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Tradeoffs of warm adaptation in aquatic ectotherms: live fast, die young?

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Abstract

In the face of a changing climate, questions regarding sub-lethal effects of elevated habitat temperature on the physiology of ectotherms remain unanswered. In particular, long-term responses of ectotherms to the warming trend in tropical regions are unknown, and significantly understudied due primarily to the difficulties in specimen and community traceability. In freshwater lakes employed as cooling reservoirs for power plants, increased physiological stress from high water temperature can lead to an increase in mortality, reduce growth and potentially alter the community structure of fishes. Throughout this study, we employ this highly tractable system to assess how elevated thermal regimes can alter the physiology and consequently the ecology of aquatic species. We documented a significantly reduced lifespan, growth performance, and a shift in the age structure towards younger individuals in the thermally-impacted population of bluegill (*Lepomis macrochirus*) in Coffeen Lake in Illinois, compared to a non-impacted control group (Lake Mattoon). Average age calculated for the Lake Mattoon population was 2.42 years, whereas the average age of bluegill from Coffeen Lake was only 0.96 years. The average specimen mass in Lake Mattoon was more than six times that of Coffeen Lake average (Mattoon = 60.26g; Coffeen = 9.42g). During laboratory cross-acclimation studies of bluegill from Lake Mattoon at 17.5 and 35.0°C, citrate synthase activity obtained from white muscle was regulated through acclimation, whereas cold-acclimated specimens exhibited twice the activity at 25°C, if compared to CS activity values from warm-acclimated specimens. This study raises the questions about the causal relationships between physiological performance and habitat temperature, in particular how thresholds in an organism's physiology may modulate their community structure, and consequently their ecological success.

Key-words: bluegill, teleostei, temperature, warm adaptation, physiological ecology, aging, metabolism, growth, *Lepomis*, accelerated senescence

1. Introduction

In the face of a changing climate, the adaptive capacity of many species to rising temperature regimes remains unclear. Physiological adaptation to rising habitat temperatures is likely to occur and attenuate the effects on a species' energetics (Hochachka and Somero, 2002). However, short-term physiological adaptation is energetically expensive, and likely not an effective long-term strategy to cope with the effects of global warming. In temperate regions, for example, shifts of population centers of marine fishes towards proximal, colder regions are well documented (Perry et al., 2005; Pörtner et al., 2001; Pörtner and Farrell, 2008). This accumulating body of evidence suggests that species with a capacity to move towards colder regions may temporarily escape the present warming trends. On the other hand, due to the thermally homogeneous nature of tropical regions, population shifts like those observed in temperate regions are unlikely (Urban et al., 2012). In addition, tropical species are often found within the upper thermal maximum, and further warm adaptation of those species already at their upper thermal limit might come with an energetic cost as a tradeoff (Gunderson and Leal, 2012; Huey et al., 2009; Stork et al., 2009; Tewksbury et al., 2008). This energetic cost is associated with activities contributing to behavioral thermoregulation, rising costs of minimum metabolic activity as well as a potential reduction in the mitochondrial energy transduction efficiency (Divakaruni and Brand, 2011). As a result, species adapted to year-round elevated temperatures are more susceptible to further habitat warming, and thus more likely to show direct signs of how organism-level thermal physiology influences upper-level processes such as growth, community structure and reproductive performance (Angilletta, 2009).

Although a robust body of literature has unveiled the links between the physiological thresholds and the ecology of terrestrial species in a changing climate, long-term responses of aquatic species facing elevated temperatures in tropical regions remains understudied (Roessig et al., 2004). A laboratory acclimation study of a tropical reef fish (*Acanthochromis polyacanthus*) indicated a high variability of acclimation capacity for this species (Donelson and Munday, 2012), and the authors conclude that the thermal metabolic reaction norm may not be a good indicator of the species' acclimation ability.

Therefore, a more tractable field study system may be instructive to evaluate population-level, cross-generational responses to rising temperature in aquatic ectotherms.

Analogous to tropical aquatic systems, freshwater lakes employed as cooling reservoirs for power plants are characterized by year-round elevated temperatures, compared to lakes that are not anthropogenically impacted. Elevated water temperatures in these systems can lead to increased physiological stress and mortality in fish assemblages, unless thermal refuges are available (De Stasio et al., 1996). Fishes able to survive in thermally-impacted lakes without thermal refuge are forced to physiologically adapt to suboptimal temperatures.

Aquatic organisms have the ability to adapt to environmental changes, and it is possible to raise or lower tolerable temperatures through acclimation (Cossins and Bowler, 1987; Hochachka and Somero, 1968; Somero, 2002; Somero, 2004; Tarzwell, 1970). Environmental temperature can alter various components of the metabolic machinery, including enzyme catalytic properties and phospholipid membrane stability. Short-term acclimation is often characterized by a quantitative strategy, where biochemical reactions are regulated via changes in the abundance of the enzyme catalyzing the reaction. This has been observed in fishes, where thermal acclimation induced changes in key enzyme concentrations is often observed within days or weeks of thermal acclimation (Cossins and Bowler, 1987; Hochachka and Somero, 1968; Hochachka and Somero, 2002; Shaklee et al., 1977; Sidell et al., 1973; Somero, 2004).

The biological purpose of metabolic compensation is to shift energy allocation from metabolism to growth (e.g. reproductive and/or somatic). In essence, an organism will be able to operate with an energetic surplus over a temperature range influenced by the width of the fitness thermal reaction norm (Angilletta Jr et al., 2003; Angilletta, 2009). This reaction norm is classically illustrated as a thermal tolerance polygon (Brett, 1956; Brett and Groves, 1979; Brett, 1952; Eme and Bennett, 2009), where the size of the tolerance polygon is a direct reflection of the organism thermal window of tolerance. Within an organism's thermal tolerance window, metabolic adjustments allow for the allocation of energy towards somatic and reproductive growth. However, physiological compensation may come with an energetic cost

to organisms experiencing suboptimal temperatures such as those organisms inhabiting thermally-impacted lakes.

Bluegill, *Lepomis macrochirus* (Rafinesque, 1819) is a centrarchid that is ubiquitous in reservoirs of North America. *L. macrochirus* are often one of the dominant species in cooling reservoirs. This dominance is primarily due to their well-documented ability to withstand and survive elevated temperatures (Holland et al., 1974; Pierce and Wissing, 1974). For example, Holland et al. (1974) investigated the acclimation capacity of *L. macrochirus* from various cooling reservoirs and found that individuals can rapidly adjust their physiology and acclimate to temperatures ranging from 25 – 35°C. The critical thermal maximum (CTM), defined by the temperature where the organism exhibits a loss of equilibrium, obtained for these individuals increased with increasing acclimation temperature, with CTM registered as high as 42.8 °C. As an example of the differences in thermal regimes between cooling reservoirs and natural lakes, the average temperature of a thermally-impacted reservoir in the mid-western US (Coffeen Lake, Donnellson, IL) was 36.67°C, 9.66°C above the average water temperature in non-impacted lakes (Lake Mattoon, Mattoon, IL) during the 2012 summer season (Martinez, unpublished). This observed difference is further amplified during the winter season, which could lead to even more pronounced effects of temperature in the aquatic community. Thus, cooling reservoirs such as Coffeen Lake may serve as useful study systems to judge long-term, cross-generational effects of elevated temperature regimes in aquatic species, including *L. macrochirus*.

The primary goals of this study were two-fold; 1) to employ an integrative framework evaluating the sub-lethal effects of warm adaptation of an ubiquitous eurytherm and 2) to evaluate the usefulness of power cooling reservoirs as long-term experiments to judge the consequences of climate change in aquatic species. We hypothesized that due to the prevalence of elevated temperature in thermally-impacted lakes, a reduction on growth performance and longevity will become tradeoffs of surviving this thermal regime.

In the present study we found evidence of a severe shift in the community structure and physiology of the bluegill, *Lepomis macrochirus*, characterized by younger individuals in a population exposed to elevated thermal regime. We documented significant differences in growth rate, age structure, and lifespan

between a thermally-impacted population of *L. macrochirus*, compared to a non-impacted control lake. In addition, we address potential physiological and biochemical mechanisms underlying our findings, to provide a mechanistic basis to the differences in growth rates and population structure found in this study. Considering the rapid increase of 1.35°C in marine ecosystems during the past 25 years (Belkin, 2009), power-cooling reservoirs may serve as tractable systems to judge consequences of climate change on the physiology of aquatic ectotherms.

2. Methodology

2.1 Chemicals. All chemicals for enzymatic measurements were purchased from Sigma-Aldrich (St. Louis, MO) or Fisher Scientific (Fair Lawn, NJ). Water for solution preparation was purified with a Milli-Q Reagent Water System (Billerica, MA) to an electrical resistance of 18 mΩ.

2.2 Study sites. Coffeen Lake is a 4.5 km^2 power-cooling reservoir, 4.8 km east–northeast of Donnellson, and approximately 3.2 km west–southwest of Coffeen, Illinois. Since 1972 the reservoir has supplied cooling water to a power station with a generating capacity of 945 MW of electricity. About 73% of the surface water of Coffeen Lake is affected by heated discharge through a cooling loop covering approximately 6.6 km, resulting in an average annual surface water temperature of 22.7°C. Our control lake was Lake Mattoon, a 4.2 km^2 water reservoir located in Mattoon, IL. Annual water temperature in Lake Mattoon range from 0.3 °C to 32.9 °C. Annual water temperatures are substantially higher in Coffeen Lake and range from 6.5 °C to 42.9 °C (data not shown).

2.3 Specimen collection. Both Lake Mattoon and Coffeen Lake were sampled during August 2011 using pulsed DC electrofishing (Gutreuter et al., 1995). Water temperatures within the sampling depth of our electrofishing rig ranged 29.7°C to 36.1°C in Coffeen Lake and 21.2°C to 27.5°C in Lake Mattoon. Sampling consisted of two, 15-min transects, randomly selected from five separate sites on both Lakes. Sampling by DC electrofishing was done using a Wisconsin rig, which consisted of dropper electrodes suspended at equal intervals from a horizontal ring (Reynolds, 1996). All collected *L. macrochirus* specimens were kept for age determination. During each sampling event, specimens were kept in aerated 90 L coolers filled with lake water for transport to the fisheries laboratory at Eastern Illinois University. Upon arrival, 30 specimens were randomly sampled from the pool for thermal acclimation experiments. The remaining specimens were weighed to the nearest 0.01 g, total length (TL) was determined to the nearest millimeter.

Sagittal otoliths were excised for aging purposes (Maceina and Betsill, 1987). Otoliths were removed by disconnecting the operculum and accessing the cranial chamber anteriorly. Whole otoliths were placed in immersion oil and viewed with a stereo microscope under low magnification (7 – 40 x) using reflected light (Colombo et al., 2010). Age of fish was estimated by counting the number of annuli (visual growth bands), using two independent readers. Disagreements on ages were corrected by a consensus among the two readers. All procedures were performed in compliance with the Eastern Illinois University Institutional Animal Care and Use Committee (approved protocol #12-002).

2.4 Thermal acclimation studies. Individual specimens collected from both Coffeen Lake and Lake Mattoon were acclimated at two thermal regimes to assess the effects of temperature on routine metabolism. To achieve this, 10 - 15 individuals from each location were acclimated for a period of 30 days to 17.5°C or 30.0°C ± 1.0°C. Acclimation tanks consisted in 114 L glass aquaria (one aquarium at 17.5°C, one aquarium at 30.0°C), each connected to a custom biological filtration system to condition the water prior and during acclimation. A split-tank design was employed, where specimens from each population were separated by a screen within each temperature treatment. Water quality parameters were monitored every 48 h, and periodical water changes were performed to reduce waste accumulation. Since a correlation between protein and caloric intakes and O_2 consumption has been reported previously (Schalles and Wissing, 1976), specimens were fed *ad libitum* with high lipid and protein food pellets (Wardley fish pellets, Hartz Mountain Corporation, Secaucus, NJ), and remaining unconsumed food pellets were removed.

2.5 Critical Thermal Maxima (CTM) measurements. Individual specimens were placed in a 10-liter container with circulating water controlled by a thermal ramp-capable water bath (NesLab RTE, Thermo Fisher, Fair Lawn, NJ). Heating ramp was configured to 0.3ºC min^{-1}. CTM was obtained according to Holland *et al*. (1974). Briefly, the temperature where the onset of balance loss (fish loosing upright position) was observed constituted a critical thermal maxima data point.

176 *2.7 Whole animal respiration.* Oxygen-consumption rates were determined following the methods
177 described by Torres and Somero (1988), with minor modifications. Individuals were placed in a sealed
178 water-jacketed acrylic chamber filled with dechlorinated tap water. The rectangular chambers were
179 constructed of Lucite® and contained a perforated Lucite false-bottom that isolated the fish from a stirring
180 bar. A low stirring speed (30 RPM approx.) was used to minimize disturbance. All experiments took
181 place in the dark, with brief periods of observation in low light. Oxygen partial-pressure was continuously
182 monitored using Clark-type, polarographic oxygen electrodes (Clark Jr, 1956). Temperature was
183 maintained at each thermal regime (17.5 and 30.0°C ± 0.1 °C) using a circulating refrigerated water-bath
184 (Forma Scientific, Model 2067), as an individual bluegill reduced oxygen levels to intermediate (~80 mm
185 Hg) partial pressures. Electrodes were calibrated using air- and nitrogen-saturated water at the
186 experimental temperature (Torres et al., 1979). Run times varied from 1-3 h, depending on the specimen
187 size and overall activity. Streptomycin and neomycin (each at 25 mg L^{-1}) were added to the water prior to
188 experimentation to minimize microbial growth. To control for possible oxygen consumption by
189 microorganisms, an individual was removed after selected runs, its volume was replaced with freshwater,
190 and oxygen consumption was again measured for 1 h. In all cases microbial oxygen consumption was
191 negligibly low (< 5%).

192 Data were recorded using a computer-controlled digital data-logging system. Each oxygen probe was
193 scanned once per second, its signal averaged over a period of 1 minute, and then recorded. Data obtained
194 during the first half hour were discarded due to the activity of the fish after its introduction into the
195 chamber. All 1-min average points thereafter, down to an oxygen partial-pressure (P_{O_2}) of 80 mm Hg,
196 were plotted and a linear regression fitted to produce a routine respiration rate for each individual in mg
197 O_2 hr^{-1} Kg wet $mass^{-1}$. After respirometry trials, specimens were immediately processed for enzyme
198 activity measurements. Due to the physical trauma exerted during handling and the short acclimation
199 period (30 min) to the chamber, respiration rates reported in this study should be regarded as routine
200 metabolic rates.

2.8 Citrate synthase and lactate dehydrogenase activity measurements. Epaxial muscle tissue of *L. macrochirus* was excised from fresh specimens, flash frozen in liquid nitrogen and stored at -80°C for enzymatic characterization. Frozen tissue was processed as described (Childress and Somero, 1979; Torres and Somero, 1988). Briefly, a piece of frozen and skinned epaxial muscle (200 mg) was thawed in 1.0 mL of ice-cold homogenizing medium containing 50 mM imidazole/HCl buffer (10 mM, pH = 7.2 at 20°C). Tissue was homogenized manually in a 7 mL, ice-cold Duall® glass homogenizer having ground glass contact surfaces (Kontes, Vineland, New Jersey). The homogenates were centrifuged at 2,500 *g* for 10 min at 4°C to pellet undisrupted tissue. The supernatant was used for enzyme analysis.

To evaluate both anaerobic as well as aerobic metabolic capacity of white muscle from *L. macrochirus*, the activity of two intermediary enzymes were assayed. Citrate synthase (CS) and L-lactate dehydrogenase (LDH) enzymatic activity was assayed with supernatants of freshly homogenized muscle tissue, following Childress and Somero (1979) with minor modifications (Torres et al., 2012). Activities of both enzymes were assayed at an intermediate temperature of 25°C, in a temperature controlled Varian Cary IE UV/Vis spectrophotometer, coupled with computer-based analysis software (Cary, North Carolina). CS activity was assayed in a solution of 42.5 mM Imidazole buffer (pH = 7.2 at 20°C), 0.2 mM DTNB, 1.5 mM $MgCl_2 \cdot 6H_2O$, and 124 µM acetyl-CoA. To 1 mL of the assay solution, 40 µL of homogenate supernatant was added, and the absorbance at 412 nm was monitored until reaching a plateau. Background NADH oxidation was monitored from 2-4 minutes and was negligible prior to addition of oxaloacetate. The enzymatic reaction was initiated by adding 12.5 µL of 40 mM oxaloacetate, and the increase in absorbance, as the reduced acetyl CoA reacts with DTNB, was monitored for 4 min. Considering that the molar absorbance coefficient for TNB at 412nm is 13.6 $cm^2/\mu mol$ (Ellman, 1959; Eyer et al., 2003), the following formula was deduced for the calculation of the catalytic concentration: $U/ml = \Delta A/min \times 4.89$.

For LDH, 10 µL of fresh homogenate was added to 1 mL assay medium consisting of 80 mM imidazole buffer (pH = 7.2 at 20°C), 5.0 mM sodium pyruvate and 0.15 mM NADH. LDH activity was determined

by quantifying the decrease in absorbance at 340 nm resulting from the oxidation of NADH for 60 seconds, immediately after adding the fresh homogenate. Considering that the molar absorbance coefficient for NAD at 340nm is 6.22cm^2/µmol (McComb et al., 1976), the following formula was deduced for the calculation of the catalytic concentration: U/ml = ΔA/min x 10.73.

2.9 Statistical analyses. Enzyme activity and critical thermal maxima data were analyzed with an unpaired t-test. Life history (TL, mass, age) and metabolic rate data were analyzed with a two-way analysis of variance (ANOVA) followed by a pairwise comparison of groups between sampled populations (Holm-Sidak method). SigmaPlot 12.5 (Systat Software Inc., San Jose, CA) was used for the analyses.

3. Results

3.1 Population structure. Two populations of *L. macrochirus* with disparate thermal regimes were sampled, with the objective of describing the overall size and age structure in both fish communities. Although the catch per unit of effort, expressed as individuals captured per hour of sampling effort, were similar between locations (Table 1), population structure results derived from the samples were strikingly different from each other. As shown in Fig. 1, size and age frequency distribution of *L. macrochirus* inhabiting a thermally impacted lake showed significant differences from those specimens living in non-impacted conditions (two-way ANOVA, $P < 0.001$). It is worth noting that no specimens older than two years were found in Coffeen Lake, whereas specimens up to 5 years were commonly observed in Lake Mattoon. In conjunction with total length (TL) and age, population differences were strikingly reflected in mass differences between fish from both populations (two-way ANOVA, $P < 0.001$). The average mass in Lake Mattoon was more than 6 times that of Coffeen Lake average.

In addition to differences in the age distribution within a given population, differences in size and mass were found, particularly within age-2 fish (Fig. 2). Average mass of age-2 *L. macrochirus* from Lake Mattoon was found to be more than triple of the observed mass in Coffeen Lake specimens (Fig. 2b). Furthermore, we found a significant increase in total length of age-2 *L. macrochirus* from Lake Mattoon (Fig. 2a; two-way ANOVA; $P < 0.001$). Although significant differences were found in age corrected size between populations, there was a strong correlation ($r^2 = 0.99$) between mass at length in both populations (Fig. 3).

3.2 Critical thermal maxima. Tolerance towards increasing water temperature, after a 30 day acclimation period at 17.5°C, was similar between both populations. Average CTM for both populations was 40.56 ± 0.29 °C (Table 2).

3.3 Metabolism. Aerobic metabolism of *L. macrochirus* acclimated to 17.5°C and 30.0°C did not significantly differ among treatments or locations. Acute response of oxygen consumption rate to a 10-

263 degree change in temperature (Q_{10}) was measured in Lake Mattoon specimens acclimated to 17.5°C,
264 which averaged to a Q_{10} of 1.8 ± 0.04 (n = 3; ± SEM).
265
266 *3.4 Enzyme activity*. Key aerobic and glycolytic enzyme activities at a fixed temperature (25°C) were
267 determined for Lake Mattoon specimens acclimated to 17.5°C and 30.0°C. As shown in Table 4,
268 regulation of CS is apparent between acclimation regimes, where cold-acclimated specimens exhibited
269 twice the activity at 25°C, if compared to CS values from warm-acclimated specimens. A calculated
270 enzyme activity derived using the mean Q_{10} reported previously is reported for each acclimation
271 temperature. Citrate synthase activity indicates a regulatory response through the course of acclimation,
272 where cold-acclimated specimens expressed a higher CS activity than warm-acclimated specimens.
273 However, LDH activity results did not show evidence of such temperature-dependent regulation (Table
274 4).
275

4. Discussion

4.1 Population structure: thermally impacted vs. non-impacted L. macrochirus. Thermal regimes of habitats have profound implications in aquatic ectotherms, both in freshwater as well as saltwater systems. We found both age-size and age-mass structures were significantly different between the thermally altered Coffeen Lake and the undisturbed Lake Mattoon population, with a trend of a smaller, younger population inhabiting Coffeen Lake (Fig.1). From the temperature size rule perspective, which postulates that elevated habitat temperatures favor growth in ectotherms (Atkinson et al., 1996), results obtained from Coffeen Lake are puzzling since no growth enhancement was observed at elevated temperatures. Although this relationship has been confirmed and often generalized to many ectothermic taxa this 'rule' should only be interpreted cautionary on a species-specific basis (Angilletta and Dunham, 2003), as exemplified by our data.

Growth rates obtained for *L. macrochirus* inhabiting Lake Mattoon, showed a pronounced spike between ages one and two (Fig. 2). This spike in growth rate was not documented in Coffeen Lake specimens, where growth rates were found to be rather constant between age classes 1 and 2. Werner and Hall (1988) documented an ontogenic shift in the diet in *L. macrochirus*, where specimens 80 mm or larger shift from feeding within vegetation to feed upon planktonic prey items. This shift towards open water feeding coincides with the observed shift in growth rates between year one and two in Lake Mattoon specimens. Moreover, this shift involves an additional energetic cost of locomotion associated with foraging as well as predator avoidance, and our study suggest that Coffeen Lake specimens a) do not display a shift in prey selection b) pelagic prey availability might be limited during the winter months or c) that the energetic requirements of a warm thermal regime and additional foraging energetic requirements might balance out the energetic benefit of the ontogenic diet shift observed in non-impacted populations. A study evaluating all three aforementioned aspects is currently underway.

4.2 Thermal response at whole-organism and sub-cellular levels. Oxygen consumption rates obtained for *L. macrochirus* fall between metabolic rates reported previously (30.7 to 160.9 mg O_2 kg^{-1} hr^{-1}) for the

same species (Pierce and Wissing, 1974; Schalles and Wissing, 1976). Interestingly, Pierce and Wissing (1974) reported temperature-dependent oxygen consumption rates that reflect a similar Q_{10} as the acute response we observed in our study. However, these differences in respiration rates with temperature were not evident in the post-acclimation respiration rates obtained in our study, and may be attributed to differences in the acclimation period between studies (14 days vs. 30 days). In addition, oxygen consumption rates reported in our study are in the higher end of the range reported for *L. macrochirus*, which could be attributed to the type of respirometric apparatus (continuous flow vs. closed system), where our closed–chamber respirometric apparatus does not allow of a more extended chamber acclimation period before the trial. Although no visible stress was observed for the specimens, handling stress and a short chamber acclimation period could have masked subtle responses to the thermal regime.

Metabolic homeostasis observed in cross acclimated *L. macrochirus* involved a biochemical reconfiguration that includes alterations in the abundance of key aerobic enzymes but not in the anaerobic enzyme LDH (Table 4). A quantitative strategy was adopted in the *L. macrochirus* specimens studied, where metabolic control was modulated by regulating CS levels (Table 4). This quantitative compensatory mechanism has been widely documented in aquatic organisms (see Hazel and Prosser (1974); Somero (2004) for review), and explains to an extent why no significant differences were observed in oxygen consumption rates for whole organisms. Citrate synthase, along with 2-oxoglutarate dehydrogenase, constitute flux-regulating checkpoints in the citric acid cycle (Newsholme and Crabtree, 1981), which could in turn regulate NADH and $FADH_2$ supply into the Electron Transport System (ETS). Acclimation induced regulation of CS has been documented at both transcriptional and enzyme levels in temperate fishes (Lucassen et al., 2006; Lucassen et al., 2003), showing that changes in mRNA for CS and enzymes activity occurred as soon as 3 - 5 days of acclimation.

Short-term acclimation responses to temperature are physiologically costly, potentially posing an energetic constraint to those populations already at their upper thermal limit (Pörtner 2001; Pörtner 2002; Pörtner et al. 2006). In fishes, slight increases in water temperatures are known to induce shifts in

population structure, and a reduction in growth as well as reproductive output (Perry et al., 2005; Pörtner et al., 2001). At *pejus* (i.e. getting worse) temperatures, compensatory responses could tap on the energetic surplus otherwise allocated to both somatic and reproductive growth, compromising the ecological success of a given population. Results obtain in this study suggest that *L. macrochirus* inhabiting Coffeen Lake are experiencing such *pejus* temperatures, reflected on their short life span and small sizes.

4.3 Tradeoffs in a warming world - live fast and die young? Our study may provide insights into the consequences of warmer thermal regimes on fish populations. Analog to marine species with little or no thermal refuge, fish population in Coffeen Lake are unable to avoid thermal stress by moving into a habitat that is not impacted by the increase in ambient temperature. We observed an overall smaller, younger population structure as a tradeoff for survival in warm waters. In fact, this study placed into perspective an often overlooked repercussion of thermal adaptation in fishes; an accelerated senescence as a tradeoff for survival. Most studies dealing with thermal tolerance in teleosts focus primarily on critical thermal limits (Eme et al., 2011; Mora and Ospina, 2001; Mora and Ospina, 2002; Ospina and Mora, 2004; Rajaguru and Ramachandran, 2001), metabolism (Brett, 1952; Somero and DeVries, 1967) and growth (Baras et al., 2001; Mwangangi and Mutungi, 1994). Those studies that have dealt with senescence in teleosts do so without considering temperature as an effector (Finch, 1998; Reznick et al., 2002). Currently, various thermal tolerance models contemplate mitochondrial function and oxidative stress, and mitochondrial senescence in invertebrates (Philipp et al., 2005a; Philipp et al., 2005b), but a model that relates temperature with mitochondrial senescence for fishes is currently lacking.

The uncoupling of mitochondrial respiration and ATP formation, either by uncoupling proteins or by intrinsic membrane proton leakage, has been shown to act as a safety valve to reduce the formation of reactive oxygen species (ROS), thus reducing oxidative stress. This “uncoupling to survive” strategy (Brand, 2000) reduces the mitochondrial energy transduction efficiency, and could explain to a certain extent the differences in size and mass of *L. macrochirus* between lakes if proton leak significantly

352 reduces overall ATP-production with only little impact on ROS production at elevated temperatures.
353 However, ATP-coupled respiration must be employed, even at high temperatures, in order to meet the
354 minimal energetic requirements of the organism. At high temperatures such as those found in Coffeen
355 Lake, mitochondrial respiration could result in moderate ROS formation, leading to the accelerated
356 senescence of *L. macrochirus*. Further studies on mitochondrial thermal tolerance and oxidative stress
357 that consider the mitochondrial membrane potential will be highly insightful to confirm this hypothesis
358 and are currently under study.

359

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Author Contributions

EM performed specimen collection, respirometry data collection and processing, enzyme activity measurements and contributed to manuscript drafting. AP preformed specimen collection, age determinations and manuscript drafting. MAM provided laboratory infrastructure, participated in CTM measurements, experimental design advice, data analysis and manuscript preparation. RC provided laboratory infrastructure, field collection gear and instrumentation for age determination, and contributed to the experimental conception, data analysis and manuscript preparation.

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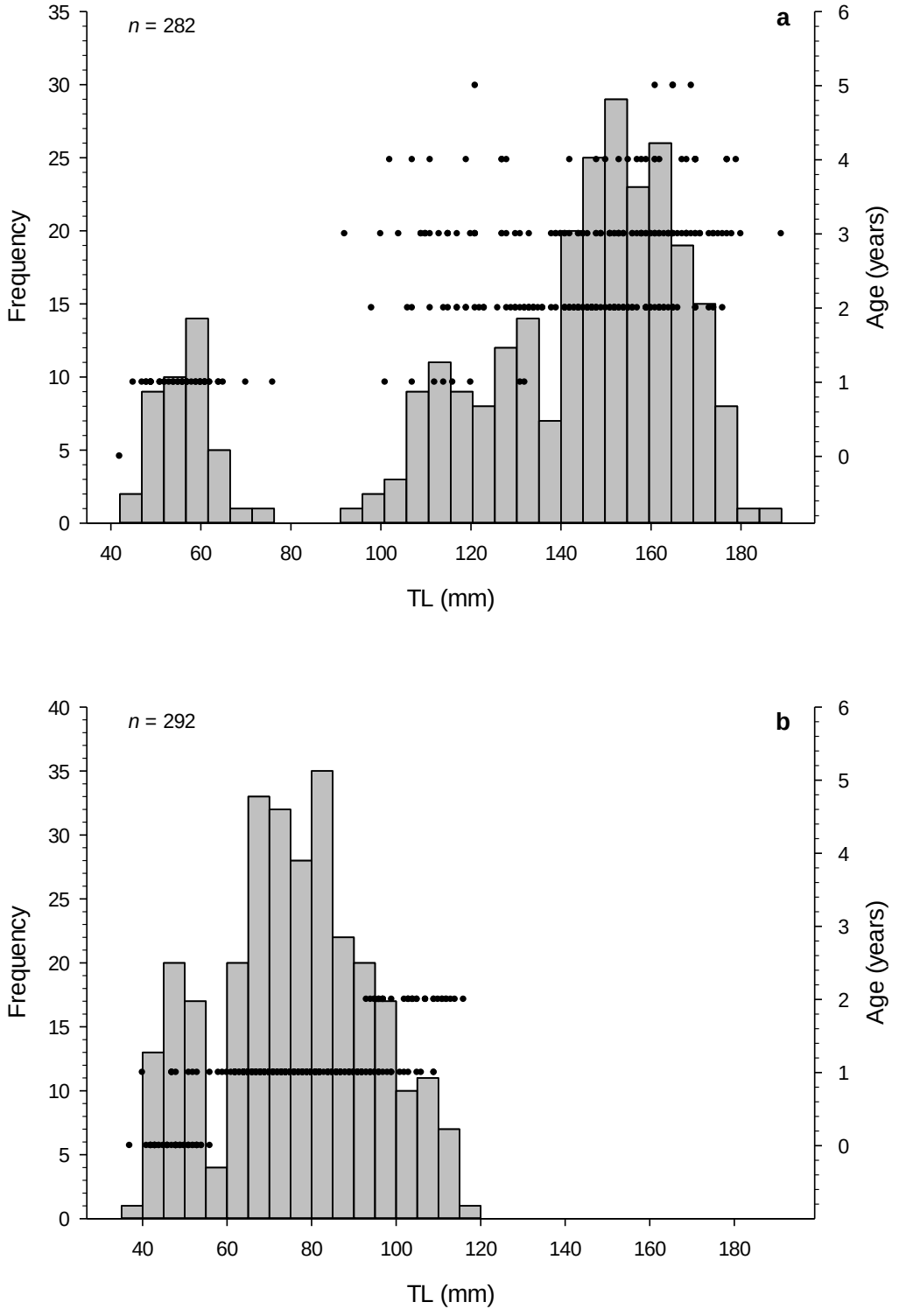
Figure Legends

Figure 1: Age and total length (TL) distribution of *Lepomis macrochirus* collected from Lake Mattoon (**a**) and Coffeen Lake (**b**).

Figure 2: Average size and mass as a function of age class for *Lepomis macrochirus* from a thermally impacted lake (○; Coffeen Lake) and a control lake (●; Lake Mattoon). Biomass accumulation rates are shown to increase after the first year in Lake Mattoon specimens (n = 285 - 291, ± SEM). Statistically significant differences within age classes are shown with an asterisk (*; two-way ANOVA; $P < 0.001$).

Figure 3: Mass – total length relations of bluegill, *L. macrochirus*, from a thermally impacted (○ Coffeen Lake) lake and a control (● Lake Mattoon) lake.

539 **Figure 1**



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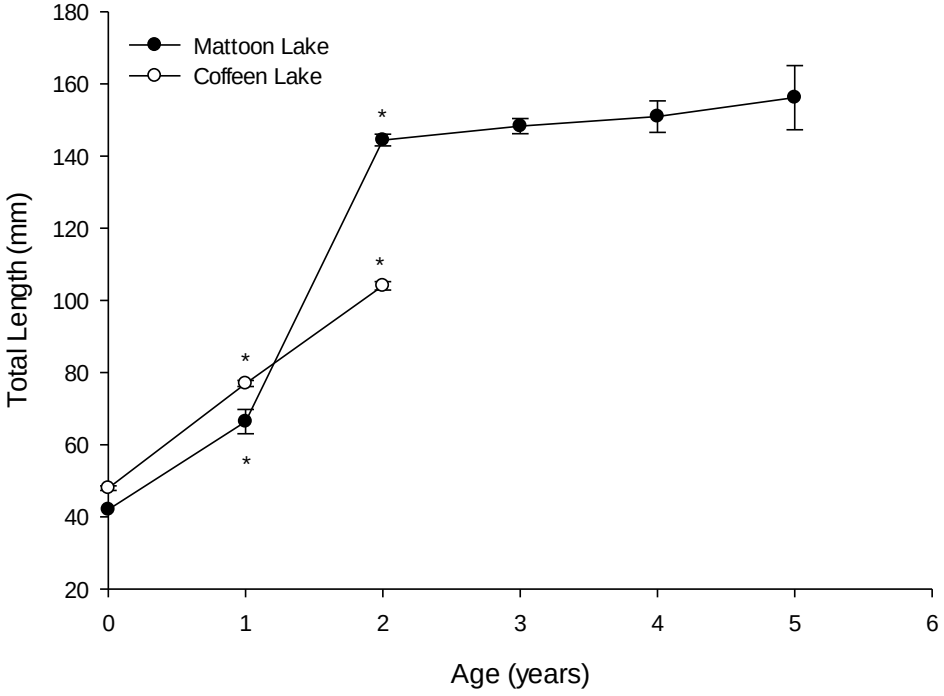
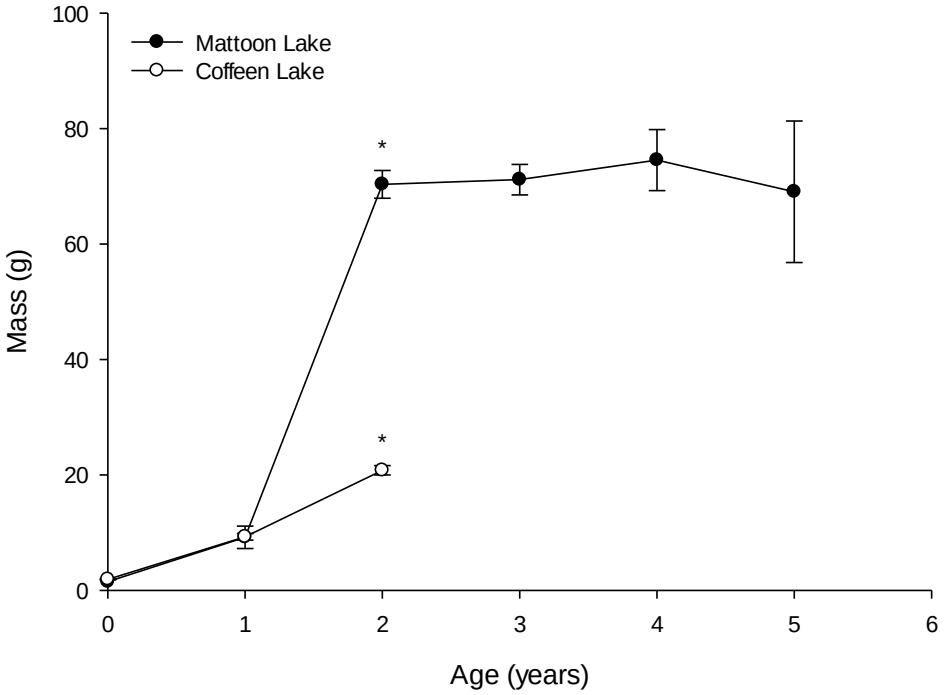
542 **Figure 2:**





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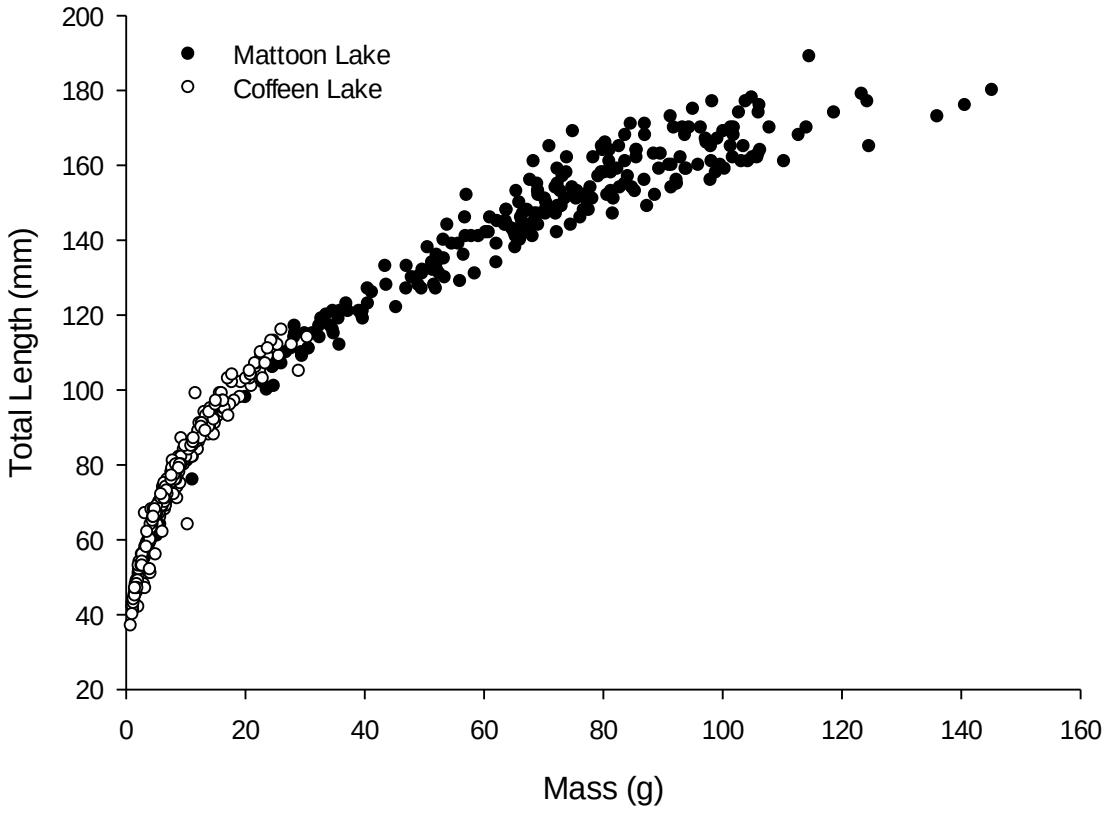
545 **Figure 3:**



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548 Table 1: Population structure of *L. macrochirus* sampled from a thermally impacted (Coffeen lake), and a
549 non-impacted lake (Lake Mattoon). Average total length (TL) and wet mass (WM) were obtained for all
550 specimens collected. The catch per unit effort (CPUE) was evaluated for both sampling sites. CPUE is
551 expressed as the number of individual *L. macrochirus* captured per hour of electrofishing (ind h^{-1})
552
553

Location	**Avg. Age (yrs)**	**Avg. TL (mm)**	**Avg. WM (g)**	**CPUE* (ind h^{-1} ±SEM)**
Coffeen Lake *N* = 291	0.96	75.35	9.42	293 ± 98.19 (*n* = 4)
Lake Mattoon *N* = 285	2.42	134.84	60.26	292 ± 86.24 (*n* = 4)

554

555
556 Table 2: Critical thermal maxima of two populations of *L. macrochirus* acclimated to 17.5°C.
557

Location	Avg. mass (g) (min-max)	Avg. CTM (°C) (min-max)*
Mattoon (*n* = 5)	67.72 (33.2-90.6)	40.06 ± 0.503 (38.2-41.1)
Coffeen (*n* = 5)	23.8(10.2-31.5)	41.08 ± 0.12 (40.7-41.3)

558
559 *No significant differences were found between population (t-test, P = 0.08, 95%CI). CTM = Critical
560 thermal maximum
561
562

Table 3: Oxygen consumption of bluegill *Lepomis macrochirus* acclimated to 17.5°C and 30°C collected from a thermally impacted (Coffeen Lake) and control lake (Lake Mattoon).

Location	Acclimation Temperature (°C)	Sample size (n)	Respiration rate*
Lake Mattoon	17.5	7	129.14 ± 16.19
Lake Mattoon	30.0	11	143.91 ± 10.80
Coffeen Lake	17.5	9	160.06 ± 12.02
Coffeen Lake	30.0	8	136.02 ± 11.30

*No significant differences were found among acclimation temperatures or populations (two-way ANOVA, P = 0.131). Average respiration rates are expressed in mg O_2 hr^{-1} Kg wet $mass^{-1}$ ± SEM.

570 Table 4: Lactate Dehydrogenase (LDH) and Citrate Synthase (CS) relative activities from white epaxial
571 muscle of bluegill obtained from Lake Mattoon and acclimated to 17.5°C or 30°C.
572

Acclimation Temperature (°C)	Sample Size	Activity (U)	Calc. Act. (U)
Lactate Dehydrogenase			
17.5	4	0.437±0.111	0.281
30.0	4	0.436±0.0532	0.585
Citrate Synthase			
17.5	4	0.898±0.101	0.578
30.0	4	0.369±0.0863	0.495

573

574 Enzyme activity was measured at 25°C, Units are µmol substrate converted to product min^{-1}. Calculated
575 activity at acclimation temperature was obtained using a Q_{10} of 1.8, derived from respirometric
576 measurements (n = 4, ± SEM).
577