Critical thermal limits for *Astatotilapia piceata*, a possibly extinct in the wild Lake Victoria cichlid fish

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Abstract. Establishing thermal limits for species of conservation concern is important for husbandry outside the native range. The pitch-black fulu, *Astatotilapia piceata* is a pseudocrenilabrine Lake Victoria cichlid which is possibly extinct in the wild. To establish whether *A. piceata* can be cultured extensively in outdoor settings in the U.S. state of Florida and in other suitable habitats, the study experimentally determined the critical thermal limits (minimum and maximum) for *A. piceata*. The critical thermal minimum was 11.6°C, while the maximum was 37.1°C, resulting in a thermal tolerance range of 25.5°C. These results indicate culture would be possible in subtropical regions worldwide. In Florida, specifically, year-round outdoor culture would be restricted to the southern peninsula.

Key Words: critical thermal maximum, critical thermal minimum, pitch-black fulu, thermal tolerance.

Introduction. In this technical note we identify the critical thermal limits for the pitch-black fulu, *Astatotilapia piceata* (*Haplochromis piceatus*; *Yssicchromis piceatus*), a species first described in 1969 (Greenwood & Gee 1969), which is possibly extinct in the wild (Hemdal & McMullin 2013) (Figure 1). *A. piceata* is just one member of the Lake Victoria pseudocrenilabrine (Tribe Haplochromini) cichlid species flock, an evolutionary radiation that may have numbered ca. 500 endemic species prior to a mass extinction event that peaked during the mid-1980's (Ogutu-Ohwayo 1990; Kaufman 1992; Witte et al 2007). Ecologically, this species was an epibenthic to midwater zooplanktivore, feeding largely on the aquatic larvae of dipteran flies. Its associates in the water column prior to limnological and community reorganization included about one dozen species of the more morphologically specialized zooplanktivores in the genus *Yssichromis* plus a variety of other zooplanktivorous and insectivorous taxa. *A. piceata* underwent a diel vertical migration in Mwanza Gulf, where it has been most thoroughly studied in the wild (Goldschmidt et al 1990). A captive stock of *A. piceata* was established in Leiden, Netherlands by the Haplochromine Ecology Study Team (HEST) during the 1980's and 1990's. Descendants of this population were incorporated along with several other haplochromines into a conservation captive breeding program for the Lake Victoria haplochromines, the Lake Victoria Fishes Species Survival Program (LVSSP). Surplus stock from the LVSSP was the source of the animals used in this study.

Figure 1. Male (background) and female (foreground) *Astatotilapia piceata*.

Critical thermal methods utilize rapid temperature changes (increases or decreases) and identify sublethal endpoints (e.g., loss of righting response or onset of muscle spasms) corresponding to critical thermal limits (Beitinger et al 2000). Methodologies utilizing sublethal endpoints are necessary for *A. piceata* and other species of conservation concern. These data are useful in population management as well as in the development of repatriation activities.

Many Lake Victoria haplochromine species are likely extinct globally or at least in the wild, with additional species highly threatened (Kaufman 1992; Witte et al 1992; Okechi et al 2022). Species extinctions and declines are attributable to numerous threats, including eutrophication that leads to hypoxia, habitat loss, and changes to water clarity that can disrupt sexual selection and lead to hybrid swarms and *de novo* clades (Alexander et al 2017; Seehausen et al 1997), fisheries exploitation causing population reductions and fisheries induced evolution (Heino et al 2015), and the introduction of non-native species such as Nile perch, *Lates niloticus*, which compete with and prey upon Lake Victoria cichlids (Witte et al 1992).

In response to species and population declines, the Association of Zoos and Aquariums (AZA) established the Lake Victoria haplochromine Species Survival Plan (VSSP), which was the first formal SSP devoted to fishes (Chandler et al 2001). The VSSP coordinated propagation of *A. piceata* among others, and zoos and aquaria achieved success in increasing the captive population of this species nearly three-fold (Hemdal & Mcmullin 2013). Still, this species was downgraded from critically endangered to vulnerable in 2010 (IUCN 2012), which may have led to reduced participation in the VSSP, and possibly challenges for maintaining *A. piceata* in captive propagation. Changing conservation status, the possibility of domestication-induced trait change for species undergoing long term culture (Bilio 2007), along with changing institutional priorities which may affect VSSP participation, necessitated a relaxed reliance on ex-situ breeding programs among those tools available to battle species extinction. Experimentally determining critical thermal limits can assist in identifying thermal conditions suitable to *A. piceata* (and other Lake Victoria cichlids) outside Lake Victoria to ease the burden of care on VSSP facilities and eliminate domestication-induced maladaptation. Most of the East African "lowlands" are suitable in temperature regime as this is where the fishes evolved. However, the VSSP has begun experimental ex situ work on reintroduction and community assembly in other areas prior to implementing repatriation efforts within the native range of these fishes and knowledge of thermal thresholds is critical to both the conduct and interpretation of such experiments.

Material and Method. Fish were acquired from VSSP participants in the U.S. and Canada. Following shipment to the University of Florida, Tropical Aquaculture Laboratory (TAL), the fish were first quarantined for two weeks. Fish were then transferred to indoor vats and acclimated to ~25°C for at least two weeks. Fish were fed *ad libitum* daily on a 1.6 mm commercial pelleted diet (Purina Aquamax, crude protein-50%, crude fat-16%, crude fiber-3%, Purina Animal Nutrition, Arden Hills, Minnesota, USA) and the uneaten portion was siphoned. Water quality was monitored throughout the acclimation period. Following acclimation, fish were transferred to individual tanks (54.6 cm length × 31.1 cm width × 34.3 cm height; 58.2 L) whereupon critical thermal limits were determined (Beitinger et al 2000). Critical temperature minima (CTMin) and maxima (CTMax) were experimentally determined on 18 individuals per trial (CTMax or CTMin); fish were not reused in subsequent trials. CTMin was estimated by gravity feeding water from a 1,700 L cold water reservoir chilled to ~5°C into the experimental tanks. Water temperature was decreased 1°C per minute until loss of equilibrium, which we describe as a loss of righting response after prodding. Water temperature at loss of equilibrium was then recorded. One fish died following the CTMin trial and was excluded from subsequent interpretations. For the CTMax trials, temperature was raised 1°C per minute using individual heaters until loss of equilibrium was noted as above. Total length (mm) was then measured and recorded.

Results and Discussion. Total length varied from 87 to 108 mm (mean = 10.0 mm; SE = 1.5) for the CTMin trial, from 84 to 110 mm for the CTMax trial (10.3 mm; 1.5), and was similar between trials ($F_{1,34}$ = 1.3; p = 0.264). CTMin varied from 10.7 to 12.6°C (mean = 11.6°C; SD = 0.55; Figure 2.) and CTMax varied from 36.2 to 37.8°C (37.1℃; 0.38). The thermal tolerance range (TTR = CTmax - CTmin) was 25.5°C.

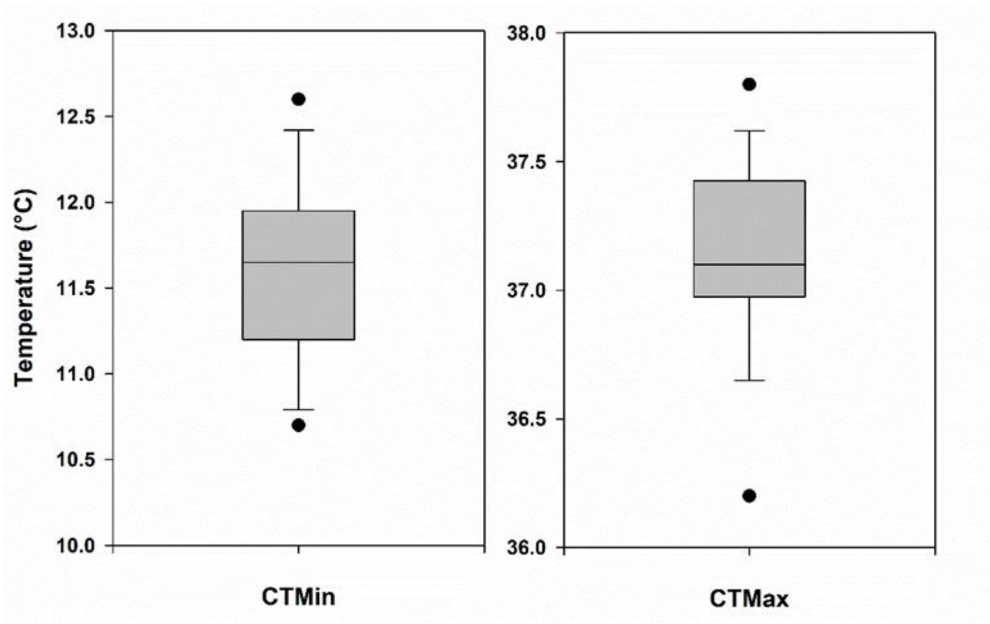

Figure 2. Critical thermal minimum (CTMin) and maximum (CTMax) for *Astatotilapia piceata*.

These results indicate that CTMin, but not CTMax, would likely limit where A. piceata can be cultured or utilized in conservation experiments outdoors. Indeed, outdoor experimental and conservation work with this and similar species would be limited to tropical and subtropical regions of southern Florida, regions which exhibit January mean minimum temperatures >13°C (Lawson et al 2015). Lake Victoria is relatively thermostable (Nyboer & Chapman 2017), which could have led to the reduced CTMin and TTR, limiting the conditions under which East African haplochromines can be studied in the US in outdoor settings.

Novel solutions to culture needs must be evaluated because long term culture of species in captivity can have drawbacks including domestication and changing institutional focus. Domestication can lead to reduced genetic diversity and maladapted phenotypes upon return to the wild (Heino et al 2015). Further, long term captive production can be cost prohibitive and subject to changes in institutional focus and external forces shaping the zoo and aquarium industry. For example, the recent pandemic may lead to reduced investment in conservation for zoos and aquaria that experienced a dramatic reduction or near complete absence of paid attendance. For this reason, the VSSP is in the process of expanding its attention from the maintenance of ex-situ populations alone, to the development of new knowledge and methodologies of utility in East Africa. A primary focus on maintenance in the wild and repatriation where necessary should be the primary goals of all freshwater fish conservation programs. Unfortunately, climate change, a global freshwater crisis, habitat destruction, and other anthropogenic changes are conspiring to make conservation and restoration in the wild ever more challenging. To some extent, then, programs such as the VSSP's experimental repatriation research have a useful role to play.

For species whose continued survival demands captive maintenance and propagation, solutions must be sought to ease the burden of care. These solutions can include moving some species or population segments to less intensive culture systems (i.e., outdoor settings) and getting species such as *A. piceata* into the hands of dedicated cichlid producers and hobbyists (Maceda-Veiga et al 2014). Aquarium hobbyists can conceivably play an important role in this context, but the ability of the hobbyist community to maintain endangered species over the long term, with proper attention to conservation genetic and husbandry requirements, is proving even more limited than for professional institutions. Under the appropriate thermal conditions, however, endangered Lake Victorian cichlids could be maintained with minimal institutional inputs. For example, it has been suggested that urban and suburban stormwater ponds can serve as refugia for regionally endangered fishes (Schaeffer et al 2012), which could be extended to regions outside the native range if those species exhibit low risk of invasion. This is true of the African rift lake cichlids, which have not been successful in establishing themselves in the wilds of Florida despite decades of production and many opportunities for escape (Shafland et al 2008).

Conclusions. The critical thermal limits identified in the study, 11.6 and 37.1°C, suggest that year-round, outdoor survival would be restricted to tropical and subtropical regions worldwide, including the southern peninsula of Florida. For species that are extinct in the wild novel solutions must be entertained which maximize the probability of long-term survival. Ideally, these solutions would minimize intensive captive propagation, which can have unintended consequences on the traits captive fish. Identifying the critical thermal limits *A. piceata* is a necessary step to identify regions suitable for outdoor culture and introductions.

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Conflict of interest. The authors declare no conflicts of interest.

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