8-9-2022

Effects of temperature on growth, metabolic rate, and lower dissolved oxygen tolerance of Speckled Peacock Bass Cichla temensis

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Effects of temperature on growth, metabolic rate, and lower dissolved oxygen tolerance of Speckled Peacock Bass *Cichla temensis*

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A Thesis
Submitted to the Faculty of
Mississippi State University
in Partial Fulfillment of the Requirements
for the Degree of Master of Science
in Wildlife, Fisheries and Aquaculture
in the Department of Wildlife, Fisheries and Aquaculture

Mississippi State, Mississippi

August 2022

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Date of Degree: August 9, 2022

Institution: Mississippi State University

Major Field: Wildlife, Fisheries and Aquaculture

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Title of Study: Effects of temperature on growth, metabolic rate, and lower dissolved oxygen tolerance of Speckled Peacock Bass *Cichla temensis*

Pages in Study: 51

Candidate for Degree of Master of Science

I examined the effects of temperature (25, 30, and 35°C) on growth, standard metabolic rate (SMR), and lower dissolved oxygen tolerance (LDOT) of juvenile Speckled Peacock Bass *Cichla temensis*. Fish were acclimated to 150-L aquaria for 7 weeks before the growth, SMR, and LDOT experiments. The growth study lasted 58 days and fish acclimated to 25 and 30°C displayed similar growth rates, while fish acclimated to 35°C had very poor growth rates. The SMR and LDOT experiments were performed using intermittent respirometers. Fish acclimated to 25°C had the lowest SMR, followed by 30°C, and finally 35°C. The highest LDOT was observed at 25°C, followed by 30°C, and finally 35°C. Collectively, these results suggest that 25-30°C is within the thermal optima of Speckled Peacock Bass for grow-out and survival in an aquaculture setting. Further, I recommend maintaining dissolved oxygen concentrations at or near saturation.

DEDICATION

I would like to dedicate my thesis to my parents, Manuel Coffill and Jessica Rivera, as well as my siblings, Josiel Coffill-Rivera and Jeiska Alanis Coffill-Rivera.

ACKNOWLEDGEMENTS

I would like to thank Puerto Rico Department of Natural and Environmental Resources (Project SFR F-69.3 [F17AF00057]) for funding. I also thank Mississippi Agricultural and Forestry Experimental Station and U.S. Department of Agriculture (USDA) Agricultural Research Service (Grant# 58-6066-5-042) for facility funding, and USDA National Institute of Food and Agriculture (Grant# 1005154) for providing funding for Peter J. Allen. I would also thank Jacob A. Moreland, Abby J. Vaughn, Joshua J. Neary, Brandon J. Gerhart, Amanda Daulong, Alicia Santiago, and the South Farm Aquaculture Facility staff for their assistance and support with fish care and experiments; Aqua Imports for sourcing the fish used in this study. Finally, I'd like to thank my committee members, Dr. J. Wesley Neal, Dr. Peter J. Allen, and Dr. Michael V. McGee for all their support, insight, and guidance throughout my journey in completing this thesis.

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INTRODUCTION

Speckled Peacock Bass *Cichla temensis* are recreationally important South American sportfish found in floodplain rivers and their tributaries (Holley et al. 2008). They are primarily found in the Negro, Orinoco, Branco, and Madeira Rivers, along with many other tributaries in Colombia, Venezuela, and Brazil (Kullander and Ferreira 2006). Speckled Peacock Bass are the largest species of the *Cichla* genus, which currently contains 16 recognized species (Kullander and Ferreira 2006; Sabaj et al. 2020). In the Rio Negro, outfitters provide fishing services for targeting Speckled Peacock Bass, which attracts many anglers to the region annually, and contributes $5-6 million in expenditures (Holley et al. 2008). In addition to having value to recreational anglers, Speckled Peacock Bass are also an important species to commercial and subsistence fisheries (Campos et al. 2020).

Speckled Peacock Bass are generalist piscivores that tend to occupy sandy and rocky banks of main river channels, offshore open-water habitats, and deeper littoral areas in lagoons (Jepsen et al. 1997; Winemiller et al. 1997; Montaña et al. 2007). In the Rio Negro, the maximum age and length observed are 9 years old and 77 cm standard length, respectively (Holley et al. 2008). Speckled Peacock Bass reach sexual maturity around ages 1-3 (Jepsen et al. 1999; Campos et al. 2015). Spawning of *Cichla* spp. has been observed to correlate with seasonal changes, occurring during the transition from dry to wet season, around March to May (Jepsen et al. 1999; Montaña et al. 2007). Based on gonadosomatic indices of several species of the *Cichla* genus, including Speckled Peacock Bass, spawning may occur year-round (Chellappa et al. 2003; Montaña et al. 2007). Many *Cichla* spp., including Speckled Peacock Bass, have highly invested spawning behaviors, including nest construction and parental guarding (Zaret 1980; Hoeinghaus et al. 2006; Campos et al. 2015). Spawning occurs over horizontal substrate, such as tree trunks (Zaret 1980).

Due to their large sizes and aggressive predatory behaviors, Speckled Peacock Bass are a good candidate for introduction as a biological control agent, specifically for invasive cichlids (Neal et al. 2017). Speckled Peacock Bass have been introduced into South American reservoirs, such as Guri Reservoir in Venezuela and Balbina Reservoir in Brazil, and have successfully improved sport fisheries (Williams et al. 1998; Nascimiento et al. 2001). Further, Speckled Peacock Bass were introduced into freshwater systems in Texas and Florida (USA) during the late 20th century by state agencies to establish sport fisheries (Guest et al. 1979; Howells and Garrett 1992). However, cold winter temperatures inhibited successful establishment.

The establishment of freshwater fisheries in Puerto Rico reservoirs has depended heavily on the introduction of non-native fish, partly due to the volcanic island's relatively young age and limited native fish communities (Neal et al. 2004; Neal et al. 2009). Non-native fish stocking in Puerto Rico reservoirs dates to the early 1900s and established introduced sportfish include Largemouth Bass *Micropterus salmoides* and Butterfly Peacock Bass *Cichla ocellaris* (Neal et al. 2004; Neal et al. 2009). Over the last few decades, several species of New World cichlids have started appearing in reservoir surveys, including Jaguar Guapote *Parachromis managuensis* and Red Devil Cichlid *Amphilophus labiatum* (Neal et al. 2008; Neal et al. 2009; Bies et al. 2016). These species compete with sportfish for spawning areas, food and space resources, demonstrate aggressive behavior towards other species, and can alter aquatic vegetation (Fuller et al. 1999). New World cichlids have been implicated in the decline of Largemouth Bass and other sunfishes within Puerto Rico reservoirs (Neal et al. 2017). Butterfly Peacock Bass have been shown to consume these New World cichlids, but their potential of serving as a biological control agent is limited by their gape width and relatively small maximum body size (Neal et al. 2017). Without adequate predation pressure, New World cichlids will continue their expansion within reservoirs and colonize additional reservoirs in Puerto Rico, negatively affecting Largemouth Bass and other species (Neal et al. 2017).

To combat the effect of New World cichlids in reservoirs, Puerto Rico Department of Natural and Environmental Resources (DNER) is considering the introduction of Speckled Peacock Bass, which have been shown to feed on New World cichlids and have a larger gape width and predator effect than Butterfly Peacock Bass (Neal et al. 2017). An additional benefit of this introduction would be the creation of a new sport fishery for local anglers with the potential for drawing tourism by nonresident anglers (Neal et al. 2017). Reservoirs in Puerto Rico offer an abundance of prey and presumably adequate environmental conditions for Speckled Peacock Bass. Sympatry between Speckled Peacock Bass and Butterfly Peacock Bass has been observed in Brazil (Petrere 1986) and, given that Butterfly Peacock Bass have thrived in Puerto Rico since their introduction in 1967 (Neal et al. 2004), this suggests that Speckled Peacock Bass could do the same. Surface water temperature of reservoirs in Puerto Rico averages around 27°C (Neal et al. 2009), which is similar to surface water temperatures observed in water bodies where Speckled Peacock Bass are established (Winemiller et al. 1997; Williams et al. 1998). Conversely, anoxia is often present in Puerto Rico reservoirs at depths over 3 m (Neal et al. 2009) and may limit habitat selection of Speckled Peacock Bass.

Introductory stocking of Speckled Peacock Bass into reservoirs will require appropriate hatchery protocols that optimize spawning conditions, survival, and growth. Speckled Peacock Bass require elevated dissolved oxygen levels and are sensitive to low temperatures (Tavares-Dias et al. 2011), but the metabolic response to these environmental conditions has not been evaluated. There is limited literature regarding metabolism, dissolved oxygen requirements, and temperature preferences of *Cichla spp*. However, it has been demonstrated that *Cichla monoculus,* a pelagic, active swimming fish, has a resting metabolic rate five-fold greater than that of *Pinirampus pirinampu,* the most active benthic fish in the Amazon River basin (Duncan 2020). Temperature variation is of fundamental importance to the ecology of fishes, as metabolism and all associated activities are directly subject to thermokinetic influences (Evans 1990). In addition, hypoxia can cause detrimental physiological disturbances in fish, depending on the severity (Hvas and Oppedal 2019). Pilot research with Speckled Peacock Bass in Puerto Rico demonstrated this species is also especially sensitive to handling stress (J. W. Neal, Pers. Comm.). Handling techniques can induce physical, hypoxic, temperature, and osmotic shock (Mazeaud et al. 1977), and it is important to minimize these stressors during tank-holding procedures (Guy and Allen 2018).

Proper hatchery protocols will promote successful rearing and production of Speckled Peacock Bass for subsequent introduction into reservoirs. The goal of this thesis was to improve culture methods of Speckled Peacock Bass by conducting directed research that fulfilled the following objectives:

1. Evaluate the effects of temperature on growth of Speckled Peacock Bass acclimated to 25, 30, and 35°C (Chapter II), and

2. Evaluate the effects of temperature on metabolic rate and lower dissolved oxygen tolerance of Speckled Peacock Bass acclimated to 25, 30, and 35°C (Chapter III).

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I

EFFECTS OF TEMPERATURE ON GROWTH OF SPECKLED PEACOCK BASS *CICHLA TEMENSIS*

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2.1 Abstract

Speckled Peacock Bass *Cichla temensis* are a popular sport and food fish that generate significant angling tourism and utilitarian harvest within their natural range. This popularity and value make this species a potential aquaculture candidate for both fisheries enhancement and food fish production. However, appropriate hatchery protocols for handling, spawning, and grow-out have not been determined. Speckled Peacock Bass have been documented to have high sensitivity to temperature extremes and low concentrations of dissolved oxygen, but the growth response to these physical variables has not been formerly evaluated. In this study, the effects of temperature (25, 30, and 35°C) on growth of juvenile Speckled Peacock Bass (mean ± standard error total length: 139 ± 1 mm, and wet weight: 29.73 ± 0.64 g) were evaluated during an 8-week period. Fish were acclimated and reared in 150-L aquaria for 7 weeks prior to the growth study. Total length and weight gains were similar for fish acclimated to 25 and 30°C (25 ± 1 mm, 20.03 ± 1.65 g, and 26 ± 2 mm, 19.17 ± 1.90 g, respectively), while fish acclimated to 35°C had lower growth rates (7 ± 1 mm, and 0.79 ± 0.55 g). Specific growth rate (SGR) in terms of weight and

Fulton's condition factor (K_f) at the end of experimentation were similar for fish acclimated to 25 and 30°C (0.87 ± 0.05 %/day, 1.10 ± 0.02, and 0.85 ± 0.06 %/day, 1.08 ± 0.02, respectively), while fish acclimated to 35°C displayed lower SGR and K_f (0.04 ± 0.03 %/day and 0.97 ± 0.02, respectively). Survival was >90% among all treatments. These results indicate juvenile Speckled Peacock Bass are sensitive to temperatures near 35°C; therefore, we recommend rearing juvenile Speckled Peacock Bass at 25-30°C for more rapid growth.

2.2 Introduction

Speckled Peacock Bass *Cichla temensis* are a species of South American freshwater fish that support valuable recreational, commercial, and subsistence fisheries (Holley et al. 2008; Campos et al. 2020). Their popularity as a sportfish is derived from their large body size, aggressive predatory behavior, colorful appearance, and acrobatic aerial displays when hooked (Winemiller et al. 2021). Speckled Peacock Bass have been introduced into South American reservoirs, such as Guri Reservoir in Venezuela and Balbina Reservoir in Brazil, and have successfully improved sport fisheries (Williams et al. 1998; Nascimiento et al. 2001). This species is currently under consideration to diversify sport fishing opportunities and for use as a biological control agent for invasive New World cichlids in Caribbean reservoirs (Neal et al. 2017). As a food fish, Speckled Peacock Bass are valued in both subsistence and commercial fisheries, and are commonly found in fish markets within their range (Winemiller et al. 2021). For these reasons, Speckled Peacock Bass are a candidate for aquaculture for conservation, management, and food fish production (Neal et al. 2017).

Husbandry of any fish species requires an understanding of optimal environmental conditions for grow-out and survival. Temperature is of fundamental importance to the physiology of fish, as metabolism and all associated activities, including growth, are directly subject to thermokinetic influences (Evans 1990). Temperature governs metabolic rate (Fry 1947), which influences numerous physiological processes and can directly affect growth by controlling food passage time, nutrient and caloric requirements, and feed consumption (Tidwell et al. 1999; Mayfield and Cech 2004; Handeland et al. 2008). The effects of temperature on growth have been well documented (Brett 1971; McCauley 1977; Gaylord et al. 2003; Righton et al. 2010). Fish tend to exhibit optimal growth within a certain temperature range, and some species exhibit ontogenetic changes in thermal optimum (Gadomski and Caddell 1991; Imsland et al. 2001). Temperatures above and below the thermal optimum result in suboptimal physiological condition. Therefore, it is essential to know the thermal optimum when designing aquaculture protocols, as maintaining proper temperatures in hatchery settings allows for optimal production, grow-out, and survival.

As a tropical-evolved species, Speckled Peacock Bass are particularly sensitive to low temperatures (Tavares-Dias et al. 2011). For example, Speckled Peacock Bass were introduced into freshwater systems in Texas and Florida (USA) during the late 20th century by state agencies to create a sport fishery (Guest et al. 1979; Howells and Garrett 1992), but cold winter temperatures inhibited successful establishment. However, controlled studies on the thermal requirements of this genus are lacking. This information is needed to create aquaculture protocols that promote successful rearing and production of Speckled Peacock Bass. Therefore, this study examined the effect of a range of temperatures on growth of juvenile Speckled Peacock Bass. The specific objective was to evaluate the influence of temperature on growth and condition of juvenile Speckled Peacock Bass acclimated to 25, 30, and 35°C.

2.3 Methods

2.3.1 Acclimation

Wild juvenile Speckled Peacock Bass were captured and transported from the Inírida River, Colombia by a commercial supplier to Mississippi State University's South Farm Aquaculture Facility. Fish were randomly distributed into three 150-L aquaria (92 cm x 46 cm x 41 cm, n=15-16 fish/tank) at 29 ± 1°C for 7 weeks before initiating experiments. Tanks were wrapped in insultation to help maintain target temperatures and covered to prevent escapement and disturbance from outside activity. Submersible aquarium heaters were used to maintain temperature. Covers were removed only during feeding, water changes, or other maintenance. Fish were fed to satiation once daily using live Fathead Minnow *Pimephales promelas* during the first two weeks of acclimation, and then transitioned to small pieces of fresh catfish *Ictalurus* spp. fed every other day for the remainder of acclimation and during experimental trials. Temperatures in aquaria were maintained at treatment levels of 25 ± 1°C, 30 ± 1°C, and 35 ± 1°C during experimental period.

Water quality parameters (temperature, dissolved oxygen, conductivity, and salinity) were monitored daily (Pro2030, YSI Inc., Yellow Springs, OH, USA); pH (EcoSense pH100A, YSI Inc.), ammonia, nitrite (DR/850 Portable Colorimeter, Hach Company, Loveland, CO, USA), alkalinity, and hardness (AQ-2, LaMotte Company, Chestertown, MD, USA) were recorded weekly and a weekly 50% water change was performed in the rearing tanks to minimize ammonia and nitrite accumulation.

2.3.2 Temperature Trials

This research evaluated temperatures of 25, 30, and 35°C. The thermal regime of waterbodies within the native range of Speckled Peacock Bass ranges 27-31°C (Winemiller et al.

1997; Williams et al. 1998) and this species is known to be sensitive to temperatures <20°C (Guest et al. 1979; Howells and Garrett 1992). Therefore, endpoints of the native thermal regime (25 and 30°C) were selected. The upper temperature (35°C) was selected because Speckled Peacock Bass utilize shallow lagoon habitats that tend to be warmer than river channels (Jepsen et al. 1997), and climate models suggest that periods of higher temperatures in South America will become more common (Torres et al. 2021).

At the end of the 7-week acclimation period, food was withheld for 48 hours, then fish were anesthetized in a buffered anesthetic bath (150 mg/L tricaine methanesulfonate [MS-222], 9 g/L NaCl, and 400 mg/L $NaHCO_3$), measured for total length (nearest mm), and weighed wet (nearest 0.01 g). All fish were implanted with a passive integrated transponder (PIT) tag (8 mm long x 1.4 mm in diameter, 0.03 g, 134.2-kHz ISO FDXB, Biomark Inc., Boise, ID, USA) in the left dorsal musculature (anterior to dorsal fin) using an implanter (MK165, Biomark, Inc.) with a 16-gauge, 50-mm stainless steel injector needle (N165, Biomark, Inc.). Fish were randomly assigned to tanks (n=15-16 fish/tank). This was day 0 of the experiment.

Temperature within each tank was adjusted to assigned treatment temperatures of 25, 30, and 35°C at a rate of 1°C/day. Tanks reached target temperatures by Day 5. Tanks remained covered to prevent disturbance except during feeding, water changes, maintenance, or sampling. Food was withheld for 48 hours prior to sampling on day 30 and 58. During sampling, all fish per tank were netted and placed in a buffered anesthetic bath (150 mg/L tricaine methanesulfonate [MS-222], 9 g/L NaCl, and 400 mg/L $NaHCO_3$). Individual identification was obtained from PIT tags, and each fish was measured for total length (nearest mm) and weighed wet (nearest 0.01 g).

2.3.3 Statistical Analyses

Survival (%) was determined for each treatment group as

$$Survival\ (\%) = \frac{\#\ of\ initial\ fish}{\#\ of\ final\ fish}\ x\ 100 \tag{2.1}$$

Linear relationships were established for length and weight over time. In addition, specific growth rates in terms of wet weight were determined using equation used by Cui et al. (1997):

$$SGR = 100\ x\ \frac{(log_e W_f - log_e W_i)}{d} \tag{2.2}$$

where W_f and W_i are the final and initial weights, respectively, and d was the duration of experiment.

Fulton's Condition Factor (K) was calculated for individual fish following Neumann et al. (2012):

$$K = \frac{W}{L^3} \times 100{,}000 \tag{2.3}$$

where W is the wet weight (g) and L is the total length (mm). Fulton's condition factor at the end of experimentation (K_f) was reported, as well as changes from initial to final condition factor (Δ K).

Statistical analyses were performed using RStudio 4.0.3 (R Core Team 2020) at a significance level of a = 0.05. Normality and homogeneity of variance were tested on residuals with Shapiro-Wilk and Levene's tests, respectively. Data were log_{10} transformed to meet these parametric assumptions as needed. A one-way analysis of variance (ANOVA) was used to compare water quality variables and changes in total length, wet weight, SGR, K_f, and Δ K among temperature treatments (25, 30, and 35°C). A two-way ANOVA was used to compare total length and weight among temperature and collection periods (0, 30, and 58 days). Following a significant ANOVA, group means were compared using Tukey's honestly significant difference (HSD) test. Data are reported as mean ± standard error (SE).

2.4 Results

Water quality variables were maintained at adequate levels for survival and growth (Boyd and Pillai 1984; Table 2.1). Temperature ($F_{2,96}$=284.99, P<0.001), dissolved oxygen ($F_{2,81}$=122.00, P<0.001), percent saturation ($F_{2,81}$=40.23, P<0.001), conductivity ($F_{2,90}$=10.97, P<0.001), and salinity ($F_{2,93}$=10.56, P<0.001) were different among treatments, while pH ($F_{2,18}$=0.20, P=0.82), ammonia ($F_{2,17}$=0.53, P=0.60), nitrite ($F_{2,15}$=1.50, P=0.26), alkalinity ($F_{2,18}$=0.11, P=0.89), and hardness ($F_{2,18}$=0.73, P=0.50) were similar (Table 2.2).

There was no difference in initial total length ($F_{2,43}$=0.23, P=0.79) and wet weight ($F_{2,43}$=0.17, P=0.84) among temperature treatments (Figures 2.1 and 2.2). Mean (± SE) initial total length and wet weight of all temperatures (25, 30 and 35°C) combined were 139 ± 1 mm and 29.73 ± 0.64 g, respectively. Growth in total length and weight differed between treatments ($F_{4,126}$=7.75, P<0.001 and $F_{4,126}$=9.76, P<0.001, respectively); this difference was due to lower observed growth for fish acclimated to 35°C compared to fish acclimated 25 and 30°C (Figures 2.1 and 2.2). Similar patterns were found for SGR ($F_{2,41}$=83.22, P<0.001), K_f ($F_{2,41}$=17.16, P<0.001), and Δ K ($F_{2,41}$=11.29, P<0.001; Table 2.3). Survival was >90% among all treatments

(Table 2.3). Growth rates (mm/day TL) were compared to other reported growth rates for juvenile Speckled Peacock Bass (Table 2.4).

2.5 Discussion

Growth of juvenile Speckled Peacock Bass was most rapid at both 25 and 30°C, and slowed significantly at 35°C. These findings suggest that the thermal optimum for juvenile growth is between 25 and 30°C. Although optimum temperature range for growth can vary with ontogeny in many species (e.g., Komoroske et al. 2014), a similar thermal regime was reported for sub-adult and adult Speckled Peacock Bass from a reservoir population (27-30°C; Williams et al. 1998). This suggests Speckled Peacock Bass are adapted to stable, warm water temperatures consistent with their tropical origin, and that significant ontogenetic differences are unlikely. For example, water temperature of the Amazon River between Manaus and Belem, Brazil, varies little (28.8-30.0°C) annually, or with depth due to mixing by turbulence (USGS 1963). Long-term temperature data from tributary rivers of the Amazon River and Orinoco River where Speckled Peacock Bass are distributed are lacking in the literature, but the few reported values further support our findings. For example, Winemiller et al. (1997) reported February temperatures from the Pasimoni River and Cinaruco River ranged 26.0 to 31.0°C.

Whereas this species evolved within stable temperature environments, it is not surprising that elevated temperatures of 35°C resulted in reduced growth. Water temperatures within the native range of this species uncommonly reach 35°C in most locations and prolonged periods at this temperature are unlikely. Further, growth rates at 35°C appeared to decline as the experiment progressed, with most observed growth occurring during the first half of the 58-day experiment. This may indicate that this species can tolerate short-term exposure to elevated temperature, but that prolonged exposure compounds the temperature effect (e.g., Wehrly et al. 2007). Temperature tolerance studies on juvenile Butterfly Peacock Bass (85-140 mm TL) indicated a lower and upper lethal temperature of 16-17°C and 38-39°C, respectively (Guest et al. 1979). Further, lower temperature tolerance experiments on two juvenile Speckled Peacock Bass (76 mm TL, 4 g wet weight and 134 mm TL, 24 g wet weight) resulted in mortality occurring at 16°C (Rutledge and Lyons 1976). Collectively, these results suggest that *Cichla* spp. are sensitive to temperatures below 20°C and above 35°C, although temperature was not evaluated for 20-25°C or >35°C.

Oxygen saturation was maintained at a minimum of 80% in all treatments during the growth study, yet the reduction in solubility with increasing temperature resulted in 21% less oxygen available at 35°C compared to 25°C. Pörtner (2001, 2002) suggested that the upper critical thermal limit may be determined by the inability of the cardiorespiratory system to meet tissue oxygen demand. As temperature increases, metabolic oxygen demand increases while oxygen saturation concentration decreases. Thus, as fish approach the upper lethal temperature, biological function becomes impaired due to lack of oxygen. Effects may include a reduction in organ system function (e.g., Anttila et al. 2013), impairment of movement capacity for feeding and predator avoidance, and reduced immune capacity (Materna 2001). Further, as metabolic demand for energy and oxygen increases, less energy can be allocated to growth. As a species approaches its upper temperature limit, growth will slow and fish will begin to lose mass (e.g., Deslauriers et al. 2017).

Observed growth rates were lower than those reported in the literature. Juvenile Speckled Peacock Bass growth rates ranged 0.12-0.43 mm/day and 0.01-0.34 g/day across treatments when fed to satiation once per two-day period. This equated to a daily ration between 2.1% and

3.7% body weight per day (wet weight), and we hypothesize that growth rates would improve if feeding occurred daily and daily ration was increased. For comparison, adult wild caught Speckled Peacock Bass (158-720 mm SL and 0.1-6.8 kg wet weight) in the Cinaruco River exhibited first year growth of 0.9 mm/day based on extrapolation from length-weight relationships (Jepsen et al. 1999). Likewise, growth rates of pond-reared Speckled Peacock Bass in Texas were reported to range 0.43-2.03 mm/day during the first 6 months after hatching (Rutledge and Lyons 1976), and growth rates of larval Speckled Peacock Bass (≤100 mm TL) were reported to range 0.8-1.0 mm/day (Braga 1953).

Temperature also influenced condition of Speckled Peacock Bass. K_f for fish acclimated to 25 and 30°C were similar and above 1.00, while fish acclimated to 35°C had K_f lower than 1.00 and displayed the poorest Δ K. These results support those of Tavares-Dias et al. (2011), who reported relative condition factor (Kn) to be ideal (mean = 1.00; SD = 0.04) for farmed Speckled Peacock Bass (16.5-27 cm TL and 40-220 g wet weight) acclimated to 27.8-29.0°C. Condition provides an index of well-being and is generally used to infer food availability (Schramm and Willis 2012). We fed to satiation and controlled all environmental factors; thus, we contend that condition can be used as an indicator of temperature stress, manifested either in terms of consumption rate or conversion efficiency. Average daily consumption was 0.91, 1.10, and 0.63 g/fish/day at 25, 30, and 35°C, respectively. Whereas each treatment was fed to satiation, these results suggest that consumption rate might have been affected by temperature.

This was the first documentation of the influence of holding temperature on juvenile Speckled Peacock Bass growth and condition. Additional research is needed on feed ration, potential for feed training on extruded pellets, and the effects of lower temperatures. Results from these trials indicate that the optimal temperature range for growth of Speckled Peacock

Bass is 25-30°C. We recommend future husbandry of this species target consistent water temperatures within this range and maintain dissolved oxygen concentration near saturation.

2.6 Acknowledgements

We thank Puerto Rico Department of Natural and Environmental Resources (Project SFR F-69.3 [F17AF00057]) for funding. We also thank Mississippi Agricultural and Forestry Experiment Station and U.S. Department of Agriculture (USDA) Agricultural Research Service (Grant# 58-6066-5-042) for facility funding, and USDA National Institute of Food and Agriculture (Grant# 1005154) for providing funding for Peter J. Allen. We also thank Jacob A. Moreland, Abby J. Vaughn, Joshua J. Neary, Brandon J. Gerhart, Amanda Daulong, Alicia Santiago, and the South Farm Aquaculture Facility staff for their assistance and support with fish care and experiments; Aqua Imports for sourcing the fish used in this study. This research was conducted in compliance with provisions established in Mississippi State University Institutional Animal Care and Use Committee.

2.7 Tables

Table 2.1 Mean (± SE) water quality variables of Speckled Peacock Bass *Cichla temensis* rearing tanks during 7-week acclimation period.

Water Quality Variables	**Mean ± SE**
Temperature (°C)	28.9 ± 0.1
Dissolved Oxygen (mg/L)	6.65 ± 0.03
Dissolved Oxygen (% saturation)	86.20 ± 0.30
Conductivity (mS/cm)	0.85 ± 0.03
Salinity (ppt)	0.48 ± 0.01
pH	7.44 ± 0.09

Table 2.2 Mean (± SE) water quality variables of Speckled Peacock Bass *Cichla temensis* rearing tanks during experimental period.

Treatment	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% saturation)	Conductivity (mS/cm)	Salinity (ppt)	pH	TAN (mg/L)	NH_3 (mg/L)	NO_2^- (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
25°C	7.29 ± 0.07[a]	89.96 ± 0.55[a]	1.60 ± 0.04[a]	0.80 ± 0.02[a]	7.01 ± 0.26	0.46 ± 0.09	0.003 ± 0.001	0.58 ± 0.15	42 ± 7	152 ± 9
30°C	6.27 ± 0.05[b]	83.31 ± 0.70[b]	1.93 ± 0.06[b]	0.97 ± 0.03[b]	6.77 ± 0.26	0.49 ± 0.07	0.003 ± 0.000	0.25 ± 0.10	38 ± 7	173 ± 14
35°C	5.76 ± 0.07[c]	81.82 ± 0.63[b]	1.89 ± 0.06[b]	0.96 ± 0.03[b]	6.90 ± 0.29	0.39 ± 0.08	0.004 ± 0.001	0.33 ± 0.14	38 ± 7	164 ± 13

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$). TAN denotes total ammonia nitrogen. Alkalinity and Hardness are read as mg/L of $CaCO_3$.

Table 2.3 Mean (± SE) biometrics of Speckled Peacock Bass *Cichla temensis* during experimental period.

Treatment	Δ total Length (mm)	Δ wet weight (g)	SGR (%/day)	K_f	Δ K	Survival (%)	N_i
25°C	25 ± 1[a]	20.03 ± 1.65[a]	0.87 ± 0.05[a]	1.10 ± 0.02[a]	0.01 ± 0.02[a]	100	16
30°C	26 ± 2[a]	19.17 ± 1.90[a]	0.85 ± 0.06[a]	1.08 ± 0.02[a]	-0.02 ± 0.02[a]	93	15
35°C	7 ± 1[b]	0.79 ± 0.55[b]	0.04 ± 0.03[b]	0.97 ± 0.02[b]	-0.13 ± 0.02[b]	93	15

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$). Δ denotes change in variable for the duration of the experiment (58 days). $_f$ denotes variable at end of experimentation, while $_i$ denotes variable at beginning of experimentation.

Table 2.4 Reported juvenile Speckled Peacock Bass *Cichla temensis* growth rates.

Source	**Growth Rate (mm/day TL)**	**Total Length (mm)**	**Age (yr)**
This study	0.12-0.43	130-160	-
Braga et al. 1953	0.8-1.0	≤100	-
Rutledge and Lyons 1976	0.43-2.03	-	0-0.5
Jepsen et al. 1999	0.9	-	0-1

2.8 Figures

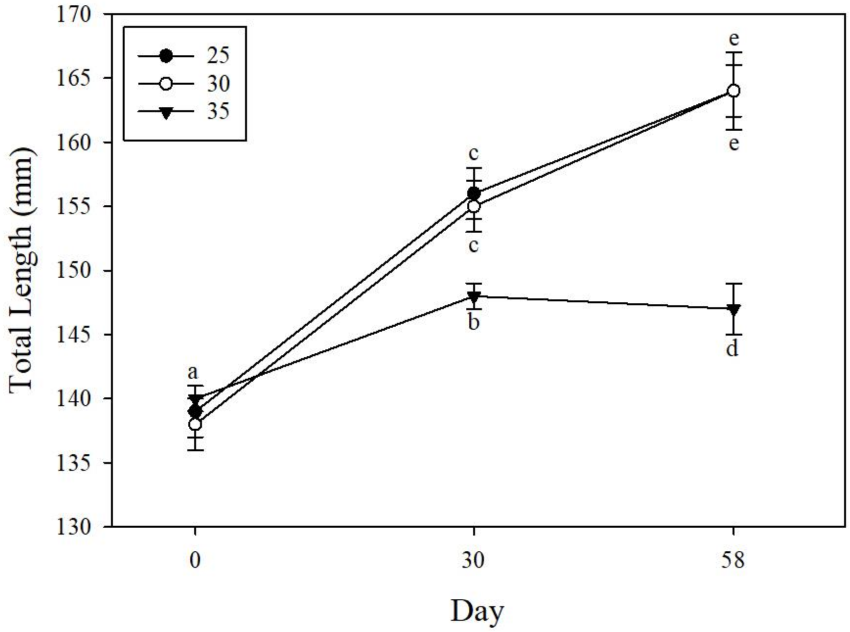

Figure 2.1 Total length of Speckled Peacock Bass *Cichla temensis* acclimated to 25, 30, and 35°C.

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$, n=14-16/temperature).

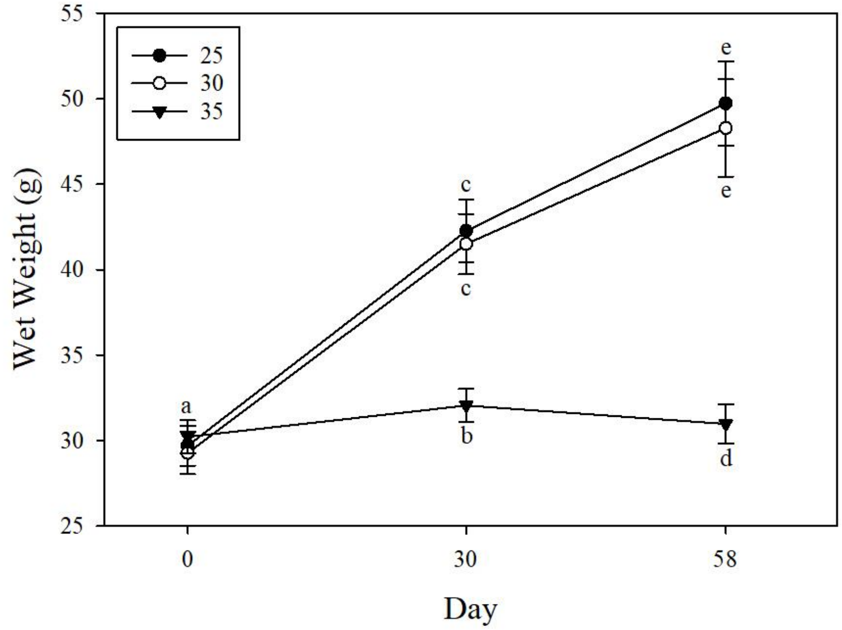

Figure 2.2 Figure 2.2 Total weight of Speckled Peacock Bass *Cichla temensis* acclimated to 25, 30, and 35°C.

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$, n=14-16/temperature).

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I

EFFECTS OF TEMPERATURE ON METABOLIC RATE AND LOWER DISSOLVED OXYGEN TOLERANCE OF SPECKLED PEACOCK BASS *CICHLA TEMENSIS*

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3.1 Abstract

Speckled Peacock Bass *Cichla temensis* are a popular sport and food fish that generate significant angling tourism and utilitarian harvest within their range. This popularity and value make this species a potential aquaculture candidate for both fisheries enhancement and food fish production. However, appropriate hatchery protocols for handling, spawning, and grow-out have not been determined. Speckled Peacock Bass have been documented to have high sensitivity to extreme temperatures and low concentrations of dissolved oxygen, but the metabolic underpinnings to these physical variables have not been evaluated. In this study, the effects of temperature (25, 30, and 35°C) on standard metabolic rate (SMR) and lower dissolved oxygen tolerance (LDOT) of juvenile Speckled Peacock Bass (mean ± standard error total length: 153 ± 2 mm, and wet weight: 39.09 ± 1.37 g) were evaluated using intermittent respirometers after an acclimation period of 2 weeks. Speckled Peacock Bass had the highest SMR at 35°C (345.56 ± 19.89 mg O_2/kg/hr), followed by 30°C (208.16 ± 12.45 mg O_2/kg/hr), and 25°C (144.09 ± 10.43 mg O_2/kg/hr). Correspondingly, the Q_{10}, or rate of increase in aerobic metabolic rate (MO_2)

relative to 10°C, for 30-35°C was also greater (2.76) than from 25-30°C (2.08). Similarly, Speckled Peacock Bass were the most sensitive to hypoxia at the warmest temperature, with a LDOT at a pO_2 of 90 mmHg (4.13 mg/L) at 35°C compared to pO_2's of 45 mmHg (2.22 mg/L) and 30 mmHg (1.61 mg/L) at 30°C and 25°C, respectively. These results indicate Speckled Peacock Bass are sensitive to temperatures near 35°C; therefore, we recommend rearing Speckled Peacock Bass at 25-30°C with dissolved oxygen concentration approaching saturation.

3.2 Introduction

An understanding of environmental adaptation of any fish species is useful for inference into ecological limitations and for guiding management and husbandry practices. Temperature is of fundamental importance to the physiology of fish, as metabolism and all associated activities are directly subject to thermokinetic influences (Evans 1990). In ectothermic fish, temperature governs metabolic rate (Fry 1947), which influences numerous physiological processes such as growth, spawning, digestion time, oxygen consumption, and blood chemistry (Kirk 1974; Wurtsbaugh and Davis 1977; Adams et al. 1982; Schurmann and Steffensen 1997; Gomiero and Braga 2004; Mayfield and Cech 2004; Fontaine et al. 2007). Further, temperature directly influences oxygen solubility and subsequent availability to fish.

Hypoxia can cause detrimental physiological disturbances in fish, depending on severity (Hvas and Oppedal 2019). Because of this importance, numerous studies have investigated hypoxic influences on teleosts, including the influence on metabolic processes (Cruz-Neto and Steffensen 1997; Aboagye and Allen 2014; Rogers et al. 2016; Li et al. 2018). Hypoxia can affect oxygen availability, limiting aerobic ATP production, and leading to less efficient anaerobic metabolism (Richards 2009). Generally, hypoxia sensitivity increases as temperature increases because oxygen solubility decreases with temperature; thus, metabolic demands become more challenging to meet (Vaquer-Sunyer and Duarte 2011). Active, higher trophic level fish species have higher metabolic demands than lower level or benthic species, and therefore display increased sensitivity to temperature and dissolved oxygen concentrations (Duncan 2020). For example, *Cichla monoculus,* an active piscivorous species, has a resting metabolic rate five-fold greater than that of *Pinirampus pirinampu,* the most active benthic fish in the Amazon River basin (Duncan 2020). Thus, an understanding of the effects of temperature on metabolic rate and hypoxia sensitivity is important for basic inference into ecology and useful for management and aquaculture.

The effects of temperature and dissolved oxygen on physiological and biochemical processes of some cichlid species (family Cichlidae) have been determined (Muusze et al. 1998; Luna-Figueroa et al. 2003; Chippari-Gomes et al. 2005; Zaragoza et al. 2008; Schofield et al. 2010; De Boeck et al. 2013; Kochhann et al. 2015; Pereira et al. 2018; Burggren et al. 2019). Many species demonstrate tolerance to hypoxic and anoxic environments because they are able to reduce metabolic expenditure in part through increased reliance on anaerobic metabolism. This includes Mayan Cichlid *Cichlasoma urophthalmus,* Oscar *Astronotus ocellatus,* Bumblebee Oscar *Astronotus crassipinnis,* Blue/Brown Discus *Symphysodon aequifasciatus,* and Pearl Cichlid *Geophagus brasiliensis.* Most of these cichlids inhabit tropical and sub-tropical waters, such as the Amazonian watershed, where temperature and dissolved oxygen concentrations fluctuate on a diel and seasonal basis (Caraballo et al. 2014; Sahoo et al. 2016). Less research has addressed the physiological response of the *Cichla* genus of cichlids to temperature and oxygen extremes.

Speckled Peacock Bass *Cichla temensis* are an important ecological community member with top-down effects on their ecosystems as a keystone predator (Winemiller et al. 1997;

Hoeinghaus et al. 2006; Aguiar-Santos et al. 2018). The use of this species for conservation, management, and food fish production has growing interest because of the valuable recreational, commercial, and subsistence fisheries they support (Holley et al. 2008; Neal et al. 2017; Campos et al. 2020). In South America, this species and related congeners are commonly harvested and commercially sold in fish markets (Winemiller et al. 2021). Fisheries managers have introduced Speckled Peacock Bass into reservoirs where they provide sport fishing opportunities (Williams et al. 1998; Nascimiento et al. 2001), and this species is currently under consideration to diversify sport fishing and serve as a biological control agent for invasive species in Puerto Rico reservoirs (Neal et al. 2017).

Specific thermal requirements and metabolic demands have not been empirically evaluated for Speckled Peacock Bass, which are important for guiding management and introduction. Tropical cichlids are particularly sensitive to low temperatures (Tavares-Dias et al. 2011), which limits their potential for establishment outside of tropical regions (Guest et al. 1979; Howells and Garrett 1992). All fish species exhibit an optimal temperature range for efficient physiological performance, with temperatures above and below this range resulting in suboptimal physiological condition manifested in slow growth, reduced condition, and potentially other deleterious effects. Likewise, all species demonstrate reduced performance when oxygen levels are insufficient. Therefore, this research examined the effects of temperature and dissolved oxygen on Speckled Peacock Bass. The specific objective was to evaluate the influence of temperature on metabolism and lower dissolved oxygen tolerance of juvenile Speckled Peacock Bass acclimated to 25, 30, and 35°C.

3.3 Methods

3.3.1 Acclimation

Wild juvenile Speckled Peacock Bass were captured and transported from the Inírida River, Colombia by a commercial supplier to Mississippi State University's South Farm Aquaculture Facility for experimentation. Fish were randomly distributed into three 150-L aquaria (92 cm x 46 cm x 41 cm, n=15-16 fish/tank) at 29 ± 1°C for 7 weeks before initiating experiments. Tanks were wrapped in insultation to help maintain target temperatures and covered to prevent escapement and disturbance from outside activity. Submersible aquarium heaters were used to maintain temperature. Covers were removed only during feeding, water changes, or other maintenance. Fish were fed to satiation once daily using live Fathead Minnows *Pimephales promelas* during the first two weeks of acclimation, and then transitioned to small pieces of fresh catfish *Ictalurus* spp. every other day for the remainder of acclimation and during experimental trials. Temperature in aquaria was maintained at treatment levels of 25 ± 1°C, 30 ± 1°C, and 35 ± 1°C during experimental period.

Water quality parameters (temperature, dissolved oxygen, conductivity, and salinity) were monitored daily (Pro2030, YSI Inc., Yellow Springs, OH, USA). pH (EcoSense pH100A, YSI Inc.), ammonia, nitrite (DR/850 Portable Colorimeter, Hach Company, Loveland, CO, USA), alkalinity, and hardness (AQ-2, LaMotte Company, Chestertown, MD, USA) were recorded weekly and a weekly 50% water change was performed in the rearing tanks to minimize ammonia and nitrite accumulation. Water quality variables during acclimation were maintained at adequate levels for survival and growth (Boyd and Pillai 1984) (Table 3.1).

This research evaluated temperatures of 25, 30, and 35°C. The thermal regime of waterbodies within the native range of Speckled Peacock Bass ranges 27-31°C (Winemiller et al.

1997; Williams et al. 1998) and this species is known to be sensitive to temperatures <20°C (Guest et al. 1979; Howells and Garrett 1992). Therefore, endpoints of the native thermal regime (25 and 30°C) were selected. The upper temperature (35°C) was selected because Speckled Peacock Bass utilize shallow lagoon habitats that tend to be warmer than river channels (Jepsen et al. 1997), and climate models suggest that periods of higher temperatures in South America will become more common (Torres et al. 2021).

3.3.2 Standard Metabolic Rate (SMR) and Q_{10}

Temperature within each tank was adjusted to assigned treatment temperatures of 25, 30, and 35°C at a rate of 1°C/day. Tanks reached target temperatures within five days, and afterwards fish were acclimated for two weeks before undergoing respirometry experimentation. Prior to placement into a respirometer, food was withheld for 48 hours to minimize post-prandial metabolic effects. Fish were anesthetized in a buffered anesthetic bath (150 mg/L tricaine methanesulfonate [MS-222], 9 g/L NaCl, and 400 mg/L $NaHCO_3$), measured for total length (nearest mm) and wet weight (nearest 0.01 g), then placed into a 2.24-L intermittent respirometer chamber (Loligo Systems, Denmark). Each respirometry system consisted of a fiber optic oxygen sensor, a temperature sensor, a recirculating pump, and a flush pump that were controlled by computer software (AutoResp2, Loligo Systems). Four systems were used simultaneously with each connected to a separate channel of an oxygen meter (Witrox 4, Loligo Systems). Fiber optic oxygen sensors were calibrated daily prior to experiments using water treated with sodium sulfite to obtain 0% air saturation or an air stone to achieve 100% oxygen saturation at experimental temperatures.

Respirometry cycles consisted of a flush period (9 min), wait period (1 min), and a measurement period (8 min), which were repeatedly performed over 18 to 24 hours. For each fish, a mean of the three lowest MO_2 values was used to determine SMR (McKenzie 2001; Cutts et al. 2002; Roche et al. 2013). To ensure steadiness in MO_2 measurements being used to calculate SMR, an acclimation time of 6 hours was determined by plotting MO_2 as a function of time and determining the initial decline in MO_2 (Chabot et al. 2016) after all trials were concluded. To prevent microbial oxygen consumption, the respirometer chambers and holding tank were treated weekly with sodium hypochlorite (50 mg/L) for 30 minutes, then rinsed with freshwater and drained three times. Blank runs (no fish in the respirometers) were performed at each temperature to verify microbial respiration did not affect pO_2 during experiments. The same number of fish (n=12) were measured at each temperature treatment. All SMR experiments were performed under normoxic conditions (>120 mmHg).

The rate of increase in MO_2 of Speckled Peacock Bass related to a 10°C increase in temperature (Q_{10}) was calculated using the mean SMR for each temperature. Each Q_{10} served as an index of thermal sensitivity, which expresses the effect of temperature change on an organism's overall metabolism (Di Santo and Bennett, 2011). Each Q_{10} was determined following Schmidt-Nielsen (1997):

$$Q_{10} = \left(\frac{K_2}{K_1}\right)^{\frac{10}{T_2 - T_1}}, \quad (3.1)$$

where K_2 is the mean SMR at the higher temperature (T_2) and K_1 is the mean SMR at the lower temperature (T_1).

3.3.3 Lower Dissolved Oxygen Tolerance (LDOT)

For each fish, after SMR trials concluded, lower dissolved oxygen tolerance (LDOT) trials were conducted at the same temperatures (25, 30, and 35°C). Immediately following the

SMR trial, full oxygen saturation within the respirometry chambers was achieved, then oxygen saturation was dropped in stepwise fashion following the methods of Aboagye and Allen (2014). Nitrogen was bubbled into the respirometer holding tank until desired oxygen tension was achieved within chambers (105, 90, 75, 60, 45, and 30 mmHg). At each oxygen tension, two MO_2 measurements were obtained using the same cycle times used for the SMR measurements, for a total of approximately 36 minutes at each oxygen tension.

A pilot study evaluating juvenile Speckled Peacock Bass LDOT found that fish displayed a behavior of sustained rapid gill ventilation frequency and erratic swimming behavior immediately prior to loss of equilibrium; however, fish did not recover after loss of equilibrium. Therefore, LDOT was determined using these behavioral markers, resulting in no mortality. Because of mortality associated with hypoxia, once LDOT was determined in one of the four chambers, oxygen was reintroduced into the respirometer holding tank via forced air and air stones until full oxygen saturation was achieved in each respirometry chamber (~30-60 min), at which point fish were removed from the chambers. The oxygen tension at which the first fish reached LDOT was assigned to all four fish. The same number of fish (n=8) were measured at each temperature treatment.

3.3.4 Statistical Analyses

Statistical analyses were performed using RStudio 4.0.3 (R Core Team 2020) at a significance level of $a = 0.05$. Normality and homogeneity of variance were tested on residuals with Shapiro-Wilk and Levene's tests, respectively. The SMR data were $\log_{10}$ transformed to meet these parametric assumptions. A one-way analysis of variance (ANOVA) was used to compare SMR at each temperature treatment (25, 30, and 35°C) and MO_2 at each oxygen tension for each temperature. Following a significant ANOVA, group means were compared using

Tukey's honestly significant difference (HSD) test. Data are reported as mean ± standard error (SE).

3.4 Results

Standard metabolic rate differed among temperature treatments ($F_{1,34}$=84.68, P<0.001). The SMR of Speckled Peacock Bass acclimated to 25°C was lower than 30°C, which was lower than 35°C (Fig. 3.1). The Q_{10} for 25-30°C, 30-35°C, and 25-35°C were all between 2-3, with a higher Q_{10} from 30-35°C (Fig. 3.1).

The LDOT of Speckled Peacock Bass was significantly different among all temperature treatments ($F_{1,4}$=48, P<0.01), with the greatest LDOT in fish acclimated to 25°C, and the least LDOT in fish acclimated to 35°C (Fig. 3.2). Within temperature treatments, MO_2 did not change with decreasing pO_2 with the exception of fish acclimated to 25°C ($F_{1,54}$=14.64, P<0.001), where a significant drop in MO_2 was observed between 120 and 30 mmHg (Fig. 3.3).

3.5 Discussion

In Speckled Peacock Bass, aerobic metabolic demands and sensitivity to hypoxia increased with temperature. These fish likely allocate more energy to metabolism and require more dissolved oxygen at higher temperatures. Conversely, Speckled Peacock Bass demonstrated decreased metabolic demands and elevated resistance to hypoxia at lower temperatures, as oxygen solubility was greater and saturation levels increased. Williams et al. (1998) reported that sub-adult and adult Speckled Peacock Bass in a South American reservoir preferred water temperatures ranging 27-30°C, and data from the current study suggests that physiological performance was optimized between 25-30°C. Although we did not examine lower or higher temperature tolerances, studies on juvenile Butterfly Peacock Bass *Cichla ocellaris*

(85-140 mm TL) indicated a lower and upper lethal temperature of 16-17°C and 38-39°C, respectively (Guest et al. 1979). Further, lower temperature tolerance experiments on two juvenile Speckled Peacock Bass (76 mm TL, 4 g wet weight and 134 mm TL, 24 g wet weight) resulted in mortality occurring at 16°C (Rutledge and Lyons 1976). Collectively, these results suggest that *Cichla* spp. are sensitive to temperatures <20 and ≥35°C, and are adapted to stable, warm water temperatures consistent with their tropical origin.

There is limited information on the effects of temperature on metabolic rate and LDOT of *Cichla* spp., but these measurements are correlated with changes in temperature for other species (Chippari-Gomes et al. 2005; Aboagye and Allen 2014; Kochhann et al. 2015). The one published study involving *Cichla* compared the resting metabolic rate of wild caught *Cichla monoculus* at 27.5 ± 0.8°C to other fish species in the same locations, and determined that RMR was higher than other species (Duncan 2020). This suggests Speckled Peacock Bass have a relatively high metabolic rate, as congeners share similar life history strategies. Likewise, there is substantial variability within the family Cichlidae in terms of hypoxia and temperature tolerance (Muusze et al. 1998; Luna-Figueroa et al. 2003; Chippari-Gomes et al. 2005; Zaragoza et al. 2008; Schofield et al. 2010; De Boeck et al. 2013; Kochhann et al. 2015; Pereira et al. 2018; Burggren et al. 2019), and evidence suggests that members of the *Cichla* genus are more sensitive than other genera. Sensitivity to upper temperatures and hypoxia appear to be due to elevated SMR and poor capacity for anaerobic metabolism. These traits may be reflective of tradeoffs incurred by the keystone predator role of species in the genus, which typically occupy the highest trophic level and grow to large body sizes in their respective ecosystems.

Values for Q_{10} of Speckled Peacock Bass increased with temperature, particularly at upper temperatures. Q_{10} was near 2 for 25-30°C and approached 3 for 30-35°C, indicating a doubling or tripling of metabolic rates, respectively. In a similar study with Mayan Cichlid, a fellow member of Cichlidae, the Q_{10} for acclimation temperatures of 23, 28, and 33°C (2.56 ± 0.21, 1.89 ± 0.15, and 2.2 ± 0.1, respectively) were lower than Speckled Peacock Bass acclimated to 25, 30, and 35°C (Burggren et al. 2019). Mayan Cichlid are efficient anaerobes that can tolerate hypoxic environments and temperatures as low as 11°C (Schofield et al. 2010; Burggren et al 2019), compared to 16°C for Speckled Peacock Bass (Rutledge and Lyons 1976). The SMR and LDOT at the temperatures examined suggest that Speckled Peacock Bass are not as hypoxia tolerant as Mayan Cichlid. Elevated metabolic demands and decreased LDOT at 35°C suggest that this temperature is not optimal for growth and survival of Speckled Peacock Bass.

Speckled Peacock Bass demonstrated a decrease in hypoxia tolerance with an increase in temperature. This relationship has been demonstrated in most fishes, and can be observed by an increase in the oxygen tension at which aerobic metabolism can no longer be supported (P_{crit}) (Rogers et al. 2016). In the current study, at each temperature, a steady decline in MO_2 was observed as pO_2 decreased, although differences among oxygen tensions were only present at 25°C between 30 and 120 mmHg. A significant decrease in MO_2 was also observed in Oscar acclimated to 28°C starting at 30 mmHg (Muusze et al. 1998). However, Oscar tolerated lower oxygen tensions than Speckled Peacock Bass in these experiments, including anoxia tolerance for 16 hours. Similarly, a significantly different MO_2 was observed in Mayan Cichlid acclimated to 23, 28, and 33°C (occurring ~15-40 mmHg; Burggren et al. 2019). Like Oscar, Mayan Cichlid also tolerated much lower oxygen tensions than Speckled Peacock Bass in the current study. Hypoxia tolerance is often linked to modifications to gill surface area, respiration, tissue O_2 demands, and hemoglobin-O_2 binding affinity (P_{50}) (Mandic et al. 2009). These modifications coupled with a wide temperature tolerance, collectively allow hypoxia tolerant fishes, such as

Mayan Cichlid and Oscar, to inhabit a wide range of habitats and often be linked with being invasive species (Burggren et al. 2019). Notably, in a pilot study determining Speckled Peacock Bass LDOT by loss of equilibrium, fish were unable to recover and died shortly after experiments. These findings further support that Speckled Peacock Bass are more sensitive to hypoxia than other members of Cichlidae.

This study represents the first documentation of the influence of acclimation temperature on juvenile Speckled Peacock Bass metabolic rate and lower dissolved oxygen tolerance. Results from these trials indicated that the optimal temperature range for metabolic demands of Speckled Peacock Bass is 25-30°C. Although temperature was not evaluated below 25°C, lower temperatures are outside the species normal habitat range. Thus, we recommend future husbandry of this species target consistent water temperatures between 25 and 30°C and maintain dissolved oxygen near saturation.

3.6 Acknowledgements

We thank Puerto Rico Department of Natural and Environmental Resources (Project SFR F-69.3 [F17AF00057]) for funding. We also thank Mississippi Agricultural and Forestry Experiment Station and U.S. Department of Agriculture (USDA) Agricultural Research Service (Grant# 58-6066-5-042) for facility funding, and USDA National Institute of Food and Agriculture (Grant# 1005154) for providing funding for Peter J. Allen. We also thank Jacob A. Moreland, Abby J. Vaughn, Joshua J. Neary, Brandon J. Gerhart, Amanda Daulong, Alicia Santiago, and the South Farm Aquaculture Facility staff for their assistance and support with fish care and experiments; Aqua Imports for sourcing the fish used in this study. This research was conducted in compliance with provisions established in Mississippi State University Institutional Animal Care and Use Committee.

3.7 Tables

Table 3.1 Mean (± SE) water quality variables of Speckled Peacock Bass *Cichla temensis* rearing tanks during acclimation period.

Treatment	Dissolved Oxygen (mg/L)	Dissolved Oxygen (% saturation)	Conductivity (mS/cm)	Salinity (ppt)	pH	TAN (mg/L)	NH_3 (mg/L)	NO_2^- (mg/L)	Alkalinity (mg/L)	Hardness (mg/L)
25°C	7.29 ± 0.07[a]	89.96 ± 0.55[a]	1.60 ± 0.04[a]	0.80 ± 0.02[a]	7.01 ± 0.26	0.46 ± 0.09	0.003 ± 0.001	0.58 ± 0.15	42 ± 7	152 ± 9
30°C	6.27 ± 0.05[b]	83.31 ± 0.70[b]	1.93 ± 0.06[b]	0.97 ± 0.03[b]	6.77 ± 0.26	0.49 ± 0.07	0.003 ± 0.000	0.25 ± 0.10	38 ± 7	173 ± 14
35°C	5.76 ± 0.07[c]	81.82 ± 0.63[b]	1.89 ± 0.06[b]	0.96 ± 0.03[b]	6.90 ± 0.29	0.39 ± 0.08	0.004 ± 0.001	0.33 ± 0.14	38 ± 7	164 ± 13

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$). TAN denotes total ammonia nitrogen. Alkalinity and Hardness are read as mg/L of $CaCO_3$.

Table 3.2 Mean (± SE) total length and wet weight of juvenile Speckled Peacock Bass *Cichla temensis* acclimated to 25, 30, and 35°C.

Temperature (°C)	Total Length (mm)	Weight (g)
25	158 ± 2^a	43.56 ± 2.22^a
30	156 ± 3^a	41.52 ± 2.16^a
35	146 ± 2^b	32.18 ± 1.27^b

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$, n=12/temperature).

3.8 Figures

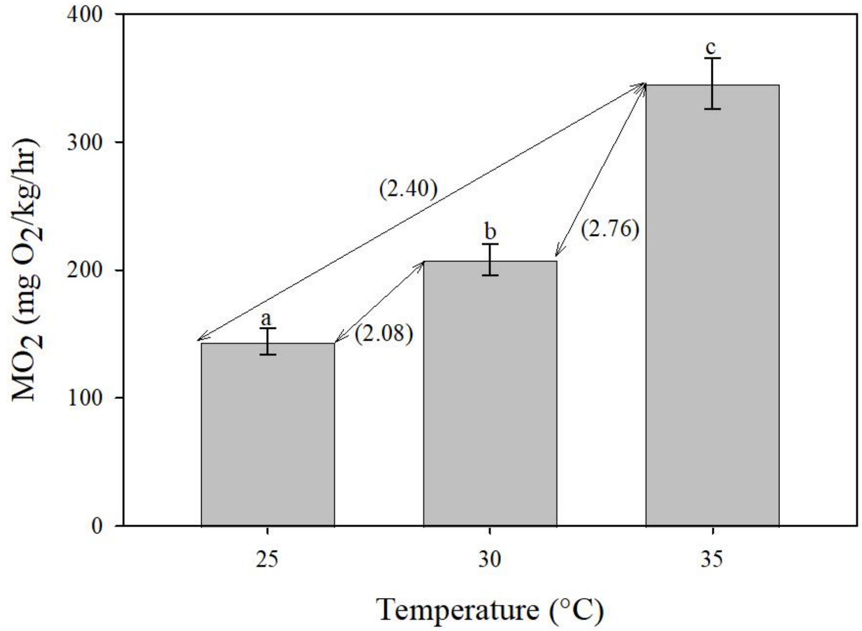

Figure 3.1 Comparison of standard metabolic rate and associated Q_{10} (parentheses) of Speckled Peacock Bass *Cichla temensis* acclimated to 25, 30, and 35°C.

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$, n=12/temperature).

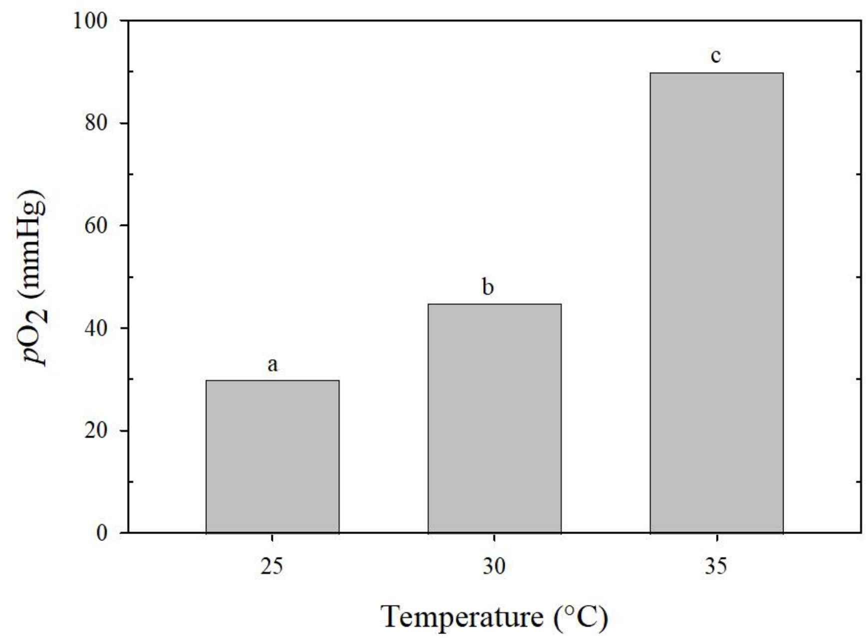

Figure 3.3 Comparison of lower dissolved oxygen tolerance of Speckled Peacock Bass *Cichla temensis* acclimated to 25, 30, and 35°C.

Different letters indicate statistical differences among temperature treatments (ANOVA; $P<0.05$, n=2 groups of 4 fish/temperature).

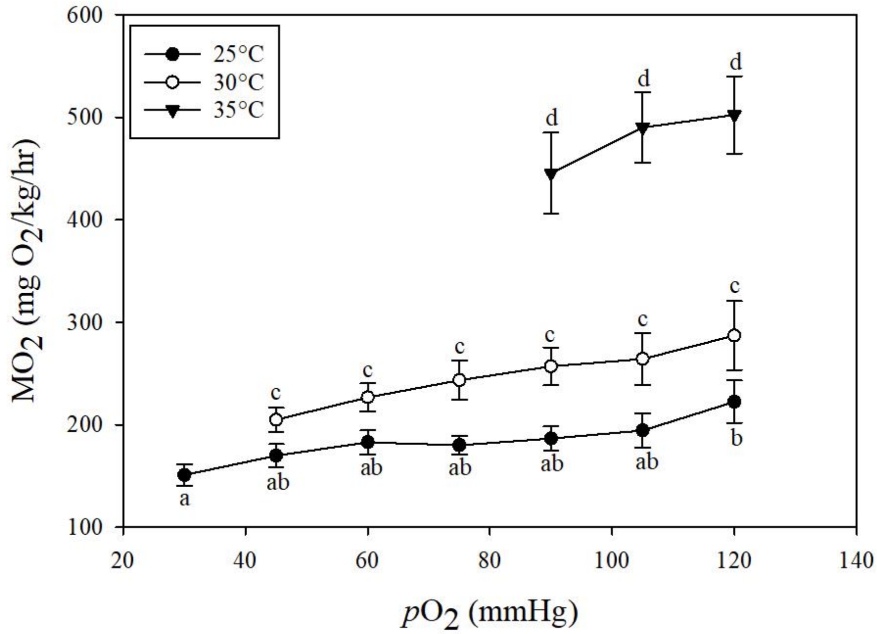

Figure 3.5 Mean (± SE) metabolic rate of Speckled Peacock Bass *Cichla temensis* exposed to graded hypoxia at 25, 30, and 35°C.

Lower Dissolved Oxygen Tolerance of Speckled Peacock Bass at 25, 30, and 35°C was 30, 45, and 90 mmHg, respectively. Different letters indicate statistical differences within temperature treatments and oxygen tensions (ANOVA; $P < 0.05$, n=8/temperature).

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