Maccullochella peelii peelii*) (Lintermans 2007; McDowall 1996).

2.2 Fish collection and accommodation

Southern pygmy perch were collected from a number of disconnected pools along Castle Creek at Gooram Hall near Euroa in Central Victoria (Figure 1). Southern pygmy perch are locally abundant within this system. The fish were collected using single-pass backpack electrofishing (Smith Root LR24 with 25.0 cm diameter anode ring). Each pool was fished in such a way as to avoid removing all Southern pygmy perch individuals from any one pool. Most of the Southern pygmy perch collected were relatively small (1.5–3.5 cm) as this was the dominant size-class present when sampling was undertaken. Juvenile Redfin perch were collected from Lake Moodemere in North-East Victoria (Figure 1) using a fine-mesh seine net. The Redfin perch that were collected ranged in size from approximately 10–15 cm.

Fish of both species were transported to the Murray–Darling Freshwater Research Centre (MDFRC) in Wodonga and were housed in recirculating aquaria systems. Southern pygmy perch were held at low densities in 50 x 55 x 50 cm (l x w x h) housing tanks containing macrophytes and rocky substrate. Redfin perch were held individually in 45 x 25 x 30 cm (l x w x h) housing tanks containing rocky substrate only. Fish were acclimated over several weeks before being used in experimental trials. Water temperature was maintained at 25 °C (±1.0 °C) and lighting was set to a 12:12 h light:dark photoperiod.

Southern pygmy perch were fed on a diet of vitamin-enriched bloodworms three times daily. Redfin perch were fed once daily a combination of Eastern Gambusia (*Gambusia holbrooki* — a small-bodied introduced species) and vitamin-enriched bloodworms.

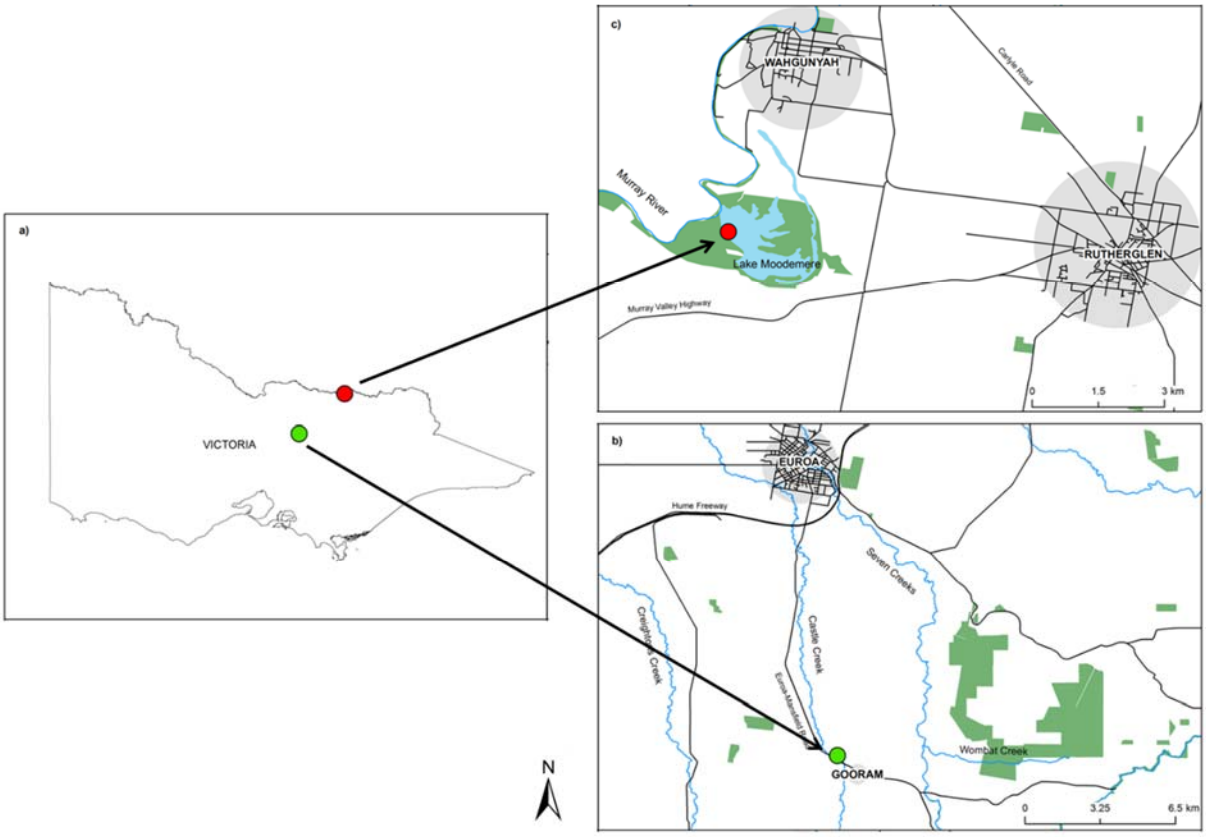

Figure 1. Maps showing the location of Southern pygmy perch (green circles) and Redfin perch (red circles) collection sites. (a) Victoria, (b) Castle Creek at Gooram Hall near Euroa, and (c) Lake Moodemere near Rutherglen. Map created using ESRI ArcMap 10.2. Spatial data sourced from Victorian Government Data Directory website (http://www.data.vic.gov.au).

2.3 Habitat preference

The apparatus used to assess habitat preferences of Southern pygmy perch was a large, acrylic, circular habitat-preference tank (Blaze Displays, Victoria, Australia): 130 cm (o.d.) x 60 cm (high) (o.d. = outside diameter; Figure 2). By using a circular design, we eliminated the influence of tank edges on habitat preference. That is, with this novel design any effect of the tank is constant across all habitat types; no individual habitat type is associated with greater tank surfaces that may impart bias on habitat association. The tank consisted of a doughnut-shaped outer compartment with a transparent inner circular compartment. Redfin perch occupied the inner compartment while Southern pygmy perch occupied the outer compartment. As such, Redfin perch were able to see all habitat patches at any time.

The bottom of the tank was covered with a 5 cm deep layer of washed sand, and then filled with treated tap water to a depth of a 40 cm. The outer compartment was divided into 4 evenly-sized quadrants, which represented four distinct habitat patches: two open water patches, one macrophyte patch and one woody debris patch (Figure 2). The use of two open water patches ensured that the macrophyte and wood patches were always separated by an open water patch, thus avoiding the possibility of movements at the edge of one habitat biasing inferences of habitat preference.

The macrophyte patch was constructed using individual strands of four different plastic aquarium plants ranging in length from 30–40 cm, selected to resemble the types of plants that have been found to be utilised by Southern pygmy perch (*Potamogeton* spp.; *Myriophyllum* spp.; *Ludwigia* spp.). The woody debris patches were constructed using River Red Gum (*Eucalyptus camaldulensis* Dehn.) sticks (ca. 40 cm length) that were collected from the floodplain adjacent to the fish collection sites. Densities of sticks and macrophytes were equal in all trials (1 stem or stick per 5 cm^2). To ensure that the distribution and density of macrophytes and sticks was equal within and among trials, each macrophyte stem and each stick was secured to a plastic grid. These grids were completely buried in the sand to avoid creating additional habitat complexity (Figure 2). Sticks and artificial macrophytes were soaked for ca. 21 days prior to being attached to the grid. For each trial, the position of the first open habitat patch (i.e. Open 1) was randomly selected and the remaining habitat patches were placed in the order Wood, Open 2, Macrophyte, thus patch location was randomised among trials.

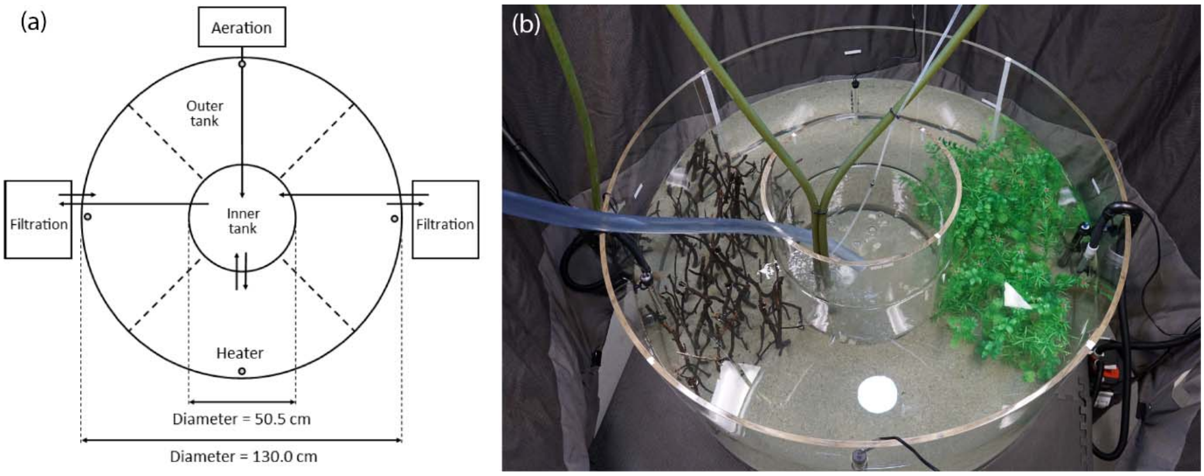

Figure 2. (a) Schematic of the aerial view of the habitat preference tank used for behavioural trials. The barrier between the inner and outer compartments was transparent. Dashed lines delineate four quadrants within which habitat types can be setup. Small circles within the outer compartment indicate positions of four heaters. (b) Photo of the habitat preference tank setup with three habitat types: macrophytes, woody debris and open. Maximum volume of tank filled to top: 760 L. Volume of the outer compartment with a water height of 40 cm: 435 L. Volume of the inner compartment with water height 40 cm: 80 L.

Water was pumped between the two compartments so that Southern pygmy perch were subjected to both visual and chemical cues during trials of treatments involving piscivore stimuli. We did not want any Redfin perch chemical cues present during trials of Southern pygmy perch habitat preference in the absence of Redfin perch. However, due to the large volume of the tank, complete water changes between every trial were not feasible, thus prohibiting a completely randomised order of trials. Instead, trials were grouped into four blocks; two blocks each of Redfin perch presence and absence trials, with five replicates per block (i.e. 10 replicates per treatment). Redfin perch presence/absence was switched between consecutive blocks, to minimise the confounding influence of lab-acclimation duration. A full water change was undertaken between each experimental block.

The habitat selection tank was located in a controlled temperature room and water temperature was maintained at 25 °C (±1.0 °C). The tank was surrounded by a dark curtain with four small holes cut out to enable observation of each habitat patch without the fish being aware of the observer. A single light was placed above the tank and set to a 12:12 h light:dark photoperiod.

Individual Southern pygmy perch and Redfin perch required for a trial were randomly selected from housing aquaria. Individuals were not reused among trials. Four Southern pygmy perch were placed in the outer compartment of the experimental tank 2.5 days prior to the measurement of habitat preference. For trials requiring Redfin perch presence, two Redfin perch were introduced to the inner compartment at this time also. For all trials, an opaque, flexible plastic sheet lined the inner compartment during this 2.5 day acclimation period; this sheet eliminated visual contact between piscivore and prey during acclimation, so that Southern pygmy perch had the opportunity to explore all habitat types before being subject to piscivory stimuli. Southern pygmy perch were fed once daily during acclimation by placing a quarter of a cube of frozen bloodworms into the centre of each quadrant.

On days when habitat preference was measured, an observer entered the controlled temperature room at 9.15 am, leaving all lights switched off, except the one above the tank. The observer then removed the screen from the inner tank and sat motionless for 15 minutes to allow the fish to re-settle. The observer then recorded general observations (behaviour, movement and habitat selection) for a 5 minute period before counting the number of fish in each habitat patch. The observer then waited another 5 minutes before counting the number of Southern pygmy perch in each patch again. This was repeated for a total of 70 minutes, forming seven distribution ‘snapshots’ per trial.

Fish were then removed and placed in a separate housing tank. Water quality was checked at the conclusion of each trial and the macrophytes and woody debris were sterilised and dried weekly to minimise epiphytic growth on the macrophytes and sticks.

2.4 Metabolism experiment

Respirometry trials were conducted to determine whether structural habitat complexity (artificial macrophytes) reduces the stress or affects the activity rates — as indicated by increased metabolic rates — of Southern pygmy perch in the presence and absence of piscivores (Redfin perch). A two-factor, crossed experimental design was used, whereby the metabolic rates of Southern pygmy perch were measured in the presence and absence of macrophytes crossed with the presence and absence of piscivores, resulting in four treatments: (1) no macrophytes + no piscivore; (2) no macrophytes + piscivore; (3) macrophytes + no piscivore; (4) macrophytes + piscivore. For convenience, these treatments are referred to as, respectively, nMnP, nMP, MnP, MP. Four or five replicate trials were completed per treatment, and the order of treatments was randomised.

The key components of the experimental apparatus were an experimental aquarium within which respiration chambers were situated, and an adjoining aquarium that could accommodate Redfin perch, depending on the treatment (Figure 3).

Computerised, intermittent-flow respirometry was used to determine the metabolic rates of Southern pygmy perch (Steffensen 1989). Within each individual trial, time series of oxygen tension were recorded from three glass respirometers (two blanks; one containing fish), each connected to an oxygen sensor, flush circuit and recirculation circuit (Figure 3). Chamber dimensions were: 11.2 cm long x 2.8 cm i.d. (i.d. = internal diameter; 69.3 mL total respirometer volume, including recirculation circuit). Relative to the size of the fish, these chambers were larger than what might be used to estimate standard metabolic rate (SMR). Oversized chambers were used to allow increased fish activity in response to treatments. The chambers were submersed in the experimental aquarium

(60 x 30 x 30 cm; l x w x h) containing water (water depth = 24 cm) that was continuously filtered and maintained at 25 °C ± 0.5 °C.

The piscivore aquarium (water depth = 24 cm) was placed lengthways against one side of the experimental aquarium. The three remaining aquarium walls of both the piscivore and ambient tanks were lined with EVA foam to minimise disturbance to all fish. Filtration, aeration and temperature control of the piscivore aquarium were maintained by continuous water exchange between the experimental and piscivore aquaria via two pumps. Thus, for trials involving piscivore stimuli, the Southern pygmy perch were exposed to both visual and chemical stimuli. The water of the entire experimental apparatus was replaced between trials.

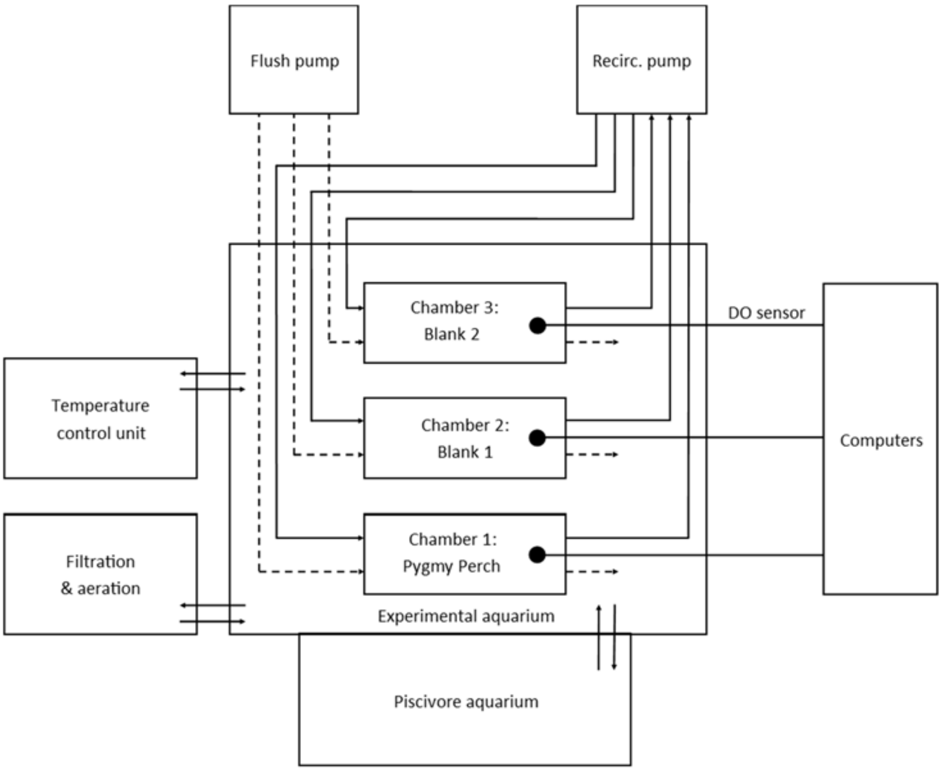

Figure 3. Diagram of the computerised intermittent-flow respirometry apparatus. The flush circuit provided respirometers with fresh water from the ambient tank, whereas the recirculation circuit recirculated water within the respirometers. The housing unit that held the fibre-optic DO (dissolved oxygen) sensors against the glass chambers is not shown in diagram.

Fibre-optic sensors were used to determine oxygen tension inside chambers (PreSens, Regensberg, Germany). A housing unit (Loligo Systems, Tjele, Denmark) was used to ensure stable positioning of the fibre cables alongside sensor spots. The flush and recirculation circuits were constructed from Tygon Chem tubing (Masterflex, Gelsenkirchen, Germany), chosen for its durability and extremely low oxygen permeability. Peristaltic pumps (Masterflex, Gelsenkirchen, Germany) controlled water movement through flush and recirculation circuits, and were automated using computers (Loligo Systems, Tjele, Denmark).

Mass-specific metabolic rate (MO_2; mg O_2 kg^{-1} h^{-1}) measurements took place as part of measurement 'loops', each of which consisted of a 'flush', 'wait' and 'measurement' phase (Figure 4). During the flush phase, the flush and recirculation circuits were activated, providing the respirometers with fresh water from the experimental aquarium. During the wait phase, the flush circuit was deactivated, but the recirculation circuit remained activated, recirculating water within the chamber and allowing for oxygen depletion to commence. The wait phase was of sufficient duration for the rate of oxygen decline to stabilise. During the measurement phase, the recirculation circuit remained active, allowing for an individual MO_2 measurement. Durations of the flush, wait and measurement phases were 300, 220 and 200 seconds, respectively.

Individual trials were completed over a four day period, during which the apparatus was under a 12:12 h light:dark schedule. Trials commenced at 8.00 am on Day 1, at which time an opaque screen was placed between the experimental and piscivore aquaria, and one Southern pygmy perch was placed in a chamber, leaving the other two chambers (blanks) empty. If the trial belonged to either MP or MnP treatments, then four stands of plastic aquarium macrophytes were placed against the chambers. For trials belonging to the MP or nMP treatments, two Redfin perch were introduced to the piscivore aquarium at 9.00 am on Day 2. For piscivore treatments, Redfin perch were given a 3 hour acclimation period, after which the screen between the two tanks was removed. The MO_2 data used to test the hypotheses presented in the Introduction was collected over a 12 hour observation period while the aquaria were lit on Day 3. Given our phase times for measurement loops, this 12 hour observation period yielded up to 60 individual MO_2 estimates for analyses, per trial.

In-between trials, respirometers were sterilised and aquaria were cleaned using a commercial chlorine-based disinfectant, then thoroughly rinsed.

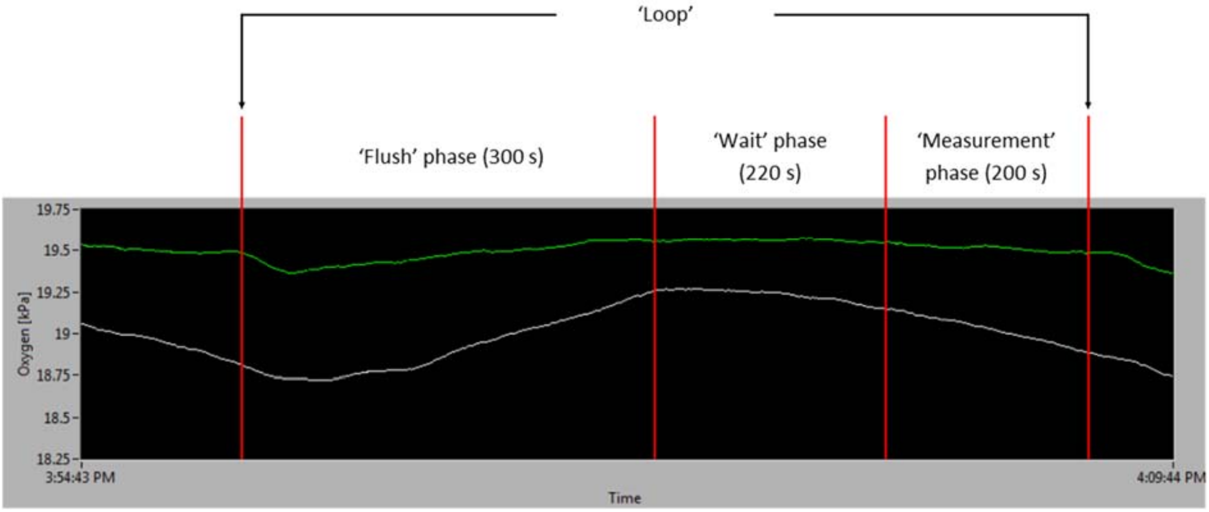

Figure 4. Screenshot from AutoResp software (Loligo Systems, Tjele, Denmark) showing the three consecutive 'phases' that comprised of a single 'loop': a 'flush', 'wait' and 'measurement' phase. $\dot{M}O_2$ (mg O_2 kg$_{-1}$ h$_{-1}$) was determined at the conclusion of the 'measurement' phase. White and green lines show the real-time partial pressure of oxygen (kPa) in chamber 1 (Southern pygmy perch present) and chamber 2 (empty 'blank' to account for background respiration), respectively.

2.5 Data analysis

2.5.1 Habitat preference

Individual observations corresponding to trials were estimated as the mean number of fish in each of the four habitat patches, calculated across the seven snapshots of distribution. For each of the 20 trials (2 treatments x 10 replicates), the total number of Southern pygmy perch observed in each of the four habitats was calculated over the seven snapshots of the distribution. As there were four Southern pygmy perch in each trial, this meant that the totals for each habitat could range between 0 and 28. The habitats 'Open 1 ' and 'Open 2' were kept separate for the analysis, even though they only differed in rotational order rather than in terms of actual habitat.

The distribution of the 28 pygmy perch over the four habitats produced a multinomially distributed dataset, which was analysed by fitting a generalised linear model with Poisson errors and log link, with the constraint that the sum of the observations across habitats is the multinomial total (McCullagh & Nelder 1989). This produced an analysis of deviance in which the first term adjusts for differences in total counts between trails. This was zero in the current case, as all trials had 28 observations of Southern pigmy perch; however, 19 degrees of freedom were removed from the total. This allowed differences between the four habitats and the habitat x piscivore interaction to be tested, and for means and standard errors to be produced for each of the eight habitat x piscivore presence/absence combinations and for the four habitats averaged over piscivore presence/absence.

2.5.2 *Metabolism*

All MO_2 values were adjusted to have the same mass (0.6 g; mean mass of Southern pygmy perch used in trials), following methods explained elsewhere (Stoffels 2015; Stoffels *et al.* 2016). Oxygen consumption rates, MO_2 (mg O_2 kg^{-1} h^{-1}), were calculated as:

$$MO_2 = -(\Delta O_f - \Delta O_b)V_{resp}\alpha B^{-1}$$

where ΔO_f is the rate of change in oxygen tension (kPa h^{-1}) due to fish respiration, ΔO_b is the background rate of change in oxygen tension (a moving-average time series calculated from both blanks), V_{resp} is the volume of the respirometer (L; minus the volume of the fish, measured by displacement in a measuring cylinder), α is the solubility of oxygen in the water at a known temperature and salinity (mg O_2 L^{-1} kPa^{-1}) and B is the mass of the individual (kg).

The objective was to determine whether treatments had an effect on the shapes of the distributions of MO_2 values obtained for each fish over the 12 hour observation period. If certain treatments increase activity or stress, then this should be manifest as a change in the shape of the MO_2 distribution; positive skew should be evident. Specifically, MO_2 distributions from individuals with increased activity and/or stress levels should have higher medians and 3rd quartiles (75th percentiles). Two-factor ANOVAs (factors: plant presence/absence; piscivore presence/absence) were carried out on log-transformed medians and 3rd quartiles obtained from the MO_2 distributions of each trail. In addition, all MO_2 values recorded from the observation period, within each treatment, were pooled so that the overall shapes of the MO_2 distributions could be broadly compared and contrasted. Kernel-smoothed density functions were used to visualise treatment-specific MO_2 distributions.

3 Results

3.1 Habitat preference

The analysis of deviance of total counts of Southern pygmy perch in each habitat showed that there was a highly significant difference between the four habitats ($F_{3,54} = 35.47$, $P<0.001$) and no interaction between habitat and presence/absence of piscivore ($F_{3,54} = 0.07$, $P = 0.976$). The mean numbers of Southern pygmy perch in each habitat at each snapshot were Open1 0.25 (SE 0.10); Open2 0.10 (0.06); Plants 2.61 (0.31); Wood 1.04 (0.20). The means in each habitat for both absence and presence of piscivores and the ten replicate values for the ten replicate trails are presented in Figure 5. Consequently:

- Mean numbers of Southern pygmy perch were significantly higher in the macrophyte habitat patches than in the woody or open habitat patches.

- Mean numbers of Southern pygmy perch were significantly higher in the woody habitat patches than in the open patches.
- These patterns were identical in the presence and absence of Redfin perch.

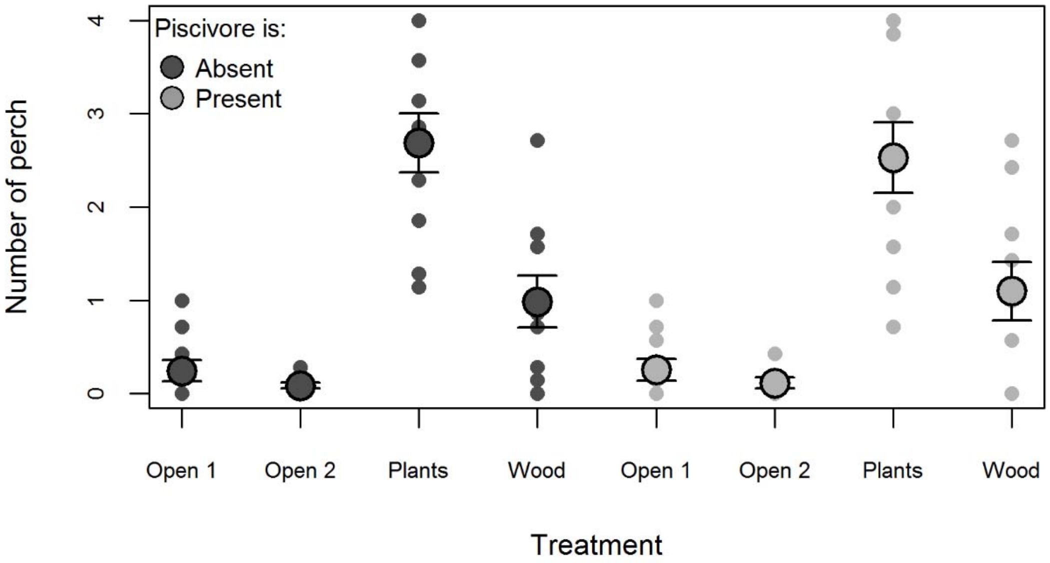

Figure 5. Habitat selection of Southern pygmy perch in the presence and absence of piscivore cues. Mean numbers (+/- SE) of Southern pygmy perch within each of four quadrants (three habitat types) are presented, following 2.5 days of acclimation to the circular habitat-selection tank. Densities of macrophyte and woody stems were equal in all trials. Smaller dots present the mean number of Southern pygmy perch within each quadrant, within each of ten replicate trials per treatment. The values of the smaller dots was calculated as the mean number of perch within each quadrant, calculated over seven observations spaced 10 minutes apart, following the acclimation period.

3.2 Metabolism

Between 56 and 60 individual MO_2 measurements were obtained from all three chambers, during Day 3, within each trial. Occasionally, less than 60 MO_2 estimates were obtained when certain estimates were omitted after yielding $R^2 < 0.9$. The range of R^2 values associated with retained MO_2 measurements across all trials was 0.97 to 0.99, while the overall mean R^2 was 0.99.

Discordant with our hypothesis, median and 3rd quartiles for MO_2s did not follow the expected pattern of MnP < MP < nMnP < nMP (Figure 6; Median ANOVA piscivore main effect: F = 0.10, P = 0.76; Median ANOVA interaction effect: F = 0.004, P = 0.79; 3rd quartile ANOVA piscivore main effect: F = 0.04, P = 0.85; 3rd quartile ANOVA interaction effect: F = 0.09, P = 0.76). Contrasting with the hypothesis posed, a main effect of macrophyte presence on metabolic rates was clear (Figure 6; Median ANOVA macrophytes main effect: F = 6.17, P = 0.03; 3rd quartile ANOVA macrophytes main effect: F = 6.89, P = 0.02). The density functions of the macrophyte treatments both had a larger proportion of MO_2 values in the right-hand tail of the distribution, indicating elevated metabolic rates, on average (Figure 6a). Mean median MO_2 values across treatments were 161 (nMnP), 156 (nMP), 273 (MnP) and 261 (MP) mg O_2, kg^{-1} h^{-1} (Figure 6b). Mean 3rd quartile MO_2 values across treatments were 222 (nMnP), 239 (nMP), 322 (MnP) and 323 (MP) mg O_2, kg^{-1} h^{-1} (Figure 6c).

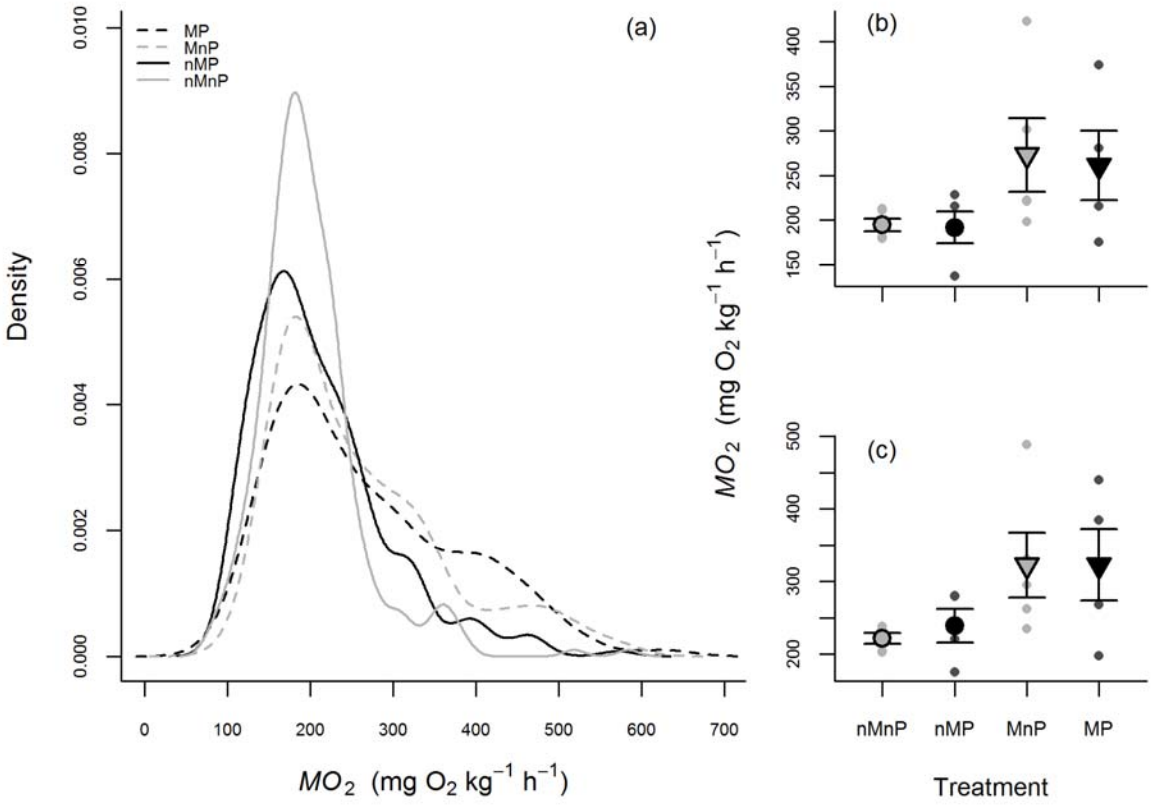

Figure 6. Effects of piscivore and aquatic macrophyte presence/absence on the distributions of mass-specific metabolic rates of Southern pygmy perch. (a) Kernel-smoothed density functions of pooled MO_2 values for each of the four treatments. The area under each curve equals one. In treatment acronyms 'n' refers to 'no'; 'M' refers to 'Macrophytes'; 'P' refers to 'Piscivore'. Hence, nMnP, nMP, MnP and MP refer, respectively, to 'no macrophytes + no piscivores', 'no macrophytes + piscivores', 'macrophytes + no piscivores' and 'macrophytes + piscivores'. In (b) and (c), mean (+/- SE), median and 3rd quartile (respectively) MO_2 values of individual Southern pygmy perch from within each treatment are presented. Black and grey characters represent piscivore-absent and -present treatments, respectively. Circles and triangles represent treatments where macrophytes were absent and present, respectively.

4 Discussion

The implications of this work for the management of Southern pygmy perch can be summarised as follows: (1) macrophytes appear to be a key habitat component for Southern pygmy perch;
(2) predator avoidance is strongly habitat-related.

4.1 Macrophytes appear to be a key habitat component for Southern pygmy perch

The experiments conducted here indicate that macrophytes represent a key, if not critical habitat for Southern pygmy perch. Southern pygmy perch preferentially selected structured habitats over open ones and demonstrated a clear preference for macrophyte habitat over woody habitat. We predicted that metabolic rates would be higher in open habitats than in structured ones, as a result of increased levels of stress. However, we found the opposite pattern whereby metabolic rates were significantly higher when macrophytes were present than in open habitat. High metabolic rates can occur as a result of several mechanisms, most commonly stress and increased activity levels (Cech Jr. 1990; Wedemeyer *et al.* 1990). Given the results from the habitat selection trials, it is highly unlikely that Southern pygmy perch are more stressed in the presence of macrophytes, but rather the results suggest significant differences in relation to activity levels. Specifically, Southern pygmy perch were observed to display reduced movement when macrophytes were absent. The reasons for this are most likely related to predation risk and will be discussed further below. However, these results suggest that processes essential for survival and growth (e.g. foraging movements) as well as reproduction may be negatively impacted if structured habitat is not available.

The results from this project strongly suggest that whilst Southern pygmy perch may use non-macrophyte habitats such as those containing wood, macrophytes represent their preferred habitat type. Consequently, management of existing Southern pygmy perch sites should focus on maintaining or creating significant areas of aquatic vegetation. Similarly, in the event that Southern pygmy perch are translocated to new sites, site selection should be strongly based on the presence of aquatic vegetation, as well as other habitat features that are thought to be important such as low velocities and relatively shallow depths (less than 1 m) (Humphries 1995; Kuiter *et al.* 1996).

- **Knowledge need:** A number of studies have demonstrated that macrophyte type, density, architectural configuration and area, as well as the presence of multiple forms of aquatic vegetation, may be important determinants of the suitability of macrophyte habitat for different species of fish (e.g. Adams *et al.* 2004; Grenouillet *et al.* 2002; Okun & Mehner 2005; Pettit *et al.* 2016). A study by Warfe and Barmuta (2004) suggested that plant density may be important for Southern pygmy perch. However, the implications of different plant densities on Southern pygmy perch are not known and we have no knowledge regarding the influence of other factors such as macrophyte type, architecture, diversity or location of macrophytes within the waterbody on habitat suitability for Southern pygmy perch. These may be important drivers of growth, survival and reproduction.
- **Knowledge need:** Whilst we have established the importance of structured habitat, particularly macrophytes, as important habitat for Southern pygmy perch, it is not clear what the implications of little or no macrophytes are for the species. For example, how does the presence or absence of macrophytes affect foraging rates, reproductive output and success? Also, what are the implications for growth and carrying capacity for this species?

4.2 Predator avoidance is strongly habitat related

It was predicted that the presence of predators would influence metabolic rates and potentially habitat selection patterns. However, these predictions were not supported by the results as neither habitat selection nor metabolic rates varied significantly in the presence or absence of Redfin perch. Whilst this suggests that Southern pygmy perch do not respond to predators, the results are most likely a result of both lack of predator recognition and innate anti-predator habitat selection patterns. The Southern pygmy perch that were used for this study were collected from a system where Redfin perch are absent. Consequently, it is possible that they have not learnt to recognise this species as a potential threat. Evidence showing that fish populations from areas with low predation risk as well as fish reared in a laboratory setting display reduced predator avoidance behaviours indicates that predator avoidance is not always innate, but in some cases is learnt (Kelley & Magurran 2003). This suggests that Southern pygmy perch, not previously exposed to Redfin perch, may be particularly susceptible to predation by this species.

Whilst the metabolic rate of Southern pygmy perch did not vary significantly in relation to predator presence or absence, as noted in the section above, there were significant differences in metabolic rates with respect to the presence or absence of structure, which we believe are related to decreased activity in open habitats. We hypothesise that this is related to predator avoidance. Predator avoidance behaviours typically include decreased swimming activity and foraging as well as a decrease in mating behaviours (Fonner & Woodley 2015; Lawler 1989; Lehtiniemi 2005). Given that predator avoidance is cited as a key factor in the use of structured habitats by fish (Matthews 1998), we suggest that the results from this study indicate that Southern pygmy perch largely perceive the level of predation risk based on habitat availability. Most native piscivorous predators are strongly associated with woody debris rather than with aquatic vegetation (Lintermans, 2007). Consequently it is likely the perception of predation risk based on the availability of aquatic vegetation has evolved in response to the habitat use of native predators. However, unlike many native predators, Redfin perch are strongly associated with aquatic vegetation (Lintermans 2007), thus posing a greater predation threat to Southern pygmy perch.

In summary, the results from this study are suggestive of a high predation risk to Southern pygmy perch if predators, which utilise aquatic vegetation as habitat, are able to access areas containing Southern pygmy perch. This risk will may be even greater if the predator in question is one that Southern pygmy perch have not previously been exposed to.

- **Knowledge need:** In order to determine whether Southern pygmy perch can co-exist with key predators such as Redfin perch, increased knowledge is needed regarding (1) the effectiveness of macrophytes as refuge habitat for Southern pygmy perch against predators which also utilise these habitats, and (2) the ability of Southern pygmy perch to quickly learn avoidance behaviours with respect to predators that they are not familiar with.

References

Adams AJ, Locascio JV, Robbins BD (2004) Microhabitat use by a post-settlement stage estuarine fish: evidence from relative abundance and predation among habitats. *Journal of Experimental Marine Biology and Ecology* **299**, 17-33.

Bond NR, Lake PS (2003) Characterizing fish-habitat associations in streams as the first step in ecological restoration. *Austral Ecology* **28**, 611-621.

Cech Jr. JJ (1990) Respirometry. In: *Methods for Fish Biology* (eds. Schreck CB, Moyle PB), pp. 335-362. American Fisheries Society, Bethesda, Maryland.

Closs GP, Balcombe SR, Driver P, McNeil DG, Shirley MJ (2005) The importance of floodplain wetlands to Murray–Darling fish: What's there? What do we know? What do we need to know? In: *Native fish and wetlands in the Murray Darling Basin: Action Plan, knowledge gaps and supporting papers. (published in)Native Fish And Wetlands In The Murray–Darling Basin – Canberra Workshop, 7–8 June 2005* Murray–Darling Basin Commission, Canberra.

Cook BD, Bunn SE, Hughes JM (2007) Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable Southern pygmy perch (*Nannoperca australis*). *Biological Conservation* **138**, 60-72.

Crook DA, Robertson AI (1999) Relationships between riverine fish and woody debris: implications for lowland rivers. *Marine and Freshwater Research* **50**, 941-953.

Dexter T, Bond N, Hale R, Reich P (2014) Dispersal and recruitment of fish in an intermittent stream network. *Austral Ecology* **39**, 225-235.

Fonner CW, Woodley SK (2015) Testing the predation stress hypothesis: behavioural and hormonal responses to predator cues in Allegheny Mountain dusky salamanders. *Behaviour* **152**, 797-819.

Grenouillet G, Pont D, Seip KL (2002) Abundance and species richness as a function of food resources and vegetation structure: juvenile fish assemblages in rivers. *Ecography* **25**, 641-650.

Hammer M (2009) Status assessment for nationally listed freshwater fishes in south east South Australia during extreme drought, spring 2008. Report to Department for Environment and Heritage, South Australian Government. Aquasave Consulatants, Adelaide.

Hammer MP (2002) *Recovery outline for the Southern pygmy perch in the Mount Lofty Ranges, South Australia* Department of Environmental Biology, University of Adelaide and Native Fish Australia (SA) Incorporated.

Humphries P (1995) Life history, food and habitat of Southern pygmy perch, *Nannoperca australis*, in the Macquarie River, Tasmania. *Marine and Freshwater Research* **46**, 1159-1169.

Kelley JL, Magurran AE (2003) Learned predator recognition and antipredator responses in fishes. *Fish and Fisheries* **4**, 216-226.

King AJ, Ramsey D, Baumgartner L*, et al.* (2009) Environmental Requirements for Managing Successful Fish Recruitment in the Murray River Valley - Review of Existing Knowledge. In: *Arthur Rylah Institute for Environmental Research Technical Report Series No. 197*, Heidelberg, Victoria.

Kuiter R, Humphries P, Arthington A (1996) Family Nannopercidae: Pygmy perches. In: *Freshwater Fishes of South-Eastern Australia* (ed. McDowall RM), pp. 168-175. Reed Books, Chatswood, NSW.

Lawler SP (1989) Behavioural responses to predators and predation risk in four species of larval anurans. *Animal Behaviour* **38**, 1039-1047.

Lehtiniemi M (2005) Swim or hide: predator cues cause species specific reactions in young fish larvae. *Journal of Fish Biology* **66**, 1285-1299.

Lintermans M (2007) *Fishes of the Murray–Darling Basin: An Introductory Guide* Murray–Darling Basin Commission, Canberra.

Matthews WJ (1998) *Patterns in Freshwater Fish Ecology* Chapman & Hall, New York.

McDowall RM (1996) *Freshwater Fishes of South-Eastern Australia* Reed Books, Sydney, Australia.

Morrongiello JR, Bond NR, Crook DA, Wong BBM (2012) Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. *Journal of Animal Ecology* **81**, 806-817.

Okun N, Mehner T (2005) Distribution and feeding of juvenile fish on invertebrates in littoral reeds. *Ecology of Freshwater Fish* **14**, 139-149.

Pettit NE, Ward DP, Adame MF, Valdez D, Bunn SE (2016) Influence of aquatic plant architecture on epiphyte biomass on a tropical river floodplain. *Aquatic Botany* **129**, 35-43.

Steffensen JF (1989) Some errors in respirometry of aquatic breathers: How to avoid and correct for them. *Fish Physiology and Biochemistry* **6**, 49-59.

Stoffels RJ (2015) Physiological trade-offs along a fast-slow lifestyle continuum in fishes: What do they tell us about resistance and resilience to hypoxia? *Plos One* **10**, e0130303.

Stoffels RJ, Richardson AJ, Vogel MT, Coates SP, Muller WJ (2016) What do metabolic rates tell us about thermal niches? Mechanisms driving crayfish distributions along an altitudinal gradient. *Oecologia* **180**, 45-54.

Stoffels RJ, Weatherman KE (2016) Will heat and hypoxia in floodplain wetlands give alien mosquitofish (*Gambusia holbrooki)* the edge over endemic pygmy perch (*Nannoperca australis*)? Final Report prepared for the Goulburn-Broken Catchment Management Authority by The Murray–Darling Freshwater Research Centre, MDFRC Publication 94/2015, August, 22pp.

Tonkin Z, King AJ, Mahoney J (2008) Effects of flooding on recruitment and dispersal of the Southern pygmy perch (*Nannoperca australis*) at a Murray River floodplain wetland. *Ecological Management & Restoration* **9**, 196-201.

Warfe DM, Barmuta LA (2004) Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia* **141**, 171-178.

Wedemeyer GA, Barton BA, McLeay DJ (1990) Stress and acclimation. In: *Methods for Fish Biology* (eds. Schreck CB, Moyle PB). American Fisheries Society, Bethesda, Maryland.

Woodward GMA, Malone BS (2002) Patterns of abundance and habitat use by *Nannoperca obscura* (Yarra pygmy perch) and *Nannoperca australis* (southern pygmy perch). *Proceedings of the Royal Society of Victoria* **114**, 31-72.

Appendix A: Literature review: the role of structural habitat for Southern pygmy perch (*Nannoperca australis)*

Introduction

The Southern pygmy perch (*Nannoperca australis*) is a small-bodied species that historically was widely distributed throughout the Murray and lower Murrumbidgee systems, as well as coastal Victoria and northern Tasmania (Hammer 2002; Lintermans 2007). The species has experienced dramatic declines in range and abundance and is now listed as endangered in NSW and South Australia (Lintermans 2007). In NSW, there are only three known populations, one in the Lachlan Catchment and two in the Murray Catchment near Holbrook and Albury (Lintermans 2007). Currently, management of the two Murray populations is focused on maintaining the limited number of existing populations with a view to establishing new populations via translocations to suitable habitats in the future. Southern pygmy perch appear to have very limited dispersal (Cook *et al.* 2007; Dexter *et al.* 2014), but locally, can breed up to good numbers quickly if good quality habitat is available (Hammer 2002). Consequently, management actions are most likely to be successful if they are focused on local-scale habitat maintenance/improvement and threat abatement. Therefore, an understanding of the critical habitat requirements of Southern pygmy perch is vital.

It is generally thought that aquatic macrophytes are a critical habitat feature for Southern pygmy perch (Hammer 2002; Humphries 1995; King *et al.* 2009). However, robust scientific evidence for this is lacking and there is anecdotal evidence of self-sustaining Southern pygmy perch populations occurring in habitats with little or no macrophytes. It is not currently known whether Southern pygmy perch are associated specifically with macrophytes or whether other structural habitat types, such as snags, can fulfil the same functional requirements. In addition, it is not known under what circumstances macrophytes or other structural habitat types are used as habitat and whether this represents a true habitat requirement. Given that this is currently a key habitat factor which is likely to be considered in the management of current and future Southern pygmy perch populations, having a clear understanding of the functional role of structural habitat is important. This review will (1) summarise current knowledge regarding Southern pygmy perch; (2) examine the functional role of macrophytes and other key structural habitat types and asses their likely importance in light of current knowledge regarding the species; and (3) develop a conceptual model describing the potential relationships between Southern pygmy perch, physical habitat structure and key biotic processes such as food production and predation.

Southern pygmy perch

Species description

The Southern pygmy perch is a relatively short-lived species which inhabits a range of still or slow-flowing waters in streams, lakes and wetlands (Kuiter *et al.* 1996; Lintermans 2007). Individuals can live for more than 5 years and reach a maximum length of approximately 8.5 cm; however, populations generally comprise of fish that are 1 or 2 years old with a maximum length of 6.5 cm (Hammer 2002; Kuiter *et al.* 1996; Lintermans 2007). Southern pygmy perch mature within their first year and usually spawn between September and January, producing non-adhesive demersal eggs, which are scattered over aquatic vegetation or the benthos (Humphries 1995; Kuiter *et al.* 1996; Lintermans 2007). Early-stage larvae are poorly developed and are associated with the benthos (Morrongiello *et al.* 2012).

Southern pygmy perch are most commonly described as being associated with relatively shallow, low velocity habitats with dense cover (Hammer 2009; Humphries 1995; Kuiter *et al.* 1996). Whilst some authors have noted that Southern pygmy perch can occur in association with woody debris or rocks (Bond & Lake 2003; Hammer 2009), the majority of studies suggest that Southern pygmy perch occur almost exclusively in aquatic vegetation (Humphries 1995; Kuiter *et al.* 1996; Lintermans 2007; Woodward & Malone 2002). This may be submerged aquatic vegetation (Hammer 2009; Humphries 1995) or emergent and overhanging edge vegetation (Hammer 2009). Newly inundated edge vegetation has been proposed as being particularly important as larval and juvenile habitat (Hammer 2009; King *et al.* 2009) and as refuge habitat for all life-stages during high flows (Hammer 2002).

Southern pygmy perch consume epibenthic and epiphytic invertebrates that include small crustaceans such as amphipods, cladocerans, ostracods and copepods, as well as aquatic insect larvae (e.g. chironomid, mayfly, hemipteran and mosquito larvae) (Humphries 1995; Kuiter *et al.* 1996; Woodward & Malone 2002).The diet of smaller individuals tends to be dominated by microcrustaceans, whereas larger individuals consume a greater proportion of larger prey such as macroinvertebrates (Humphries 1995).

Key threats

Habitat degradation and loss, and introduced species are thought to represent the most significant threats to Southern pygmy perch populations. Southern pygmy perch habitat can be adversely impacted by changes in hydrological regimes, stock access, land reclamation, loss of riparian vegetation, sedimentation and channel erosion, which can result in disconnection and drying of floodplain habitats, poor water quality, hydro-geomorphological changes (e.g. depth and velocity of stream habitat) and loss of aquatic and fringing vegetation (Hammer 2009; Hammer 2002; Lintermans 2007). In addition, predation by introduced species such as Redfin perch (*Perca fluviatilis*) and trout, and competition and predation by Eastern gambusia (*Gambusia holbrooki*) (Macdonald *et al.* 2012) can be a significant source of mortality. Mortality occurs directly, but can also occur indirectly, for example if Southern pygmy perch are forced into marginal habitat to avoid predation and/or competition.

The functional role of structural habitat for Southern pygmy perch

Physical habitat is important in structuring assemblages in rivers and streams (Matthews 1998). Many studies have demonstrated that microhabitat characteristics, such as substrate type, the amount and type of instream structure, area and depth, play an important role in determining species distribution and abundance patterns (e.g. Gorman 1987; Greenberg 1991; Gozlan *et al*. 1999; Vadas & Orth 2000; Boys & Thoms 2006; Mesquita *et al*. 2006).

Habitat complexity has been found to be positively correlated with both species richness and the abundance of individual species (Petry *et al*. 2003; Cooperman and Markle 2004; Boys & Thoms 2006), and is attributed to the presence of a range of physical elements such as macrophytes, terrestrial vegetation, woody debris and complex substrates (e.g. large boulders (Matthews, 1998; Crook and Robertson, 1999; Boys and Thoms, 2006). Habitat structure can affect resource or niche availability, the amount of surface area for living, competitive interactions, predator-prey relationships, food availability and foraging efficiency (Schlosser 1987; Werner & Hall 1988; Tabor & Wurtsbaugh 1991; Lehtiniemi 2005; Okun & Mehner 2005; Willis *et al*. 2005), with prey density having been reported to be positively correlated with structural complexity (Cooperman and Markle 2004; Okun & Mehner 2005; Okun *et al*. 2005; Garner 1996*).* However, experimental studies have shown that fish foraging efficiency is decreased in highly complex habitats, where prey organisms such as zooplankton and macroinvertebrates also use these habitats to evade predators (Werner & Gilliam 1984; Tátrai & Herzig 1993; Stansfield *et al*. 1997; Balayla & Moss 2003; Harrison *et al*. 2005).

Aquatic vegetation provides important habitat for fish, particularly for early-life-stages and small-bodied species (Matthews 1998). The significance of macrophytes has been attributed to reduced velocities within and around beds (Cooperman & Markle 2004) and greater food availability, with prey densities often highly correlated with plant density (Balcombe & Closs 2004; Cooperman &

Markle 2004; Okun & Mehner 2005; Okun *et al*. 2005). In addition, aquatic vegetation has been shown to be an effective refuge from predators for many species (Tabor & Wurtsbaugh 1991; Gregory & Levings 1996; Cooperman & Markle 2004; Harrison *et al*. 2005; Lehtiniemi 2005; Okun & Mehner 2005). However, foraging efficiency may be decreased in highly complex habitats, as prey organisms such as zooplankton and macroinvertebrates also use these habitats to evade predators (Werner & Gilliam 1984; Tátrai & Herzig 1993; Stansfield *et al*. 1997; Balayla & Moss 2003; Harrison *et al*. 2005). Thus, for species that are both predators and prey, the use of aquatic vegetation is seen as a trade-off between foraging efficiency and refuge from predation (Werner & Gilliam 1984; Werner & Hall 1988; Tátrai & Herzig 1993).

Aquatic and fringing vegetation is thought to function as shelter from high flows and also as spawning habitat for Southern pygmy perch (Tonkin *et al*. 2008; Hammer 2009). However, on a day-to-day basis, aquatic and fringing vegetation are thought to provide both a refuge from predation by piscivorous fish such as Redfin perch, and foraging habitat due to their increased invertebrate densities (Kuiter *et al.* 1996; Hammer 2002; Closs *et al.* 2005; Humphries 1995; Warfe & Barmuta 2004). This is potentially supported by the observation that Southern pygmy perch can alter their foraging strategy within macrophytes depending on the density of cover. Warfe and Barmuta (2004) found that at low macrophyte densities, Southern pygmy perch utilised a sit-and-wait strategy, whereby they remained motionless except to dart out and capture prey, whereas at higher densities, fish swam amongst plant stems consuming prey items as they were encountered.

Other types of instream structure, such as snags, or woody debris, also play an important role in providing structure and habitat complexity in freshwater systems (Matthews 1998; Crook & Robertson 1999). Snag cover influences the quality of food and habitat resources by contributing to overall habitat complexity and like aquatic vegetation, it is also believed to play an important role in providing refuge from high current velocities, as spawning sites for a number of species and as camouflage from predators or prey (Matthews 1998; Crook & Robertson 1999). For a number of species, snags also provide excellent foraging substrates, since high densities of macroinvertebrates and algae are often associated with snags (Crook & Robertson 1999). It is not known whether microinvertebrates also occur in higher densities around snags.

It would seem that snag habitat has the capacity to offer similar benefits to Southern pygmy perch as aquatic and fringing vegetation; however, there is little documented evidence of Southern pygmy perch being strongly associated with coarse or fine woody debris. Snags are frequently used as habitat by juveniles and adults of larger species (Crook & Robertson 1999) which may represent a predation threat to Southern pygmy perch. Consequently, snags may be a riskier habitat for Southern pygmy perch than aquatic vegetation. Alternatively, the invertebrate community that is associated with snags may differ markedly from that of aquatic vegetation and may be less suitable for Southern pygmy perch. However, given the relative paucity of studies that have investigated the habitat associations of Southern pygmy perch, it is also possible that snags do in fact represent suitable Southern pygmy perch habitat, but this has not yet been documented by any studies. This is important information to have as it would have implications for habitat management for existing populations and would also strongly affect the selection of possible translocation sites.

Approaches for assessing habitat requirements

Habitat requirements are frequently inferred from correlational studies that link high abundances of a particular species with specific habitat attributes (Rosenfeld 2003). Whilst correlations between species can be valuable in suggesting possible habitat variables of importance, they can only elucidate positive or negative habitat selection in a given system at a given time (Mitchell 2005). Observed patterns in habitat use may change depending on habitat availability, time of day, season, environmental conditions, as well as between populations, depending on food availability and biotic interactions (Cantu & Winemiller 1997; David & Closs 2003; Rosenfeld 2003; Mitchell 2005). In addition, habitat associations can be misleading because suitable habitats may be unoccupied if densities are very low, whereas unsuitable habitats may be occupied and operating as sinks when densities are high. Consequently, habitat associations are indicative of habitat selection rather than habitat requirement, which can be defined as those features of the environment which are necessary for the persistence of individuals or populations (Rosenfeld 2003).

Habitat preference is a far stronger indicator of habitat requirements. This is because preference is a function of species ecology, physiology, and behaviour (Rosenfeld 2003). Habitat preference is best determined using habitat choice experiments in which extraneous factors (e.g. predation risk, competition, availability of different habitats) are controlled. These types of experiments have been frequently used in order to assess the relative importance of different habitat variables such as temperature, light, food and cover (e.g. Connolly 1994; Bevelhimer 1996; Jordan 1996; Goddard 1997; Mackenzie 1998; Ohman 1998; Schofield 2003; Crawley and Hyndes 2007; Feary 2007; Pratchett 2008).

Conceptual model

A conceptual model depicting the potential linkages and relationships between habitat structure and key processes that may underpin Southern pygmy perch population dynamics is shown below (Fig. 1). In this model, a variety of different habitat types, including rocks, woody debris, submerged macrophytes, fringing vegetation and overhanging riparian vegetation can contribute to structural habitat complexity. The complexity that arises from these habitats can mediate key processes such as predation rates, foraging and spawning success and washout rates during high flow events. Virtually all aspects of this conceptual model are untested for Southern pygmy perch, the notable exception being work undertaken by Warfe and Barmuta (2004) which showed that aspects of habitat structure can influence foraging success. Key knowledge gaps include whether different types of structural habitats affect population processes equally, the functional role that habitat structure plays in Southern pygmy perch population dynamics and the relative importance of habitat structure versus other drivers for population processes.

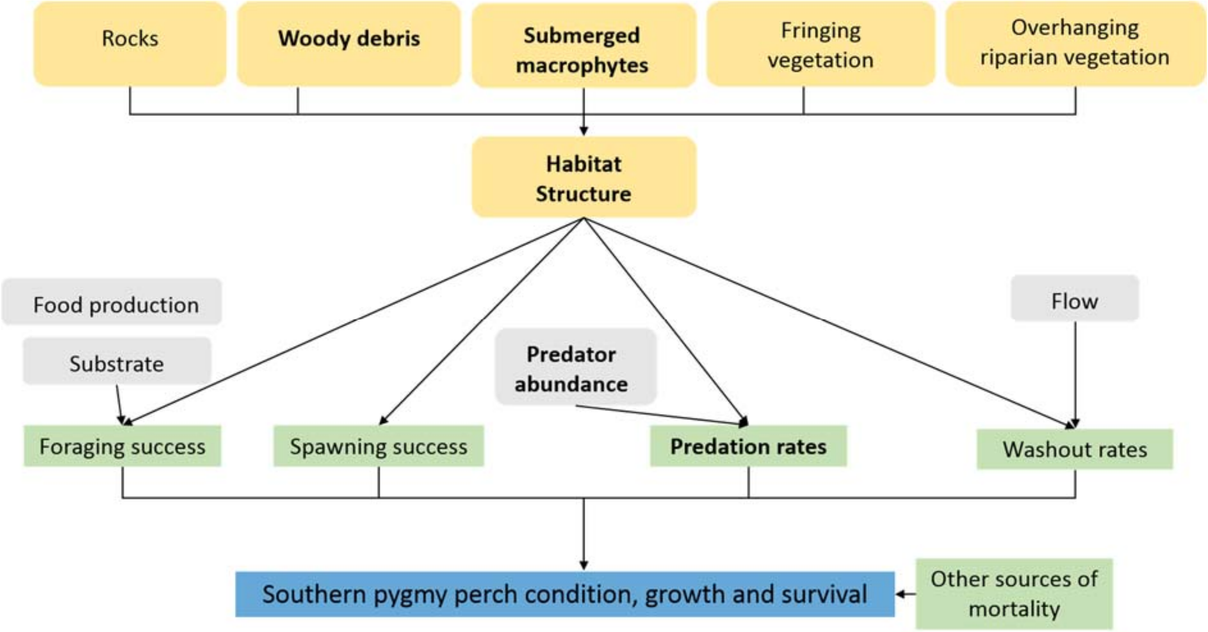

Figure A1. Conceptual model showing the potential relationship between structural habitat (yellow boxes) and key processes (green boxes) that may underpin Southern pygmy perch population dynamics. Key drivers that are not related to habitat structure are shown in grey. Bold text denotes parameters and/or relationships that will be investigated as part of this project.

References

Balayla DJ, Moss B (2003) Spatial patterns and population dynamics of plant-associated microcrustacea (Cladocera) in an English shallow Lake (Little Mere, Cheshire). *Aquatic Ecology* **37**, 417–435.

Balcombe SR, Closs GP (2004) Spatial relationships and temporal variability in a littoral macrophyte fish assemblage. *Marine and Freshwater Research* **55**, 609–617.

Bevelhimer MS (1996) Relative importance of temperature, food, and physical structure to habitat choice by smallmouth bass in laboratory experiments. *Transactions of the American Fisheries Society* **125(2)**, 274–283.

Bond NR, Lake PS (2003) Characterising fish-habitat associations in streams as the first step in ecological restoration. *Austral Ecology* **28**, 611–621.

Boys CA, Thoms MC (2006) A large-scale, hierarchical approach for assessing habitat associations of fish assemblages in large dryland rivers. *Hydrobiologia* **572**, 11–31.

Cantu NEV, Winemiller KO (1997) Structure and habitat associations of Devils River fish assemblages. *The Southwestern Naturalist*, **42(3)**, 265–278.

Closs GP, Balcombe SR, Driver P, McNeil DG, Shirley MJ (2005) The importance of floodplain wetlands to Murray–Darling fish: What's there? What do we know? What do we need to know. In: Phillip B (ed) *Native fish and wetlands in the Murray–Darling Basin: Action plan, knowledge gaps and supporting papers.* Murray–Darling Basin Commission, Canberra, pp 14–28.

Connolly RM (1994) The role of seagrass as preferred habitat for juvenile *Sillaginodes punctata* (Cuv. & Val.)(Sillaginidae, Pisces): habitat selection or feeding? *Journal of Experimental Marine Biology and Ecology* ***180***(1), 39–47.

Cook BD, Bunn SE, Hughes JM (2007) Molecular genetic and stable isotope signatures reveal complementary patterns of population connectivity in the regionally vulnerable Southern pygmy perch (*Nannoperca australis*). *Biological Conservation* **138**, 60–72.

Cooperman MS, Markle DF (2004) Abundance, size, and feeding success of larval shortnose suckers and Lost River suckers from different habitats of the littoral zone of Upper Klamath Lake. *Environmental Biology of Fishes* **71**, 365–377.

Crawley KR, Hyndes GA (2007) The role of different types of detached macrophytes in the food and habitat choice of a surf-zone inhabiting amphipod. *Marine Biology* **151(4)**, 1433–1443.

Crowder LB, Cooper WE (1982) Habitat structural complexity and the interaction between bluegills and their prey. *Ecology* **63**, 1802–1813.

David BO, Closs GP (2003) Seasonal variation in diel activity and microhabitat use of an endemic New Zealand stream-dwelling galaxiid fish. *Freshwater Biology* **48**, 1765–1781.

Dexter T, Bond N, Hale R, Reich P (2014) Dispersal and recruitment of fish in an intermittent stream network. *Austral Ecology* **39**, 225–235.

Feary DA, Almany GR, McCormick MI, Jones GP (2007). Habitat choice, recruitment and the response of coral reef fishes to coral degradation. *Oecologia* **153(3)**, 727–737.

Garner P (1996) Microhabitat use and diet of 0+ cyprinid fishes in a lentic, regulated reach of the River Great Ouse, England. *Journal of Fish Biology* **48**, 367–382.

Goddard K, Mathis A (1997) Microhabitat preferences of longear sunfish: low light intensity versus submerged cover. *Environmental Biology of Fishes* **49**, 495–499

Gorman OT (1987) Habitat segregation in an assemblage of minnows in an Ozark stream. In: Matthews WJ, Heins DC (eds) *Community and Evolutionary Ecology of North American Stream Fishes*. University of Oklahoma Press, Norman, Oklahoma, pp 33–41.

Gozlan RE, Mastrorillo S, Copp GH, Lek S (1999) Predicting the structure and diversity of young-of-the-year fish assemblages in large rivers. *Freshwater Biology* **41**, 809–820.

Greenberg LA (1991) Habitat use and feeding-behaviour of thirteen species of benthic stream fishes. *Environmental Biology of Fishes* **31**, 389–401.

Gregory RS, Levings CD (1996) The effects of turbidity and vegetation on the risk of juvenile salmonids, *Oncorhynchus* spp., to predation by adult cutthroat trout, *O.Clarkii*. *Environmental Biology of Fishes* **47**, 279–288.

Hammer M (2009) Status assessment for nationally listed freshwater fishes in south east South Australia during extreme drought, spring 2008. Report to Department for Environment and Heritage, South Australian Government. Aquasave Consultants, Adelaide.

Hammer MP (2002) *Recovery outline for the Southern pygmy perch in the Mount Lofty Ranges, South Australia*. Department of Environmental Biology, University of Adelaide and Native Fish Australia (SA) Incorporated.

Harrison SSC, Bradley DC, Harris IT (2005) Uncoupling strong predator-prey interactions in streams: the role of marginal macrophytes. *Oikos* **108**, 433–448.

Humphries P (1995) Life history, food and habitat of Southern pygmy perch, *Nannoperca australis*, in the Macquarie River, Tasmania. *Marine and Freshwater Research* **46**, 1159–1169.

Jordan F, Bartolini M, Nelson C, Patterson PE, Soulen HL (1997). Risk of predation affects habitat selection by the pinfish *Lagodon rhomboides* (Linnaeus). *Journal of Experimental Marine Biology and Ecology* **208(1)**, 45–56.

King AJ, Ramsey D, Baumgartner L, Humphries P, Jones M, Koehn J, Lyon J, Mallen-Cooper M, Meredith S, Vilizzi L, Ye Q, Zampatti B (2009) Environmental requirements for managing successful fish recruitment in the Murray River Valley — review of existing knowledge. In: *Arthur Rylah Institute for Environmental Research Technical Report Series No. 197*, Heidelberg, Victoria.

Kuiter R, Humphries P, Arthington A (1996) Family Nannopercidae: Pygmy perches. In: McDowall RM (ed) *Freshwater Fishes of South-Eastern Australia*. Reed Books, Chatswood, NSW, pp 168–175.

Lehtiniemi M (2005) Swim or hide: predator cues cause species specific reactions in young fish larvae. *Journal of Fish Biology* **66**, 1285–1299.

Levin PS (1991) Effects of microhabitat on recruitment variation in a Gulf of Maine reef fish. *Marine Ecology Progress Series* **75**, 183–189.

Lintermans M (2007) *Fishes of the Murray–Darling Basin: an introductory guide* Murray–Darling Basin Commission, Canberra.

Macdonald JI, Tonkin ZD, Ramsey DSL, Kaus AK, King AK, Crook DA (2012) Do invasive Eastern gambusia (*Gambusia holbrooki*) shape wetland fish assemblage structure in south-eastern Australia? *Marine and Freshwater Research* **63**, 659–671.

MacKenzie AR, Greenberg L (1998) The influence of instream cover and predation risk on microhabitat selection of stone loach *Barbatula barbatula* (L.). *Ecology of Freshwater Fish* **7**, 87–94.

Matthews WJ (1998) *Patterns in freshwater fish ecology* Chapman & Hall, New York.

Mesquita N, Coelho MM, Filomena MM (2006) Spatial variation in fish assemblages across small Mediterranean drainages: effects of habitat and landscape context. *Environmental Biology of Fishes* **77**, 105–120.

Mitchell SC (2005) How useful is the concept of habitat? — a critique. *Oikos* **110**, 634–638.

Morrongiello JR, Bond NR, Crook DA, Wong BBM (2012) Spatial variation in egg size and egg number reflects trade-offs and bet-hedging in a freshwater fish. *Journal of Animal Ecology* **81**, 806–817.

Öhman MC, Munday PL, Jones GP, Caley MJ (1998) Settlement strategies and distribution patterns of coral-reef fishes. *Journal of Experimental Marine Biology and Ecology* **225(2)**, 219–238.

Okun N, Lewin WC, Mehner T (2005) Top-down and bottom-up impacts of juvenile fish in a littoral reed stand. *Freshwater Biology* **50**, 798–812.

Okun N, Mehner T (2005) Distribution and feeding of juvenile fish on invertebrates in littoral reeds. *Ecology of Freshwater Fish* **14**, 139–149.

Pratchett MS, Berumen ML, Marnane MJ, Eagle JV, Pratchett DJ (2008). Habitat associations of juvenile versus adult butterflyfishes. *Coral Reefs* **27(3)**, 541–551.

Petry P, Bayley PB, Markle DF (2003) Relationships between fish assemblages, macrophytes and environmental gradients in the Amazon River floodplain. *Journal of Fish Biology* **63**, 547–579.

Rosenfeld J (2003) Assessing the habitat requirements of stream fishes: an overview and evaluation of different approaches. *Transactions of the American Fisheries Society* **132**, 953–968.

Schlosser IJ (1987) The role of predation in age-related and size-related habitat use by stream fishes. *Ecology* **68**, 651–659.

Schofield PJ (2003). Habitat selection of two gobies (*Microgobius gulosus*, *Gobiosoma robustum*): influence of structural complexity, competitive interactions, and presence of a predator. *Journal of Experimental Marine Biology and Ecology* **288(1)**, 125–137.

Stansfield JH, Perrow MR, Tench LD, Jowitt AJD, Taylor AAL (1997) Submerged macrophytes as refuges for grazing Cladocera against fish predation: observations on seasonal changes in relation to macrophyte cover and predation pressure. *Hydrobiologia* **342**, 229–240.

Tabor RA, Wurtsbaugh WA (1991) Predation risk and the importance of cover for juvenile rainbow trout in lentic systems. *Transactions of the American Fisheries Society* **120**, 728–738.

Tatrai I, Herzig A (1995) Effect of habitat structure on the feeding efficiency of young stages of razor fish (*Pelecus cultratus* (L)) — an experimental approach. *Hydrobiologia* **299**, 75–81.

Vadas RL, Jr., Orth DJ (2000) Habitat use of fish communities in a Virginia stream system. *Environmental Biology of Fishes* **59**, 253–269.

Warfe DM, Barmuta LA (2004) Habitat structural complexity mediates the foraging success of multiple predator species. *Oecologia* **141**, 171–178.

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size structured populations. *Annual Review of Ecology and Systematics* **15**, 393–425.

Werner EE, Hall DJ (1988) Ontogenetic habitat shifts in bluegill — the foraging rate predation risk trade-off. *Ecology* **69**, 1352–1366.

Willis SC, Winemiller KO, Lopez-Fernandez H (2005) Habitat structural complexity and morphological diversity of fish assemblages in a Neotropical floodplain river. *Oecologia* **142**, 284–295.

Woodward GMA, Malone BS (2002) Patterns of abundance and habitat use by *Nannoperca obscura* (Yarra pygmy perch) and *Nannoperca australis* (Southern pygmy perch). *Proceedings of the Royal Society of Victoria* **114**, 31–72.

Woodward GMA (2005) Factors influencing the distribution and abundance of *Nannoperca obscura* (Yarra pygmy perch) and *Nannoperca australis* (Southern pygmy perch). PhD Thesis, La Trobe University, Bundoora, Victoria.