Navigating a future for threatened freshwater fish in the CLLMM region in the face of environmental change

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Title page photo credit: Soft-release enclosure at Clayton Bay (Sylvia Zukowski)

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Executive Summary

The Coorong Lower Lakes and Murray Mouth (CLLMM) region supports more than 35 freshwater fish species, including several threatened small-bodied species. Already at risk, small-bodied fishes were severely impacted by the prolonged Millennium Drought (2001–2010) and will be vulnerable to the impacts of environmental change in the region. Some of the most affected fish species were the southern pygmy perch and Yarra pygmy perch. A range of conservation actions have been implemented in the attempt to recover these two species. Among these actions are improved water level management, habitat restoration, alien species control, and translocations to reestablish resilient, connected populations to help secure the long-term survival of the species. Despite 17 years of interventions, both species remain precarious, and key questions remain about how to achieve self-sustaining populations.

To help inform future management of wild and ex situ pygmy perch populations, reintroduction ecology, and the use of water for the environment, this project built on ongoing collaborations and conservation actions, and used 15+ years of data on southern pygmy perch from the CLLMM and Eastern Mount Lofty Ranges (EMLR) regions, modelling, otolith aging, and field experiments to investigate how life history processes (e.g. reproduction) and population demographics are associated with biotic and abiotic variables.

Population modelling aimed to determine trends (changes) in southern pygmy perch abundance and recruitment over time (phase 1), and the drivers of these trends (phase 2). Broadly, relative abundance of southern pygmy perch declined in the EMLR and increased in the Lower Lakes over the study period (2007–2025). These findings indicate that the effects of environmental change may be evident in the EMLR population, with fish abundance declining in a continuing drying climate and limited water resource system. Although the Lower Lakes population underwent considerable disturbance during and after the Millennium Drought, the continuing positive trajectory of this population indicates this region may confer resilience to this species in the face of environmental change. In the EMLR, however, the southern pygmy perch population is likely to continue to decline and urgent intervention is required.

The second phase of characterising trends in southern pygmy perch populations in the EMLR and Lower Lakes was to determine which environmental variables may be driving the population trends using empirical and model methods. In the EMLR, at the regional scale, modelling of the relative abundance of southern pygmy perch, in relation to potential regional environmental drivers, revealed that the Antarctic Oscillation (AAO), with a time lag of two years, was the most influential driver, with empirical analysis showing a positive AAO drove a decrease in southern pygmy perch and that this relationship strengthened after the Millennium Drought (2009). This result highlights the compromised long-term sustainability of southern pygmy perch, and other small-bodied fish species in the EMLR under current and future changing climates where positive AAO conditions will be more common.

In the Lower Lakes, modelling of the relative abundance of southern pygmy perch in relation to regional environmental driver data revealed that mean lake level, with a lag time of six months (previous September), was found to be the most influential driver with a positive relationship, where elevated lake water levels promote increased abundance. As the key driver of relative abundance of southern pygmy perch in the Lower Lakes, the importance of lake water level management, via River Murray flow and barrage operations, is paramount. The optimal conditions to promote recruitment in Lower Lakes southern pygmy perch populations is approximately a 0.8 m Australian Height Datum (AHD) lake water level in September.

In both the EMLR and Lower Lakes, the density of aquatic macrophytes present was found to be the most influential site level, local driver of southern pygmy perch relative abundance compared to the other suite of drivers tested. A strong positive relationship was found where increasing macrophyte density drove an increase in southern pygmy perch abundance.

These modelling results, of site-scale drivers of southern pygmy perch abundance, led to a field experiment to investigate how aquatic macrophyte density may influence the recruitment and abundance of southern pygmy perch in the Lower Lakes. Although Southern pygmy perch were not recaptured during field experiments, results revealed early-stage juveniles from other native fish species were only found in high vegetation enclosures, and that most adult native fishes were found in high vegetation enclosures. No significant differences were observed between water parameters in high and low vegetation enclosures; thus, water physico-chemistry may not be a contributing factor to variability in fish abundances between treatments.

The use of otoliths to gain knowledge about southern pygmy perch can help inform how hydrological manipulations of lake water levels will influence the timing of breeding and the outcomes of recruitment (e.g. growth rates). This study is the first to provide credible age estimates of the Vulnerable MDB population of southern pygmy perch. Daily increments were found to be interpretable in most southern pygmy perch up to ~50 mm total length. Daily age estimated hatch dates were predominantly in spring and summer. September and December were the most frequent months of hatching, closely followed by January. Quantification of hatch dates, estimated from age, are potentially informative for the conservation of southern pygmy perch particularly in the Lower Lakes. For example, knowing the time of spawning can inform the precise timing of environmental watering interventions to maximise reproduction and recruitment. This type of watering intervention is frequently undertaken by the Department of Environment and Water (DEW). The modelling chapter of this report shows the optimal conditions to promote recruitment of Lower Lakes southern pygmy perch populations is approximately 0.8 m AHD lake water level in September. The hatch dates in the current study were most numerous in September, which suggests the optimal conditions revealed by the model are strongly related to spawning and recruitment. Together, these findings provide a clear evidence base to guide future conservation actions for threatened small-bodied fish in the CLLMM region

Acknowledgements

We acknowledge the cultural connections to the land and connected waters, as well as the cultural significance of waterbirds that exist between people of the Ngarrindjeri and Boandik Nations across the Coorong, Lower Lakes, and Murray Mouth region. We recognise that First Nations peoples' spiritual, social, cultural, and economic practices come from their lands and waters, and they continue to maintain their cultural heritage, economies, languages, and laws which are of ongoing importance. We are committed to fostering and nurturing ongoing relationships with First Nations communities in the CLLMM region as we collectively work toward healthy, resilient and thriving lands, waters, ecosystems and communities across the region.

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Na zdrowie fish.

Tanya, Les and Alac helping with field experiments.

1 Introduction

Amid a global biodiversity crisis (Harrison et al. 2018), freshwater fishes are disproportionately at risk (Darwall and Freyhof 2016). For example, 28% of freshwater fish species assessed for the International Union for Conservation of Nature (IUCN) Red List of Threatened Species are considered threatened with extinction (Tickner et al. 2020). In certain regions of the world, such as Australia, freshwater fish species have a heightened level of threat (Lintermans et al. 2024). Threats to freshwater fishes include: habitat loss and degradation, invasive species, over-exploitation, pesticides, pollution, water abstraction and flow alteration, and climate change (Arthington et al. 2016; Darwall and Freyhof 2016; Dudgeon et al. 2006).

The expansive Murray-Darling Basin (MDB) is now home to almost 60 species of native freshwater fishes (Lintermans 2023) and importantly almost 80% of the Basin's small-bodied freshwater fish species - those obtaining a maximum total length (TL) of less than 150 mm – are found in the lower Murray River, including the Coorong Lower Lakes and Murray Mouth (CLLMM) region (Wedderburn et al. 2017b). Small-bodied fishes typically possess traits, such as limited dispersal, short longevity and small ranges, that make them inherently at risk, which is exacerbated when species decline to small, fragmented populations (Hammer et al. 2009; Kopf et al. 2017; Liu et al. 2017; Olden et al. 2007). Yet, freshwater fishes of the MDB have been severely impacted, with native fish populations estimated to be at only 10% of pre-European settlement levels and almost three-quarters of fish species recognised as either rare or threatened on State, Territory, or National listings (Lintermans 2023; Lintermans et al. 2024). The River Murray downstream of the Darling River, and associated aquatic and floodplain systems, (e.g. the lower Murray River Ecological Community) is now also listed as Critically Endangered (DCCEEW 2026), implying that the habitat supporting species is equally at risk.

Freshwater fishes of the lower Murray River were substantially impacted by the prolonged Millennium Drought (2001–2009) (Wedderburn et al. 2012a; Wedderburn et al. 2017b). In the CLLMM region during the drought, the water level of the Lower Lakes receded to more than -1 m below sea level, which was accompanied by significant reductions in submerged aquatic vegetation cover, disconnection of fringing vegetation and elevated salinities (Kingsford et al. 2011; Mosley et al. 2012), leading to declines of freshwater fishes (Wedderburn et al. 2012a). Similarly, in the nearby Eastern Mount Lofty Ranges (EMLR) tributary streams, diminished stream flow resulted in reduced habitat availability and pool permanency. The reduced water availability and habitat, substantially impacted freshwater fishes across this period (Hammer et al. 2013; Wedderburn et al. 2012b; Whiterod et al. 2015; Zampatti et al. 2010). Two of the most impacted species were the nationally Vulnerable southern pygmy perch *Nannoperca australis* (MDB population) and its congener, nationally Endangered Yarra pygmy perch *Nannoperca obscura*. Both species experienced a contraction in range and a reduction in abundance, and remain in peril. Indeed, Yarra pygmy perch may be the first freshwater fish to have become extinct from the MDB (Wedderburn et al. 2022).

Since the Millennium Drought, conservation translocations have been a critical aspect of the management of southern pygmy perch and Yarra pygmy perch (see Hammer et al. 2013). Initially, to establish ex situ maintenance and breeding, as well as surrogate populations, small numbers of each species were removed from known locations during the Millennium Drought. This form of *assisted colonisation* with individuals moved to isolated waterbodies mostly outside of their natural range, provided a safeguard for the species. Following the drought, through the use of ex situ management, reintroductions of fish from surrogate wetlands and targeted water management in the Lower Lakes, some improvement has been made in southern pygmy perch populations, For instance, reintroductions continue to drive the regional recovery of southern pygmy perch following the loss of the species during the Millennium Drought (Marshall et al. 2022). Yet, other species such as Yarra pygmy perch have not re-established (Wedderburn et al. 2022). The long-term viability of these threatened fishes across the region remains tenuous. Continued targeted conservation efforts of the target species are therefore required in the Lower Lakes, to ensure long term self sustaining populations. However, questions remain about best reintroduction scenarios to ensure maximum population growth.

The impacts of climate change across in the Murray-Darling Basin, including in the CLLMM region, are forecast to be significant (Robertson et al. 2021; Whetton and Chiew 2021). In the CLLMM region, climate change will manifest through reducing freshwater inflows, warming of the land and water, more frequent and extreme disturbance events, and increasing sea level, which will lead to a cascade of ecological impacts over coming decades (Rees et al. 2022). Previous articulation of trajectories of environmental and ecological change emphasised the vulnerability of the CLLMM region to future change in climate (Grigg et al. 2022). This change will threaten the suitability of habitats that support threatened freshwater fish species in the CLLMM region; an anticipated to change under future climates. It is therefore paramount to simultaneously build resilience in populations and explore adaptation pathways to facilitate the long-term viability of threatened freshwater fish species.

The objective of this project is to advance understanding of the conservation ecology of southern pygmy perch and Yarra pygmy perch across the CLLMM region in the face of environmental change. This improved understanding is achieved by investigating:

- relationships between southern pygmy perch biotic metrics (i.e. recruitment strength, abundance and distribution) and abiotic metrics (i.e. managed water levels and habitat) using modelling and data from southern pygmy perch sampled in the CLLMM and EMLR region over the past 15 years (Figures 1 and 2),
- otolith increment formation to infer daily age and growth and explore what information may be gained from use of the method in determining drivers of recruitment, and
- a trial reintroduction release using parameters informed by modelling outcomes.

The outcome of the project will be used to inform management of wild and ex situ pygmy perch populations, reintroduction ecology, and the use of water for the environment.

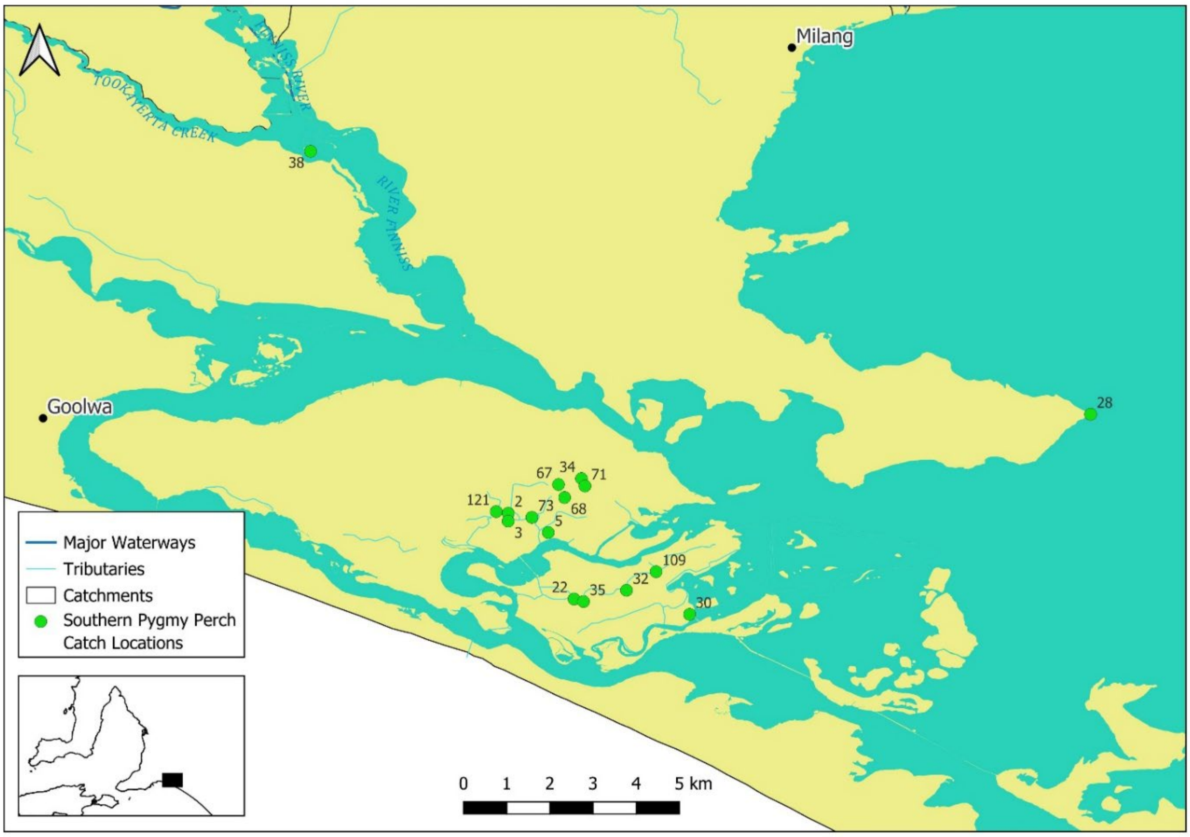

Figure 1.1. Southern pygmy perch sites sampled in the CLLMM region.

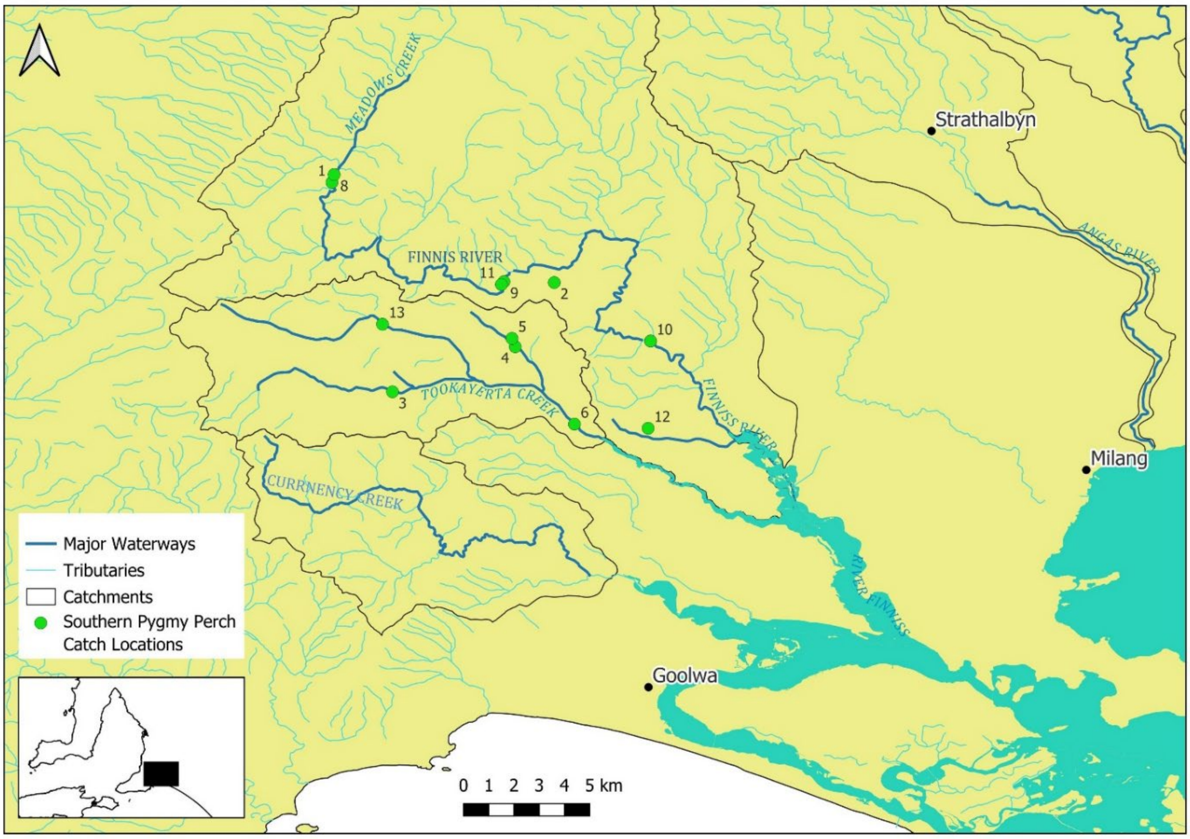

Figure 1.2. Southern pygmy perch sites sampled in the EMLR region.

2 Characterisation of southern pygmy perch population trends in the CLLMM

2.1 Introduction

The objective of this component was to characterise temporal trends in abundance and recruitment of southern pygmy perch *Nannoperca australis* in the Coorong, Lower Lakes and Murray Mouth (CLLMM) region, and to identify environmental variables driving trends in abundance. Analyses were conducted using long-term monitoring data collected from two regions: the Eastern Mount Lofty Ranges (EMLR) and the Lower Lakes.

Temporal population abundance trends were assessed using both empirical and modelled analyses. Empirical analyses provide insight into observed changes in abundance and size structure, while model-based analyses provide an assessment of underlying population trends by accounting for variation in sampling effort, spatial structure, and overdispersion in catch data.

Further, understanding recruitment of southern pygmy perch via size structure (length frequency analysis) provides an understanding of the dynamics of population trends (Crook et al. 2023). For example, poor recruitment contributing to a negative population trend should be identifiable in the relevant size structure analysis.

Recruitment is defined in this component as the successful addition of young-of-the-year (YOY) individuals to the population, inferred from size-frequency structure and temporal changes in relative abundance. Recruitment patterns were examined qualitatively using length-frequency distributions through time, in conjunction with modelled estimates of relative abundance.

Drivers of southern pygmy perch population trends were determined via empirical and modelled analyses. Candidate drivers (environmental correlates) of population trends were examined at both regional and site scales, to facilitate separation of broad drivers (e.g. climatic) and localised drivers (e.g. pool water depth).

2.2 Methods

2.2.1 Data acquisition, cleaning, and formatting

The southern pygmy perch population characterisation was done on long term monitoring data from two CLLMM projects assessing the health of threatened fish species, one in the EMLR, and the other in the Lower Lakes. All data analysis was done in R version 4.4.1 (R Core Team 2024). Population data was recorded during autumn monitoring work for between 2006 and 2025 for the EMLR and 2008 to 2025 for the Lower Lakes. The criteria for a sampling site to be included in the analyses was Southern pygmy perch were caught at least once, which saw seven and 16 sites included from the EMLR and Lower Lakes, respectively (see Figures 1.1 and 1.2). The method of sampling fish was backpack electrofishing and small fyke nets for the EMLR and Lower

Lakes, respectively. Hence, the data was analysed separately but in the same manner. For details on field sampling see Zukowski and Whiterod (2025) and Wedderburn and Barnes (2016a). Annual fish count, size (total length), and sampling-effort data were screened for errors, standardised by effort where appropriate, and formatted for empirical summaries and modelled analyses. Effort was defined as on time for electrofishing (EMLR) and soak time for fyke nets (Lower Lakes).

2.2.2 Analyses of population trends

Empirical analyses of population trends

Mean catch and standard error were calculated separately for each dataset (EMLR and Lower Lakes). The analysis was weighted by sampling year. To provide a relative abundance (or catch per unit effort), count data was divided by effort (electrofishing on time [seconds] and fyke net soak time [hours]).

Modelled analyses of population trends

To provide further temporal information on population trends in the EMLR and Lower Lakes, we modelled variation in southern pygmy perch relative abundance (counts offset by effort [see below]) using a Bayesian generalized additive mixed modelling (GAM) framework. The GAM has been found to be suitable for modelling Southern pygmy perch populations due to the possibility of nonlinear relationships between covariates (Wedderburn et al. 2019). Further, the Bayesian method has been found to provide increased flexibility in available error distributions (compared to frequentist methods) in another MDB fish population study (Crook et al. 2023). The response variable was the observed count (value), treated as integer-valued count data. Because the data exhibited overdispersion, counts were modelled using a negative binomial error distribution with a log link function. The Lower Lakes used a zero inflated variation of the negative binomial error distribution due to the high probability of zero southern pygmy perch sampled at a given site (e.g. stochastic nature of catches [more below]).

To account for differences in sampling effort among observations, we included the log of the effort metrics described above, such that the model estimates rates (relative abundance) rather than raw counts. This precaution ensured that expected counts scale proportionally with sampling effort.

Temporal variation was modelled as the predictor of southern pygmy perch relative abundance (observed counts offset by effort) using a smooth function of time, expressed as days since the earliest sampling date. We fitted a thin-plate regression spline with a basis dimension of k=3, allowing for a low-complexity nonlinear trend, while avoiding overfitting. Spatial structure was incorporated by including sampling site as a random intercept, allowing baseline abundance to vary among locations, while assuming a common temporal pattern. To control for repeat sampling, the Lower Lakes also had sampling date included as a random intercept.

For the EMLR the model can be written as:

$$y_i \sim \text{Negative binomial}\ (\mu_i, \phi)$$

$$\log \mu_i = \alpha + f(\text{Days}_{\ i}) + b_{\text{Site}[i]} + \log(\text{Electrical seconds}_{\ i})$$

where y_i is the observed count, μ_i is the expected rate, ϕ is the negative-binomial dispersion parameter, a is the intercept, *f(days $_i$)* is the smooth function of days since first sampling event, $b_{(\text{Site}[i]}$ is the random intercept for sampling site, and Electrical seconds $_i$ is the electrofishing time used as the effort offset.

For the Lower Lakes the model can be written as:

$$y_i \sim \text{Zero inflated negative binomial}\ (\mu_i, \phi, \pi_i)$$
$$\log \mu_i = \alpha + f(\text{Days}_{\ i}) + b_{\text{Site}[i]} + b_{\text{Date}[i]} + \log(\text{Soak hours}_{\ i})$$

where π_i is the zero-inflation probability (probability of an extra zero), $b_{(\text{Date}[i]}$ is a random intercept for sampling date, and soak hours $_i$ is the fyke net sampling effort used as a log-offset (otherwise terms are the same as previously described).

Model conditions included Markov chain Monte Carlo (MCMC) sampling with four chains, each run for 5,000 iterations, including a burn-in of 2,000 iterations. Convergence was assessed using trace plots, effective sample sizes, and the potential scale reduction factor. To reduce the risk of divergent transitions, we increased the target acceptance rate (adapt delta = 0.999). Posterior predictive checks were used to evaluate overall model fit and the adequacy of the assumed error distribution. The modelled trend would be considered significant if the posterior credible intervals exclude zero. These conditions applied to all models fitted in this study.

Empirical analyses of recruitment

Recruitment patterns of Southern pygmy perch were examined using length-frequency distributions constructed annually using the same data as the population trend analyses. Individual fish lengths (total length [mm]) were grouped into fixed-width length classes and plotted for each sampling period, allowing shifts in the size structure of the population to be visualised over time. Length-frequency graphs were used to identify the timing, strength, and progression of new cohorts entering the population.

Recruitment events were inferred from the appearance and subsequent growth of distinct modes at smaller length classes, consistent with the arrival of young-of-the-year (YOY) or newly recruited individuals (<50mm is the arbitrary size value used to infer YOY). Temporal changes in modal structure were assessed qualitatively by tracking changes in the position and relative prominence of these modes across successive sampling periods. This approach provides a visual and intuitive assessment of cohort dynamics.

To ensure comparability across sampling occasions, length-frequency plots (smooth densities) were scaled by the number of observations per year (but not by sampling effort). Plots were constructed using consistent bin widths across time. Length-frequency data were interpreted (in the discussion) in conjunction with modelled estimates of relative abundance, to provide complementary evidence for recruitment timing and intensity. At least three fish need to be in the annual length sample to form a density ridge.

2.2.3 Drivers of population trends (regional and site)

Empirical drivers of population trends

Relative abundance and candidate environmental driver data (regional and site level) were standardised using the base R function scale. This procedure centres the data by subtracting its sample mean and divides by its sample standard deviation (calculated with denominator n−1). The resulting transformed data therefore have a mean of zero and a standard deviation of one. Standardising in this way prepare the abundance data for initial graphical comparison to candidate drivers of abundance (e.g. creates a common order of magnitude). An important outcome of the empirical analysis is that it allows candidate regional abundance drivers to be preselected for inclusion in modelling (as covariates). Thus, streamlining the modelling process that can be complicated by a large suite of broadscale candidate drivers (e.g. collinearity between climatic and river flow covariates). Further, it aids model type selection (i.e. whether to fit a nonlinear or linear model).

Candidate regional environmental drivers of abundance were sourced mainly from the Department for Environment and Water, (more details are provided below). These included river and lake discharge, water levels, rainfall, and broad climatic indexes. For brevity, only those that were found to significantly drive southern pygmy perch relative abundance are described herein.

Antarctic Oscillation (AAO) (also known as Southern Annular Mode [SAM]) is a large-scale climate pattern describing the north–south movement of the westerly wind belt that circles Antarctica. In positive mode, westerlies contract poleward (often brings drier conditions to southern Australia) and negative, the opposite occurs (i.e. the westerlies and frontal systems are targeted at southern Australia). Indexed monthly AAO data (<=2025) was downloaded from NOAA Physical Sciences Laboratory (https://psl.noaa.gov/data/20thC_Rean/timeseries/monthly/SAM) on the 24/09/2025. For empirical comparison to southern pygmy perch relative abundance the monthly summaries of AAO data were also summarised as annual means (i.e. like the relative abundance of Southern pygmy perch). As it is possible that the effect of AAO may be lagged (i.e. due to its influence on the southern pygmy perch breeding success) lag times from 0 to 3 years were tested visually via fitted linear models (Figure A1). This candidate driver was only compared to EMLR southern pygmy perch relative abundance data as it is only likely to be influential in this catchment (e.g. the Lower Lakes water can have its origin in northern New South Wales and south-east Queensland component of the MDB).

The mean lake water level (Australian Height Datum, AHD, m) of Lake Alexandrina has been found previously to be a driver of southern pygmy perch abundance in the Lower Lakes (Wedderburn et al. 2019). Water levels in the Lower Lakes are influenced by the complex interaction of regulated river flows, barrage operation, evaporation, etc., and is known to affect the amount of available southern pygmy perch habitat and fish food (Wedderburn et al. 2019). Lake water level data (2006 to 2025) was downloaded from Water Data SA (https://water.data.sa.gov.au) on 27/09/2025. For empirical comparison to southern pygmy perch relative abundance, the daily mean lake water level data were also summarised as annual sampling period means (i.e. like the relative abundance of southern pygmy perch). As it is possible that the effect of lake level may be lagged (i.e. due to its influence on the southern pygmy perch breeding success) lag times of zero, six, and nine months were tested visually via fitted linear models (Figure A2). The water level driver was only deemed useful in the Lower Lakes as the EMLR southern pygmy perch population is not influence by water levels in the Lower Lakes. Linear model lag exploration analysis was done with and without the ‘Millennium Drought’ period (i.e. including 2009 to 2013 and from 2013) to detect the abnormal drought condition’s impact on results. As results were very similar, it was decided that only the ‘without Millennium Drought’ results would be presented here as the lake levels are more realistic in terms of what will (likely) occur in the future.

Site level candidate environmental drivers were recorded at the time of southern pygmy perch sampling in the EMLR and Lower Lakes, (see Zukowski and Whiterod (2025) and Wedderburn and Barnes (2016a)). These candidates are shown in Table (2.1) with summary statistics. To test for relationships between candidate drivers and southern pygmy perch relative abundance all (scaled) data was analysed graphically. Species richness was estimated by the number of fish species detected during sampling events.

Table 2.1. List of site level environmental candidate drivers for the Eastern Mount Lofty Ranges and Lower Lakes recorded at the time of southern pygmy perch sampling. Shown are the number of records, completeness out of a possible number of records (113 and 384 EMLR and Lower Lakes, respectively) and minimum, maximum, and total values where appropriate. Eastern Gambusia *Gambusia holbrooki*. Redfin Perch *Perca fluviatilis*.

POTENTIAL DRIVER	RECORDS (N)	COMPLETENESS (%)	MINIMUM	MAXIMUM	TOTAL
Eastern Mount Lofty Ranges:					
Conductivity (µs/cm)	110	97.3	250	8090	
Depth (maximum [m])	111	98.2	0.3	3.3	
Eastern gambusia (n)	113	100	0	100	464
pH	108	95.6	5.86	8.52	
Redfin perch (n)	113	100	0	11	64
Species richness	113	100	0	5	
Macrophytes (%)	110	97.3	0	30	
Temperature (°C)	110	97.3	10.1	27.8	
Transparency (m)	88	77.9	0.1	1.8	
Lower Lakes:					
Redfin perch (n)	384	100	0	4585	12,609
Eastern gambusia (n)	384	100	0	5787	64,859
Species richness	384	100	1	13	
pH	384	100	5.49	9.46	
Conductivity (µs/cm)	384	100	363	45,100	
Temperature (°C)	384	100	12.9	28.1	
Secchi (mm)	322	83.9	80	1200	
Mean depth (cm)	384	100	10	118	
Macrophytes (%)	384	100	0	100	

Note: EMLR macrophytes refers to proportional coverage of submerged macrophytes only, whereas Lower Lakes macrophytes refers to proportional coverage of submerged and emergent combined.

Modelled drivers of population trends (regional and site)

Regional models

The AAO data with the two-year lag was fitted to a (similar) Bayesian GAM as a candidate driver of EMLR southern pygmy perch abundance. The key difference was that the time (days since sampling began) covariate was replaced by the AAO, which was fitted as a linear predictor, as the empirical analysis suggested this approach was most appropriate (see Results). The other difference is that the sampling date was fitted as a random effect, which was not done in the equivalent abundance model due to the potential for collinearity with time. The model can be written as:

$$y_i \sim \text{Negative Binomial}\ (\mu_i, \phi)$$
$$\log \mu_i = \alpha + \mathrm{AAO}_{\ i} + b_{\text{Site}[i]} + b_{Date[i]} + \log(\text{Electrical seconds}_{\ i})$$

where AAO $_i$ is the annual mean Antarctic Oscillation Index lagged by 2 years (otherwise terms are the same as previously described).

The mean lake water level data from the empirical and fitted smooth analysis (above), with the six-month lag, was fitted to a (similar) Bayesian GAM as a candidate driver of Lower Lakes southern pygmy perch abundance. The key difference was that the time (days since sampling began) covariate was replaced by the lake level. Also, the Millennium Drought sensitivity analyses were conducted in a manner akin to the lag effect exploratory analyses described above, and only the 'without' analysis is presented herein, as it presents a more realistic model without negative lake levels. The model can be written as:

$$y_i \sim \text{Zero inflated negative binomial}\ (\mu_i, \phi, \pi_i)$$
$$\log \mu_i = \alpha + f(\text{Lake level}_{\ i}) + b_{\text{Site}[i]} + b_{\text{Date}[i]} + \log(\text{Soak hours}_{\ i})$$

where f(Lake level $_i$) is a smooth function of the mean Lake Alexandrina level (m AHD) lagged by 6 months (otherwise terms are the same as previously described).

Site level models

Prior to site level modelling all candidate drivers were tested for collinearity (Figures A3 and A4) Pearson's correlation coefficients were <0.6, indicating no collinearity concerns, for both the EMLR and Lower Lakes data. All site candidate drivers listed in Table 1 were fitted as smooth functions, as the relationship type with southern pygmy perch abundance was not clear post the empirical analyses. Other than the covariates, the models were the same as the regional models described above. All covariates were included as full models and the models reduced until only significant drivers remained. Only the full model equations are presented below. The Lower Lakes site model used all available data (i.e. including the 'Millennium Drought' period) as negative values were not found in site covariates.

The EMLR site model can be written as:

$$y_i \sim \text{Negative binomial}\ (\mu_i, \phi)$$

$$\log \mu_i = \alpha + f(Redfin_i) + f(Gambusia_i) + f(pH_i) + f(Conductivity_i) + f(Species\ richness_i) + f(Depth_i) + f(Transparency_i) + f(Macrophytes_i) + f(Temperature_i) + b_{Site[i]} + b_{Date[i]} + \log(Electrical\ seconds_i)$$

where f(·) denotes a smooth function for each covariate: redfin, eastern gambusia, pH, conductivity, species richness, depth, transparency, macrophytes, and temperature (otherwise terms are the same as previously described).

The Lower Lakes site model can be written as:

$$y_i \sim \text{Zero inflated negative binomial } (\mu_i, \phi, \pi_i)$$
$$\log \mu_i = \alpha + f(Conductivity_i) + f(pH_i) + f(Temperature_i) + f(Secchi_i) + f(Depth_i) + f(Macrophytes_i) + f(Species\ richness_i) + f(Gambusia_i) + f(Redfin_i) + b_{Site[i]} + b_{Date[i]} + \log(Soak\ hours_i)$$

where f(·) denotes a smooth function for each covariate: conductivity, pH, temperature, Secchi depth, macrophytes, species richness, redfin, and eastern gambusia (otherwise terms are the same as previously described).

2.3 Results

2.3.1 Analyses of population trends

Empirical analyses of population trends

Eastern Mount Lofty Ranges

In the EMLR, southern pygmy perch annual mean abundance varied between two and 50 individuals per sampling site (2,290 southern pygmy perch total, figure 2.1). Error bars indicated high degree of variability in abundance and implied a mean variance relationship (increased variability when higher mean abundance). The mean relative abundance displayed a generally similar trend to the raw data; however, it suggested that the catch per unit effort was higher in the initial three-years of sampling (2007–2009) (Figure 2.1). Abundance peaked in 2008 and was lowest in 2024 with considerable variability over time.

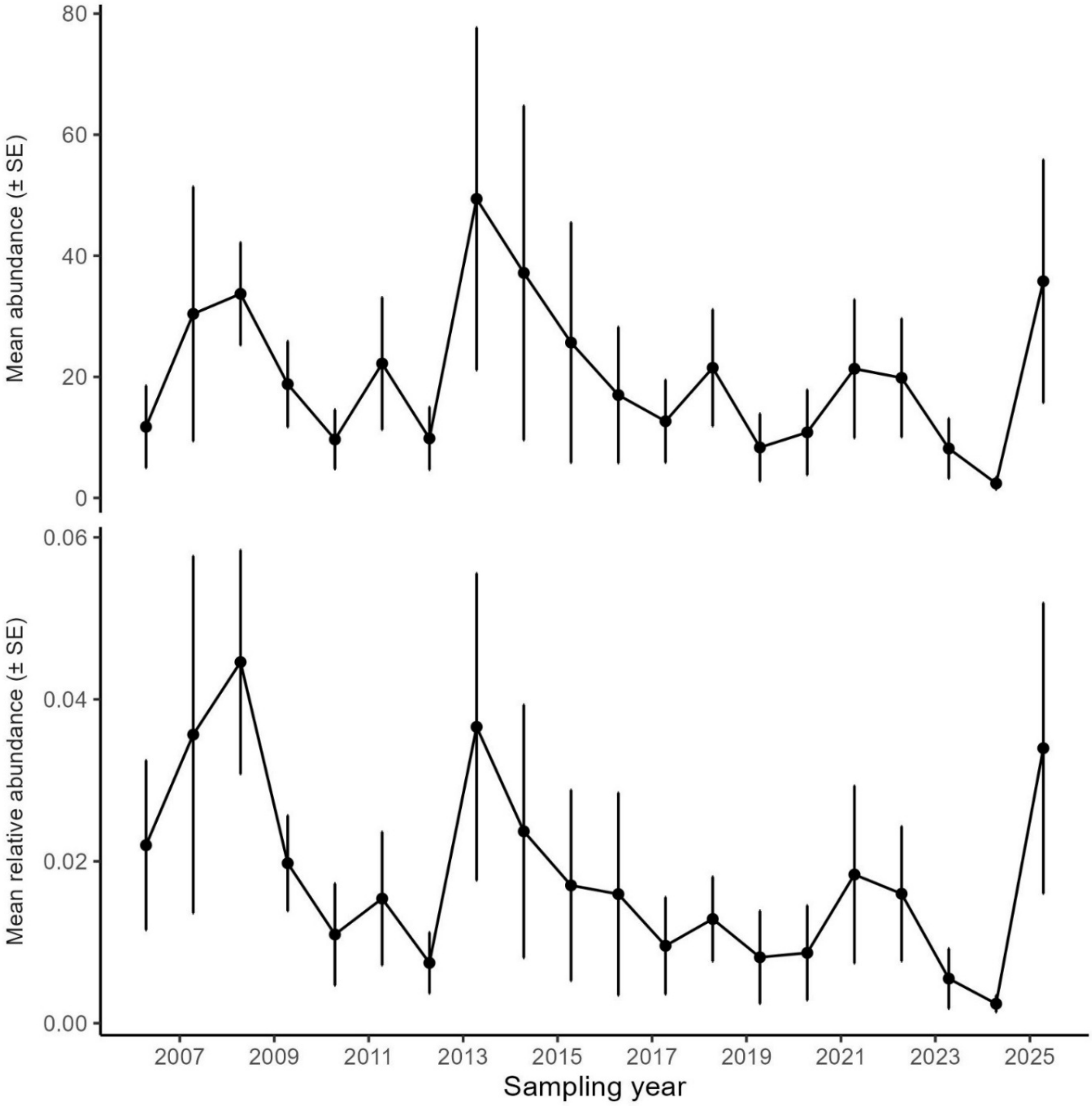

Figure 2.1. Eastern Mount Lofty Ranges southern pygmy perch annual mean abundance (top) and annual mean relative abundance (bottom) with ±1 standard error.

Lower Lakes

In the Lower Lakes, southern pygmy perch annual mean relative abundance varied between absence and 10 individuals per sampling site (1,533 southern pygmy perch total, Figure 2.2). Like the EMLR, error bars indicated the high degree of variability in abundances among sites in any given year. The mean relative abundance predominantly showed a similar trend to the raw data, indicating very similar effort over time at the sampling site level (Figure 2.2). Southern pygmy perch relative abundance peaked in 2024, and no fish were observed between 2010 and 2012.

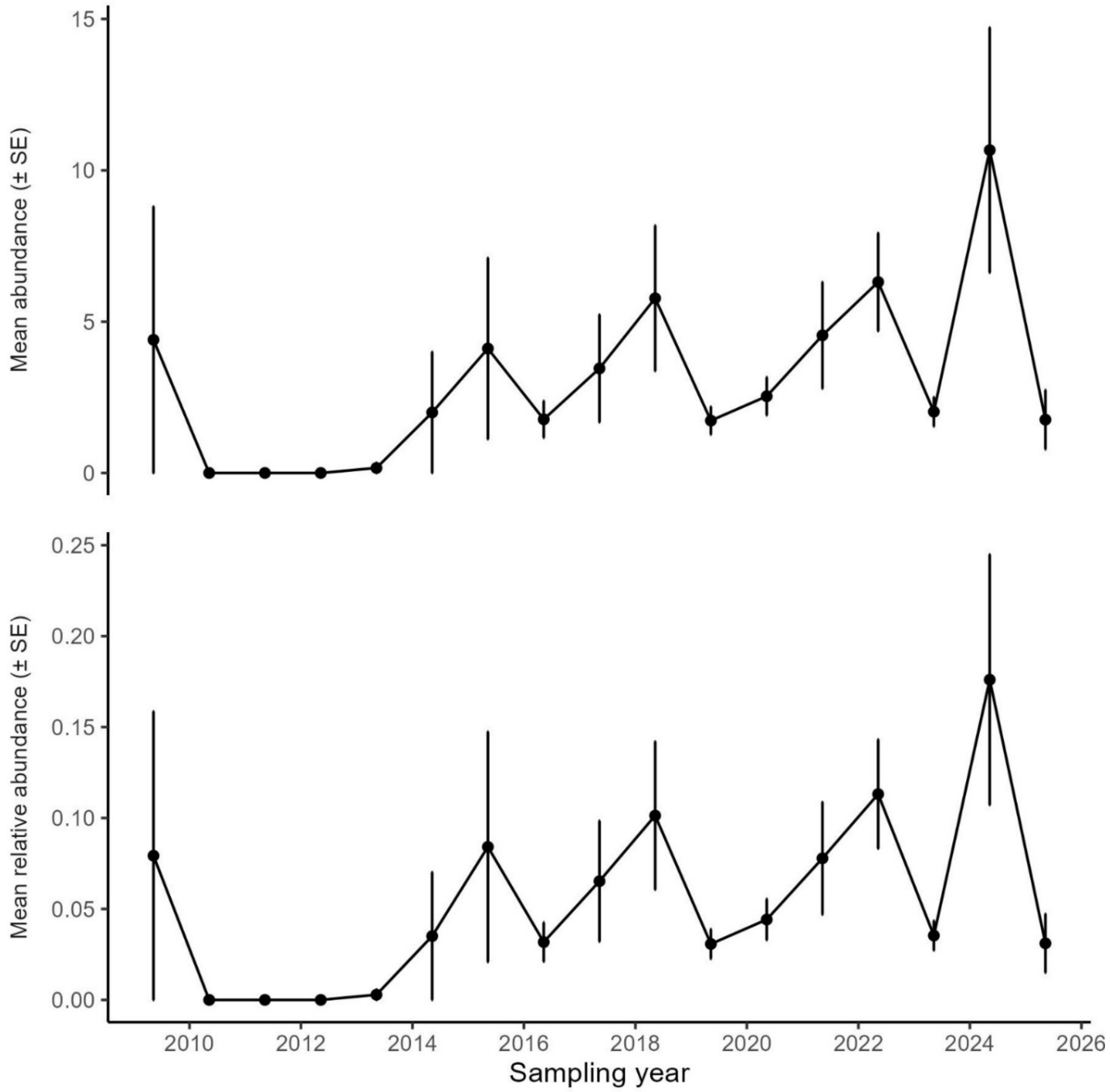

Figure 2.2. Lower Lakes southern pygmy perch annual mean abundance (top) and annual mean relative abundance (bottom) with ±1 standard error.

2.3.1.2 Modelled analyses of population trends

Eastern Mount Lofty Ranges

In the EMLR, Bayesian GAM predicted southern pygmy perch relative abundance to significantly decline over time (Figure 2.3). The credible intervals (95%) suggest high uncertainty in the predictions early in the sampling period (~ first three years). This time is when relative abundance peaked (Figure 2.3). Greater certainty in the predictions was evident from ~2013 onwards, when the model-predicted a severe decline in the population. During peak relative abundance the model-predicted one southern pygmy perch sampled every 20 seconds of electrofishing (based on 0.05 Southern pygmy perch per electrofishing second in 2006). From ~2020 the model-predicted extremely low relative abundance in the EMLR. The 2025 increase in empirical relative abundance (Figure 2.2) is not predicted by the model, but the credible interval does widen slightly.

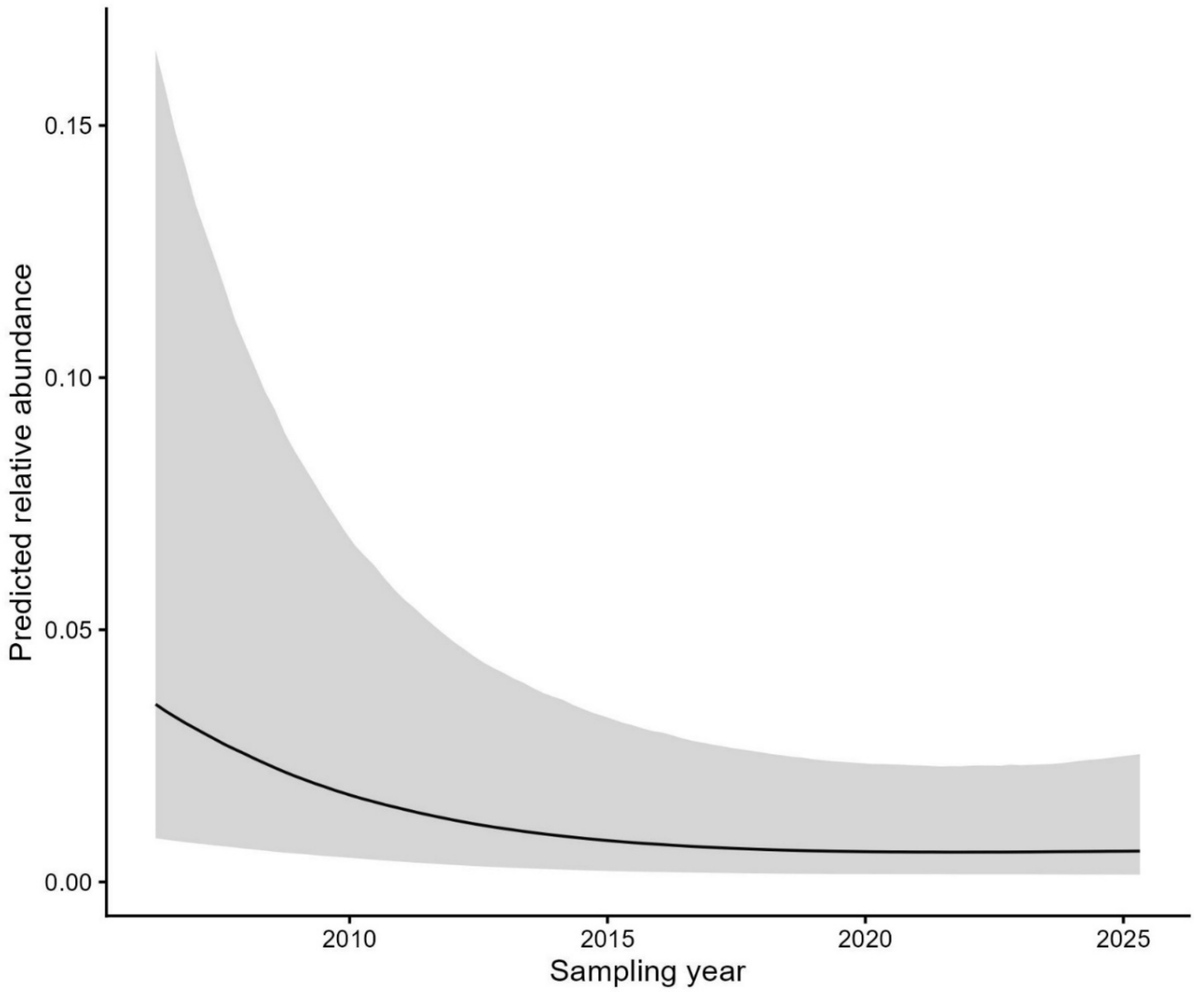

Figure 2.3. Predicted southern pygmy perch relative abundance in the Eastern Mount Lofty Ranges as a function of time (project duration). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

Lower Lakes

In the Lower Lakes, the Bayesian GAM predicted relative abundance, which mainly showed an increase of southern pygmy perch over time, although there was a decline in predicted relative abundance in the near term (from ~2021) (Figure 2.4). Nevertheless, throughout the sampling period, 95% credible intervals suggested relatively large uncertainty in the predictions, and hence lack of statistical significance. During peak relative abundance (~2020) the model is predicting that ~two southern pygmy perch were being sampled per fyke net hour. At the lowest level, less than 0.5 southern pygmy perch are predicted to be sampled an hour, with the lower credible interval suggesting there is a 95% chance of an absence of southern pygmy perch.

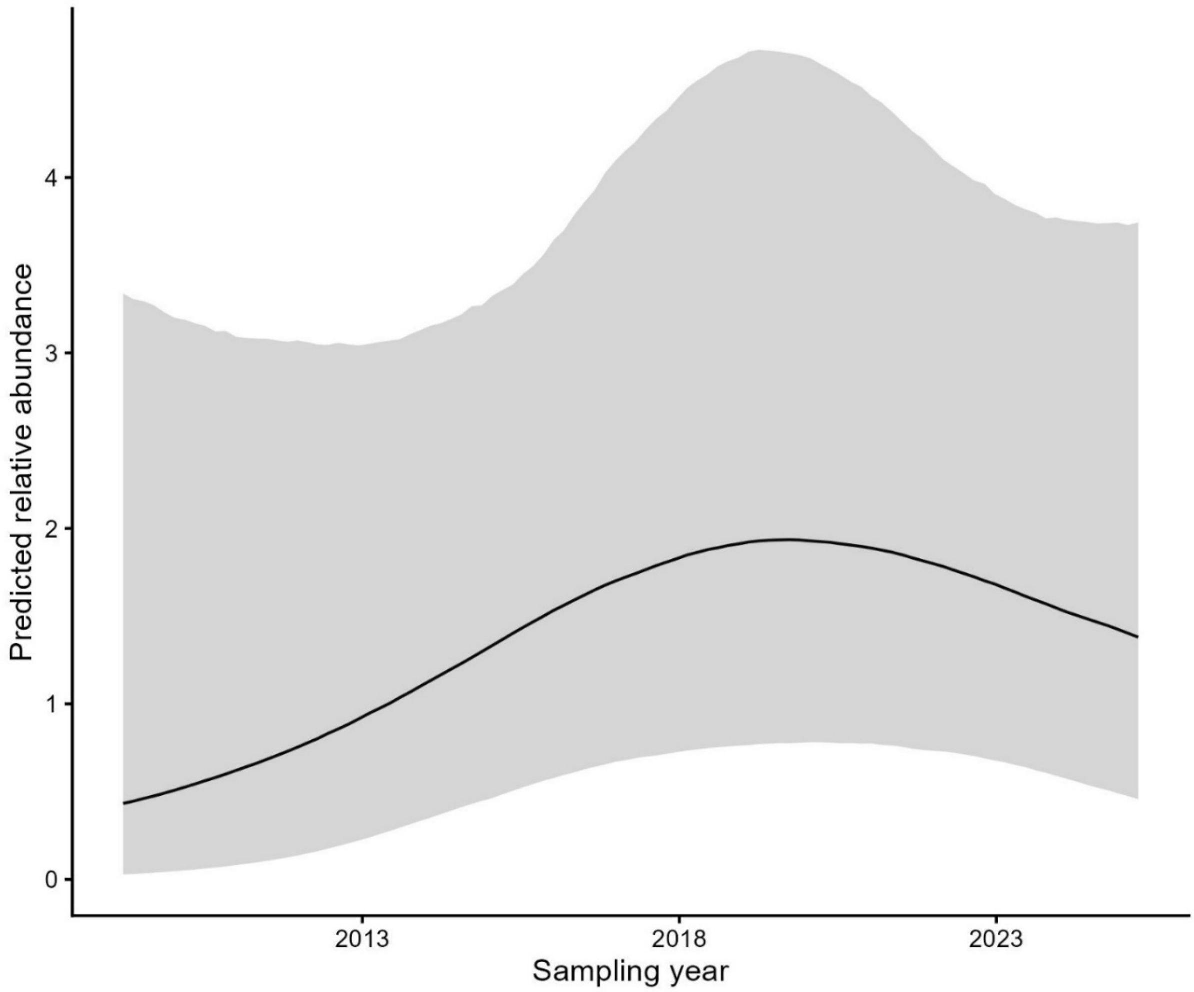

Figure 2.4. Predicted southern pygmy perch relative abundance in the Lower Lakes as a function of time (project duration). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

Empirical analyses of recruitment

Eastern Mount Lofty Ranges

The size range of EMLR southern pygmy perch was 13 to 96 mm with the centre of the size distribution at 44 mm (mean and median). The first and third quartile sizes were 37 and 52 mm, respectively. The EMLR size structure showed truncation over time (Figure 2.5). This change is mainly due to the virtual disappearance of the small southern pygmy perch size mode (~<30mm), particularly after 2016. Distributions are generally found either side of the red vertical line (indicating the arbitrary limit of the YOY southern pygmy perch size), but the main part of the distribution is normally left of the YOY size, indicating populations are predominantly putative YOY. An exception here is 2025 with a strong mode past the YOY size.

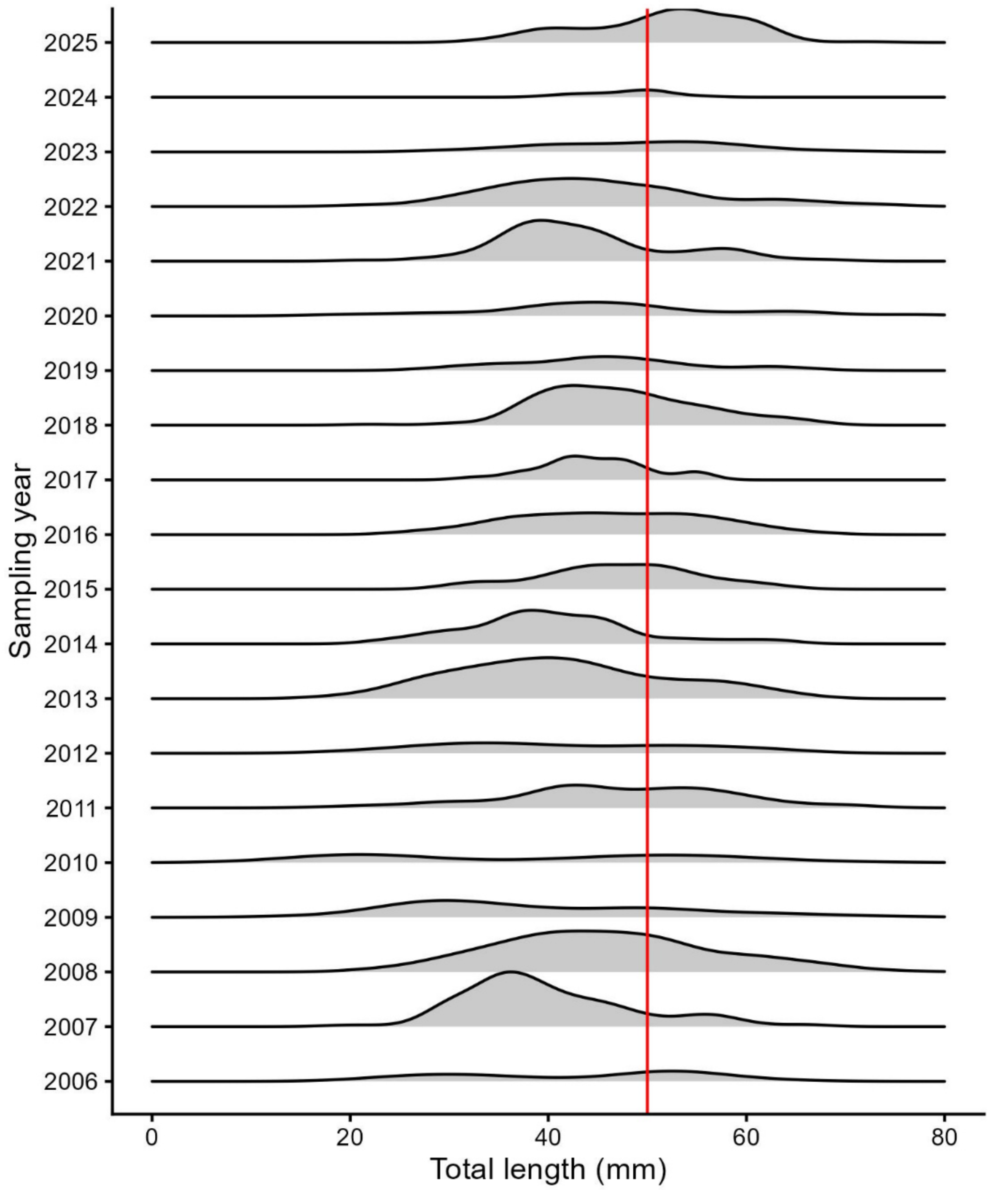

Figure 2.5. EMLR Kernel-density estimates of total length (mm) for southern pygmy perch collected during autumn surveys in the Eastern Mount Lofty Ranges, 2006–2025. Curves are weighted to annual abundance to represent population-level length structure. Higher densities are indicated by greater shaded area. The vertical red line indicates the presumed upper limit of the size of young of the year.

Lower Lakes

The size range of Lower Lakes southern pygmy perch was 21 to 77 mm, with the centre of the size distribution at ~44 mm (mean = 44 and median = 43 mm). The first and third quartile sizes were 36 and 51 mm, respectively. In contrast to the EMLR, the Lower Lakes southern pygmy perch size structure showed reducing truncation over time (Figure 2.6). After 2016, nearly all length-frequency distributions were bimodal. Distributions are mainly concentrated on the left side of the red vertical line (indicating the arbitrary limit of the YOY southern pygmy perch size) indicating populations are predominantly dominated by YOY fish. An exception was 2021 where a relatively strong mode larger than the YOY size threshold was evident. Prior to 2016, few individuals were sampled with unstable size structure indicating poor population status during this period.

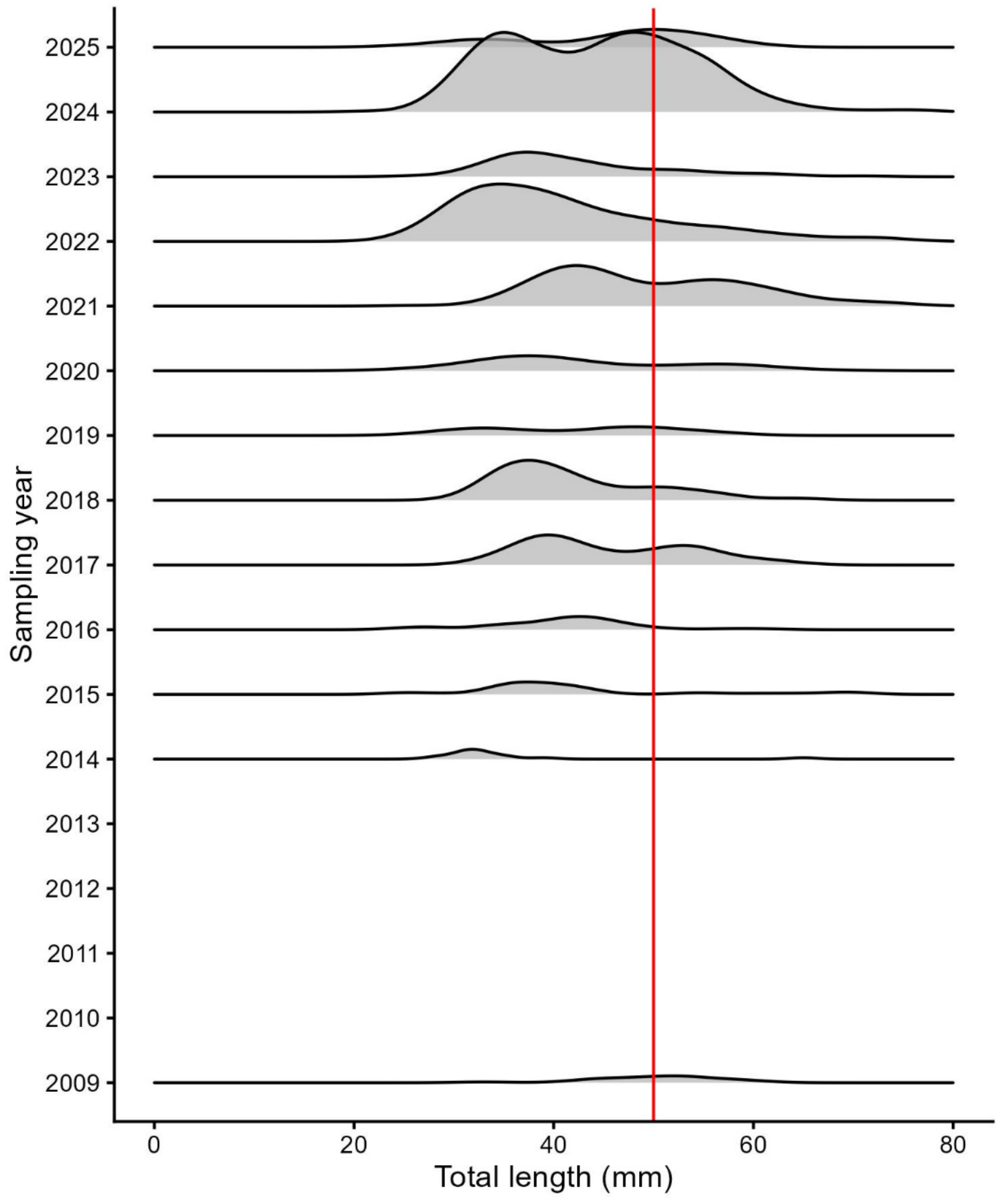

Figure 2.6. Lower Lakes Kernel-density estimates of total length (mm) for southern pygmy perch collected during autumn surveys in the Lower Lakes, 2009–2025. Curves are weighted to annual abundance to represent population-level length structure. Higher densities are indicated by greater shaded area. The vertical red line indicates the presumed upper limit of the size of young of the year.

2.3.2 Drivers of population trends

Regional drivers of population trends

Eastern Mount Lofty Ranges (empirical and modelled analyses)

In the EMLR, the AAO with a lag time of two years was found to be the most influential driver of regional Southern pygmy perch relative abundance (Figure A1). Empirical analysis showed an inverse relationship, with positive AAO driving a decrease in southern pygmy perch relative abundance (Figure 2.7). The negative relationship appeared stronger after 2009.

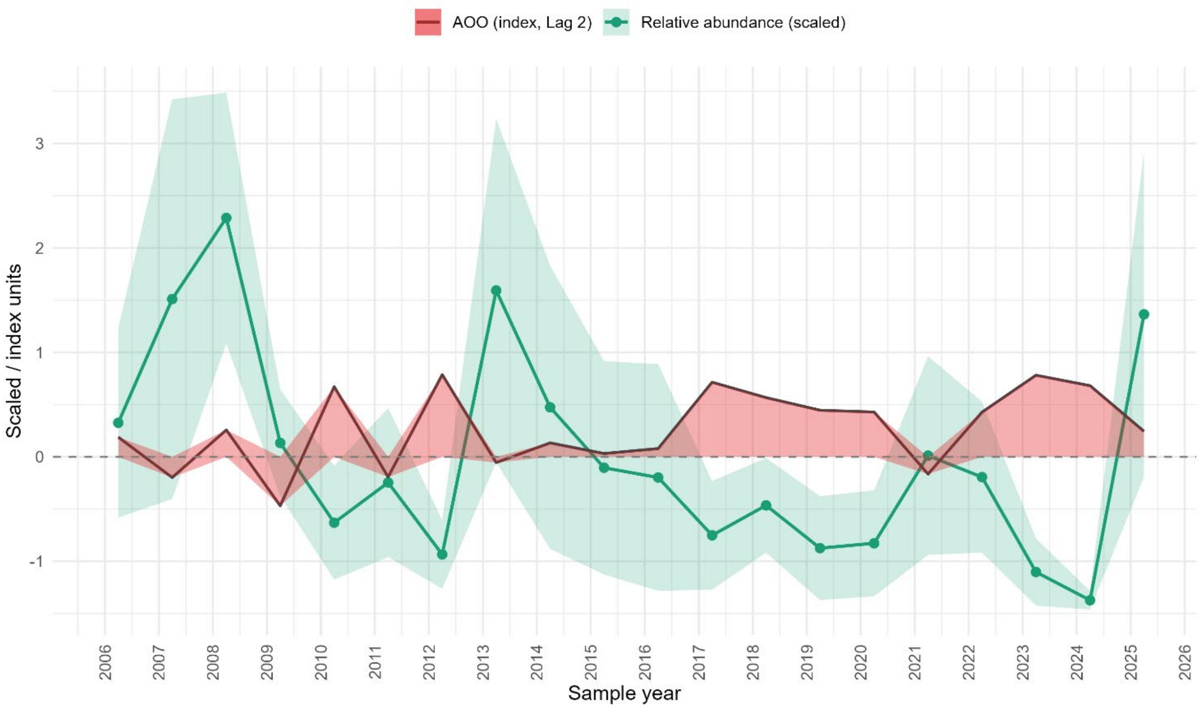

Figure 2.7. Time-series of scaled Eastern Mount Lofty Ranges southern pygmy perch relative abundance (green line and shaded uncertainty band) and the Antarctic Oscillation Index with a two-year lag (Lag 2, red polygon), shown over the full sampling period.

The model-predicted southern pygmy perch relative abundance declines monotonically as lagged mean annual AOO increased (from ~0.02 at AAO = −1 to ~0.005 southern pygmy perch per electrofishing second at AAO = 1.5), supporting the lower southern pygmy perch abundance following more positive AAO conditions theory (Figure 2.8). Uncertainty was greatest at higher predicted relative abundance, consistent with wider credible intervals in that range.

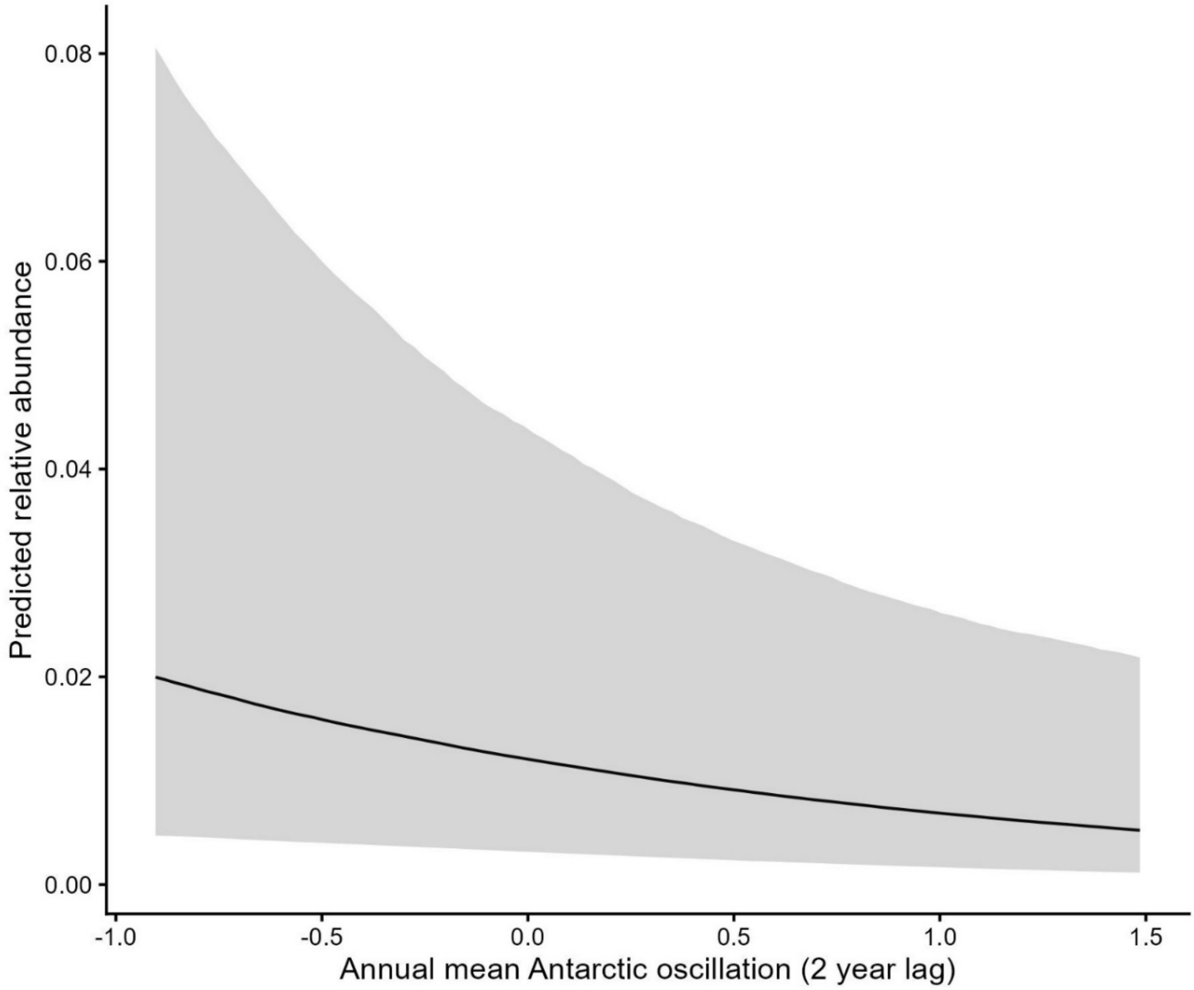

Figure 2.8. Predicted Eastern Mount Lofty Ranges southern pygmy perch relative abundance as a function of the annual mean Antarctic Oscillation Index with a two-year lag. The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

Lower Lakes (empirical and modelled analyses)

In the Lower Lakes, the most influential driver of southern pygmy perch relative abundance was the mean Lake Alexandrina water level, with a lag time of six months (previous September) (Figure A2). Empirical analysis showed a positive relationship where elevated lake levels promoted increased southern pygmy perch abundance (Figure 2.9). Abundances did, however, still fluctuate, even in times of elevated lake water level. Historically low lake levels in the period 2009 to 2011 drove a three-year collapse in southern pygmy perch.

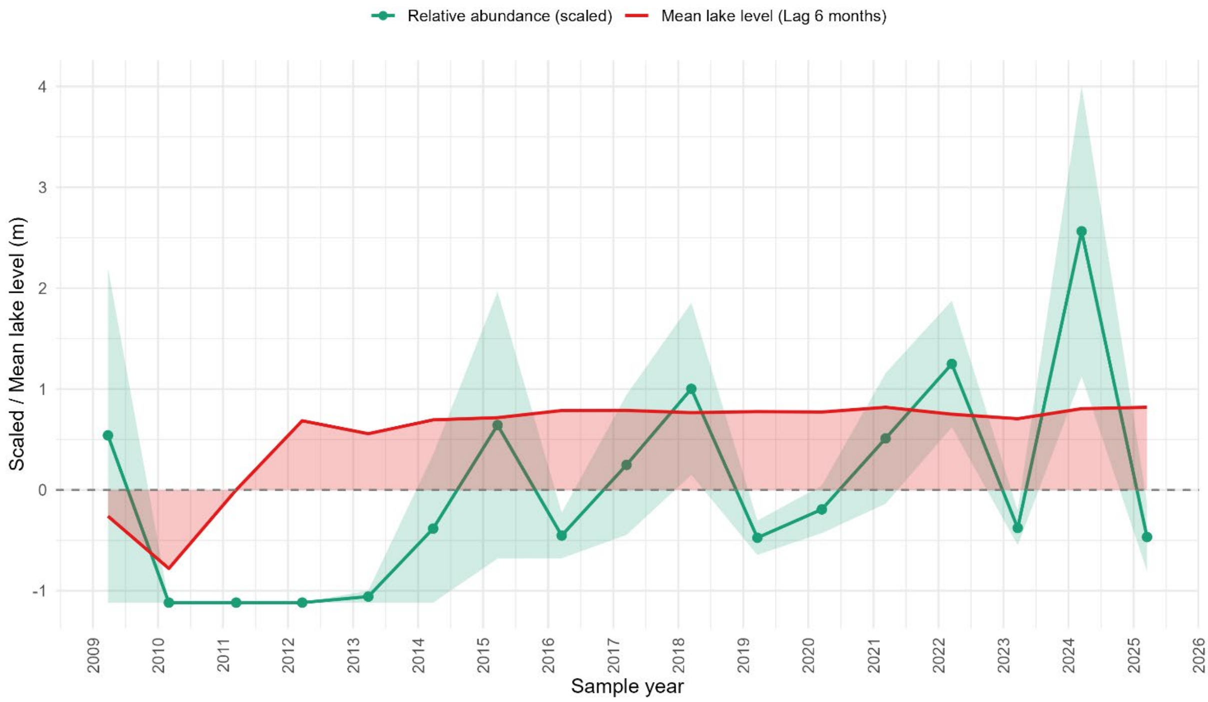

Figure 2.9. Time-series of scaled Lower Lakes southern pygmy perch relative abundance (green line and shaded uncertainty band) and the mean Lake Alexandrina water level with a 6-month lag (red polygon), shown over the full sampling period.

Model-predicted southern pygmy perch relative abundance increased with increasing September mean lake level, in a curve linear manner (i.e. a modest increase in southern pygmy perch at mid lake water levels and more pronounced at higher water levels [Figure 2.10]). The model suggested that southern pygmy perch abundance is very low at the lowest lake water levels and around two southern pygmy perch per fyke net hour at the higher lake levels (0.8m AHD). Model uncertainty increased with higher predicted relative abundance, with the credible interval widest at high levels.

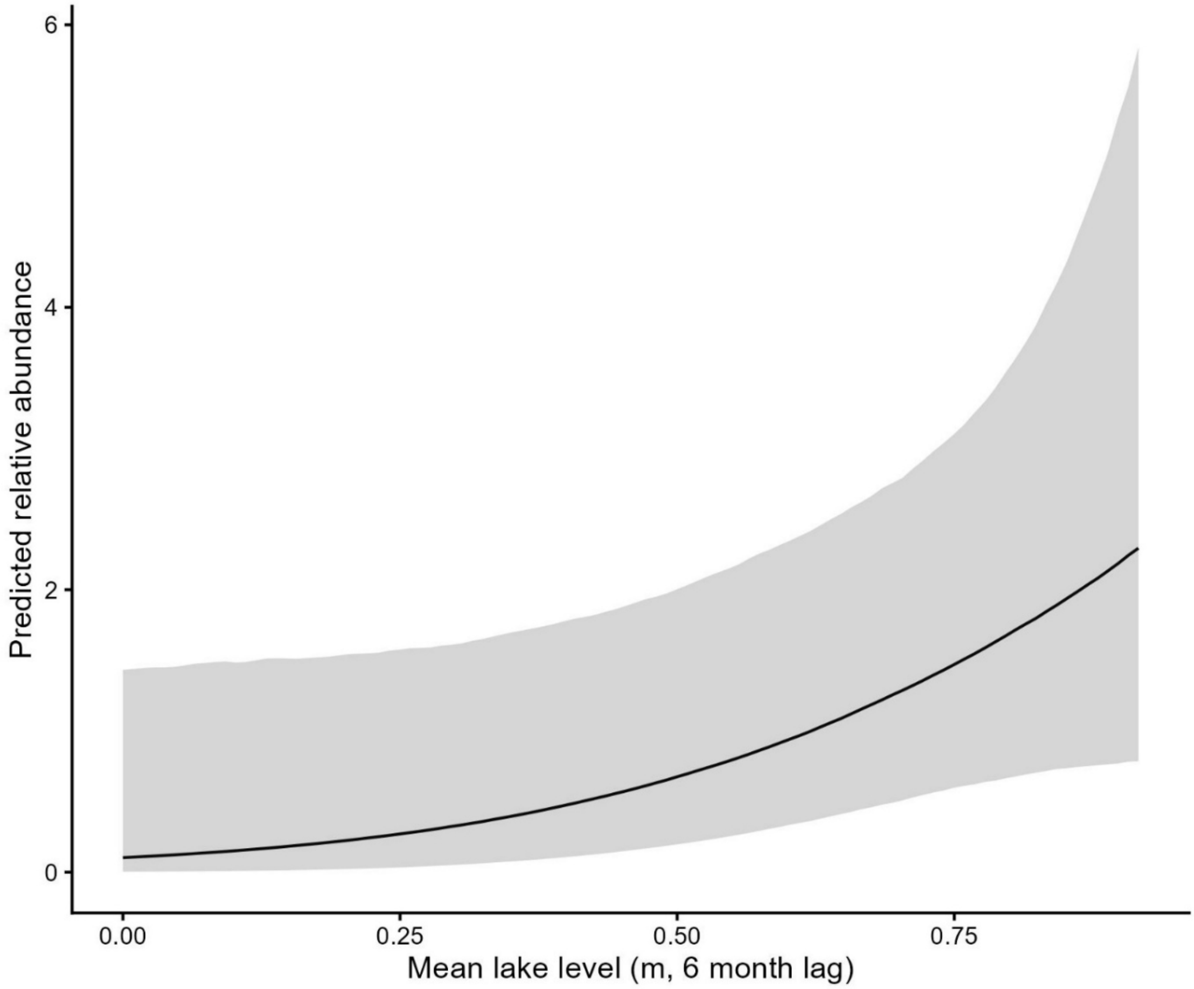

Figure 2.10. Predicted Lower Lakes southern pygmy perch relative abundance as a function of the mean Lake Alexandrina water level with a 6-month lag. The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

Site level drivers of southern pygmy perch population trends

Eastern Mount Lofty Ranges (empirical and modelled analyses)

At the site scale in the EMLR, the proportional coverage of macrophytes was found to be the most influential driver of southern pygmy perch relative abundance (Figure 2.11 and Figure A5). Empirical analysis of macrophyte coverage and southern pygmy perch relative abundance shows a positive relationship, where increasing coverage drives an increase in southern pygmy perch abundance.

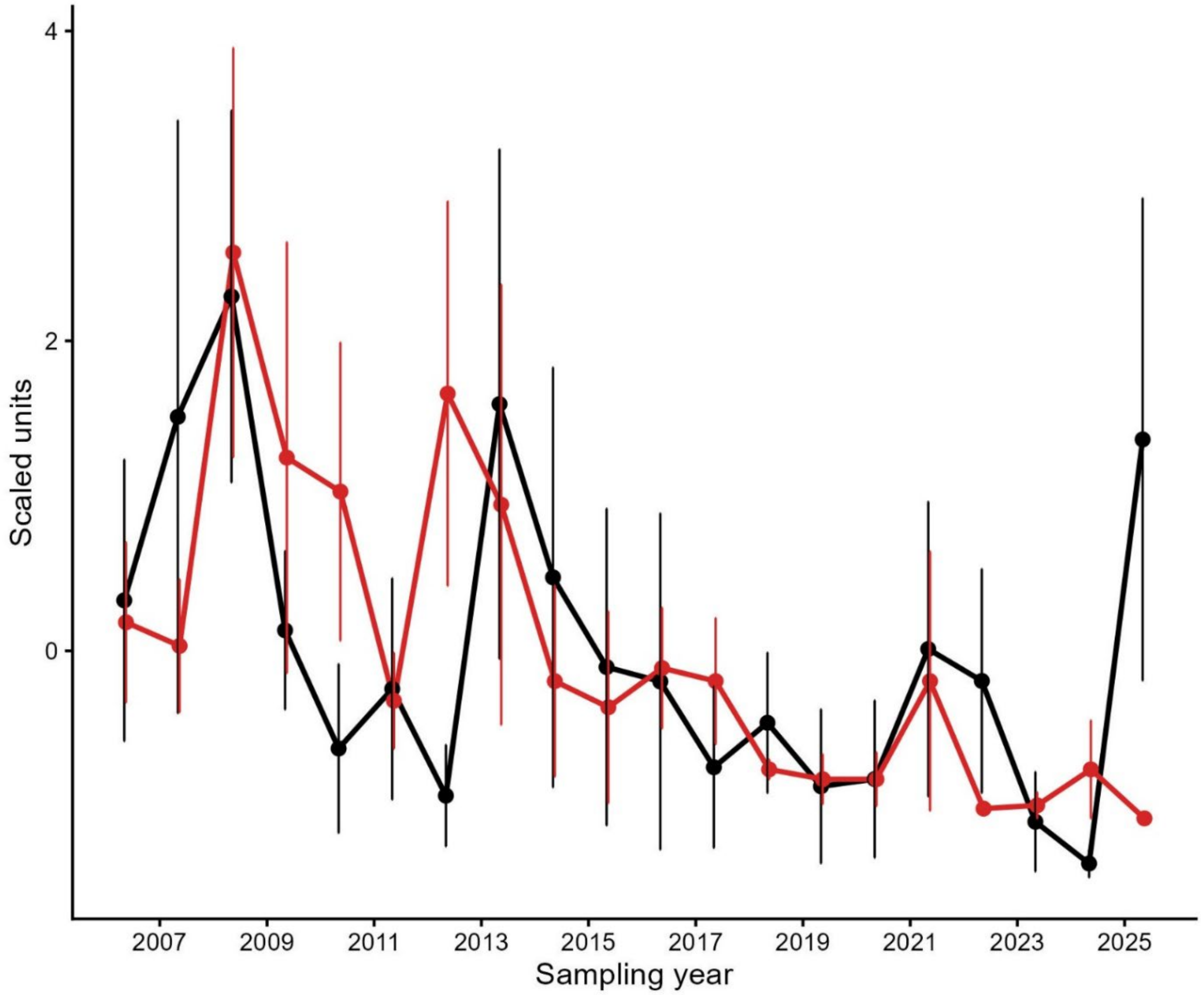

Figure 2.11. Annual trend in relative Eastern Mount Lofty Ranges southern pygmy perch abundance and macrophyte coverage (%). Shown are scaled annual means (points) with ±1 SE error bars. Macrophytes (%) (red) overlaid with southern pygmy perch relative abundance (black).

The model-predicted relative abundance also increased with increasing macrophyte coverage (particularly post 20%, Figure 2.12). The model suggests an absence of southern pygmy perch at very low coverage and almost 0.1 southern pygmy perch per electrofishing second at 30% coverage. Model uncertainty increased with higher predicted relative abundance, with the credible intervals widest at high levels.

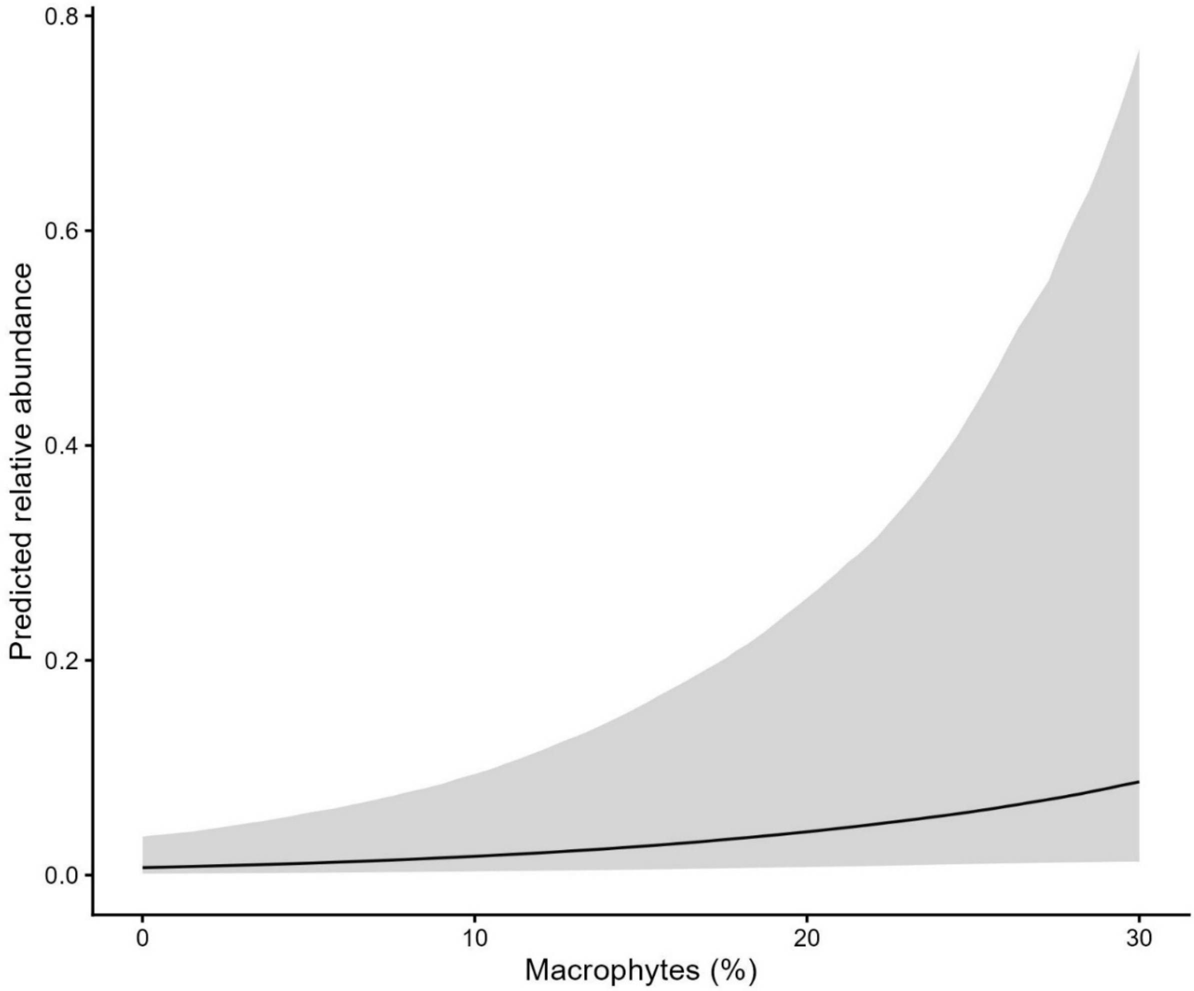

Figure 2.12. Predicted Eastern Mount Lofty Ranges southern pygmy perch relative abundance as a function of the proportion of macrophyte coverage (%). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

Lower Lakes (empirical and modelled analyses)

At the site scale in the Lower Lakes, mean site water depth, water temperature, and, proportion of macrophyte coverage were found to be the strongest drivers of southern pygmy perch abundance (Figure 2.13 and Figure A6). Empirical analysis showed the relationship between mean site water depth and southern pygmy perch abundance appears to be negative but variable (Figure 2.13). In the case of water temperature and macrophyte coverage, mainly positive relationships exist, where increases in these variables led to an increase in southern pygmy perch abundance. The relationships are, however, variable, particularly around the historic low southern pygmy perch abundances (2010 to 2013) because of the impacts of the Millennium Drought.

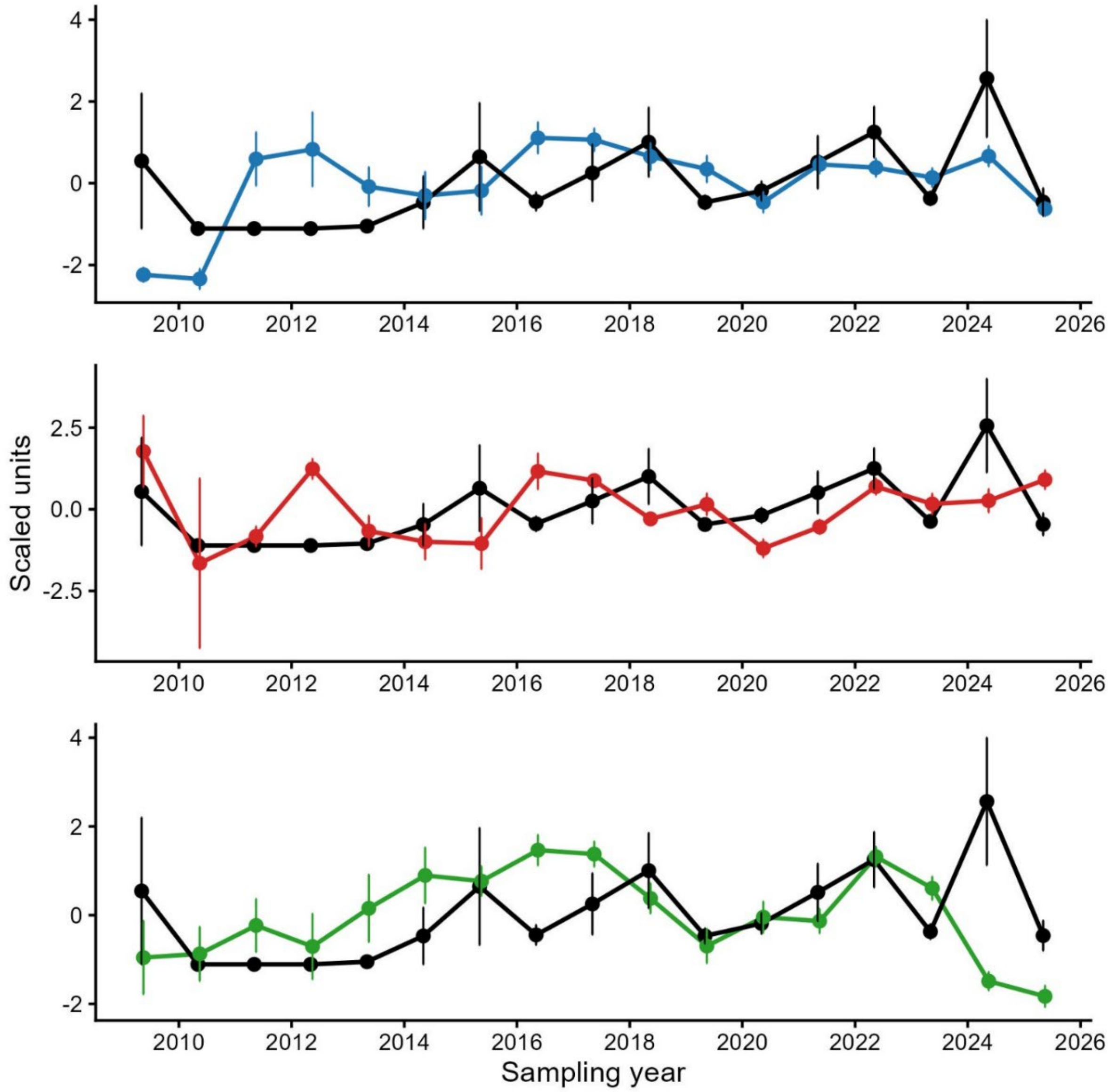

Figure 2.13. Annual trends in Lower Lakes southern pygmy perch relative abundance and environmental covariates. For brevity only those showing a relationship are shown. Each panel shows scaled annual means (points) with ±1 SE error bars. To avoid overlap, the two series in each panel are horizontally offset by a few days (relative abundance left, environmental variable right). Top: Mean depth (blue) overlaid with relative abundance (black). Middle: Water temperature (red) overlaid with relative abundance (black). Bottom: Macrophyte coverage (green) overlaid with relative abundance (black).

The model-predicted southern pygmy perch relative abundance decreased with increasing site mean depth in a curvilinear manner (Figure 2.14). The model suggests an absence of southern pygmy perch at very deep sites (>90cm). The model-predicted increases in southern pygmy perch abundance with increasing water temperature and proportion of macrophyte coverage with the highest levels of both suggesting ~five southern pygmy perch captured per fyke hour (Figures 2.15 and 2.16). In all cases, model uncertainty increased with higher southern pygmy perch abundance, with the credible interval widest at the greatest southern pygmy perch abundance.

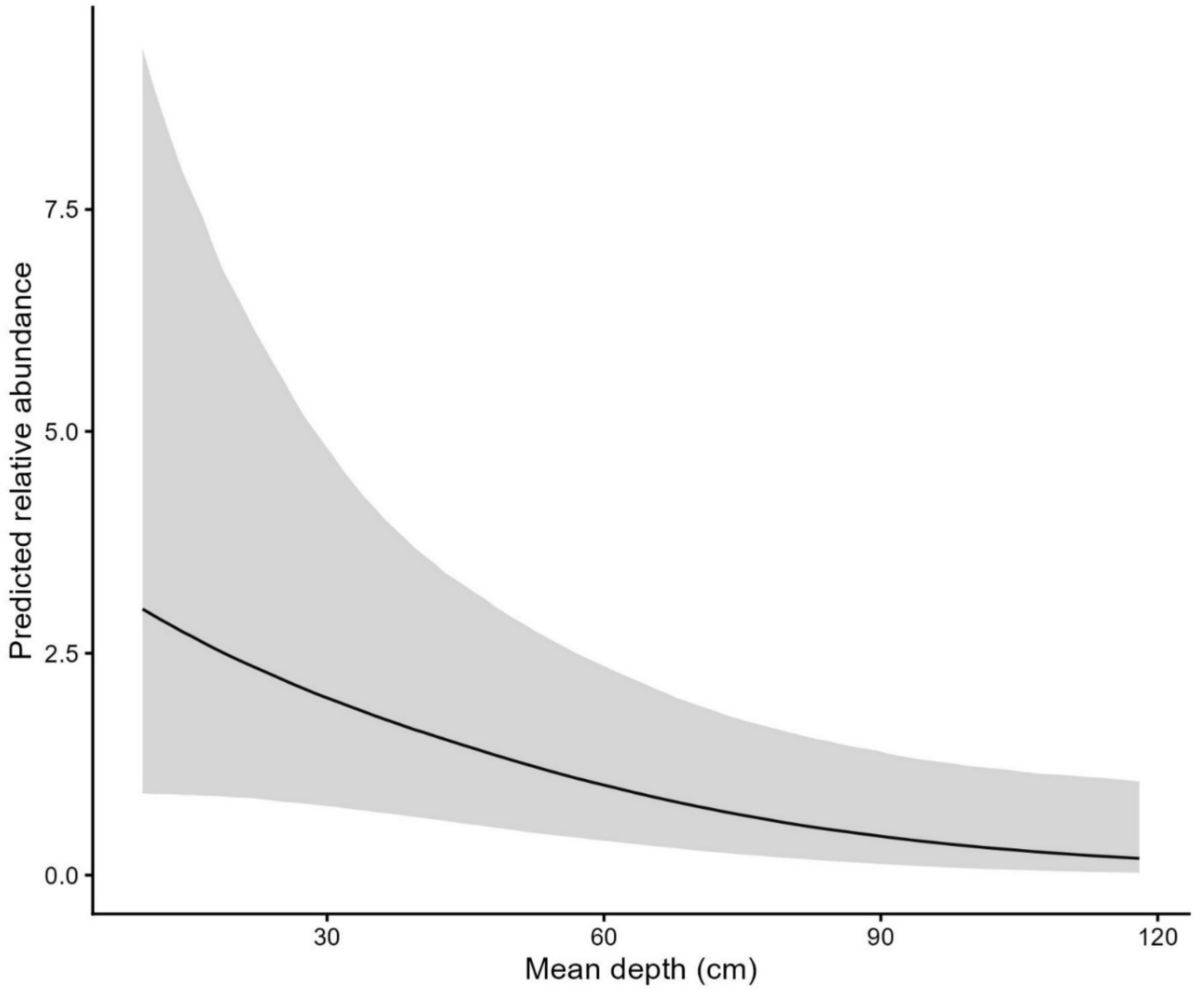

Figure 2.14. Predicted Lower Lakes southern pygmy perch relative abundance as a function of the mean sampling site depth (cm). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

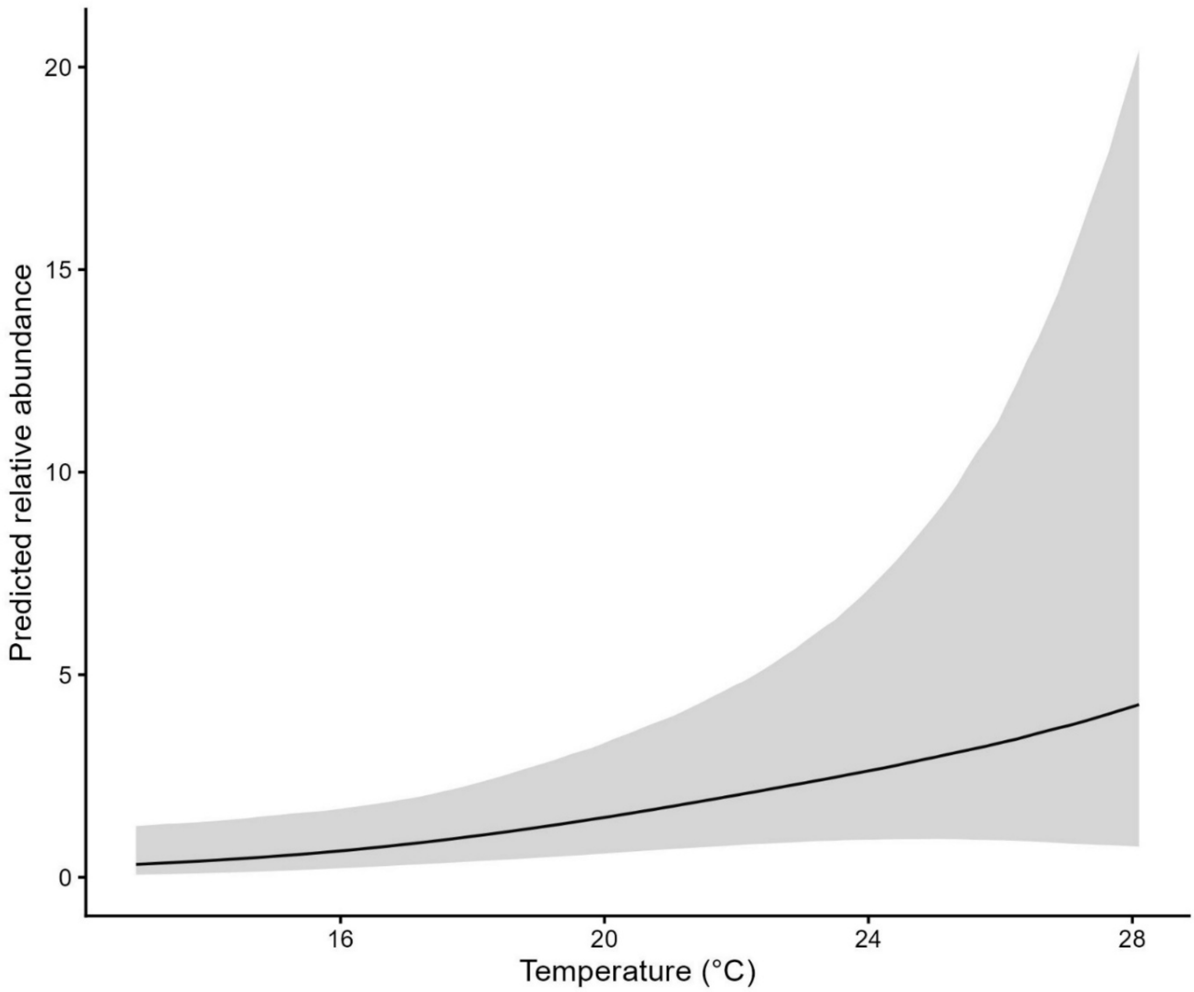

Figure 2.15. Predicted Lower Lakes southern pygmy perch relative abundance as a function of the mean water temperature (°C). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

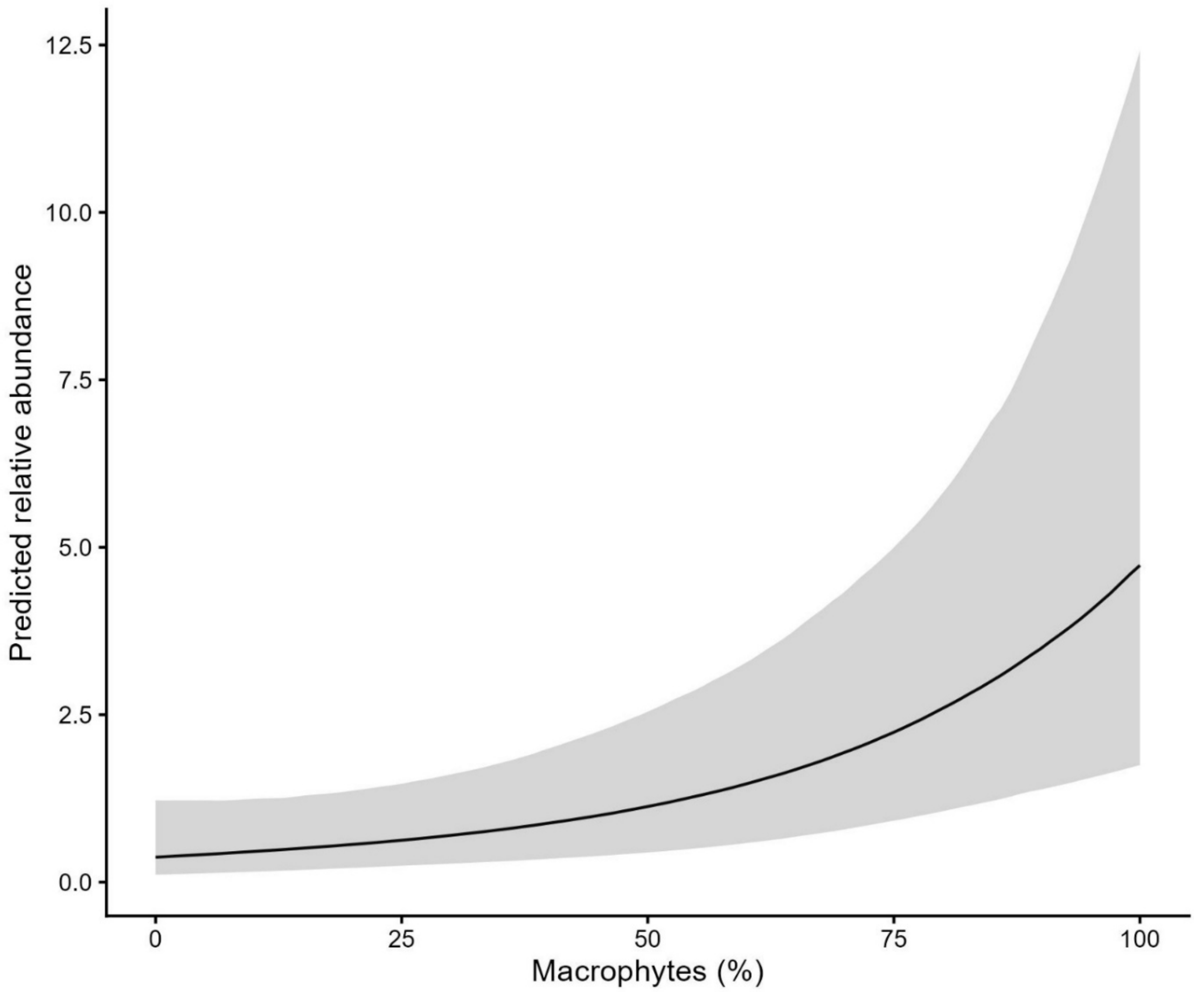

Figure 2.16. Predicted Lower Lakes southern pygmy perch relative abundance as a function of the proportion of macrophyte coverage (%). The black line shows the modelled effect; the grey ribbon indicates the 95% credible interval.

2.4 Discussion

Southern pygmy perch populations in the EMLR and Lower Lakes demonstrated differential trajectories in abundances over time. Generally, from the late 2000s to 2025, southern pygmy perch have declined in abundance in the EMLR whilst increasing in in the Lower Lakes. These trends are supported by both the empirical (abundance and size structure) and modelled analysis of relative abundance. The modelling analyses in the EMLR revealed an increase in southern pygmy perch abundance in 2025, however, this is likely an artefact of increased catchability in smaller refuge pools present during the prevailing drought conditions. Importantly, the model framework captured this dynamic change and, hence, the 2025 upward trend in abundance is not predicted by this analysis, highlighting the usefulness of combining different methods of analyses.

The Lower Lakes southern pygmy perch population was also affected by drought (the 'Millennium Drought') but early in the timeseries of data collection. During the drought, water levels in the Lower Lakes were historically low, negatively affecting southern pygmy perch and other aquatic biota (Wedderburn et al. 2014). Southern pygmy perch were detected in 2009 but absent in 2010 and 2011, despite Lower Lakes returning to normal regulated lake levels as the drought broke. The species was not detected in 2012 and very few detectable in 2013, before the analyses revealed increasing relative abundance from 2014. This recovery has been linked to reintroductions over 2011 and 2012, and subsequent recruitment of wild individuals (Marshall et al. 2022).

Abundance of southern pygmy perch in the Lower Lakes declined in the later years of the sampling period. This may be due to a significant flood event in 2022–2023 either negatively affecting catchability and/or the relative abundance for undetermined reasons. Indeed, there were declines in abundances of all freshwater fishes in fringing wetlands of the Lower Lakes over 2022–23 (Wedderburn and Barnes 2023). A possible explanation for low native freshwater fish abundances is that YOY common carp *Cyprinus carpio* abundance was extremely high over 2022–23 (38,111 common carp) compared to any preceding monitoring year (<400 common carp).

In the EMLR southern pygmy perch population, increased truncation of the size structures suggests breeding is now restricted to a smaller timeframe and/ or the number of cohorts recruiting has been reduced. Broadly, the opposite trend is seen in the Lower Lakes. Thus, patterns in size structure are consistent with, and reinforce, the population trends inferred from the abundance analyses (empirical and modelled). The arbitrary value of 50 mm to indicate YOY will be explored and quantified in Chapter 3 of this report.

The AAO driver of southern pygmy perch abundance in the EMLR may be strongest after two years due to the combined effect of aquifer recharge and the timing of southern pygmy perch breeding. Positive AAO sees frontal systems that would bring rain and cool conditions in winter pushed south out of the southeastern Australian window (Southern Annular Mode | Earth Sciences New Zealand | NIWA). Meaning rain and therefore, aquifer recharge is reduced during positive AAO. Unfortunately, positive AAO is becoming more common in recent times and is likely driven by global climate change. The size structure analysis supports a reduced breeding and recruitment success in recent times which is consistent with increased AAO positivity.

The strongest regional driver of the relative abundance of Lower Lakes southern pygmy perch appears to be the water level of Lake Alexandrina in September. The higher the water level in September, the greater the relative abundance, likely reflecting optimal timing of inundation of fringing wetland habitats that provide food and shelter. This conclusion is reinforced by the finding of macrophyte coverage as a significant driver of southern pygmy perch at the site level in both the EMLR and Lower Lakes. The presence of macrophytes increases the availability of food (i.e. zooplankton) and shelter from predators for southern pygmy perch compared to open habitats (Wedderburn et al. 2017a).

Lake Alexandrina water level depends on available water in the MDB and, as this is a large catchment, with highly regulated river systems, lake level may be somewhat independent of local climatic effects driven by the AAO. However, lake water level may be influenced by Australian eastern seaboard climatic drivers such as El Nino, but this is likely complicated by MDB water abstraction for anthropogenic use, therefore hard to test empirically and model. The water level in Lake Alexandrina is also regulated by barrage operations that can, to some extent (e.g. for short periods), be managed independently of these Basin-scale influences. This opportunity creates some (limited) flexibility to potentially manipulate lake water levels to influence habitat availability and the relative abundance of Lower Lakes southern pygmy perch.

The variables found to be significant drivers of southern pygmy perch at a site scale were water temperature and site water depth. Both may cause an increase in catchability of southern pygmy perch rather than a real impact on relative abundance. For example, increased water temperature may mean southern pygmy perch are more active, increasing their probability of being captured. Alternatively, increased site depth may reduce the probability of capture. The influence of these variables on probability of detection will be examined in future research using the Lower Lakes southern pygmy perch data, which incorporates a repeat sampling regime.

In summary, the EMLR and Lower Lakes southern pygmy perch populations are displaying different trajectories over time, which is likely due to hydrological distinctness of their catchments. The EMLR ranges is in dire need of intervention, with increased water required, to save the southern pygmy perch population, which likely describes the situation of the Lower Lakes during the height of the Millennium Drought.

3 Exploring the otolith ageing of southern pygmy perch to quantify life history parameters

3.1 Introduction

Information on the age of fish is important to quantify life history parameters (e.g. hatch dates, growth, and natural mortality), which can contribute to management actions that promote the persistence of populations (Campana and Thorrold 2001). Understanding how such life history parameters relate to environmental conditions is essential to navigate future predicted changes in climate and water availability. For example, knowledge of the environmental variables that drive spawning responses can help to prioritise and optimise water releases in the face of competing needs for water resources. This information is particularly relevant in heavily altered systems such as the MDB, where the life histories of fishes are often interrupted by river regulation (Wedderburn et al. 2017b).

In the CLLMM region, the natural hydrological regime (river flow and lake water levels is highly altered by river regulation. As such, it is important to understand how water and infrastructure management may influence the breeding and recruitment of fishes, so conservation outcomes can be maximised. Indeed, the Lower Lakes have a unique set of characteristics that means the region should be tested in isolation. For example, the magnitude and duration of lake water levels are highly managed and will have amplified influences on water level recession in fringing waterbodies. The nationally Vulnerable southern pygmy perch currently inhabits fringing wetlands of Lake Alexandrina, Hindmarsh Island, Mundoo Island, and the lower River Finniss arm of the Goolwa Channel. To ascertain the timing of key life history events and their associated environmental variables, a knowledge of the age of captured southern pygmy perch is required. Otoliths, particularly sagittal, are recognised as the most reliable structure as a source of age estimates (Campana and Thorrold 2001).

For YOY southern pygmy perch in the CLLMM, validation of daily increment formation in otoliths has not been conducted. Nevertheless, for other teleosts in the study area, local environmental conditions are associated with discernible daily growth increments (or zones) in otoliths (Bice et al. 2012; Wedderburn and Barnes 2016b; Wedderburn and Barnes 2020). The use of otoliths to gain knowledge regarding southern pygmy perch can help us understand how lake water levels may be associated with the timing of breeding, survival and growth rates. The results may also be informative for reintroductions of southern pygmy perch and Yarra pygmy perch (e.g. identify optimal timing prior to breeding).

A first step in fish age determination is to examine hard parts such as otoliths to resolve the presence of structures (increments) which may represent days or years of growth. Once increment presence is resolved, there is a need to validate the periodicity of increment formation. This validation is sometimes done by capture-mark-release-capture-examine experiments but there are a range of ways increment periodicity has been validated or (at least) corroborated. Once validated, life history parameters can be generated and inform management. Nevertheless, pre-validation of life history parameters can still be useful when treated with caution and corroborated with other lines of evidence.

Here, we seek to determine the feasibility of ageing southern pygmy perch in the CLLMM via sagittal otoliths, by determining the presence of daily growth increments, (Wedderburn et al. 2021). Next, we generate preliminary key life history parameters including hatch dates, growth rate, size of YOY, and size at maturity, to relate some of these factors to environmental parameters (e.g. temperature and lake water level). We also propose the next steps required to refine our findings to ensure managers can better understand the influence of environmental parameters on southern pygmy perch life history process in the face of environmental change.

3.2 Methods

3.2.1 Otolith collection

We used otoliths from 169 southern pygmy perch sampled from Lower Lakes sites between 2014 and 2024 (Table 3.1). Sites were mostly on Hindmarsh and Mundoo islands, where Lake Alexandrina water levels exert a strong influence on water levels and other habitat features. Fish were sampled with fyke nets during a project assessing the health of populations of threatened fish species in the region. Otoliths were removed under a dissecting microscope and placed into a small, labelled vial in preparation for laboratory processing.

Table 3.1. Sites, dates and size ranges of southern pygmy perch collection used in otolith analyses.

SITE NO.	SITE NAME	TL (MM)	COLLECTION DATES	NUMBER OF OTOLITHS
2	Premier's, Hindmarsh Is.	20–47	25/11/2019, 8/12/2020, 4/03/2022	18
3	Hunters Creek, Hindmarsh Is.	34–40	15/03/2017	10
5	Channel off Steamers Drain	17–33	9/11/2018	10
22	Mundoo Is.	30	26/03/2014	3
32	Mundoo Is.	30–72	10-11/03/2021, 15/03/2022	16
34	Shadows Lagoon, Hindmarsh Is.	32–42	22/03/2018	14
38	Black Swamp, River Finniss	26–58	23/03/2018, 4/12/2019, 25/03/2021, 23/03/2022	22
71	Well's (Manor) Channel, Hindmarsh Is.	34–40	18/03/2016	2
73	Hunters Creek, Steamers Drain	33	12/12/2019	1
109	Tea Tree Lagoon, Mundoo Is.	24–47	12/03/2021, 15/03/2022, 15/11/2022, 13/03/2024	42
121	Lawari, Hindmarsh Is.	23–34	12/11/2018, 8/12/2020	31

3.2.2 Otolith preparation

Sagittal otoliths were provided as a pair, with one otolith being arbitrarily selected for processing. Exceptions were made where samples were deemed inadequate for accurate reading due to significant fracturing and obscuring from residual tissue.

Whole otoliths were fixed distal side up, to glass slides, by immersion in crystal bond adhesive (ProSciTech Crystalbond) using a hotplate. Once set, samples were ground and distally wet polished using a sanding sheet (P800 grit) and a range of graded lapping films (30, 9 and 3 µm). Polishing achieved a transverse cross-section, or series of cross-sections, intersecting the primordium for a clear view of successive a priori daily growth increments (one light and one dark concentric band) from otolith core to the edge (Figure 3.1).

Preparation and imaging were undertaken using a Leica DMLB compound light microscope with a 100x objective and a 0.63x camera adapter (2/3", 17.5 mm) magnification, using transmitted light along with paraffin oil. Once increments became exposed, photographs were taken intermittently, as the topography of the otolith surfaces rarely allowed for all daily growth lines, between first evident concentric ring and otolith edge, to be visible in a single image.

Two large fish (>50 mm total length) were assessed as being difficult to identify daily increments via whole otolith microscopic examination. As such, these were prepared for annual ageing by mounting in epoxy resin blocks and slicing a 0.3 mm transverse section through the otolith primordium with a low-speed saw. More details on the preparation of otoliths in transverse sections can be found in Barnes and Gillanders (2013).

Figure 3.1. The process of otolith preparation.

3.2.3 Otolith ageing

Two counts of increments were conducted per otolith sample, by one experienced reader with no prior knowledge of fish biological data. The starting point for increment counting was alternated between core and edge, and counting was conducted along distinct axes each time, to capture variations in increment clarity, increment interpretation, and confounding visual artifacts.

Otolith growth increments were manually counted using ImageJ, a Java-based image processing and analysis program, to reduce human error in extensive counts. ImageJ allowed users to overlay individual points onto consecutive growth increments, providing an end sum of increment points as an output.

Otolith images were ranked for ease of readability with a score from one to five, with one indicating high clarity and five indicating a poor and likely unreadable image (Table 3.2). High quality readability otoliths (one to three) would automatically make the grade for downstream analyses, otoliths with a readability of four would be removed if they were found to be outliers during analyses, and readability five would often not be aged.

Table 3.2. Description of otolith readability scores and its effect on age accuracy.

READABILITY SCORE	DESCRIPTION	AGE ACCURACY
1	Excellent definition of light and dark bands forming an increment across the entire otolith cross section	±0 days
2	Good definition (some minor extrapolation required) of light and dark bands forming an increment across the entire otolith cross section	±1 to 2 days
3	Reasonable definition (some extrapolation required) of light and dark bands forming an increment across the entire otolith cross section	±3 to 5 days
4	Reasonable to poor definition (some extrapolation required) of light and dark bands forming an increment across the entire otolith cross section	±6 to 10 days
5	Poor definition (major extrapolation required) of light and dark bands forming an increment across the entire otolith cross section. Normally, otolith not read	±>10 days

For many samples, multiple images were required to approximate age, as growth rings closest to the nucleus often needed either more grinding or a different microscopic focus than their marginal counterparts to become evident. Using ImageJ for each otolith, the analyst chose images with the most extensive areas of clarity and counted all visible rings (Figure 3.2).

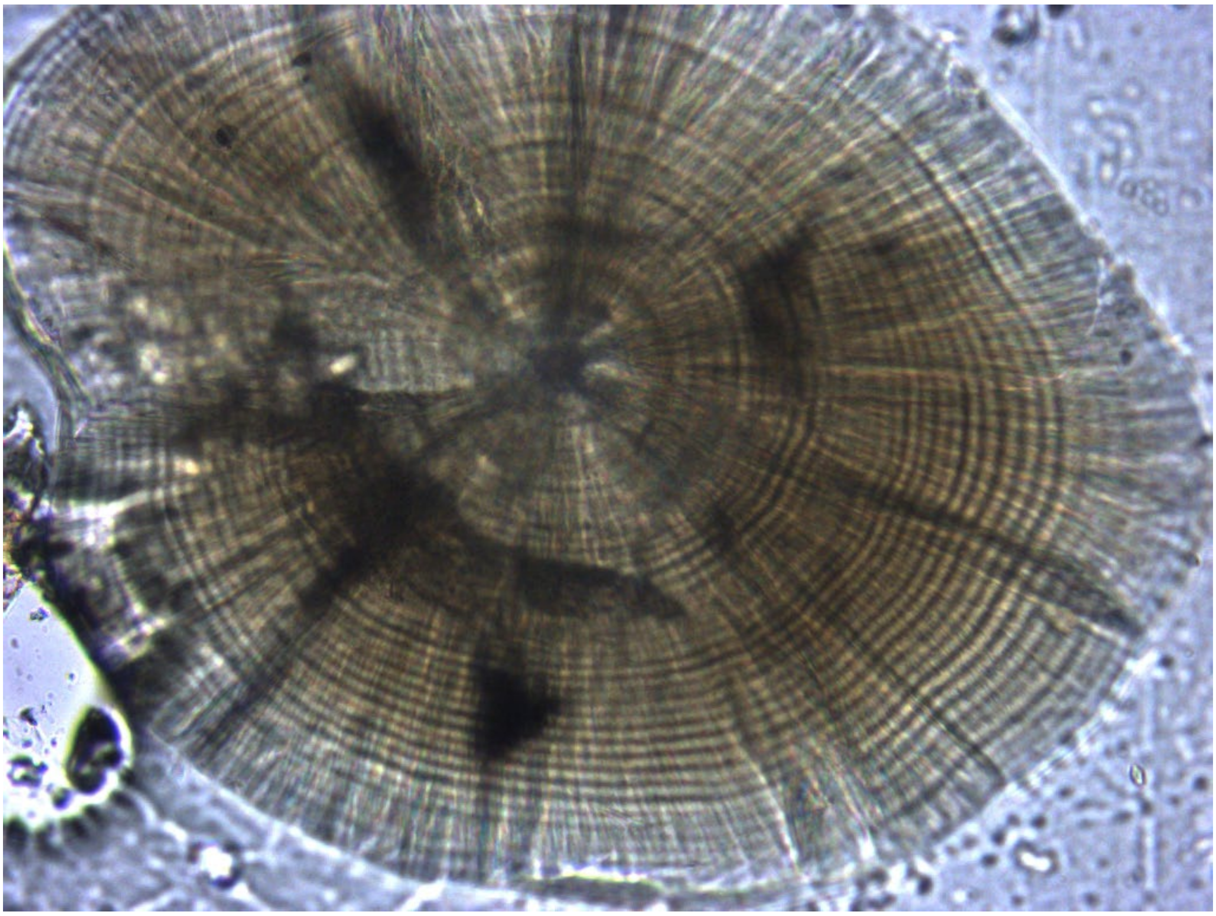

Figure 3.2. Southern pygmy perch otolith with a readability score of one. In most parts of the otolith section increments (light and dark concentric bands) are very clear (hence the excellent readability score).

3.2.4 Data analyses

Hatch date was estimated by subtracting the number of days old the fish had been estimated from increment counts in the otoliths from the sample date. Hatch dates were then reduced to months and analysed graphically to determine the frequency of birth month and thus provide a spawning season. Growth parameters were estimated using a Von Bertalanffy growth model, which uses size-at-age to estimate population growth. The equation of the model can be written:

$$\text{Total length (mm)} \sim \text{Linf} * (1 - \exp(-K * (\text{Age (days)} - t0)))$$

This model has previously been used on daily ageing data; see Stevenson and Campana (1992). The YOY length, which has been used in other analyses previously (e.g. Wedderburn and Barnes 2016a), and therefore is an important parameter to quantify, was estimated using the Von Bertalanffy (VB) growth model. Growth at different ages was extracted from the VB model via plugging the parameters into the equation:

$$dL/dt = L\infty\ Ke{-}K(t{-}t0)$$

Other growth models (e.g. logistic growth) were explored but were found to be suboptimal (i.e. produce unrealistic growth parameters [not shown]).

3.3 Results

3.3.1 Presence of increments, readability, and sample-at-age

Out of the 169 otoliths extracted, 152 yielded useable sections that provided daily age estimates. Daily increments were found to be interpretable in most southern pygmy perch to ~50 mm. Figure 3.3 shows the size distribution of aged southern pygmy perch with the two fish >50 mm prepared differently (see methods) and aged in years.

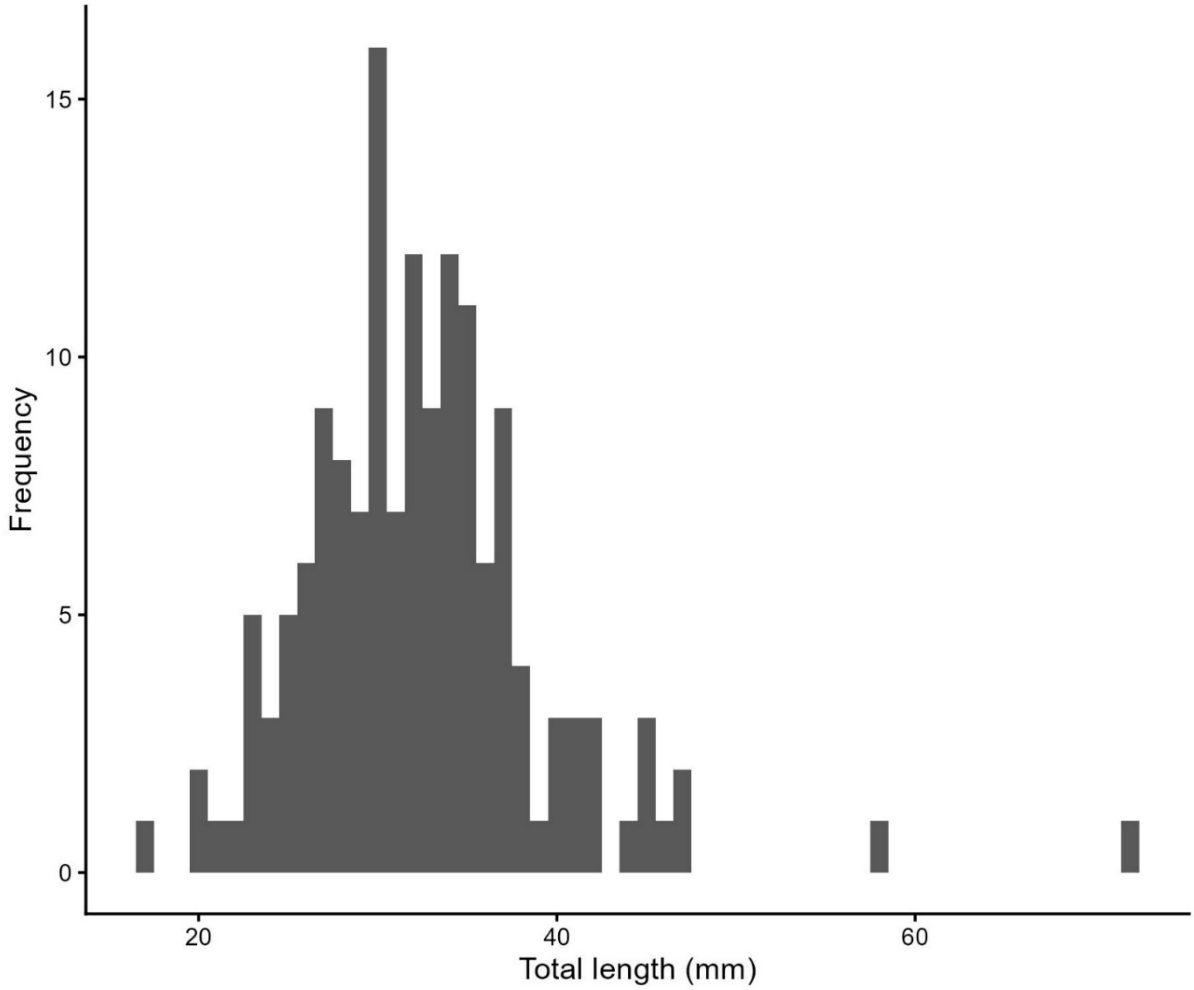

Figure 3.3. Total length (mm) of aged southern pygmy perch. Shown are all fish prepared for ageing with most prepared for daily ageing and the two fish >50 mm prepared for annual ageing.

The readability score shows a large proportion (41%) of aged southern pygmy perch yielded a good quality score of three (Figure A7) with reasonable proportions of very high to high quality readability (i.e. readability one and two were 6% and 17%, respectively). Readability of four (average quality) became more prevalent as the TL increased >30 mm (Figure 3.4) whereas the poor readability southern pygmy perch (readability five) were relatively low in numbers and spread across the <40 mm size classes.

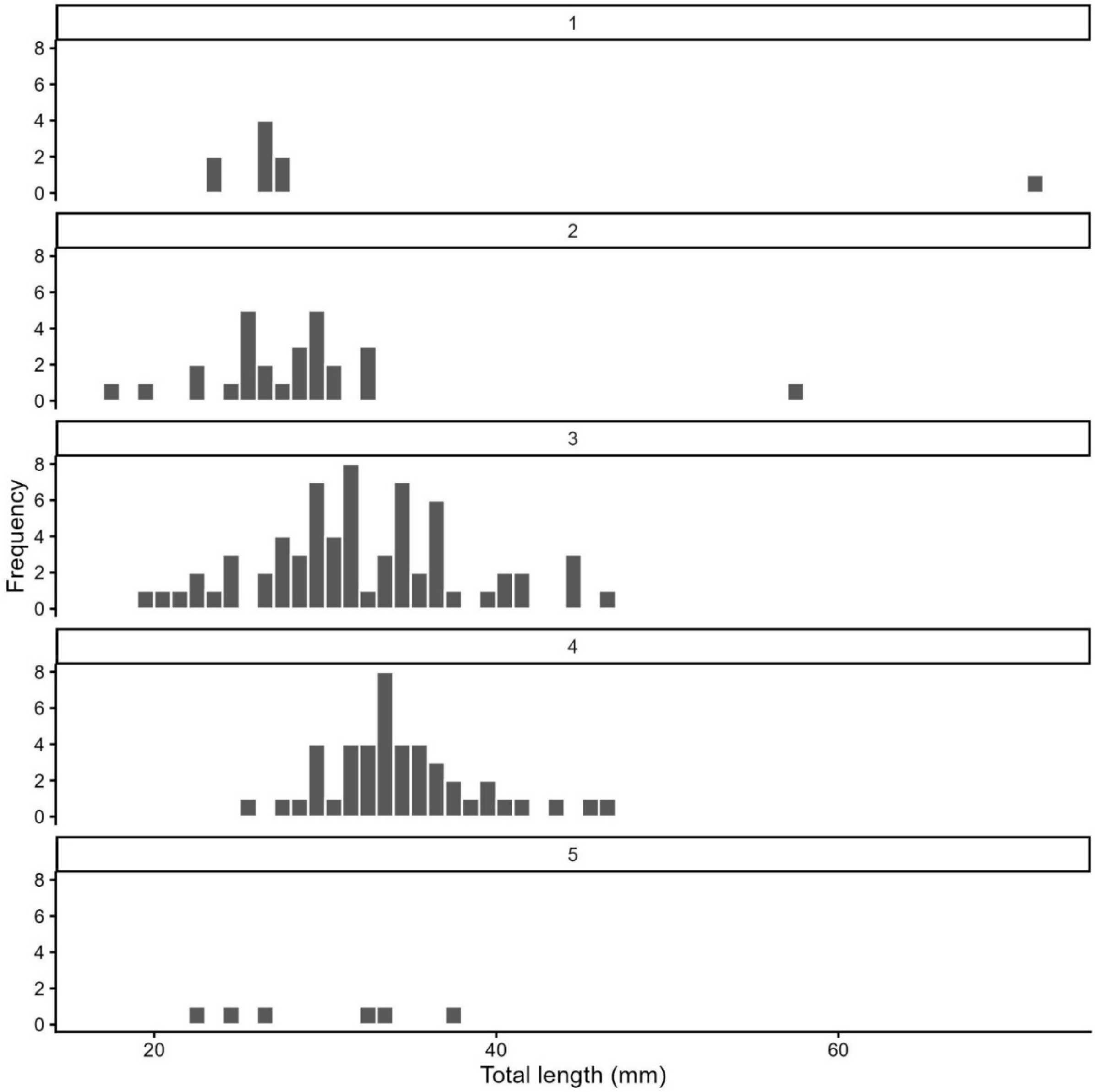

Figure 3.4. Size (TL mm) range of southern pygmy perch prepared for ageing faceted by readability. A readability score of one is the highest quality and five the lowest.

When considering only daily aged samples, the putative daily age range of southern pygmy perch was 30 to 175 days. Across the collection months of November, December, and March, the youngest distribution of southern pygmy perch was in November (median and mean age 56 days), then December (median 65 and mean 68 days old), and finally March oldest (median 82 and mean 89 days old, Figure 3.5).

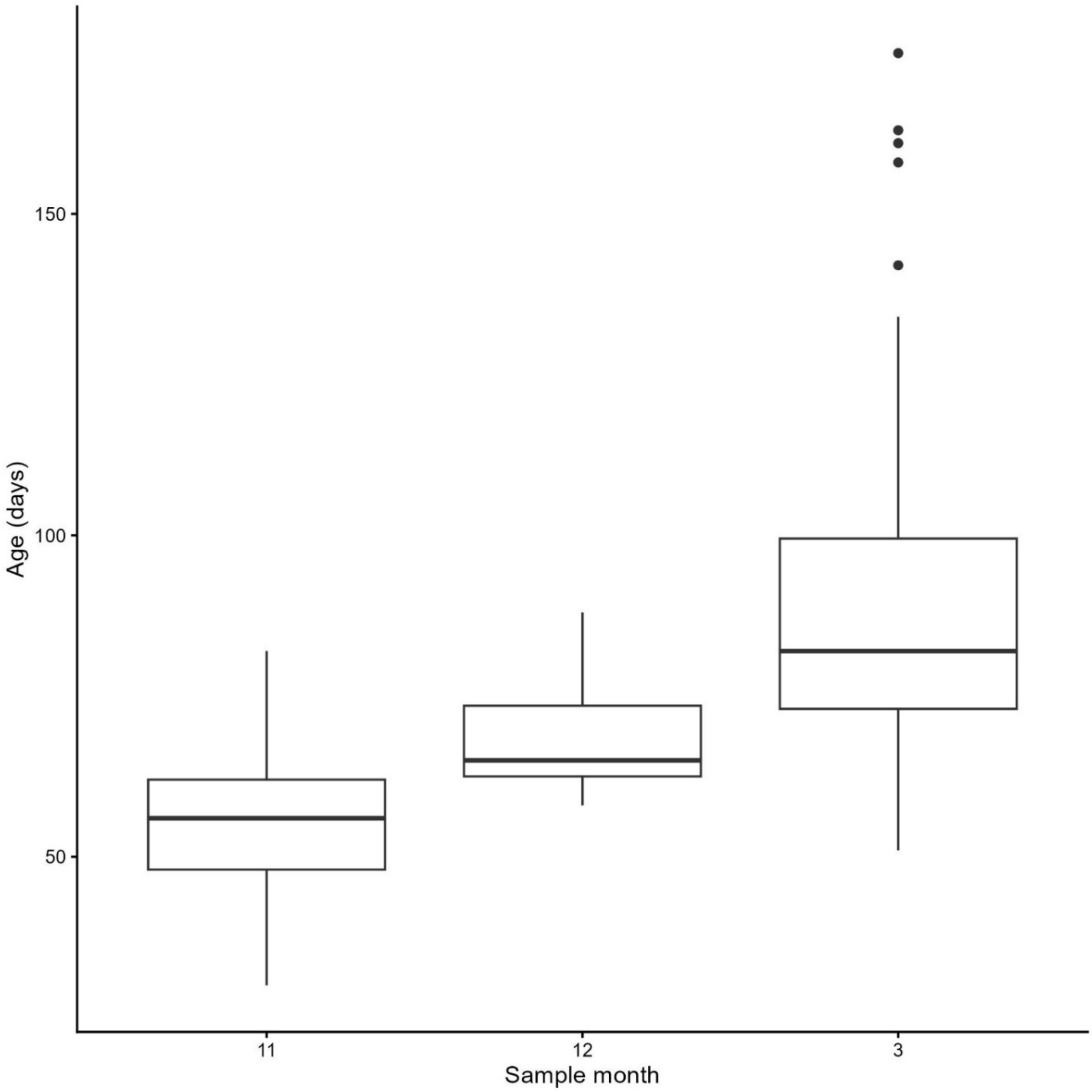

Figure 3.5. Daily age distributions of fish by collection month. Note the two fish older than suitable for daily ageing have been removed.

The years with spring and autumn sampling (i.e. 2018 and 2022) generally show two distinct age classes (Figure 3.6). However, in 2022, the spring and summer cohort overlapped in daily age at ~70 days.

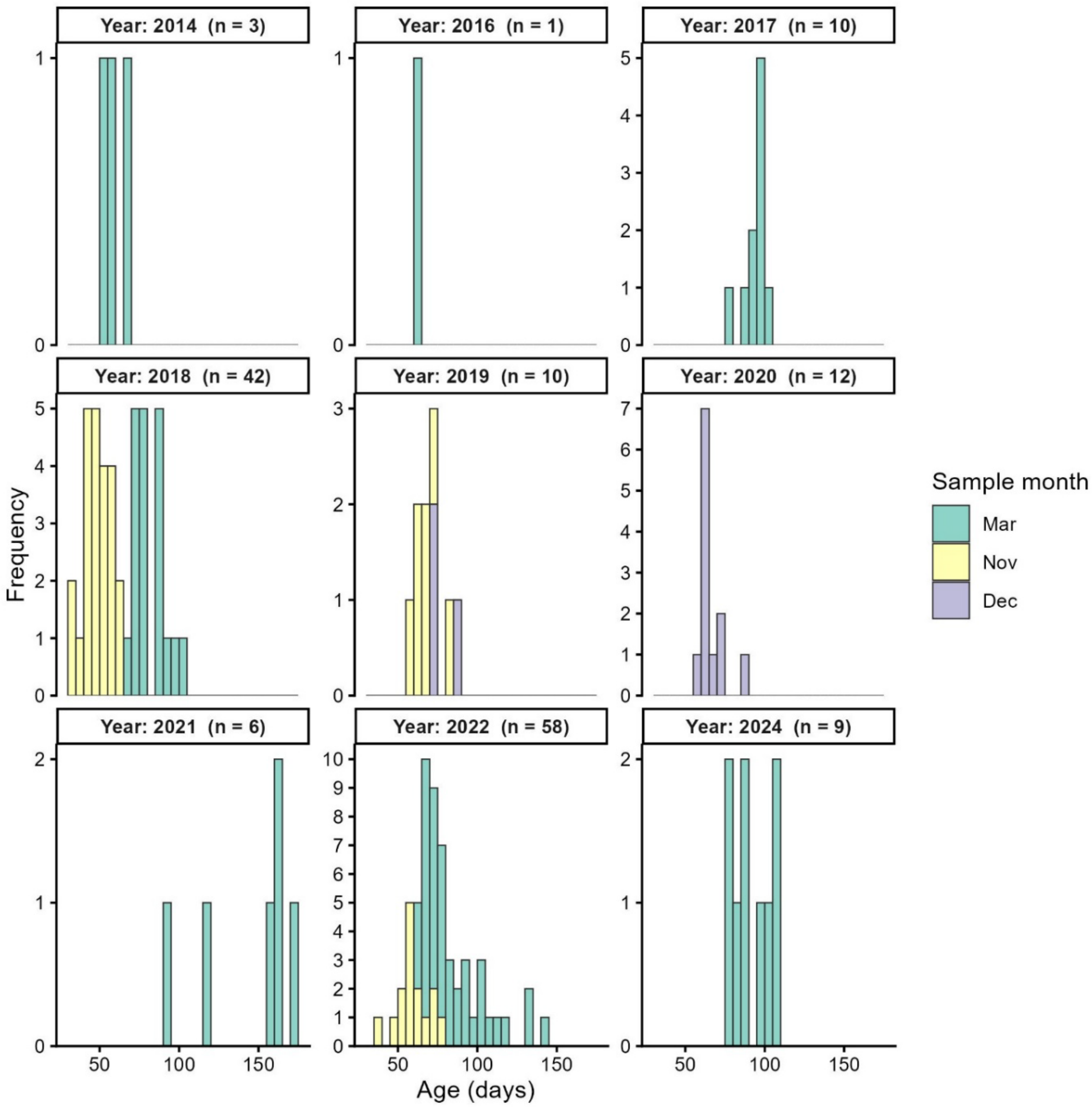

Figure 3.6. Daily age distributions (age frequencies) facetted by sample year (calendar) and showing month (Mar = March, Nov = November, Dec = December) of sampling.

3.3.2 Life history

Hatch dates

Daily age estimated hatch dates were mainly in spring and summer, with two fish estimated to be hatched in late winter (Figure 3.7). September and December were the most prolific months of hatching, closely followed by January, with other months having <20 southern pygmy perch hatched.

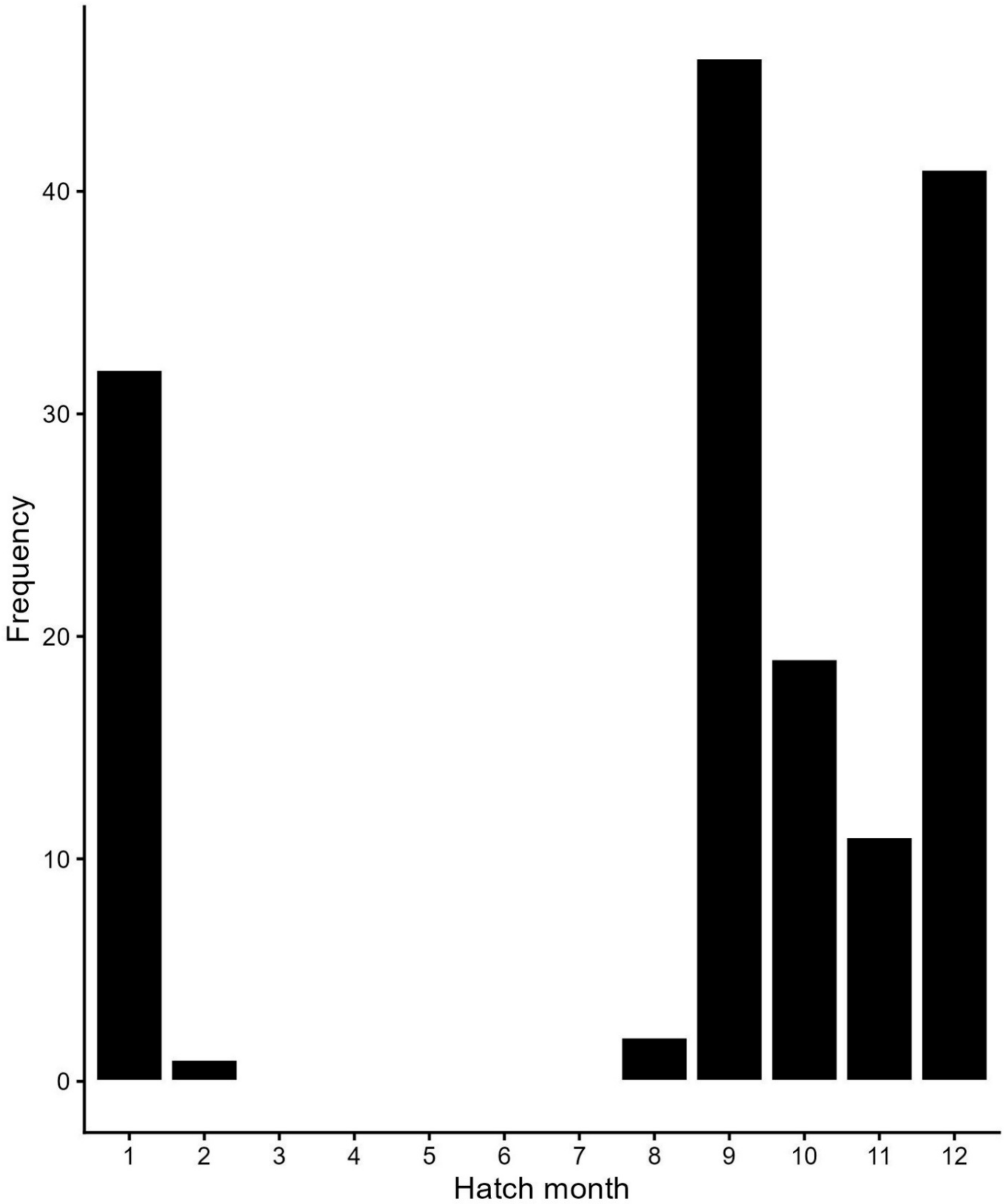

Figure 3.7. Distribution of hatch months estimated from daily ages and sample dates.

Distributions of hatch months were generally confined to adjacent months (Figure 3.8), except for 2018 and 2022 when multi season sampling is known to have occurred (Figure 3.6) within a calendar year.

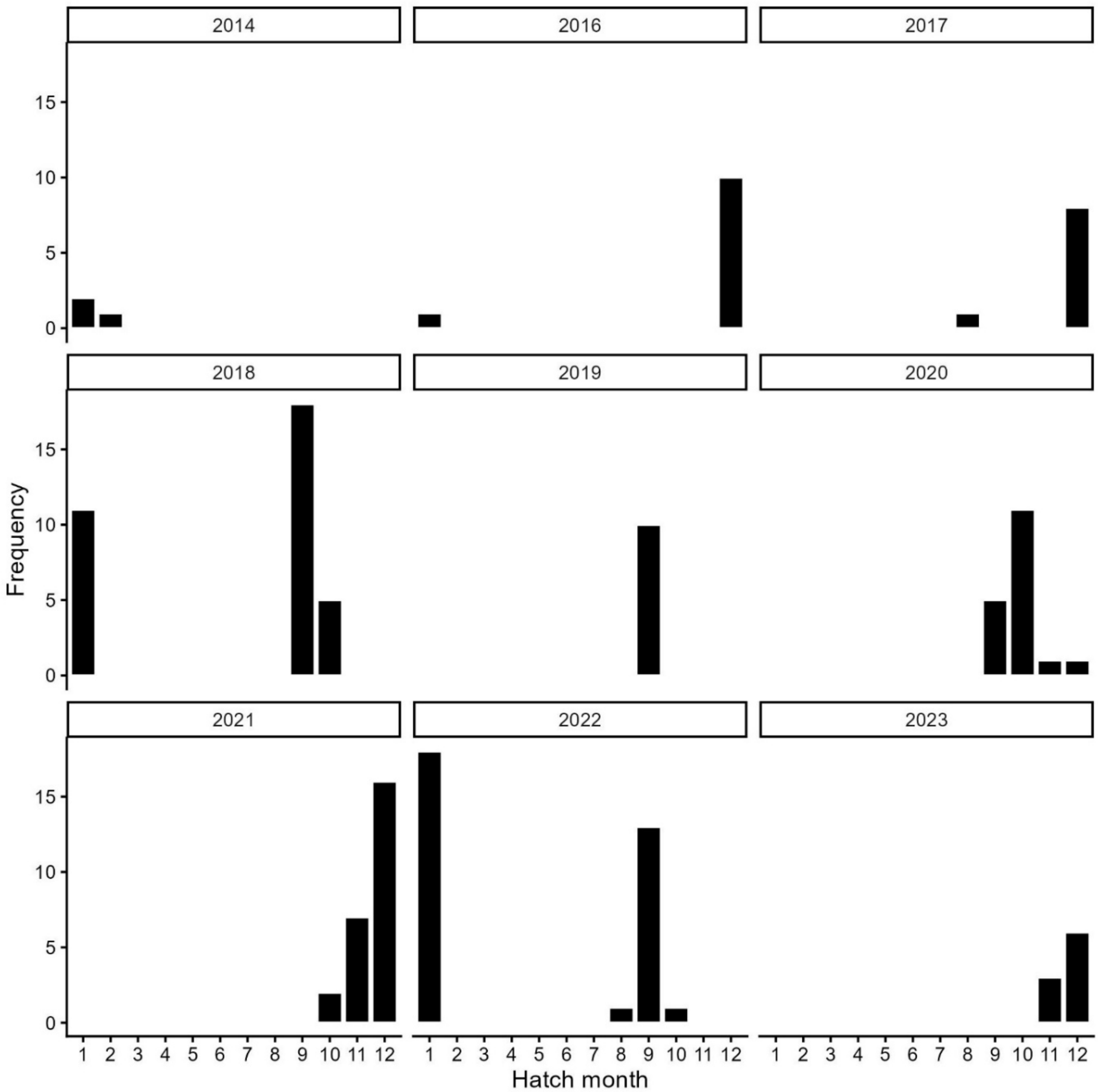

Figure 3.8. Distribution of hatch months estimated from daily ages and sample dates and facetted by hatch year.

Growth

Incorporating all available data into Von Bertalanffy modelling, growth parameters of Linf = 71 mm (TL), K = 0.0045, and t0 = -57 days were estimated (Figure 3.9). The confidence intervals widen substantially after 200 days, where few data points were present. The growth is initially rapid and then exponentially declines as size approaches an asymptote.

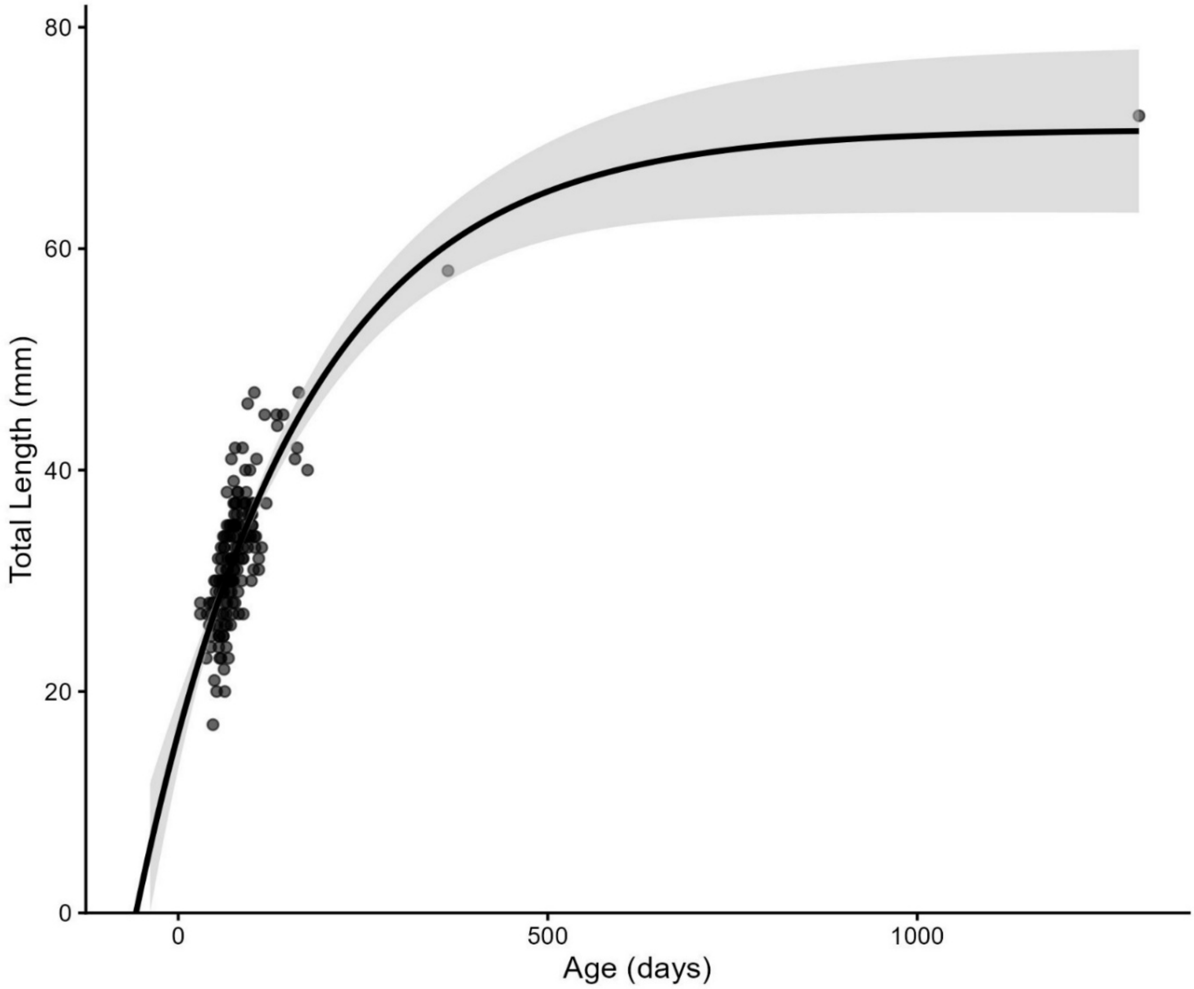

Figure 3.9. Southern pygmy perch length-at-age scatterplot fitted with Von Bertalanffy growth curve and confidence intervals (95%). Daily age of southern pygmy perch >50 mm total length has been estimated from the age in years.

When the growth curve is restricted to the data less than one year old, the Linf is only 55 mm TL, K = 0.007, and t0 = -35 days (Figure 3.10). Summer hatched fish are noticeably confined to the middle age range. In the first six months of life the growth is very rapid at first (nearly 0.5 mm/day in the first 30 days), then slowing at 100 days (~0.2 mm/day) to half the growth rate by 200 days (~0.07 mm/day).

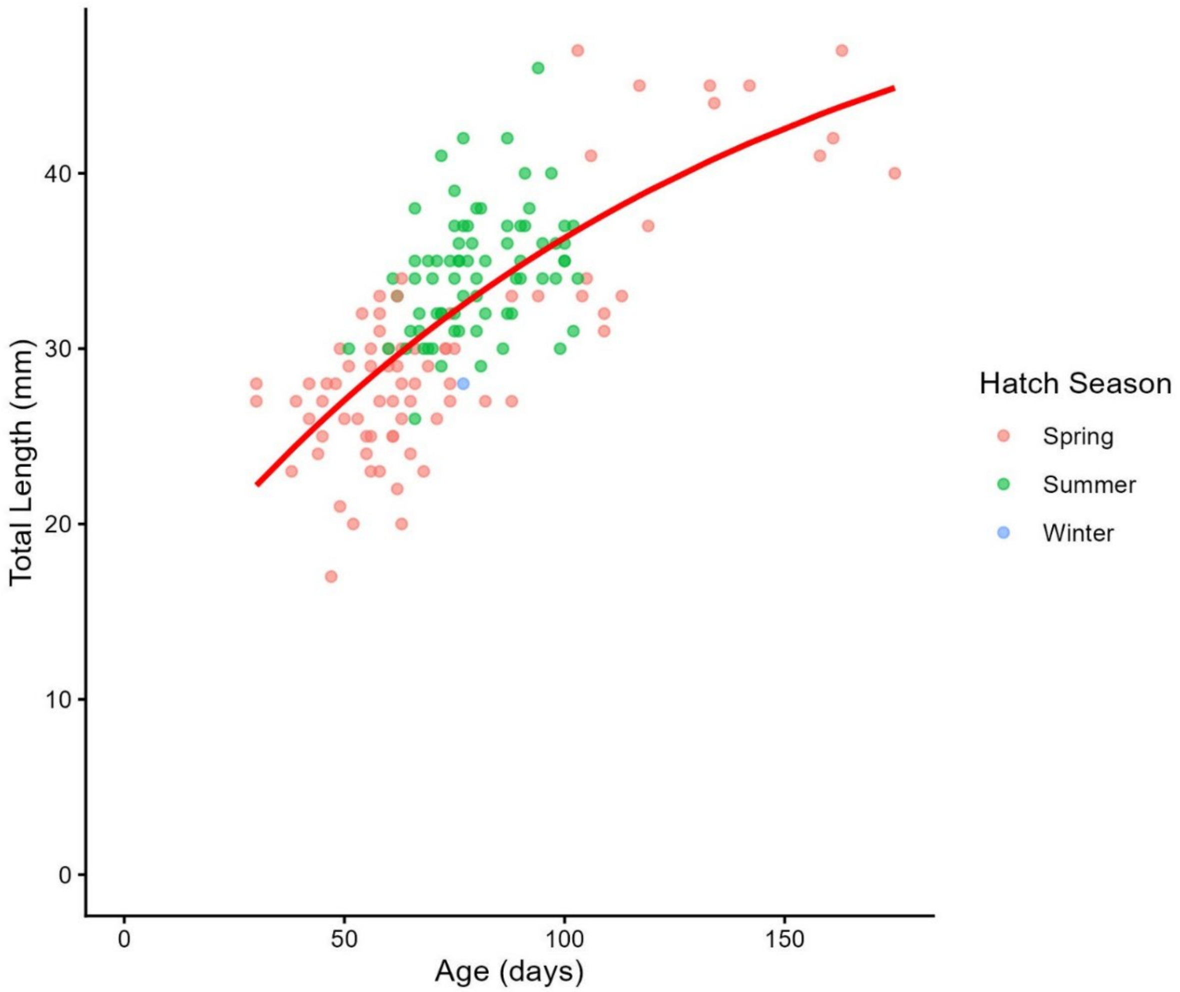

Figure 3.10. Southern pygmy perch length-at-age scatterplot fitted with Von Bertalanffy growth curve and confidence intervals (95%). Shown is only the fish less than one year old with hatch season indicated by point colour.

Young-of-the-year

Based on the growth parameters from the Von Bertalanffy model including all southern pygmy perch sampled, a young-of-the-year (YOY) fish would be <60 mm (95% CI: 57 to 64 mm, Figure 3.11). When using the reduced (<1 year) growth model parameters only (Figure 3.10) to estimate the size at 365 days, a YOY southern pygmy perch would be <=53 mm.

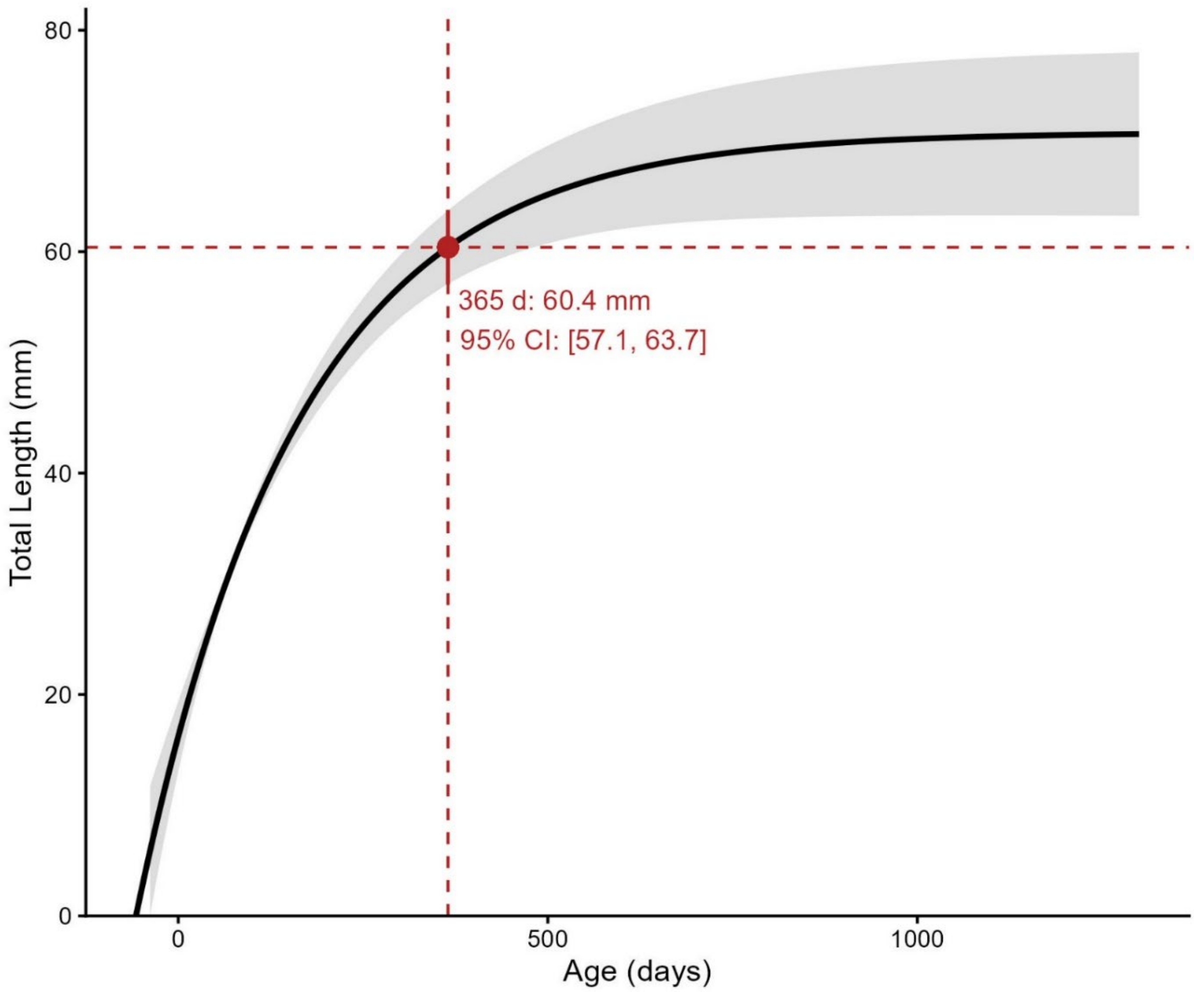

Figure 3.11 Southern pygmy perch Von Bertalanffy growth and confidence intervals (95%). Daily age of southern pygmy perch greater than 50mm has been estimated from the age in years. Shown is the total length expected at 365 days or one year to provide a cut off age when using size as a proxy for young of the year.

3.4 Discussion

The present study is the first to provide credible daily age estimates for southern pygmy perch from the CLLMM region, a vulnerable MDB population of this species. A high success rate in otolith preparation with reasonable to good readability indicates the formation of interpretable daily and annual increments in otolith structure.

Subsequent life history analyses support the interpretation of daily and annual increments. For example, the hatch dates estimated from ageing align with an expected spring and summer spawning period (Llewellyn 1974). Furthermore, southern pygmy perch age distributions were older if sampling was later in the sampling season, hatch month distributions were largely connected, and the VB growth curve follows the pattern of expected fish growth. Also, the length estimates for YOY were predominantly within the expected total length of southern pygmy perch.

Quantification of hatch dates estimated from age are potentially informative for the conservation of southern pygmy perch, particularly in the Lower Lakes. For example, understanding the time of spawning can inform the timing of environmental watering interventions, including lake level targets/manipulation to maximise reproduction and recruitment of southern pygmy perch. The modelling chapter of this report indicates the optimal conditions to promote Lower Lakes southern pygmy perch populations is an approximately 0.8 m AHD lake water level in September. The hatch dates in the current study are most numerous in September, which suggests the modelled optimal conditions are strongly related to spawning and recruitment. This finding highlights the importance of investigating multiple lines of evidence to understand population dynamics. It should be noted, however, that the hatch dates are preliminary and would benefit from a study to validate increment periodicity.

Rapid growth early in life, that slows gradually and then exponentially, was observed for Lower Lakes southern pygmy perch and is the typical growth for most fish. The VB parameters could be strengthened by including very young and old fish to drive the curve with data, rather than predictions at the lower and upper ends of the curve. The VB equation is normally fitted to length-at-age data, where the age is in years. The present study results suggest that the VB equation is also suitable to describe the growth of rapid growing short-lived fish (see Stevenson and Campana (1992) and Stocks et al. (2019)), giving reasonable parameters and insight at the daily level.

Investigating length-at-age and growth has allowed the preliminary quantification of a YOY proxy size (length, mm). The YOY size is a parameter commonly used in condition monitoring (Wedderburn and Barnes 2016a). The length-at-age data indicates YOY fish are likely <50 mm, which is the currently used cutoff limit in the YOY index in the southern pygmy perch population assessment. However, the preliminary VB analyses suggest this length could be as much as 60 to 64 mm. If this is correct, it would change the interpretation of current monitoring data analyses. For example, length frequency figures in Chapter 2 show that the right-hand tails of the length distributions are complete or only extend to approximately 60 mm, meaning the great majority of fish monitored are YOY. This conclusion means that southern pygmy perch are very rarely surviving past the age of one year, yet the (likely) age one and four-year southern pygmy perch in the current study show longer life is possible. This lack of survivorship beyond one year could be another sign of compromised population health but needs more investigation.

As the age data shows great promise to provide evidence to support decision making to ensure the persistence of southern pygmy perch, we recommend the following future research. First, validate the periodicity of increment formation at the daily and annual level. Second, implement a robust sample size sample-at-age monitoring program, which will be representative of the Lower lakes southern pygmy perch populations. Ensure the program samples more small and large fish (e.g. via length bin sampling) to fill out the VB curve with quantitative data. Third, investigate sexual dimorphism in growth and other life history parameters. Fourth, age southern pygmy perch from a variety of catchments (including surrogate dams) to test for geographical differences in growth. When this work is complete, life history parameters can then be related to environmental drivers (e.g. produce a short-term bio and chemical chronology to investigate drivers of growth). A future objective should be to investigate the association between hatch dates and lake water levels, over time. For example, some seasons appear to have prolonged spawning (e.g. 2021) of southern pygmy perch, and this may relate to extrinsic factors such a lake water level.

4 Field Experiments

4.1 Introduction

Freshwater fish species are becoming increasingly threatened globally and within Australia (Darwall and Freyhof 2016). Within the MDB, native fish species are subjected to substantial pressures, including river regulation, water extraction, invasive species and a changing climate (Lintermans 2013). The lower Murray River, including the CLLMM region, support 35 species of native freshwater fishes (Wedderburn et al. 2021), including the nationally Endangered Yarra pygmy perch and the nationally Vulnerable southern pygmy perch (MDB population) (Figure 4.1). The pygmy perch populations in this region were subject to severe pressure during the Millennium Drought (Wedderburn et al. 2012a; Wedderburn et al. 2017b). During this time, loss of obligate habitat for the pygmy perches, and increasing salinity and other unfavourable conditions, caused drastic measures to be taken. A small number of each species were removed from the Lower Lakes and relocated into private dams and wetland surrogate sites where the fish could be safeguarded and bred, with the intention of reintroduction to the Lower Lakes once the pressures had eased (Bice et al. 2014).

The reintroduction of fish species to former wild sites is a key facilitator in the re-establishment of populations in areas that have undergone, and continue to experience, stress and unfavourable conditions. Historically, fish translocations were carried out with large-bodied fish being moved between waterways to supplement numbers or introduce fish into new areas for recreational purposes (Lintermans et al. 2015). These early fish translocations were often poorly executed, with minimal concern given to the destination of these fish species and the impacts they may have on their new environment (Lintermans et al. 2015). Since these early translocations, and in the face of increasing anthropogenic threats, conservation-based translocations have become more common. Nevertheless, questions remain on how to best carry out reintroductions of native species that are in low numbers in wild populations (Todd and Lintermans 2015). Both species are a genetically isolated population within this area, with Lake Alexandrina being the only area in the MDB that Yarra pygmy perch occur (Brauer et al. 2016; Hammer et al. 2010). Due to the requirement of safeguarding the distinct genetic populations of Yarra pygmy perch and southern pygmy perch that were rescued from the Lower Lakes, fishes have been bred in both ex situ breeding facilities as well as in controlled private surrogate sites, which enabled the production of recruits from the Lower Lakes genetic populations (Bice et al. 2012; Whiterod et al. 2019; Zukowski 2025).

Wild Yarra pygmy perch in the CLLMM region were extirpated by 2009, although officially declared as such in 2020, and the southern pygmy Perch was extirpated from the Lower Lakes, however small populations persisted in neighbouring tributaries (Wedderburn et al. 2021). With reintroductions of southern pygmy perch into the Lower Lakes following the Millennium Drought, self-supporting wild populations have reestablished (Marshall et al. 2022). In contrast, the reestablishment of Yarra pygmy perch had not occurred (Wedderburn et al. 2016; Wedderburn et al. 2022) despite reintroduction and short-term persistence. Most recent reintroductions (2023-2026) are showing low numbers of recaptures of Yarra pygmy perch during post sampling periods, however self-sustaining populations have not yet been observed to successfully establish (Zukowski 2024; Zukowski 2025; Zukowski 2026). It is therefore essential to continue building knowledge of both species, as well as revise the reintroduction methods used to provide the greatest opportunity for wild population recovery.

Using otolith (Chapter 2) and modelling data (Chapter 3) collected within the EMLR and CLLMM regions, insight has been gleaned into the factors associated with the reproductive success of southern pygmy perch. Southern pygmy perch can spawn as early as August–September, and in some years, fish may spawn multiple times with recruits being observed as late as February in the EMLR (See Chapter 3). Some fish are also able to reach sexual maturity within a three-month timeframe and at lengths >30 mm (see Chapter 3). Our modelling also demonstrated a significant relationship between southern pygmy perch abundance and that of submerged vegetation (see Chapter 2). With these factors in mind, the aim of these field experiments was to compare the reintroduction viability, growth, and reproductive success of southern pygmy perch between areas of high-density submerged vegetation and low-density submerged vegetation areas within the Lower Lakes. In this experiment, fish were stocked into the experimental enclosures in September, allowing the fish to have the maximum time during the spawning season to reproduce. The length of all stocked fish was measured prior to release into enclosures to determine growth rate of any fish sampled post release and to differentiate new recruits. The results of this experiment provide information to underpin the most favourable conditions for supplementing wild populations of the species within the Lower Lakes area, and help to increase understanding of the species for areas further afield. Information gained through this field experiment may also be extended to reintroductions of the Yarra pygmy perch within the Lower Lakes area.

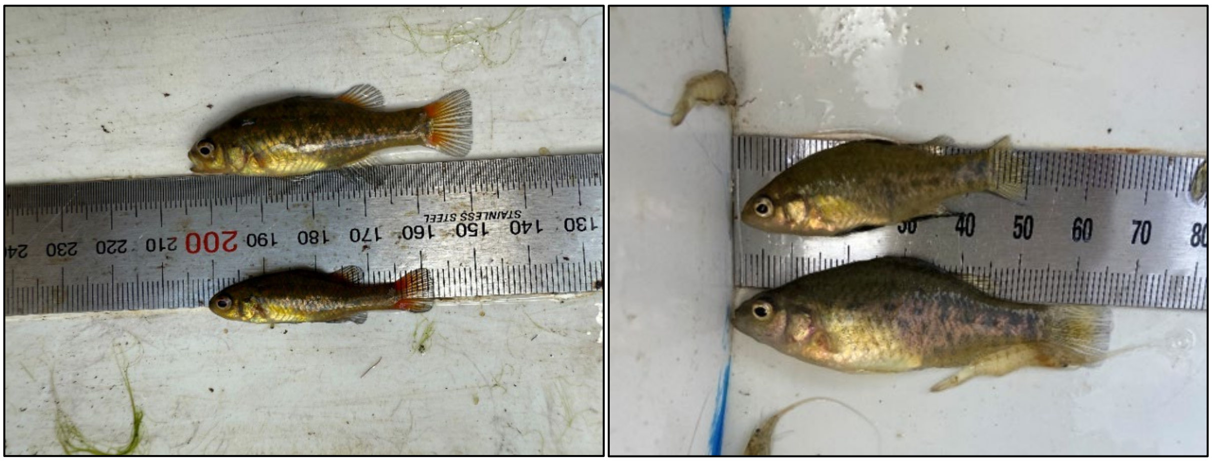

Figure 4.1. Southern pygmy perch (left) and Yarra pygmy perch (right). Note the red of the tail and fins of southern pygmy perch and the slight notch and black mottle in the eye of Yarra pygmy perch.

4.2 Methods

Effectiveness of the reintroduction between the two test conditions was measured by survivability of the mature southern pygmy perch, growth of the mature individuals during the experiment, and the spawning success and recruitment abundance of the fish. To ensure the fish had the greatest chance for a recruitment event, they were stocked in September 2025, allowing for the potential of multiple spawns within one season.

4.2.1 Site Selection

Sites for the experiment were limited to areas of historical natural distribution of southern pygmy perch within the Lower Lakes, site accessibility, landholder participation, and distance away from the barrages (Figure 4.2). Sites were required to have availability of both high- and low-density areas of submerged vegetation to allow room for the two different treatments at each site. Vegetation density was measured visually with ~20% submerged vegetation present in low-density treatments, and ~80% submerged vegetation present in high-density treatments. Sites also required a water depth of approximately one metre, to provide refuge in periods of high temperature or with falling lake levels. Two site locations were selected to minimise site specific differences affecting the results, with a total of six enclosures used at each site (3 low vegetation, 3 high vegetation), thus a total of 12 enclosures were used (Table 4.1).

Figure 4.2. Site selection undertaken by the project team, Ngarrindjeri Aboriginal Corporation rangers and landholders.

Table 4.1. Sites used in the experiment with E dictating the enclosure number, Lx for a low vegetation condition, Hx for a high vegetation condition and the percentage of cover of each habitat type for each site.

River System	Waterway	Location	Latitude	Longitude	Subsurface physical %	Subsurface biological %	Emergent %	Edge vegetation %	Shade %
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E1 (L1)	-35.486	138.907386	5	20	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E2 (L2)			5	20	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E3 (H1)			5	80	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E4 (H2)			5	80	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E5 (H3)			5	80	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 1 Marina E6 (L3)			5	20	30	70	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E1 (H1)	-35.454	138.951	5	80	60	95	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E2 (H2)			5	80	60	95	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E3 (L1)			5	20	60	95	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E4 (L2)			5	20	60	95	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E5 (L3)			5	20	60	95	0
Lake Alexandrina	Lake Alexandrina	Site 2 Richards E6 (H3)			5	80	60	95	0

4.2.2 Fish Collection

Southern pygmy perch for the experiment were sourced from a surrogate site (private wetland) where a population of the genetically distinct Murray-Darling Basin fish are maintained. These fish were originally collected during the Millennium drought from the Lower Lakes. A total of 240 individuals were collected using fyke nets and separated into aerated transport buckets with a 7:13 male to female ratio of 20 fish in each bucket (Figure 4.3). This stocking sex ratio was used due to a limited number of males available, but it did also increase potential reproductive output with more females than males. These fish were transferred to the enclosure sites and released on the same day.

Figure 4.3. Fish being sorted into buckets with 7:13 Male to Female sex ratio.

4.2.3 Enclosure Setup

Enclosures for this experiment were designed to try to minimise southern pygmy perch fry from leaving the enclosure whilst simultaneously providing protection from larger predatory fish that were present in the surrounding area and allowing zooplankton of small enough size to be food for fish to pass through. To achieve this outcome, enclosures comprised a 10 m long by 1.2 m high steel mesh, with a 6.5 mm^2 mesh size, to which 10 m of fiberglass flyscreen (mesh size 1.16 x 1.56 mm) was attached using cable ties. The mesh enclosures were installed into the experiment sites in a circle shape, with the flyscreen side on the outside, and were held in place using garden stakes (Figure 4.4). The stakes and mesh were placed in a circular shape, and the two ends of the mesh cable tied together to enclose the area (Figure 4.4). During setup the mesh was pushed into the muddy bottom of the experiment sites to try and eliminate any gaps and prevent fish movement underneath the mesh. The enclosures were set up in three high vegetation areas (>80% veg) and three low vegetation areas (<20% veg) at both experiment sites, in water that was of moderate depth (~0.8 m.) Within the enclosures, southern pygmy perch were stocked after a week of allowing the enclosures to settle, with fish being stocked at a 7:13 male to female ratio and a total of 20 fish into each enclosure.

Figure 4.4. Experiment enclosures being prepared and set up at Marina site.

4.2.4 Sampling Method

Enclosures were sampled for southern pygmy perch juveniles, using two methods: 1) a 36 µm fine mesh plankton sampling drag net and 2) a plankton dip net (mesh size 250 µm). Water quality readings including pH, electrical conductivity (µs/cm), temperature (°C), and dissolved oxygen (mg/L), were recorded at the time of sampling (YSI-556 multiprobe water quality meter). Initial sampling of the enclosures was conducted two weeks following the introduction of the southern pygmy perch. At this initial sampling event, the plankton drag net was set to 2.5 metres, the approximate distance across each of the enclosures, by placing a knot in the rope. Once the distance on the rope was set, the plankton net was lowered into an enclosure and slowly retrieved across the enclosure at a pace to keep the net just under the surface of the water, to ensure the net was above the silt on the bottom. After the net was retrieved, it was lifted to ensure all the water drained through the mesh, following which, it was washed down with ethanol into the collection jar at the bottom of the net. The collection jar was then washed with ethanol into a storage container and labelled, after which, the plankton net and collection jar were both rinsed again with distilled water before the next sample. The sampling continued, following this process, until all enclosures were sampled. During sampling, three plankton drags were also conducted on the outside of the enclosures to provide control samples of the plankton structures of the surrounding area.

After initial sampling was conducted, sampling was moved to a three weekly sampling period to better allow opportunity for fish to spawn and for free-swimming fry to be present. During subsequent sampling events the dip net method was also added, in which a fine-mesh plankton dip net was placed in each enclosure and moved at an even pace for approximately one metre, adjacent to vegetation in the enclosure, and the contents placed into a white inspection tray, following which the dip net was rinsed into the tray. The tray was then visually inspected for any fish fry, which were later identified under microscope. After inspecting each tray, the tray was emptied, and the net was rinsed again before sampling the next enclosure. This process was repeated until all enclosures had been sampled.

During the last two sampling events, bait traps, baited with dry cat food, were used to sample large juveniles that would have been able to evade the dip and plankton nets. For this method, four bait traps were placed into each enclosure for one hour, before being retrieved and captured fish identified and recorded.

At the end of the experiment, the enclosures were sampled using two dip nets moved around the outside edge of each enclosure at the same time, meeting at the same location, before being withdrawn from the enclosure and the contents identified. Similarly, each enclosure was sampled with three bait traps, for one hour set times, to provide a better chance of sampling any fish in the enclosures. After these final samples were taken, all fish caught were measured and counted to look for growth and survivorship of mature individuals, as well as the abundance of any recruits.

To determine what fish species where outside of the enclosures, and to detect any southern pygmy perch that may have escaped the enclosures, eight single-winged fyke nets were set overnight every three weeks outside of the experimental enclosures.

4.3 Results

4.3.1 Water Quality

Over the course of the experiment, some variation in water quality parameters for each test parameter was observed, however, no major changes were observed (Table 4.2). During the 10/12/2025 sampling event, water level was higher than both the previous and following sampling, however, this variation is likely due to wind during the sampling event. Water temperatures increased up to 28°C, indicating that temperatures exceeded the threshold for southern pygmy perch to breed. Water pH was marginally higher in high vegetation enclosures than in low vegetation enclosures at Site 1, but no real difference was observed at Site 2 (pH 7.5 vs 7.92 at Site 1 over the entire sampling period.) Dissolved oxygen followed the same trend, with Site 1 showing a higher dissolved oxygen in high vegetation enclosures (6.50 mg/L vs 6.31 mg/L high vs low, respectively,) but no difference was noted at Site 2. Low dissolved oxygen was noted at the Marina site on the 20/11/2025, but this was due to the water quality meter reading inaccurately and did not represent the true oxygen value.

Table 4.2. Dates of each sampling event and mean water quality parameters for all enclosures of both high and low vegetation test conditions for each site.

Date	Location	Vegetation Concentration	Plankton Drag Net	Dip Net (1m)	Bait Traps (1 Hour Replicates)	Depth max (m)	pH	Conductivity (uS/cm)	Temperature (ºC)	Dissolved oxygen @0.2m (ppm)
23/09/2025	Site 1 Marina	Low				1	7.19	2457.0	14.1	8.4
23/09/2025	Site 1 Marina	High				1	8.39	2503.0	14.5	8.8
23/09/2025	Site 2 Richards	Low				1	8.84	1179.7	14.9	8.9
23/09/2025	Site 2 Richards	High				1	9.02	1227.3	14.9	9.2
10/10/2025	Site 1 Marina	Low	Y			1	7.11	2220.3	16.1	9.3
10/10/2025	Site 1 Marina	High	Y			1	7.51	2159.3	15.3	8.4
10/10/2025	Site 2 Richards	Low	Y			1	8.09	998.7	18.0	11.6
10/10/2025	Site 2 Richards	High	Y			1	7.75	997.3	17.8	10.9
30/10/2025	Site 1 Marina	Low	Y	Y		1	7.72	1698.0	19.6	5.2
30/10/2025	Site 1 Marina	High	Y	Y		1	8.16	1687.0	19.6	5.5
30/10/2025	Site 2 Richards	Low	Y	Y		1	7.97	1016.0	20.0	6.2
30/10/2025	Site 2 Richards	High	Y	Y		1	8.43	1056.3	20.1	7.8
20/11/2025	Site 1 Marina	Low	Y	Y		1	7.14	1484.3	16.4	2.9
20/11/2025	Site 1 Marina	High	Y	Y		1	7.83	1448.7	16.7	2.8
20/11/2025	Site 2 Richards	Low	Y	Y		1	8.22	1083.0	18.7	7.4
20/11/2025	Site 2 Richards	High	Y	Y		1	8.36	1087.0	18.8	8.2
10/12/2025	Site 1 Marina	Low	Y	Y		1.2	7.15	1442.7	20.2	6.2
10/12/2025	Site 1 Marina	High	Y	Y		1.2	7.61	1434.0	20.7	7.5
10/12/2025	Site 2 Richards	Low	Y	Y		1.2	8.11	826.3	21.6	7.2

10/12/2025	Site 2 Richards	High	Y	Y		1.2	7.87	1005.0	20.9	6.4
8/01/2026	Site 1 Marina	Low		Y		1	7.97	1482.7	26.1	7.8
8/01/2026	Site 1 Marina	High		Y		1	8.09	1486.0	26.3	7.4
8/01/2026	Site 2 Richards	Low		Y		1	8.19	982.7	27.4	7.8
8/01/2026	Site 2 Richards	High		Y		1	8.02	993.0	27.3	7.7
27/01/2026	Site 1 Marina	Low	Y	Y		1	7.32	1507.0	19.1	4.4
27/01/2026	Site 1 Marina	High	Y	Y		1	7.29	1535.3	19.1	4.5
27/01/2026	Site 2 Richards	Low	Y	Y		1	7.55	966.7	19.8	4.8
27/01/2026	Site 2 Richards	High	Y	Y		1	7.41	983.0	19.9	4.6
10/02/2026	Site 1 Marina	Low	Y	Y	2x	1	8.24	1707.7	26.2	6.4
10/02/2026	Site 1 Marina	High	Y	Y	2x	1	8.61	1734.0	27.1	7.8
10/02/2026	Site 2 Richards	Low	Y	Y	2x	1	8.51	1051.3	27.1	8.2
10/02/2026	Site 2 Richards	High	Y	Y	2x	1	8.91	1090.0	26.9	8.3
4/03/2026	Site 1 Marina	Low	Y	Y	3x	1	7.66	1454.7	17.4	6.4
4/03/2026	Site 1 Marina	High	Y	Y	3x	1	7.78	1436.0	17.6	5.9
4/03/2026	Site 2 Richards	Low	Y	Y	3x	1	7.67	975.3	20.5	8.3
4/03/2026	Site 2 Richards	High	Y	Y	3x	1	7.67	994.3	20.1	7.3
					Site 1 Marina	Mean Low	7.50	1717.15	19.45	6.31
					Site 1 Marina	Mean High	7.92	1713.70	19.65	6.50
					Site 2 Richards	Mean Low	8.13	1008.85	20.87	7.83
					Site 2 Richards	Mean High	8.16	1048.15	20.74	7.83

4.3.2 Phytoplankton and dip net samples

During dip net sampling, all samples were analysed, with any small fish being identified under microscope in order to ensure accurate identification. Across the experiment most juvenile fish captured were the alien eastern gambusia *Gambusia holbrooki* (Table 4.3). Over the course of the experiment, this species was collected in all the enclosures and was noted early on in the experiment. Thirty-one Murray-Darling rainbowfish *Melanotaenia fluviatilis* fry were collected in the high vegetation enclosures along with adults of the native species flathead gudgeon *Philypnodon grandiceps*, dwarf flathead gudgeon *Philypnodon macrostomus*, carp gudgeon *Hypseleotris spp.*, and western blue-spot goby *Pseudogobius olorum*. Only one mature female southern pygmy perch (total length 62 mm) was collected across all enclosures.

For the phytoplankton sampling, all samples were collected and preserved in ethanol and will be analysed in the future during stage two of this experiment (pending funding). From this data, the two test conditions will be compared to look for patterns within the collected phytoplankton and zooplankton, which could indicate whether juvenile fish were present, even if they were not detected during dip netting or bait trapping. This data will also be useful to investigate the difference in phytoplankton and zooplankton species, and their respective productivity levels, between the two test conditions (high and low vegetation), as well as between the inside and outside of the enclosures.

Table 4.3. Overview of fish sampled inside each enclosure over the entire sampling period.

			Fish Caught							Opp Catch		
Waterway	**Location**	**Vegetation Cover**	**Southern Pygmy Perch**	**Carp Gudgeon**	**Rainbowfish Fry**	**Western Blue-spot Goby**	**Flathead Gudgeon**	**Dwarf Flathead Gudgeon**	**Eastern Gambusia**	**Yabby**	**Para**	**Macro**
Lake Alexandrina	Site 1 Marina E1 (L1)	Low							3			
Lake Alexandrina	Site 1 Marina E2 (L2)	Low							5	1		
Lake Alexandrina	Site 1 Marina E3 (H1)	High		1	6			1	34	3		
Lake Alexandrina	Site 1 Marina E4 (H2)	High			20				16	1		
Lake Alexandrina	Site 1 Marina E5 (H3)	High	1		5		3		24	1		9
Lake Alexandrina	Site 1 Marina E6 (L3)	Low							5			
Lake Alexandrina	Site 2 Richards E1 (H1)	High							10			
Lake Alexandrina	Site 2 Richards E2 (H2)	High		2		1			24			
Lake Alexandrina	Site 2 Richards E3 (L1)	Low				1			13		1	
Lake Alexandrina	Site 2 Richards E4 (L2)	Low										
Lake Alexandrina	Site 2 Richards E5 (L3)	Low							13	1		
Lake Alexandrina	Site 2 Richards E6 (H3)	High				1			8			

4.3.3 Growth / recaptures at end of experiment

The initial sizes of each southern pygmy perch that were recorded for each enclosure are shown in table 4.4. There were no southern pygmy perch sampled after the initial stocking period meaning that growth rates during this experiment were unable to be reviewed.

Table 4.4. Initial stocking sizes of southern pygmy perch for each enclosure, including minimum, maximum and mean for each sex.

Location	Sex	Initial stocking sizes Min	Max	Average
Site 1 Marina E1 (L1)	Male	51	62	57
	Female	50	76	61
Site 1 Marina E2 (L2)	Male	52	62	56
	Female	42	78	60
Site 1 Marina E3 (H1)	Male	40	61	52
	Female	47	81	60
Site 1 Marina E4 (H2)	Male	40	67	55
	Female	45	70	57
Site 1 Marina E5 (H3)	Male	45	56	53
	Female	45	68	58
Site 1 Marina E6 (L3)	Male	51	60	55
	Female	46	73	59
Site 2 Richards E1 (H1)	Male	46	61	54
	Female	45	70	56
Site 2 Richards E2 (H2)	Male	40	55	49
	Female	48	71	60
Site 2 Richards E3 (L1)	Male	47	58	52
	Female	42	70	57
Site 2 Richards E4 (L2)	Male	42	60	51
	Female	30	67	55
Site 2 Richards E5 (L3)	Male	50	65	56
	Female	37	72	55
Site 2 Richards E6 (H3)	Male	41	68	53
	Female	47	67	60

4.4 Discussion

The non-detection of southern pygmy perch post release in the experimental enclosures negates a comparison of how vegetations density may influence reintroduction success. Despite this outcome, the results and experience can be used to help inform future reintroductions. During the dip net sampling, more native species were observed in the high vegetation enclosures, potentially indicating a preference for other native fishes to high vegetation sites. Juvenile Murray-Darling rainbowfish were only sampled in high vegetation enclosures at the Marina site. Unfortunately, the non-native eastern gambusia was also more abundant within the high vegetation enclosures, as both juveniles and adults. This preference by eastern gambusia may promote unfavourable interactions with small-bodied native species.

Establishing enclosures, that required setting up in situ, may have caused some of the fish species surveyed to be trapped within the enclosure from the beginning of the experiment. Similarly, during some of the extreme and unprecedented high wind weather conditions that occurred, some of the enclosure walls dipped under the surface temporarily, providing the potential for fish into move to and out of the enclosure (with a preference for surface dwelling fish such as the eastern gambusia). Fully enclosed enclosures could be used for future fish experiments in the field, however, when testing a low versus high density of macrophytes, the bottom of the enclosure must remain open, allowing fish to potentially move underneath the enclosure if gaps are present. The addition of steel or plastic drops to the bottom of the enclosure, on all sides, to penetrate 10–15 cm into the sediment, could prevent fish movement, however, it would make installation of the enclosure more difficult. This modification could also mean that pre-existing fish present in the area could be trapped in enclosures affecting results.

Other limitations of the method described include the presence of various fish species within the enclosures, all of which have the potential to predate on eggs and early-stage juveniles of the southern pygmy perch if spawning occurred. Similarly, eastern gambusia can nip the fins of native species and could have caused stress to the southern pygmy perch, reducing their likelihood of spawning. If the southern pygmy perch did spawn, another potential cause for their absence in subsequent sampling, may have been due to the flyscreen that was used to line the enclosures. The fly screen aperture (1.16 x 1.56 mm) was selected to allow water movement through the enclosures, whilst minimizing the ability for eggs and larval fish to escape. Yet, due to wind and waves at the experiment sites, on occasions, the flyscreen was displaced, allowing the larger 6.5 mm mesh to be exposed and potentially facilitating fish movement through the walls of the enclosure.

An extension of this study would be to sort and identify the plankton samples. Plankton samples may contain small larval fishes that were unobservable, which could provide results that the dip net and bait traps were unable to collect. Even in the case that no reproduction occurred, these plankton samples will also hold key information on the plankton composition and density, and thus secondary productivity in high vegetation vs low vegetation areas within the CLLMM region; a strong indicator of food availability at future release sites. Consequently, the analysis of these samples is important and could benefit future site selection for fish releases.

5 General Discussion

Small-bodied freshwater fishes are under threat from a range of anthropogenic pressures across the CLLMM region, with ongoing population decline exacerbated by the prolonged Millennium Drought. The drought resulted in unprecedent declines in water availability and lake levels and associated loss of aquatic habitat. Amongst some of the most affected species were the southern pygmy perch and Yarra pygmy perch. A range of conservation actions have been implemented in the attempt to recover these two target species. These conservation actions include improved water level management, habitat restoration, alien species control, and translocations to reestablish resilient, connected populations, to help secure the long-term survival of the species in the face of environmental change. Nevertheless, after almost two decades of post-drought intervention, populations of both species remain in peril, with questions remaining about what is required for the species to form self-sustaining populations.

To help inform future management of wild and ex situ pygmy perch populations, reintroduction ecology, and the use of water for the environment, this project used 15 years of data on southern pygmy perch from the CLLMM and EMLR regions, and modelling, otolith aging and field experiments, to investigate how life history processes (e.g. reproduction) and population demographics and are associated with biotic and abiotic variables.

Population modelling was used to determine trends (changes) in southern pygmy perch abundance and recruitment over time (phase 1), and the drivers of these trends (phase 2). Broadly, over the study period, the relative abundance of southern pygmy perch declined in the EMLR and increased in the Lower Lakes. Southern pygmy perch mean abundance over time (2006–2025) peaked in the EMLR in 2008, and was lowest in 2024, with high variation between sites and over time. Modelled predicted relative abundance supported this conclusion with a significant decline of southern pygmy perch over time in the EMLR population from 2006–2025. In contrast, mean relative abundance in the Lower Lakes was lowest in 2010–2012 (following Millennium Drought) and peaked in 2024, with less variability observed than in the EMLR. These findings show that the effects of environmental change are highly evident in the EMLR population, with fish abundance declining in a continuing drying climate and water resource limited system. Although the Lower Lakes population underwent considerable disturbance during and after the Millennium Drought, the continuing positive trajectory of this population indicates this region may confer resilience to this species in the face of environmental change. In the EMLR, however, the southern pygmy perch population is likely to continue to decline.

The second phase of characterising trends in southern pygmy perch populations in the EMLR and Lower Lakes was to determine which environmental variables may be associated with population trends, using empirical and modelling approaches. In the EMLR, modelling of the relative abundance of southern pygmy perch in relation to potential regional environmental drivers revealed that Antarctic Oscillation (AAO), with a time lag of two years, was the most influential driver with empirical analysis showing that a positive AAO drives a decrease in southern pygmy perch and that this relationship strengthening after the Millennium Drought (2009). This conclusion is reflected in the local hydrology of the EMLR. A positive AOO pushes frontal systems south, resulting in lower rainfall and increased water extraction in the EMLR, with a concurrent decline in southern pygmy perch abundance. This result highlights the compromised long-term sustainability of southern pygmy perch, and other small-bodied fish species in the EMLR under current and future changing climates, where positive AAO conditions will be more common.

In the Lower Lakes, modelling of the relative abundance of southern pygmy perch, in relation to regional environmental drivers, revealed that mean lake water level, with a lag time of six months (previous September), was found to be the most influential driver, with a positive relationship where elevated lake levels promote increased abundance. As the key driver of relative abundance of southern pygmy perch in the Lower Lakes, the importance of lake water level management, via River Murray flow and barrage operations, is paramount. The optimal conditions to promote recruitment in Lower Lakes for southern pygmy perch populations is approximately a 0.8 m AHD lake water level in September.

In both the EMLR and Lower Lakes, the density of aquatic macrophytes was found to be the most influential site level local driver of southern pygmy perch relative abundance. A strong positive relationship was found where increasing macrophyte density is associated with increased southern pygmy perch abundance. Like native fishes, aquatic macrophytes, have also been negatively impacted by river regulation in the lower River Murray. To guide restoration of both, further studies investigating the relationship between different aquatic macrophyte species, and the relative abundance of southern pygmy perch and other native fish species, would provide important information to help guide habitat restoration programs.

Modelling of site-scale drivers of southern pygmy perch abundance led to a field experiment to investigate how aquatic macrophyte density may influence the recruitment and abundance of fish in the Lower Lakes. Although southern pygmy perch were not recaptured during field experiments, results revealed early-stage juveniles from other native fish species were only found in high density aquatic vegetation enclosures, and the highest abundances of adult native fishes were also collected in these conditions. No significant differences in water quality were measured between the high- and low-density vegetation treatments; thus, water physico-chemistry may not be a contributing factor to variability in fish abundances between treatments. Future analysis of phytoplankton samples during the next stage of this project will allow for comparison of secondary productivity between treatments, information that is important for water level, habitat, and fish conservation management.

The present study is the first to provide credible daily age estimates for southern pygmy perch from the CLLMM region, a vulnerable MDB population of this species. A high success rate in otolith preparation, with reasonable to good readability, indicates the formation of interpretable daily and annual increments in otolith structure. Future research is recommended as follows: (1) to validate the periodicity of increment formation at the daily and annual level, (2) to implement a robust sample size sample-at-age monitoring program, which will be representative of the Lower lakes southern pygmy perch populations, (3) to investigate sexual dimorphism in growth, and other life history parameters and (4) to age southern pygmy perch from a variety of catchments (including surrogate dams) to test for geographical differences in growth. When this work is complete, life history parameters can be related to environmental drivers.

5.1 Key Messages

A series of key messages arise from this project, which are summarised below:

- Broadly, over the study period, the relative abundance of southern pygmy perch declined in the EMLR and increased in the Lower Lakes.
- In the EMLR, the most influential regional driver of southern pygmy perch relative abundance was the Antarctic Oscillation (AAO) with a time lag of two years.
- In the Lower Lakes, the most influential regional driver of southern pygmy perch relative abundance was mean lake water level with a lag time of six months (previous September).
- In both the EMLR and Lower Lakes, the density of aquatic macrophytes present was found to be the most influential site level local driver of southern pygmy perch relative abundance.
- In face of environmental change, the capacity to maintain appropriate water levels and key habitat requirements for small-bodied fish species will become more challenging. Thus, there is a strong need to prioritise the specific requirements of these species during management processes, particularly during times of poor water availability.
- The EMLR and Lower Lakes regions will likely have different responses to environmental change. In the EMLR, fish conditions are likely to continue to decline without intervention. In the Lower Lakes, if water conditions can be maintained above 0.4 m at all times, and annual spring (September) freshes can be included into management plans (≥ 0.8 m), this will help to promote better breeding and recruitment responses for fishes such as southern pygmy perch.
- This project has significantly benefited from strong long-term collaborations and long-term data and fish specimen collections through other projects (15 years+), ongoing conservation work of the Big Little Fish group, involvement and support of NAC, landholder support and willingness to participate, and critical funding.
- The ongoing strong working relationship with management agencies ensures that the outcomes of this and other similar projects are readily incorporated into decision making.

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Appendix

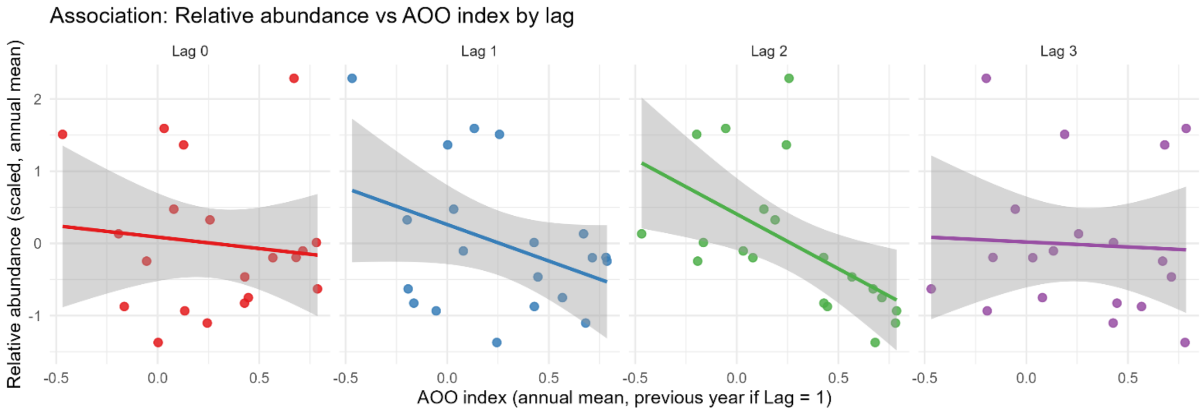

Figure A1. Relationship between scaled EMLR southern pygmy perch relative abundance and the Antarctic Oscillation (AAO) index across four temporal lags (Lag 0–3). Each panel shows annual relative abundance (scaled to mean = 0, SD = 1) plotted against the AAO index for the corresponding lag period, with fitted linear regression lines and 95% confidence intervals. The panels illustrate how contemporaneous (Lag 0) and lagged climate conditions (Lag 1–3) relate to interannual variation in abundance, highlighting the strongest negative association at Lag 2.

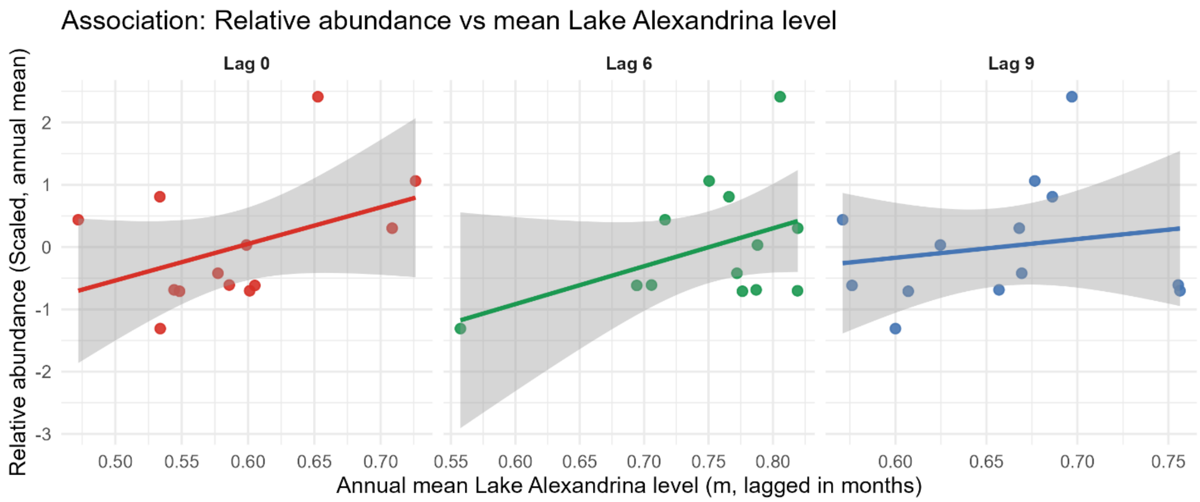

Figure A2: Relationship between scaled Lower Lakes southern pygmy perch relative abundance and the Lake Alexandrina mean water level index across three temporal lags (zero [at the time of sampling], six and 9 months). Each panel shows annual relative abundance (scaled to mean = 0, SD = 1) plotted against the mean lake level for the corresponding period, with fitted linear regression lines and 95% confidence intervals. The panels illustrate how contemporaneous (Lag 0) and lagged levels (lags 6 and 9 months) relate to interannual variation in abundance, highlighting the strongest positive association at a lag of 6 months. To remove the effect of the Millennium Drought the data was filtered to contain only 2013 onwards but post sensitivity analyses (with and without <2013).

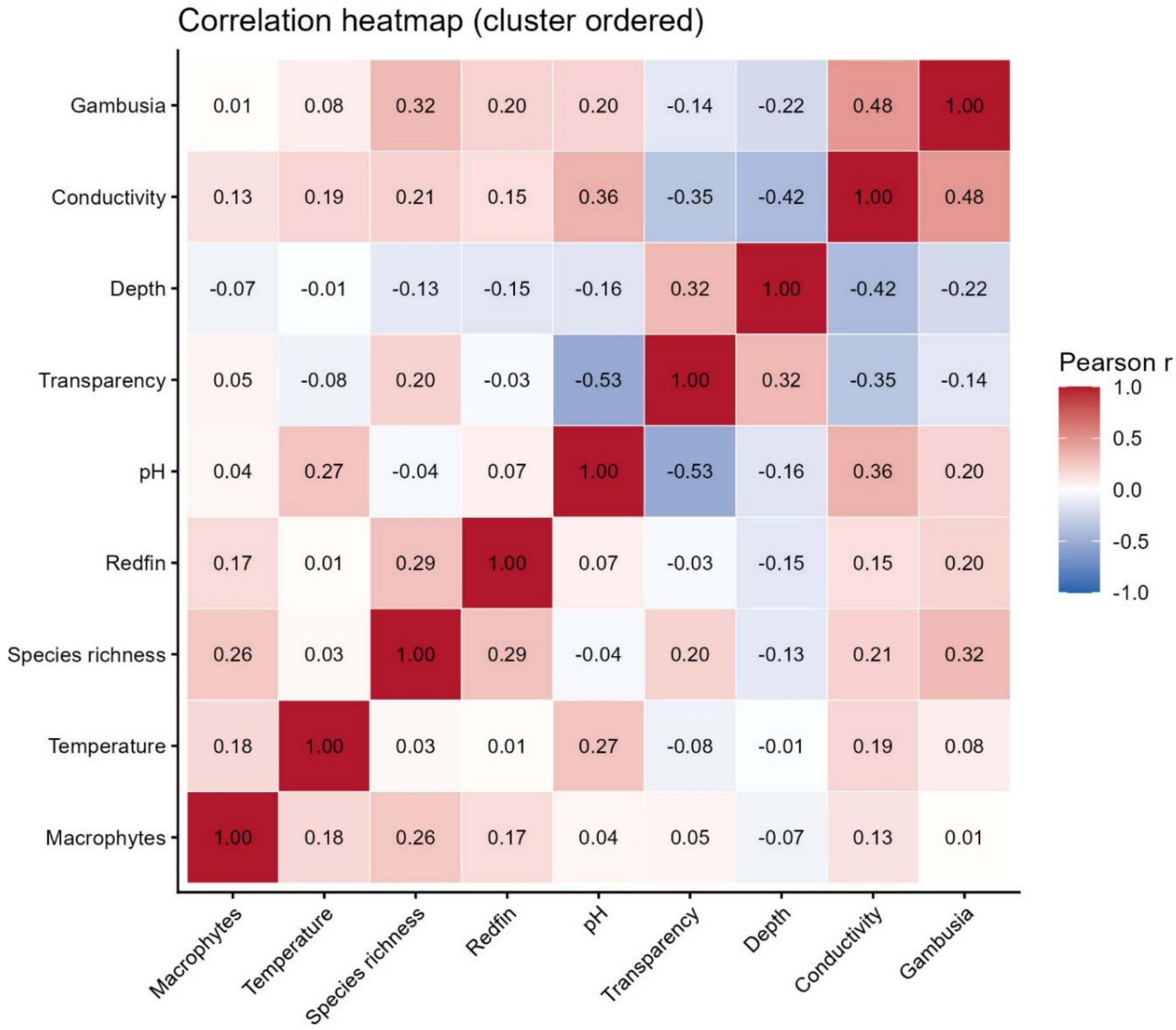

Figure A3. Collinearity among EMLR site level candidate drivers. The cluster-ordered correlation heatmap displays Pearson r with values annotated.

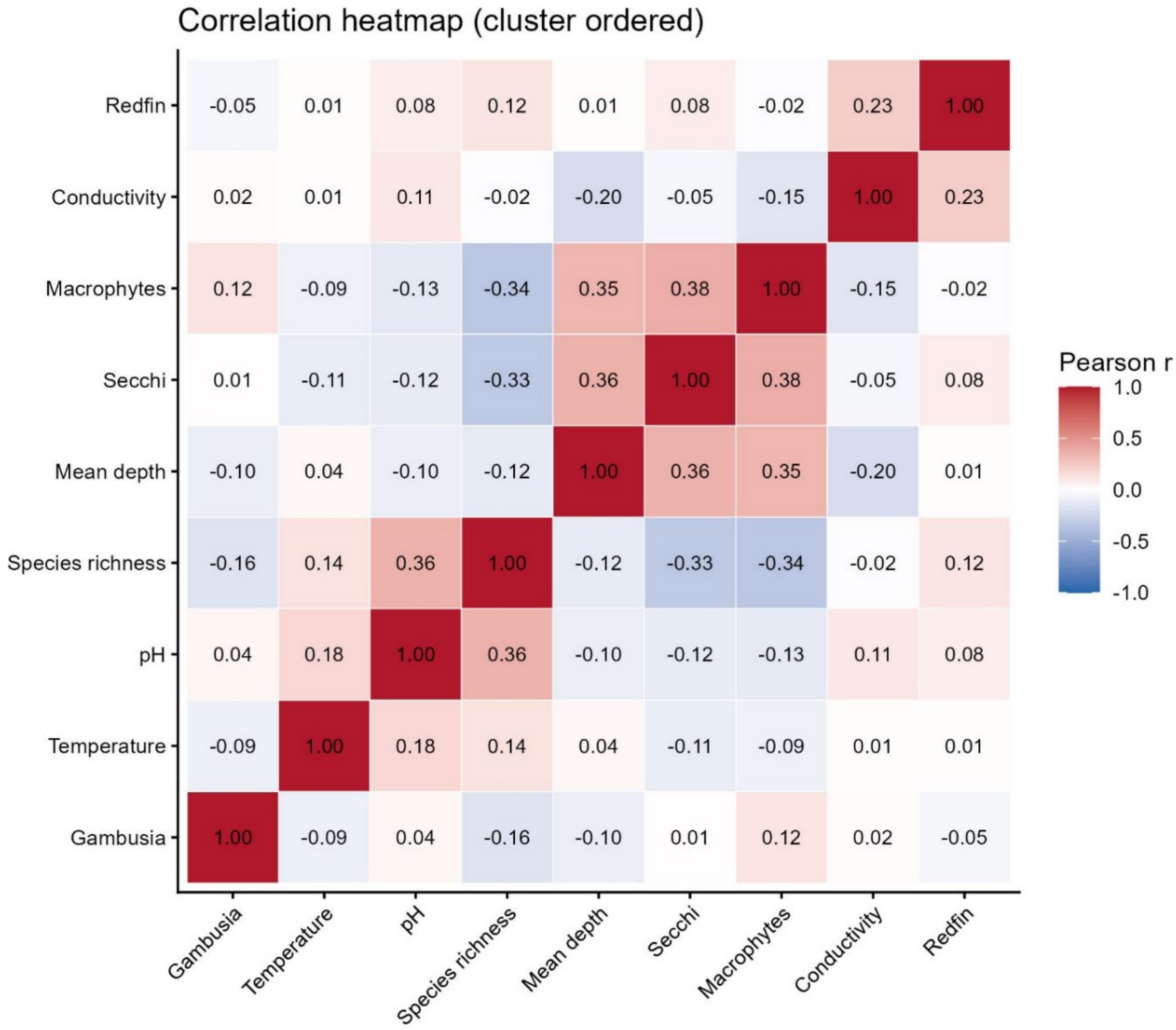

Figure A4. Collinearity among Lower Lakes site level candidate drivers. The cluster-ordered correlation heatmap displays Pearson r with values annotated.

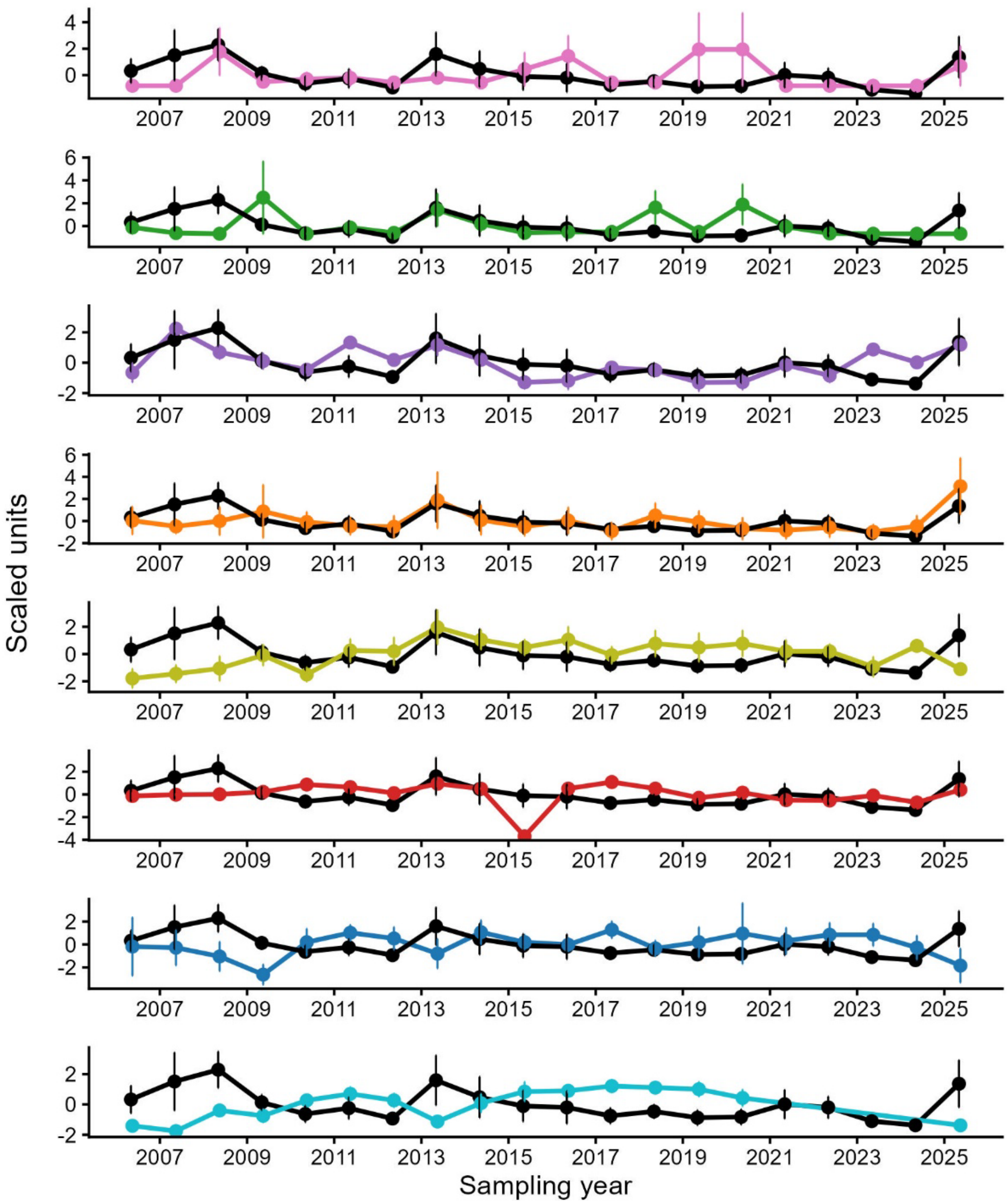

Figure A5. Annual trends in EMLR southern pygmy perch relative abundance and environmental candidate drivers of abundance. Each panel shows scaled annual means (points) with ±1 SE error bars. To avoid overlap, the two series in each panel are horizontally offset by a few days (Relative abundance left, environmental variable right). Top: Redfin (magenta) overlaid with relative abundance (black). Second: Gambusia (green) overlaid with relative abundance (black). Third: pH (purple) overlaid with relative abundance (black). Fourth: Conductivity (orange) overlaid with relative abundance (black). Fifth: Species richness (olive) overlaid with relative abundance (black). Sixth: Temperature (red) overlaid with relative abundance (black). Seventh: Depth (blue) overlaid with relative abundance (black). Bottom: Transparency (teal) overlaid with relative abundance (black).

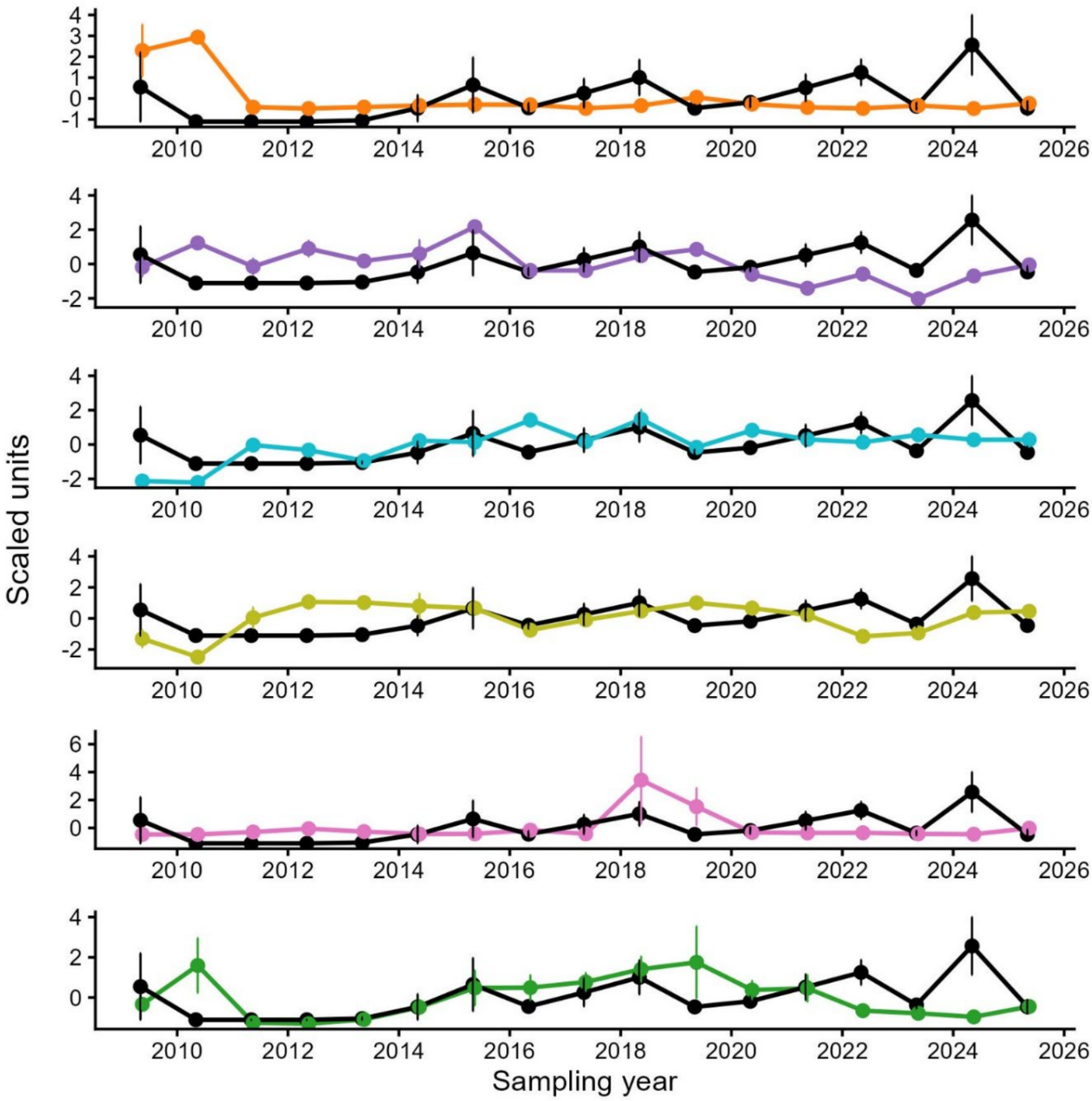

Figure A6. Annual trends in Lower Lakes relative southern pygmy perch abundance and environmental candidate drivers of abundance. Each panel shows scaled annual means (points) with ±1 SE error bars. To avoid overlap, the two series in each panel are horizontally offset by a few days (Relative abundance left, environmental variable right). Top: Conductivity (orange) overlaid with relative abundance (black). Second: pH (purple) overlaid with relative abundance (black). Third: Secchi depth (teal) overlaid with relative abundance (black). Fourth: Species richness (olive) overlaid with relative abundance (black). Fifth: Redfin (magenta) overlaid with relative abundance (black). Bottom: Gambusia (green) overlaid with relative abundance (black).

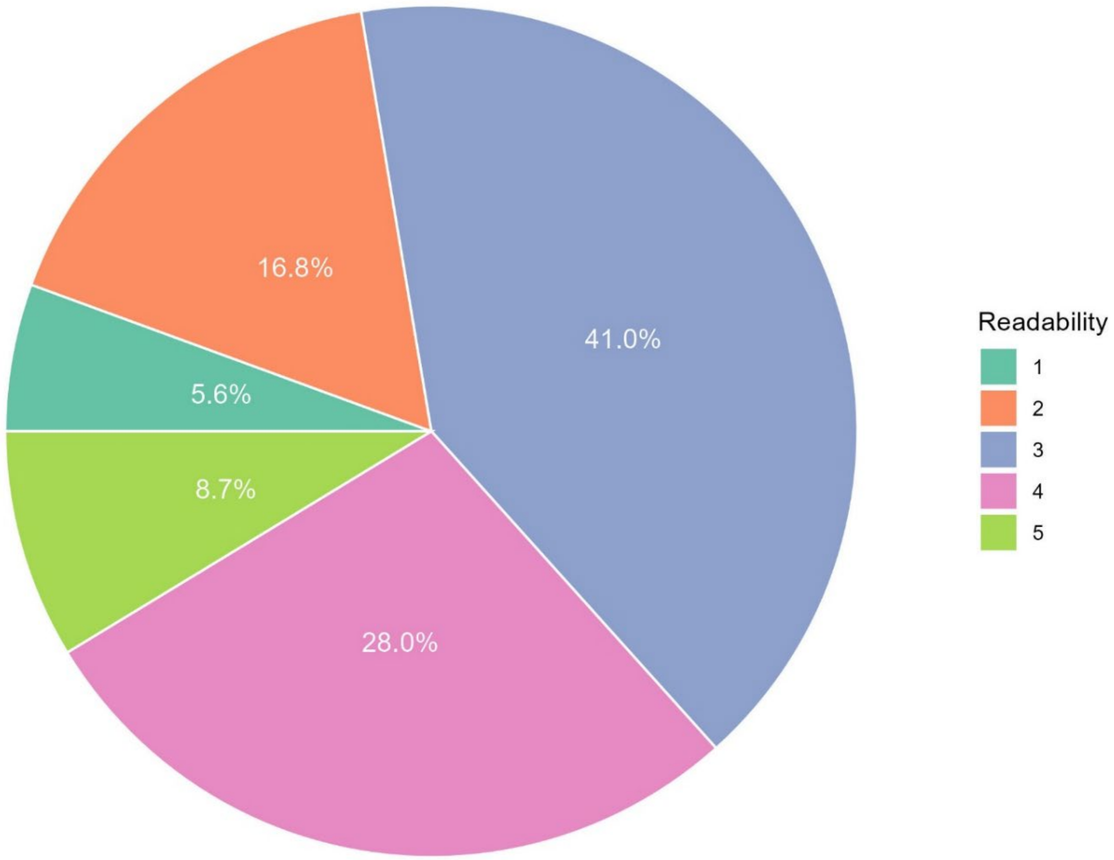

Figure A7: Proportion of southern pygmy perch sectioned otolith readability scores for all samples prepared for ageing. A readability score of one is the highest quality and five the lowest.

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