**INTRODUCTION:**

H1 – Population-specific thermal limits are set by physiological limitations in larval aerobic performance, occurring at an intra-population scale. We predict that southern populations that have historically been exposed to a greater magnitude of summer water temperature variation will have greater aerobic scope and a higher thermal limit.

H2 – Population-specific thermal limits are set by physiological limitations in larval aerobic performance, occurring at an inter-population scale. We predict that all populations will have the same aerobic scope and thermal limit, regardless of historical exposure to temperature variation across latitudes.

H3 – Plastic changes induced from egg incubation will increase larval thermal limit within a population. We predict larvae hatched from eggs incubated under warmer temperatures will have a higher upper thermal limit.

**METHODS:**

Crossing Design and Fertilization

Cisco were collected from the Apostle Islands, Lake Superior (46.85, -90.55) and Chaumont Bay, Lake Ontario (44.05, -76.20) in December 2019. Eggs and milt were stripped from 12 females and 16 males from each population and artificially fertilized under a blocked, nested full-sib, half-sib fertilization design to create a maximum of 48 families. A single fertilization block consisted of four males each paired to three unrelated females, where all offspring of a given female were full siblings (Stewart et al. 2021).

Rearing Conditions

Embryos were incubated at mean (SD) constant temperatures of 2.0 (0.5), 4.4 (0.2), 6.9 (0.2), and 8.9 (0.3)°C, and randomly placed in climate-controlled chambers (Memmert® IPP260Plus). Reconstructed fresh water was used during fertilizations and incubations (OECD ISO 6341:2012) to standardize the chemical properties of the water between populations. Full embryo incubation methods can be found in Stewart et al. 2021. After hatching, larvae were immediately photographed for morphological traits.

Newly hatched larvae were transferred to rearing tanks separated by population and incubation treatments. Larvae from Lake Superior were reared in four (4 incubation treatments x 1 replicate) 150-liter oval tanks. Larvae from Lake Ontario were reared in eight (4 incubation treatments x 2 replicates) 150-liter oval tanks. Lake Superior larvae were unreplicated – this was a practical constraint of low fertilization success and embryo survival limiting the number of available larvae for multiple rearing tanks. All rearing tanks were supplied with chilled, recirculating water maintained at 6.5°C (mean (SD) = 6.36 (1.17)). Hourly water temperatures were recorded (±0.2°C). Larvae in all rearing tanks were exposed to the same photoperiod cycle (*i.e.,*12-hr light, 12-hr dark) with gradual sunrise and sunset transitions. ﻿Full spectrum, white LED lights (AquaShift® MLA-WH) were used to simulate daylight. Dead larvae were removed and counted each day. Larvae were fed *Artemia* and transitioned to Otohime A dry feed beginning after ﻿one-week post-hatch. Food was provided *ad libitum*.

Thermal Challenge

After 60 days, up to 100 larvae from each rearing tank were exposed to an acute thermal challenge. Because all larvae did not hatch on the same day, 60-days post-hatch was calculated from the date of 50% hatching. Larvae were transferred to 5.4-liter rectangular tanks 12 hours prior to the acute thermal challenge to acclimate at 10.0°C, with 2 replicate tanks per rearing tank and 50 larvae used in each replicate tank. Larvae were acclimated at 10.0°C for 12 hours prior to the acute thermal challenge. During the thermal challenge, water temperatures were raised from 10.0°C at a constant rate of 0.5°C per 30 minutes until all larvae were deceased. Larvae were considered deceased when loss of equilibrium (LOE) was achieved and were motionless for at least 5 seconds. Once termination criteria was met, larvae were euthanized, photographed, and preserved in 95% ethanol. The elapsed time and temperature (±0.2°C) at termination of each individual was recorded and total length was measured.

Statistical Analyses

All statistical analyses were performed in R version 4.0.3 (R Core Team 2020).

*Larval Survival*

Larval survival was estimated for each rearing tank, across all families, as the percent of larvae surviving between hatching and 60 days after the date of 50% hatching. Our estimate of larval survival from Lake Superior is unreplicated. However, useful information can still be gleaned without strict statistical testing (*e.g.*, Davies and Gray 2015). Observations of single estimates of larval growth rates across populations/incubation temperatures could suggest further hypotheses and lead to more focused studies.

*Larval Growth*

Mean daily growth increment was calculated as (mean final length – mean length-at-hatch)/duration of the larval experiment. Similarly, to larval survival, our estimate of larval growth rate for Lake Superior is unreplicated. A bootstrapped growth rate estimate with 95% confidence intervals was calculated from random sampling with replacement to qualitatively compare the likelihood of differences in growth across populations and incubation temperatures. The resampling procedure was repeated 10,000 times.

*Acute Thermal Challenge*

Because the rate of temperature increase between tanks may slightly vary, estimates of LOE will be converted to cumulative exposure in degree-minutes by summing all differences between the acclimation temperature (10.0°C) and the increased temperature at each interval until LOE, considered as the upper thermal tolerance (UTT):

*UTT = ∑j(Xj- Xa);*

where *Xj*is the increased temperature every 15-minutes, *Xa*is the acclimation temperature, and *j* represents each minute up to LOE for each individual fish (*45*).