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Research Article

Cold tolerance estimates for the Rio Cauca Caecilian (*Typhlonectes natans*), a novel amphibian invader in the USA

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Abstract

Thermal tolerance can reveal the risk of establishment and spread for non-native tropical species introduced to more subtropical regions. These data are particularly important for novel introductions such as the Rio Cauca Caecilian (*Typhlonectes natans*), a species of amphibian established in Miami, Florida, United States of America (USA). To estimate its thermal tolerance *T. natans* individuals were captured with baited traps, transported to the laboratory, and acclimated to 25°C. We used chronic lethal methodology to estimate three cold tolerance endpoints: cessation of feeding, loss of equilibrium, and death. This methodology utilizes a 1°C per day temperature change which allows for stepwise reacclimation. Endpoints were 18.61°C ± 0.91, 17.08–20.56 (mean ± SD, range) for cessation of feeding, 13.61°C ± 0.81, 12.68–14.98 for loss of equilibrium and 12.45°C ± 0.49, 11.72–13.84 for death. The chronic lethal minimum temperature is relatively high for an established aquatic species in Florida, suggesting water temperature may limit its northward spread. Thermal tolerance attributes are one aspect of the risk of spread, and some information gaps remain, including salinity and desiccation tolerance, attributes that could allow movement between coastal watersheds and persistence in seasonal wetlands.

Key words: Cessation of feeding, chronic lethal minimum temperature, CLMin, Florida, loss of equilibrium

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Introduction

It may become increasingly uncommon in the age of mature global trade in live animals for a truly novel, non-native, vertebrate species to appear. However, the Rio Cauca Caecilian (*Typhlonectes natans*), a species belonging to the order Gymnophiona, an amphibian group not before found outside the native range, was recently detected by Sheehy and colleagues (2021). Following this initial detection, this species has been detected at multiple locations and is now considered established in Florida, USA (Sheehy et al. 2025). This novelty prompted interest in the potential for this species to spread in Peninsular Florida (Sheehy et al. 2021).

Gymnophionans are found in tropical regions in Africa, Asia, South America, and southern North America (Frost 2025). *Typhlonectes natans* is native to tropical regions of Colombia and northwestern Venezuela (Tapley and Acosta-Galvis 2010), suggesting that in Florida its spread would be limited to the southern warm climate regions. This has been the case for a variety of non-native fish in Florida, some of which are restricted to southern Florida and have been unable to spread north (Shafland 1995). Our objective was to estimate cold temperature thresholds, including cessation of feeding, loss of equilibrium, and death, for *T. natans* collected in the wild in Florida. These data can be used to inform whether this species will persist long term in subtropical regions of Florida and on the risk of invasion to global tropical and subtropical regions elsewhere.

Methods

We estimated the cold temperature thresholds of *T. natans* by estimating the chronic lethal minimum temperature (CLMin), which utilizes a relatively slow rate of temperature change, and has been used for amphibians (Beitinger et al. 2000; Tuckett et al. 2024). Individuals were captured using minnow traps baited with canned sausages set on 5 September 2024 during daylight hours. Six caecilians were trapped at Robert King High Park in Miami, Florida (25°46'18"N, 80°18'32"W) and 17 were captured in a residential canal connected to the C-4 canal in University Park, Florida (25°45'04"N, 80°21'36"W). These capture locations are in close proximity to where *T. natans* has been captured and identified (Sheehy et al. 2021; Sheehy et al. 2025). This sample size was deemed sufficient to estimate cold temperature thresholds by Shafland and Pestrak (1982). The animals were transported in a cooler with aeration to the University of Florida, Institute of Food and Agricultural Sciences, Tropical Aquaculture Laboratory in Ruskin, Florida where they were acclimated at 25°C in treated well water for three days. During acclimation, caecilians were prophylactically treated once with benzalkonium chloride (2 mg/L for one hour) following recommendations by Whitaker and McDermott (2018) for preventing amphibian saprolegniasis, a fungal infection to which they are susceptible (Mylniczenko 2006).

We used a recirculating system to estimate CLMin, which consisted of 10-L containers (n = 18) with snapping lids and aeration. Two tanks were randomly assigned as controls. Caecilians are known to escape from captivity through small gaps in enclosures (Tapley et al. 2022), and thus weights were placed on top of the lids and any holes in the lids were sealed. Despite that, and just prior to the start of the trial, an individual from a control tank was swapped out because it escaped and was later found on the floor alive. The recirculating system included a 253-L reservoir filled with 166 L of degassed well water; the temperature of the water in the reservoir was controlled using a water chiller (Aqualogic MT-7, San Diego, CA). Trials were conducted at a salinity of 1.5 ppt, within the range of salinity reported from the Miami River (Troxell et al. 2024), which connects to the C-4. All tanks included a 300-W heater to maintain the temperature at 25°C. For the 16 experimental containers, temperature was then decreased approximately 1°C per day (Shafland and Pestrak 1982; Tuckett et al. 2016; Tuckett et al. 2024). This methodology has been used for both fish and amphibians and allows for stepwise reacclimation during the trial (Beitinger et al. 2000; Tuckett et al. 2023; Tuckett et al. 2024).

Caecilians were fed daily (midday) with cut shrimp (approximate size < 1 cm^2) following guidance by Tapley et al. (2022). The uneaten portion was removed after 1 hour. Water quality was assessed (total ammonia nitrogen; nitrite, pH, hardness, and alkalinity) using a Hach diagnostic kit (Model FF-1A, Loveland, CO) and salinity was assessed every three days using salinity meter (Yellow Springs Instruments;

Ecosense Model EC300A; Yellow Springs, OH). Water quality parameters were stable over the course of the experiment. Temperature, health status, and onset of three thermal endpoints were visually checked at least twice daily (in the morning before feeding and in the afternoon) over the course of the experiment.

The first thermal endpoint, cessation of feeding was noted, although shrimp were offered throughout the experiment. Loss of equilibrium (LOE) for aquatic caecilians will differ from fishes, which exhibit an abrupt change (Shafland and Pestrak 1982). LOE was noted when the individual was not oriented in an upright position and did not return to that position after prodding. Death was noted when the individual was unresponsive after prodding and no movement was detected; death was later verified after removal from the water. Temperature was recorded for each endpoint using a digital thermometer (Fisherbrand, Traceable, model 15-078-181; Thermo Fisher Scientific; Waltham, MA). Caecilians were measured for total length (mm) upon death. We compared the temperature at which each of the three endpoints occurred using an analysis of variance with individual included as a random effect. Tukeys HSD was used as a post hoc test to compare end points. Size is sometimes related to thermal tolerance (Tuckett et al. 2016). Thus, the relationship between the three end points (cessation of feeding, loss of equilibrium, and death) and size (TL) was analyzed using linear regression. Significance was determined at $p < 0.05$. All statistical analyses were conducted in JMP Pro (v17.0.0; SAS Institute, Cary NC).

Results

Caecilian TL varied from 298 to 540 mm (treatment mean = 447 ± 70 SD; control mean = 404 ± 6 SD). Endpoints were not recorded for all individuals. Cessation of feeding was determined on 11, loss of equilibrium on 8, and death on 15 of the 16 individuals. One individual was removed early in the trial because it was observed to have a fungal infection, presumed to be a species of *Saprolegnia*. No other individuals presented the same condition during the trial, although one other individual had spots on the head. Cessation of feeding was difficult to identify due to irregular feeding and loss of equilibrium often went unrecorded due to inactivity. End points varied ($F_{2,30} = 217.2$; $P < 0.001$; Figure 1), were highest for cessation of feeding (mean = 18.61°C ± 0.91 SD, range = 17.08–20.56), followed by LOE (13.61°C ± 0.81,

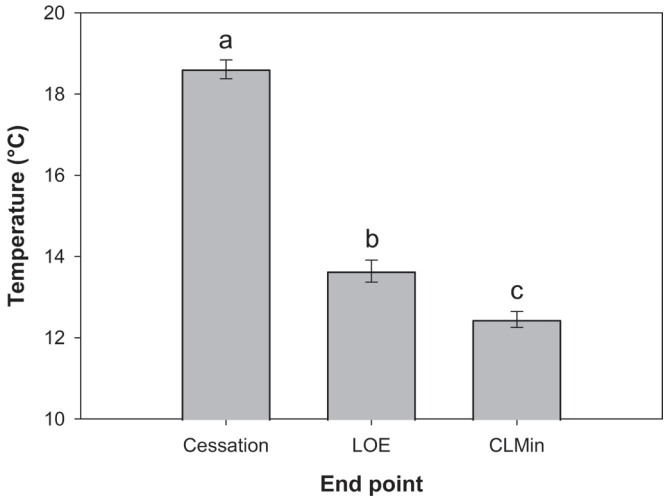

Figure 1. End points for the Rio Cauca Caecilian (*Typhlonectes natans*) collected from South Florida. End points include cessation of feeding (Cessation), loss of equilibrium (LOE), and death (CLMin). Error bars represent one standard deviation. Letters indicate differences among end points (post hoc Tukey HSD; $P < 0.05$).

12.68–14.98) and CLMin (12.45°C ± 0.49, 11.72–13.84). The two control *T. natans* continued to feed and survived to the conclusion of the trial. Body size (TL) was unrelated to cessation of feeding (r^2 = 0.10; $F_{1,9}$ = 1.0; p = 0.354) and LOE (r^2 = 0.09; $F_{1,6}$ = 0.6; p = 0.462); however, death occurred at higher temperatures in larger individuals (r^2 = 0.27; $F_{1,13}$ = 4.9; p = 0.046), which was driven by a single large individual.

Discussion

Our results suggest that northward expansion in Florida should be limited by seasonal cold temperatures. In Florida, expansion throughout the peninsula requires CLMin less than 10.0°C for non-native fishes (Shafland and Pestrak 1982). However, *T. natans* could spread throughout much of South Florida as the mean minimum temperatures in this region are above the CLMin range for this species, particularly in the deep-water canals which tend to remain warmer during cold events (Hallac et al. 2010). Temperatures in coastal canals and rivers of South Florida will usually remain over the CLMin estimate of 12.45°C (Troxell et al. 2024), but this estimate may be approached during extreme cold events, such as the one that occurred during of 2010 (Boucek and Rehage 2014).

As expected, cessation of feeding and LOE occurred at higher temperatures than CLMin. Cessation of feeding was relatively high (mean = 18.61°C) and temperatures in the Miami River (near the C-4 canal) will occasionally fall below this threshold (Troxell et al. 2024). While cessation of feeding is sublethal, temperatures below this threshold may cause stress, potentially saprolegniasis, and affect long-term survival if water temperatures remain below this threshold (Tapley et al. 2022). The estimate for LOE was ~1°C higher than CLMin, a finding which contrasts with an airbreathing fish species (Lawson et al. 2015), where LOE and CLMin occurred at the same temperature. This is likely because this species utilizes both cutaneous and pulmonary respiration (Smits and Flanagin 1994). Loss of equilibrium was also difficult to estimate. For fishes, LOE is often abrupt, and is noted by twirling, uncoordinated swimming, somersaulting, and surface bobbing (Shafland and Pestrak 1982). To our knowledge, LOE has not been examined for aquatic caecilians, and should be interpreted with caution.

There is no straightforward process for predicting the spread of aquatic species based on their CLMin and multiple approaches have been utilized. One limitation is the heterogeneous thermal landscape (Tuckett et al. 2021; Scott and Carlson 2024). Shafland and Pestrak (1982) and Lawson et al. (2015) use mean low January stream water temperature. Based on the thermal map developed by Lawson et al. (2015), caecilian spread would be limited to the Florida Peninsula south of 28 to 29°N. However, a mean low temperature can mask the impact of extreme events with infrequent return intervals. These events can include temperature declines of much less than 1°C per day (Hall-Scharf et al. 2025). Scott and Carlson (2024) use CLMin and river water temperature modeled from air temperature to identify suitable thermal habitat. Spread of non-native fishes was recently modeled for peninsular Florida using CLMin to establish a maximum potential range (Lawson et al. 2025). Other studies contrast the distribution of non-native species with known CLMin estimates (Tuckett et al. 2021; Tuckett et al. 2024). What is clear, however, is that *Typhlonectes natans* exhibits a higher CLMin than most of the established aquatic species in Florida (Lawson et al. 2015), and is similar to *Astronotus ocellatus*, which has a core range restricted to waterways south of 27°N (Nico et al. 2025).

In addition to the thermal tolerance data presented here, the following data could be useful to predict spread: 1) salinity tolerance to understand movement between coastal watersheds by using moderate salinity connections and 2) desiccation

tolerance to understand persistence within seasonal wetlands such as Everglades National Park, Florida (Pintar et al. 2023). If it exists, salinity tolerance in the Rio Cauca Caecilian could facilitate movement between coastal drainages as reported for other non-native aquatic species (Brown et al. 2007). We did find an escaped individual alive on the laboratory floor, but the elapsed time since escape is unknown and few conclusions could be drawn about their ability to resist desiccation. While our results suggest the northern spread of this species in Florida will be limited by seasonal cold temperatures, range predictions are challenging and will be simultaneously limited by species traits such as thermal, desiccation, and salinity tolerances, access to permanent and ephemerally connected pathways, and the heterogenous thermal landscape.

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Author Contribution

QMT, JRB, and JEH research conceptualization; QMT, KME, TMD, JRB, and JEH sample design and methodology; QMT, KME, and TMD investigation and data collection; QMT, KME, TMD, JRB, and JEH data analysis and interpretation; QMT and JEH ethics approval; QMT, JRB, and JEH funding provision; QMT writing – original draft; QMT, KME, TMD, JRB, and JEH writing - review & editing.

Ethics and permits

Research was completed under an approved University of Florida-Institutional Animal Use and Care Committee protocol (project# 202400000297). We thank Eric Johnson (FWC) for expediting our application of a scientific collector's permit (permit# SCSW-2024-013).

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