



Effects of suboptimal temperatures on larval and juvenile development and otolith morphology in three freshwater fishes: implications for cold water pollution in rivers

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Abstract Temperature is a key determinant that governs fish survival, reproduction, growth and metabolism. In freshwater ecosystems, anthropogenic influences have resulted in acute and prolonged temperature changes which lead to lethal and sub-lethal impacts on the biota that occupy these environments. We assessed the effects of temperature on somatic and otolith growth and development of three species of native Australian freshwater fish (silver perch *Bidyanus bidyanus*, trout cod *Maccullochella macquariensis* and golden perch *Macquaria ambigua*) to simulate how thermal pollution from the release of unseasonably cold water from thermally stratified dams in Australian freshwater

ecosystems may impact fish at critical life-history stages. Fish (31 days post-hatch) were exposed to four temperature treatments (13, 16, 20, 24 °C) for 30 days. Low temperatures resulted in reduced somatic growth, with no growth observed in silver perch and golden perch held at 13 °C over 30 days. Somatic growth was highest at the upper temperature of 24 °C. Morphological assessment of fish size reiterated that low water temperatures resulted in reduced body size, particularly in terms of body width and head size. Low temperatures were associated with reduced otolith growth in all species, however a somatic-otolith size relationship was maintained for all species in measures of otolith weight, area, length and perimeter. The sub-lethal impacts observed in our study are likely to manifest at the population level through a reduced capacity of larvae and juveniles to avoid size-dependent predation, a narrower range of prey sources due to extended gape-limited feeding and, ultimately, poorer survival and recruitment.

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Introduction

Temperature is an important regulator of the biological processes of aquatic organisms (Brett 1971). As ectotherms, fish depend on temperature as a determinant of metabolism, spawning and survival and are adapted to specific temperature ranges (Brett 1971; Jobling 1995; Beiting et al. 2000). The optimal thermal range of fish

is determined by genetic histories and the thermal histories of the ecosystems they inhabit, as such thermal tolerance can vary widely between species but can also vary intra-specifically (Beitinger et al. 2000; Gunderson and Stillman 2015; Comte and Olden 2017). Within these ranges, metabolic functions such as feed utilization, growth, reproduction and swimming ability are enhanced (Jobling 1997; Beitinger et al. 2000). Optimal temperature ranges may also vary between life-history stages within species, where efficacy of metabolic functioning is necessary to support periods of rapid growth and development or reproduction (Morita et al. 2010). Maintaining optimal environmental thermal conditions is essential for fish at early life history stages when developmental changes and rapid growth are occurring and sensitivity to suboptimal thermal conditions is high (Sogard 1997).

Growth and body size is a major determinant for survival in fish, particularly during larval and juvenile life-history stages (Childs and Clarkson 1996; Sogard 1997). Reduced body size increases predation risk (Rice et al. 1987), size-dependent competition (Fausch and White 1986) and susceptibility to temperature extremes and changes (Johnson and Evans 1990; Johnson and Evans 1996; Clarkson and Childs 2000). Temperature can affect growth potential in fish as it drives metabolic functioning, which is essential for optimal feeding efficiency, ensuring that surplus energy is attained through food intake and can be converted into somatic growth (Kitchell et al. 1977; Buentello et al. 2000). Temperature-driven reductions in growth rate and/or body size in larval and juvenile fish increases their vulnerability to numerous stressors and ultimately affects fitness and survival.

Another potentially important effect of temperature on fish is its influence on the development of otoliths (fish ear stones). Otoliths are calcified structures that develop incrementally, forming annual and daily increments that are frequently used for ageing and back-calculating the growth histories of fish (Pannella 1971; Gauldie and Nelson 1990; Morrongiello et al. 2012) and for assessing life history events and past environmental conditions of individuals and populations of fish (Campana and Neilson 1985; Jones 1992; Campana 1999; Sponaugle 2010). Otolith growth is strongly linked to somatic growth (Gagliano and McCormick 2004; Starrs et al. 2013), however under certain conditions otolith growth can decouple from somatic growth (Barber and Jenkins 2001; Folkvord et al. 2004;

Baumann et al. 2005; Tonkin et al. 2008a; Tonkin et al. 2008b). This can complicate, or even invalidate, the use of these structures in biochronological studies and as proxies for back-calculating growth histories (Morrison et al. 2019). Understanding how otolith growth and its relationship with somatic growth is affected by thermal stress is essential for the application of otolith biochronological analyses in fisheries science.

Suboptimal thermal conditions in river ecosystems can originate from numerous sources causing either a warming or cooling of the thermal regime. Warm water pollution can be caused by industrial effluents, urban run-off and global warming (Verones et al. 2010; Van Vliet et al. 2013), whereas cold water pollution can be caused by large dams that release hypolimnetic water during periods of thermal stratification (Weber et al. 2017). Suboptimal thermal conditions have been identified as a contributing factor in historical reductions in native fish populations in freshwater, estuarine and marine ecosystems; therefore managing various sources of thermal pollution is essential in improving native fish stocks in many parts of the world (Clarkson and Childs 2000; Koehn 2001; Roessig et al. 2004; Lugg and Copeland 2014).

In Australian freshwater ecosystems, thermal reductions from large dam releases can reduce river temperature by as much as 16 °C (Lugg and Copeland 2014), with thermal suppressions persisting for up to 300 km downstream (Burton 2000; Lugg and Copeland 2014). This ‘cold water pollution’ has been documented widely in Australian rivers and has had a strong, adverse impact on native fish populations (Burton 2000; Todd et al. 2005; Sherman et al. 2007; Lugg and Copeland 2014; Gray et al. 2019; Michie et al. 2020).

The aim of this study was to determine the effect of temperature on somatic growth and otolith development in early life-history stages of three species of Australian freshwater fish that occupy rivers impacted by cold-water pollution; silver perch (*Bidyanus bidyanus*), trout cod (*Maccullochella macquariensis*) and golden perch (*Macquaria ambigua*). We subjected individuals of each species to a range of water temperatures that were representative of Murray-Darling Basin rivers affected by cold water pollution. Cold water pollution is extensive within the Murray-Darling Basin, the native habitat of the three test species. Experimental temperatures were selected based on a 20-year analysis of water temperatures downstream of Burrendong Dam on the Macquarie River (Michie et al. 2020), and also

encompass temperatures observed in a number of rivers across the Murray-Darling Basin (Burton 2000; Todd et al. 2005; Sherman et al. 2007; Lugg and Copeland 2014; Gray et al. 2019). We anticipated that cooler water temperatures may reduce growth potential in the species of fish tested due to the influence of temperature on biological and metabolic processes. Further, we predicted that the threatened species being tested (silver perch and trout cod) may be more sensitive to thermal pollution given historic range reductions and population declines in heavily regulated systems. The results of our study are discussed with regard to their relevance to the management of fish populations in regions affected by thermal pollution.

Methods

Experimental set-up

Silver perch, trout cod and golden perch were acquired 31 days post-hatch (dph) from the Department of Primary Industries (DPI) Narrandera Fisheries Centre hatchery in New South Wales, Australia. Trials were run sequentially due to the availability of the fish being affected by the specific timing of breeding for each species. Individuals for each species were selected from mixed breeding pairs. Fish were transferred to experimental aquaria containing bore water of 20 °C, matching the hatchery water conditions. The experimental set-up was within a light and temperature-controlled room at the Narrandera Fisheries Centre; room temperature was maintained at approximately 25 °C and light was sustained on a 12:12 cycle. Four replicate aquaria (3 L) were maintained for each temperature treatment, they were submersed in large water baths (70 L) which were temperature controlled by water chillers (HC-300A Hailea, China) to maintain constant temperature. Each replicate was fitted with a temperature logger (HOBO Pendant TM, Onset, U.S.A., accuracy ± 0.53 °C) and an aeration system. Each experiment was conducted over 30 days, which included acclimation to the experimental temperatures (13, 16, 20 and 24 °C) over a maximum period of 4 days. Throughout the experiment, the temperature loggers within each replicate tank monitored temperature at 15-min intervals. Measurements of temperature, dissolved oxygen, pH and conductivity were taken daily. Fish were fed three times daily to satiation in accordance with

hatchery procedures on a diet of live *Artemia* (*Artemia franciscana*), which were hatched onsite. Tanks were cleaned daily, with an approximate 30% water exchange and any uneaten food removed.

Sampling procedure

Approximately 40 fish per species were removed at the beginning of the trial (day zero) to attain initial size data. Remaining fish were exposed to the temperature treatments for 30 days (approximately 15 per replicate aquaria) and were sampled at the end of the trial. At the cessation of experiments, all remaining fish were euthanized using a benzocaine overdose of 100 mg L⁻¹. Following euthanasia, photographs of each fish were taken under a dissecting microscope and camera (MC120 HD, Leica, Germany) for morphological assessment. The fish were photographed laterally in a head-to-left orientation, on a stage micrometer with a 0.01 mm scale. After photographs were taken, measurements of weight, total length, and maximum vertical mouth gape were taken with a digital Vernier calliper. Sagittal otolith pairs were removed from the fish under a dissecting microscope, washed in clean water and stored in black well microplates. Otolith dry weight (μg) was measured from one otolith from each fish. The otoliths were then photographed in high contrast under a dissecting microscope (MZ 16, Leica, Germany) and camera (Infinity 1, Lumenera, Canada) on a stage micrometre with a 0.01 mm scale; they were orientated with the rostrum to the left of the image.

Data analysis of fish growth

Measurements of fish weight, length and mouth gape were calculated as proportional change from the average size fish at day zero for each species. Specific growth rate (SGR) was calculated according to Jobling (1983) using the average initial start weight (w_i), the final weight (w_t) and the duration of the exposure (t). SGR was represented as a percent increase in body mass per day.

$$SGR = \frac{\ln(w_t) - \ln(w_i)}{t} \times 100$$

To determine differences in all measures of fish growth between initial and final measurements, and

between temperature treatments at the finish of the experiment, we conducted parametric analysis of variance (ANOVA). Where parametric assumptions were not met, a log transformation of the data was performed. Significant differences among treatments were determined using a Tukey's post hoc test. All statistical analyses were conducted in R version 3.5.1 (R Core Team 2019) with a significance level of $\alpha < 0.05$.

Image-J software (Schindelin et al. 2012) was used to take morphometric measurements of each individual fish based on a truss network protocol which measured 21 lengths between 10 homologous anatomical landmarks (Fig. 1). Anatomical landmarks refer to (1) the anterior tip of the snout on the upper jaw, (2) the origin of the scaled nape, (3) the origin of the pelvic fin, (4) the origin of the spinous dorsal fin, (5) the origin of the anal fin, (6) the origin of the soft dorsal fin, (7) the cessation of the anal fin, (8) the cessation of the soft dorsal fin, (9) the ventral origin of the caudal fin and (10) the dorsal origin of the caudal fin. Principal component analysis of the morphometric measurements was undertaken using the vegan package in R (Oksanen et al. 2010). The four measures that contributed towards the highest variation in size between treatments (>80%) were identified and used as descriptors of the first principal component.

Data analysis of otolith growth and development

Assessment of otolith growth and development was conducted in R (R Core Team 2019) using the R packages ShapeR (Libungan and Pálsson 2015a) and vegan (Oksanen et al. 2010), with methods as described by Libungan and Pálsson (2015b). Otolith outlines were extracted from digital otolith images by transforming the images to grey-scale and designating a threshold pixel value to distinguish the white otolith from the black background. Wavelet shape coefficients were extracted from the otolith outline by measuring radii from the otolith centroid to the otolith outline, the Wavelet

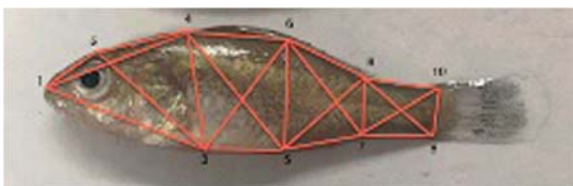


Fig. 1 Location of 10 anatomical landmarks and connected truss network measured on each fish for morphometric analysis. Pictured: golden perch (*Macquaria ambigua*)

method of analysis was selected due to its usefulness in detecting shape differences in specific regions of the otolith. Measurements of otolith area, otolith length and otolith perimeter were then extracted for each otolith with pixel calibrations applied for images taken at different magnifications.

To conduct analysis of otolith shape, the size of the otolith was normalized by dividing the otolith coordinates by the square root of the otolith area. Examination of variation in otolith shape between each treatment was conducted by plotting mean otolith shape from the previously extracted otolith outlines and using the normalized co-ordinates to remove size-bias. Wavelet coefficients were adjusted for fish length before the mean and standard deviation of all combined coefficients and the proportion of variance between groups (intraclass correlation) was plotted against the angle of the measured otolith outline (in degrees, based on polar coordinates) to determine the regions of the otolith driving variation in shape between groups.

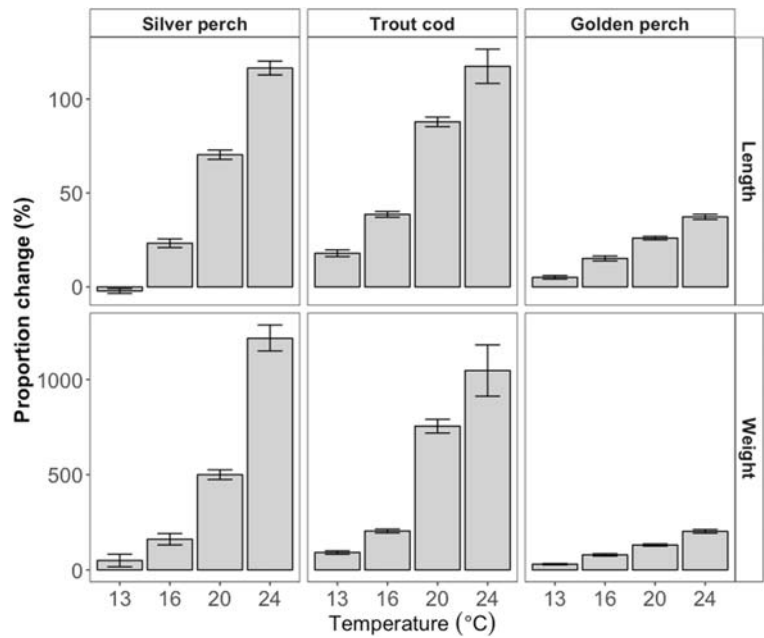
To determine if there was variation in otolith development between temperature treatments for measures of otolith weight, area, length and perimeter, group means were compared using parametric analysis of variance (ANOVA), with log transformation applied where data did not meet test assumptions. Statistical analysis of shape was conducted by applying Canonical Analysis of Principal coordinates (CAP) on the length standardized Wavelet coefficients and running an ANOVA-like permutation test using 1000 permutations to evaluate differences between the temperature treatments for each species (Libungan and Pálsson 2015b).

Results

Fish growth

Temperature had a significant effect on growth in all three species; silver perch (SP), trout cod (TC) and golden perch (GP) (Figs. 2 and 3). Temperature affected fish in terms of changes in total length (SP: $F_{4, 272} = 416.2, p < 0.001$, TC: $F_{4, 143} = 473.8, p < 0.001$, GP: $F_{4, 254} = 135.5, p < 0.001$) and weight (SP: $F_{4, 272} = 305.6, p < 0.001$, TC: $F_{4, 143} = 278.1, p < 0.001$, GP: $F_{4, 254} = 151.7, p < 0.001$). Growth in all species was highest at the maximum temperature treatment of 24 °C and lowest at the minimum temperature of 13 °C. In all species there was a significant reduction in the size of fish

Fig. 2 Growth in length and weight of silver perch, trout cod and golden perch after exposure to a range of water temperatures. Growth is measured as the proportional (%) change in size from sample fish measured on day zero after 30 days exposure to water temperatures of 13, 16, 20 and 24 °C. Error bars are calculated as standard error of the mean



(length and weight) with each incremental reduction in temperature. There was no significant difference between the somatic growth of silver perch and golden perch when held at 13 °C for 30 days compared with day zero.

Temperature affected mouth gape in silver perch ($F_{4, 272} = 243.9, p < 0.001$) trout cod ($F_{4, 143} = 104.5, p < 0.001$) and golden perch ($F_{4, 254} = 51.58,$

$p < 0.001$) (Fig. 4). In all species mouth gape was smaller in fish exposed to reduced water temperatures. No significant changes in mouth gape were found between silver perch held at 13 °C and fish measured at day zero. Similarly, there was no statistical difference between golden perch held at 13 °C and 16 °C and fish measured at day zero. A strong linear relationship was maintained between fish length (LT) and mouth gape (MG) for all

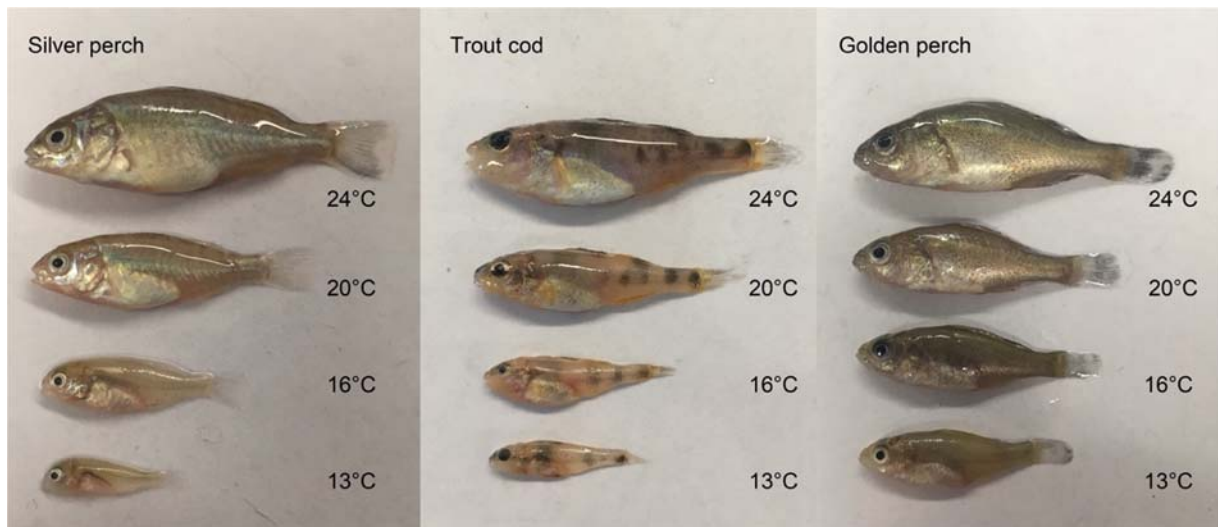
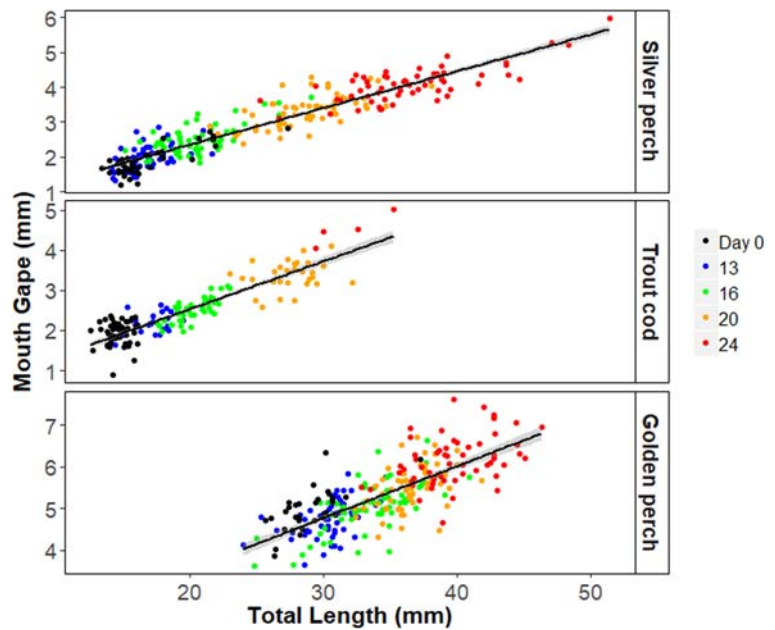


Fig. 3 Photographic comparison of silver perch, trout cod and golden perch after exposure to a range of water temperatures. Fish were exposed to water temperatures of 13, 16, 20 and 24 °C for

30 days. The fish selected for photographs were similar in length to the calculated mean for each treatment

Fig. 4 The effect of water temperature on the mouth gape (MG) of three species of fish in relation to total length (LT). Each data point represents an individual fish, measured on day zero of the experiment (black markers) or after 30 days of exposure to water temperatures of 13 °C, 16 °C, 20 °C and 24 °C (colour markers). Confidence intervals (95%) are indicated in grey



species (SP: $MG = 0.106 \cdot LT + 0.235$; $adj R^2 = 0.88$, TC: $MG = 0.120 \cdot LT + 0.138$; $adj R^2 = 0.82$, GP: $MG = 0.125 \cdot LT + 1.025$; $adj R^2 = 0.57$).

Temperature affected specific growth rate (SGR) in silver perch ($F_{3, 233} = 317.3, p < 0.001$), trout cod ($F_{3, 94} = 232.9, p < 0.001$) and golden perch ($F_{3, 225} = 126.6, p < 0.001$), although the effect was lower in golden perch (Fig. 5). SGR was minimized when water temperatures were reduced. SGR varied between all

treatments in all species except for silver perch exposed to 13 °C and 16 °C water temperatures. SGR reduced incrementally as temperature was reduced.

Temperature also affected growth as measured using a combination of morphometric traits (Fig. 6). Traits that contributed to the largest variation in size between treatments originated from measures 3, 6, 9 and 11 in all species, and these contributed >80% of the variation in size. These measures reflect size of the head and body

Fig. 5 Specific growth rate (SGR) of silver perch, trout cod and golden perch after exposure to a range of water temperatures. SGR is calculated as the percentage increase in body weight measured daily as calculated after 30 days exposure to water temperatures of 13, 16, 20 and 24 °C. Error bars are calculated as standard error of the mean

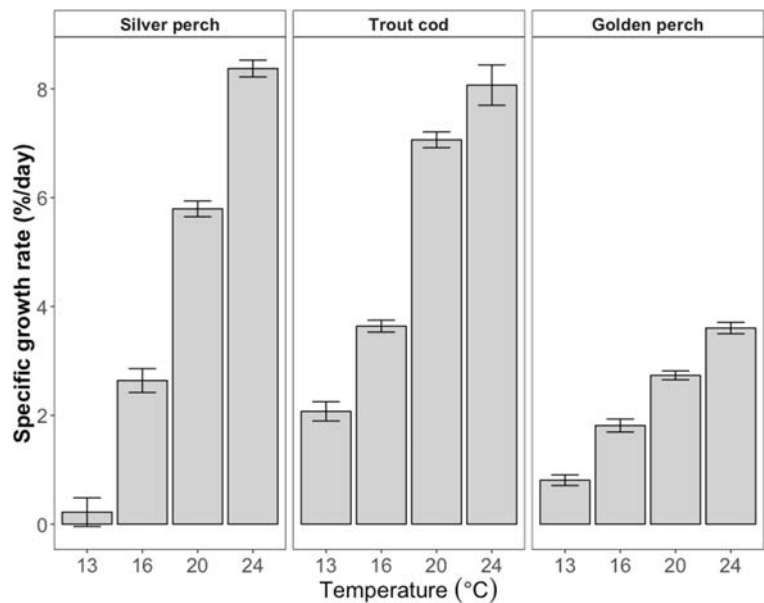
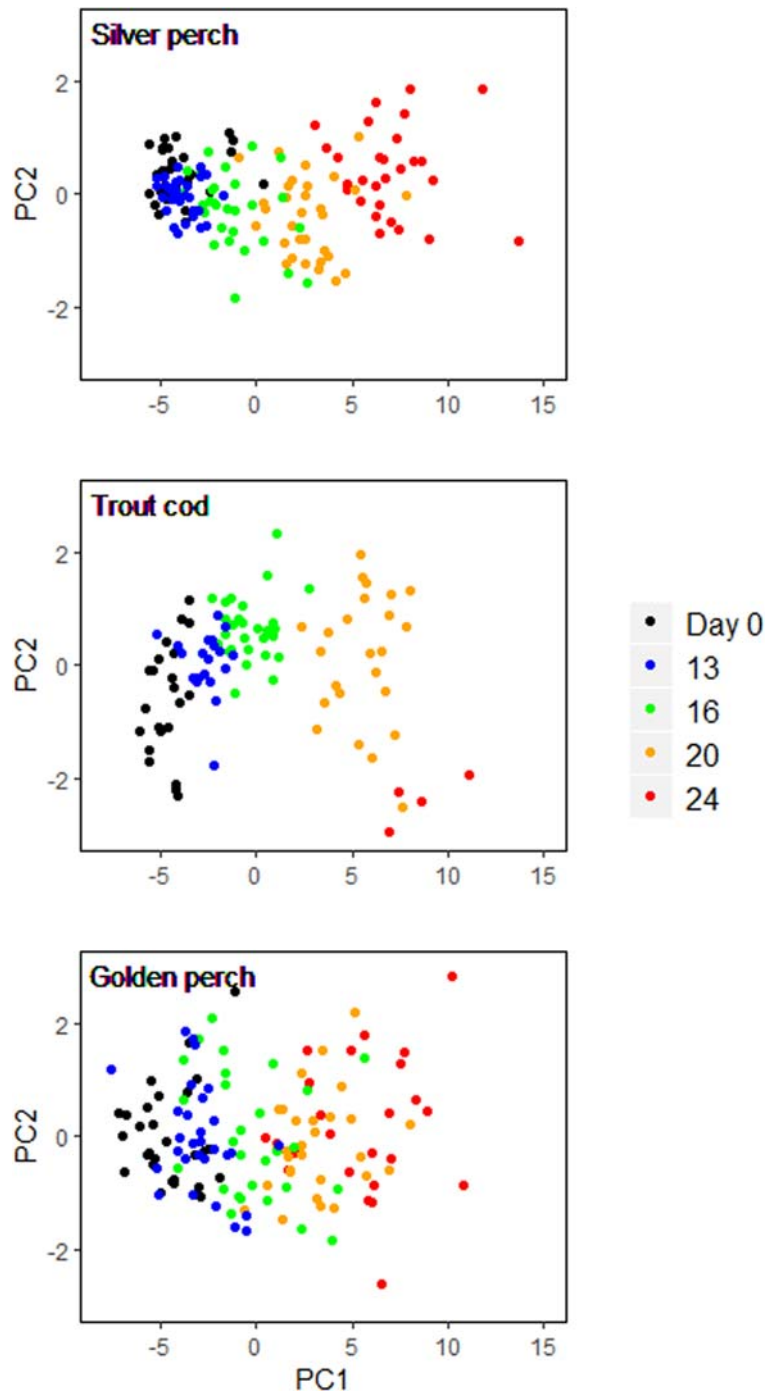


Fig. 6 Visualization of principal components analysis (PCA) indicating variation in the morphometric truss body network measurements of silver perch, trout cod and golden perch. The PCA compares groups after 30 days of exposure to water temperatures of 13, 16, 20 and 24 °C to fish as measured on day zero of the experiment



width; with measure 3 being from the anterior tip of the snout on the upper jaw to the origin of the spinous dorsal fin, measure six being from the origin of the pelvic fin to the origin of the spinous dorsal fin, measure nine being from the origin of the spinous dorsal fin to the origin of

the anal fin and measure 11 being the from the origin of the anal fin to the origin of the soft dorsal fin (Fig. 1). There was greater variation in size between treatments observed in silver perch and trout cod than there was in golden perch (Fig. 2).

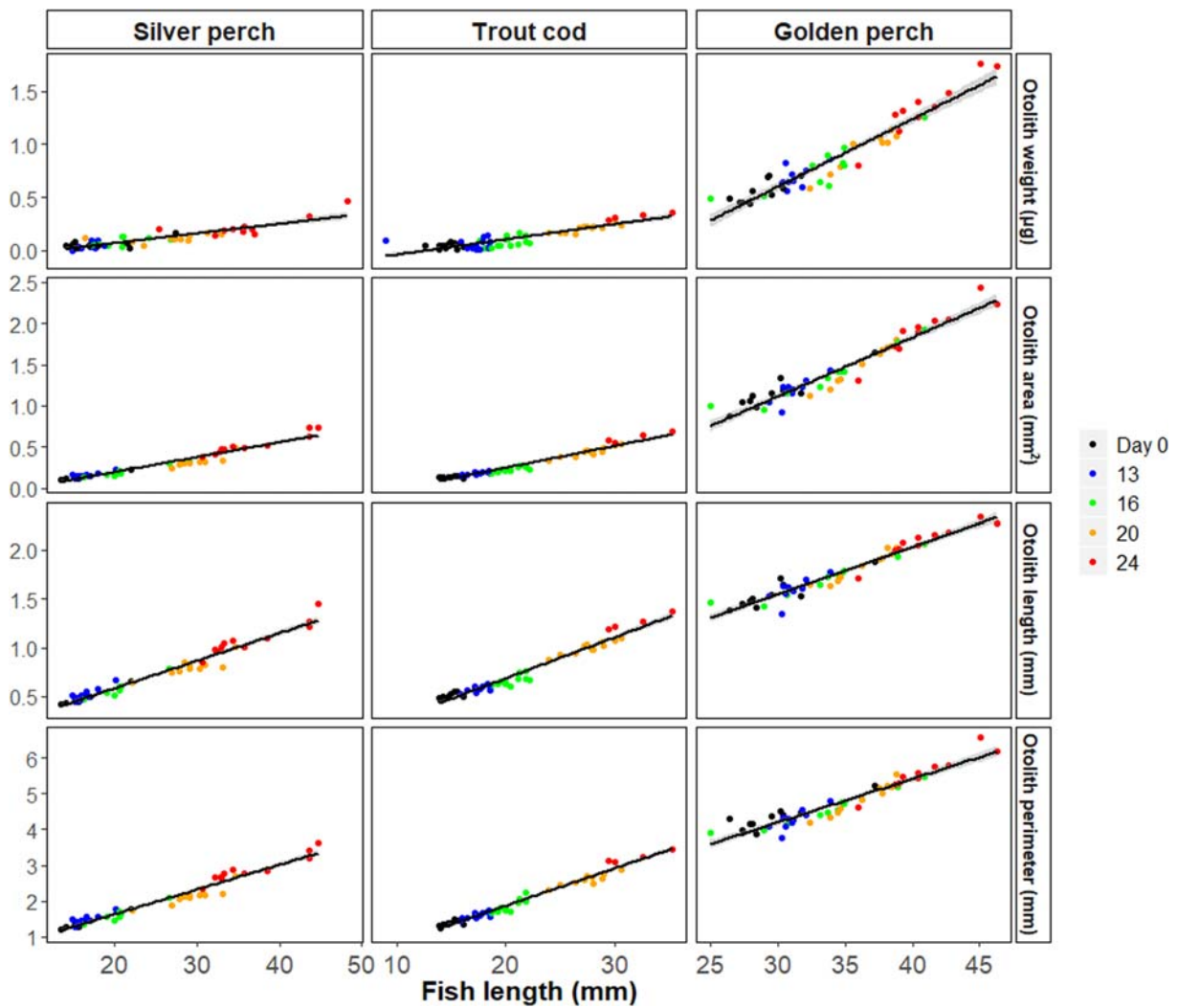


Fig. 7 The effect of water temperature on otolith weight (μg), otolith area (mm^2), otolith length (mm) and otolith perimeter (mm) of silver perch, trout cod and golden perch in relation to total length (LT). Each data point represents an individual fish,

measured on day zero of the experiment (black markers) or after 30 days of exposure to water temperatures of 13, 16, 20 and 24 $^{\circ}\text{C}$ (colour markers). Confidence intervals (95%) indicated in grey

Otolith growth

Water temperature had a significant effect on otolith weight in silver perch, trout cod and golden perch (Fig. 7 and Table 1). Reductions in water temperature equated to reduced otolith weight in all species. This pattern was also observed for the effect of water temperature on measures of otolith area, otolith length and otolith perimeter (Fig. 7). Strong linear relationships were observed between fish length (LT) and all

measures of otolith weight, otolith area, otolith length, and otolith perimeter.

Otolith development

Otolith shape varied between fish held at different temperatures in silver perch ($F_1 = 8.527, p = 0.002$), trout cod ($F_1 = 12.168, p = 0.001$) and golden perch ($F_1 = 5.761, p = 0.001$) (Fig. 8). In silver perch, the highest variation between treatments in the Wavelet coefficients

Table 1 Summary statistics of one-way ANOVA for differences between temperature treatments within measures of otolith weight (OW), otolith area (OA), otolith length (OL) and otolith perimeter

(OP). Linear regression model is included for the comparison of fish length (LT) against otolith measures irrespective of treatment group

	Measure	F value	P value	Linear regression	Adj. R ²
Silver perch	OW	$F_{4, 43} = 17.46$	$p < 0.001$	OW = 0.009* LT - 0.111	0.770
	OA	$F_{4, 47} = 72.72$	$p < 0.001$	OA = 0.018* LT - 0.162	0.937
	OL	$F_{4, 48} = 67.78$	$p < 0.001$	OL = 0.028* LT + 0.027	0.956
	OP	$F_{4, 47} = 70.49$	$p < 0.001$	OP = 0.069* LT + 0.246	0.956
Trout cod	OW	$F_{4, 52} = 68.46$	$p < 0.001$	OW = 0.014* LT - 0.182	0.787
	OA	$F_{4, 42} = 200.5$	$p < 0.001$	OA = 0.0265* LT - 0.281	0.958
	OL	$F_{4, 41} = 249.8$	$p < 0.001$	OL = 0.042* LT - 0.152	0.962
	OP	$F_{4, 41} = 164.3$	$p < 0.001$	OP = 0.105* LT - 0.221	0.973
Golden perch	OW	$F_{4, 44} = 24.71$	$p < 0.001$	OW = 0.064* LT - 1.309	0.906
	OA	$F_{4, 45} = 15.26$	$p < 0.001$	OA = 0.0265* LT - 0.281	0.958
	OL	$F_{4, 45} = 18.46$	$p < 0.001$	OL = 0.048* LT + 0.097	0.929
	OP	$F_{4, 45} = 15.08$	$p < 0.001$	OP = 0.122* LT + 0.537	0.897

(ICC) existed at angles 30–80°, 180–250° and 300° of the otolith outline indicating development towards a longer more rectangular otolith with a more developed rostrum at warmer temperature treatments. Trout cod exhibited the greatest variation in otolith shape in regions 140–240° and 350–40° of the otolith outline, again indicating rectangular development and a more developed rostrum at the warmer temperature treatments. Variation in shape in golden perch was less distinct; variation was highest at angles 260–10°.

Discussion

Slow somatic growth and reduced body size of fish during larval and juvenile life-history stages is strongly linked to mortality and delays in reaching sexual maturity (Sogard 1997). As such, environmental conditions that reduce growth potential of early life stages may have significant deleterious effects on the productivity of fish populations. In this study we demonstrated that low water temperatures reduced somatic growth in the early-life history stages of three species of Australian freshwater fish. This is significant within the context of widespread cold-water pollution downstream of thermally stratified dams with bottom-level offtakes. For example, in Australia large dams located in temperate regions have been documented to reduce water

temperatures in downstream rivers by as much as 16 °C with water temperatures as low as 12–13 °C being reported (Burton 2000; Lugg and Copeland 2014; Michie et al. 2020). We demonstrate that these temperatures would have a profound effect on individual fish growth rates and manifest significant negative impacts upon populations. All species experienced limited growth when held at 13 °C, with silver perch and golden perch experiencing no growth at this temperature over 30 days. In their native range within the Murray-Darling Basin, approximately 2000 km of collective river length can be affected by reduced water temperatures originating from large reservoirs (NSW-CWPIG 2012). Our study highlights the need for effective mitigation of thermal pollution in this region.

In a number of species, delayed ontogenetic development occurs at lower water temperatures, extending the duration of larval stages and increasing mortality risk (Clarkson and Childs 2000; Green and Fisher 2004; Kitchell et al. 1977). A number of Murray-Darling Basin native fish species have demonstrated reduced growth when exposed to suboptimal water temperatures; these species include juvenile freshwater catfish (*Tandanus tandanus*), Murray cod (*Maccullochella peelii*) (Ryan et al. 2003), silver perch (Astles et al. 2003) and spangled perch (*Leiopotherapon unicolor*) (Gehrke 1988). Juvenile silver perch exposed to natural thermal regimes were roughly twice the weight of fish

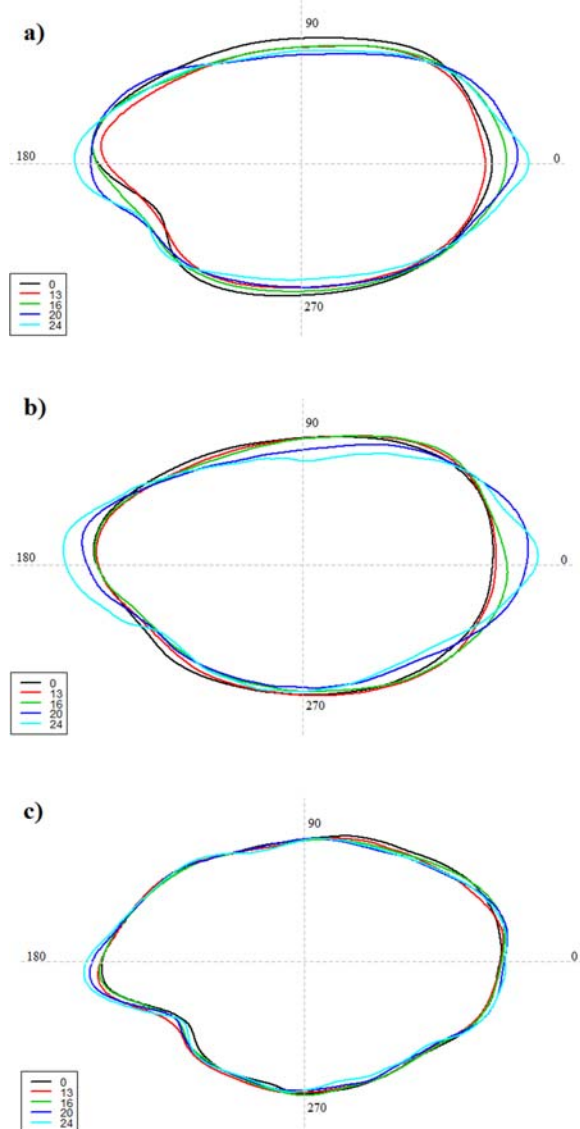


Fig. 8 Average otolith shape based on Wavelet analysis of a) silver perch, b) trout cod and c) golden perch. Otoliths were assessed prior to exposure (0) and after 30 days exposure to water temperatures of 13, 16, 20 and 24 °C. Day 0 is represented in black. Numbers represent the angles in degrees ($^{\circ}$) based on polar coordinates, with the centroid being indicated by the centre of the cross (dashed lines)

exposed to thermal conditions of a river affected by cold water pollution (11 °C difference) (Astles et al. 2003). Our analysis of silver perch indicates the increased sensitivity of this younger age group to suboptimal thermal regimes; at similar temperature exposures over a similar time frame, we demonstrated that silver perch weight was approximately 10 times higher when exposed to the simulated natural thermal regimes

compared to temperatures that can occur in a thermally polluted river. Temperature plays a similar role in the growth performance of fish throughout the world, particularly in early life-history stages. Suboptimal temperatures have caused reduced somatic growth in a number of species including greater amberjack (*Seriola dupmerili*) (Fernández-Montero et al. 2018), Atlantic cod (*Gadus morhua*) (Pérez-Casanova et al. 2009), humpback chub (*Gila cypha*), Colorado squawfish (*Ptychocheilus lucius*) (Clarkson and Childs 2000), yellowtail kingfish (*Seriola lalandi*) (Abbink et al. 2012), Atlantic halibut (*Hippoglossus hippoglossus*) (Jonassen et al. 1999) and turbot (*Scophthalmus maximus*) (Imsland et al. 1996).

Suboptimal water temperatures in aquatic ecosystems pose a significant threat to the viability of fish species as reduced body size in fish can increase their vulnerability to a number of stressors and may ultimately affect fitness and survival. Swimming ability in fish is dependent on both body size and water temperature (Wolter and Arlinghaus 2003). Body size is positively correlated with swimming ability (Fisher et al. 2005; Ojanguren and Brana 2003), particularly in terms of sustained, prolonged and maximum swimming speeds (Domenici 2001). Predator-prey interactions are directly linked to swimming speed limits and endurance in fish; reductions in the swimming performance of larval fish is likely to affect survival and growth potential of fish, through increasing predation risk and limiting their ability to capture prey (Domenici and Blake 1997; Videler and Wardle 1991; Wolter and Arlinghaus 2003). Independent of size, swimming ability in fish is often reduced at suboptimal temperatures. In golden perch, fast-start performance was considerably reduced at temperatures below 15.5 °C (Lyon et al. 2008) and sprint and sustained swimming performance was reduced in Macquarie perch (*Macquaria australasica*) at a similar thermal threshold (Starrs et al. 2011). Suboptimal water temperatures reduce swimming ability in a number of freshwater species in other regions (Childs and Clarkson 1996; Myrick and Cech 2000; Ward et al. 2002). The combined effects of decreased body size and impaired swimming ability under sub-optimal thermal conditions exacerbates predation risk and limits the ability of fish to find prey (Sogard 1997). Considering these compounding effects, we highlight the need to consider sub-

lethal responses to suboptimal thermal regimes in the management of fish populations.

Mitigation of cold water pollution has been either considered or implemented in some affected regions. The success of mitigation is usually determined by estimates of mortality or by reproductive measures (Ferrari 1987; Gray et al. 2019; Hanna et al. 1999; Sherman 2000; USDI 1999). Population modelling of Murray cod downstream of Dartmouth Dam suggested that a 5–6 °C increase in water temperature from 13 °C would be necessary to diminish the effects of thermal pollution on the reproductive success of the Murray cod in the Mitta Mitta (Todd et al. 2005). A similar study determined that mitigation of reduced water temperature pollution below Hume Dam would likely see 4–6 °C increases downstream which was predicted to improve spawning conditions and increase female population abundance by 30–300% (Sherman et al. 2007). Although such improvements in temperature would be beneficial, our results indicate that further improvements to these temperatures would be required to diminish the effects of this cold water pollution on fish growth at early life-history stages and the associated consequences to individual fitness. Our results emphasize the value in recovering water temperatures of freshwater ecosystems to natural thermal regimes and highlight the need to consider sub-lethal responses in fish to thermal pollution in future mitigation efforts.

A strong somatic-otolith size relationship was maintained in silver perch, trout cod and golden perch when fish were exposed to suboptimal water temperatures. A number of studies have reported that slower growing individuals often have larger otoliths at a given size (Secor et al. 1989; Francis et al. 1993; Wright et al. 1990). Such decoupling of somatic and otolith growth has the potential to create biases in back-calculated estimates of fish growth histories and biochronological analyses, particularly when fish in similar geographical regions can display discrepancies in the relationships determining otolith growth (Fey 2006). For some species it is proposed that increased metabolic activity, brought on by increased water temperatures, increases the rate of accretion of material in the otolith microstructure (Mosegaard et al. 1988; Wright et al. 1990; Fey 2006). Analysis that has observed decoupling between otolith and somatic growth has generally focussed on temperatures above optimal for somatic growth; in these assessments somatic growth slowed above thermal optima whilst otolith growth continued (Mosegaard et al.

1988; Wright et al. 1990; Hoff and Fuiman 1993). A potential reason for the strong coupling between somatic and otolith growth in our study might be explained by the fact that we only assessed temperatures below and within the optimal range for the study species and therefore no metabolic driven accretion of material occurred in the otolith.

In early life history stages, ontogenetic development of otolith shape tends to shift from a circular (larval) to a more irregular or elliptical shape (adult); this has been observed in a number of species including walleye pollock (*Theragra chalcogramma*) (Brown et al. 2001), windowpane (*Scophthalmus aquosus*) (Neuman et al. 2001) and plaice (*Pleuronectes platessa*) (Modin et al. 1996). The otoliths of fish held at low water temperature were more circular in shape than fish of the same age held at higher temperatures. This provides further evidence for the maintenance of a strong somatic-otolith relationship in these species when exposed to suboptimal water temperatures. Given the prevalence of otolith microstructure assessment in back-calculations of fish age and growth in current fishery research (Nishimura and Yamada 1984; Radtke 1989; Cordes and Allen 1997; Karlou-Riga 2000; Buckmeier et al. 2002; Fablet and Le Josse 2005; Bermejo et al. 2007), understanding environmental conditions and processes that affect growth and development of otoliths is essential in ensuring ongoing reliability of these techniques and the applicability of their use in management of fish populations (Campana and Neilson 1985; Sponaugle 2010). With a strong somatic-otolith size relationship being maintained by silver perch, trout cod and golden perch at suboptimal water temperatures, we validate the use of otoliths in the analysis of life-history of these species in populations that have been exposed the widespread cold water pollution that persists in their native distribution.

Conclusion

Due to the importance of body size on survival, fitness, feeding ability and overall health in fish, the sub-lethal effects of temperature on growth identified in this study will likely have further significant effects on population health (Sogard 1997). Combined with the direct impacts of temperature on swimming ability, reduced thermal regimes in freshwater ecosystems have the potential to drastically alter fish communities in regulated river

systems globally. Understanding the sub-lethal impacts of somatic and otolith growth and development experienced by freshwater fish exposed to thermal pollution is critical in the management of threatened fish populations and highlights the need for effective mitigation of sources of thermal pollution.

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