BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
MARATHON PETROLEUM COMPANY, LP	)	
Petitioner,	)	
v.	)	PCB No. 2018-049
	)	(Thermal Demonstration)
ILLINOIS ENVIRONMENTAL PROTECTION AGENCY,	)	
Respondent	)	

NOTICE OF FILING

TO:

Don Brown, Clerk of the Board
Illinois Pollution Control Board
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(VIA ELECTRONIC MAIL)

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(SEE PERSONS ON ATTACHED SERVICE LIST)

PLEASE TAKE NOTICE that I have today filed with the Office of the Clerk of the Illinois Pollution Control Board the ILLINOIS DEPARTMENT OF NATURAL RESOURCES' ANSWERS TO QUESTIONS OF THE HEARING OFFICER FOR THE ILLINOIS POLLUTION CONTROL BOARD, as ordered, dated March 10, 2020, a copy of which are herewith served upon you.

Respectfully submitted,

ILLINOIS DEPARTMENT OF NATURAL RESOURCES

By: Virginia I. Yang

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Dated: July 7, 2020

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BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

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MARATHON PETROLEUM COMPANY, LLC	)	
Petitioner,	)	
v.	)	PCB 2018-049
ILLINOIS ENVIRONMENTAL	)	(Thermal Demonstration)
PROTECTION AGENCY,	)	
Respondent,	)	

THE ILLINOIS DEPARTMENT OF NATURAL RESOURCES' ANSWERS TO QUESTIONS OF THE HEARING OFFICER FOR THE ILLINOIS POLLUTION CONTROL BOARD

NOW COMES the Illinois Department of Natural Resources (IDNR), an Interested Party to the above referenced proceedings, by and through one of its Attorneys, Virginia I. Yang, and files THE ILLINOIS DEPARTMENT OF NATURAL RESOURCES'S ANSWERS TO QUESTIONS OF THE HEARING OFFICER FOR THE ILLINOIS POLLUTION CONTROL BOARD as ordered, dated March 10, 2020 as follows:

A. Background

On March 5, 2020, the Board issued an order in this proceeding stating, "[b]ased on the current record, the Board finds that additional information is warranted in determining, among other things, whether the requested mixing zone, absent any zone of passage, would assure the protection and propagation of the bigeye chub, and if the requested thermal limits protect the biotic life in Robinson Creek. The Board requests that additional information to include IDNR's explanation of whether and, if so, how its assessment of the UIUC data has changed. Therefore, the Board will direct the hearing officer to issue an order, providing specific questions to be addressed by the participants." See PCB 18-49 Marathon Petroleum Company, LP (March 5, 2020), slip op. at 11.

B. Request for Response by IDNR

1. *Based on the review of the UIUC bioassay of the Bigeye Chub and Marathon's technical data, IDNR states that Marathon is at "high risk" for a "take" in the form of: "harassment" where the fish is forced to evacuate aquatic habitat areas in the thermal effluent of Robinson Creek beginning at 33 degrees C (91.4 degrees F); and "harm" where the fish is unable to properly swim, avoid predators, and is at increased risk of mortality beginning at 96.8 degrees F for fish acclimated to 26 degrees C (78 degrees F). 12/28/20 IDNR Rep. at 4-5.*

   Further, IDNR notes,

   "the Illinois Endangered Species Protection Act (IEPSA), 520 ILCS 10/3 (1), prohibits any person 'to possess, take ... or otherwise dispose

of any animal ... which occurs on the Illinois List", 17 Ill. Adm. Code 1010.30(a). However, the IEPSA authorizes a taking otherwise prohibited by Section 3 ... (of the IESPA) ... if that take is incidental to, and not the purpose of, the carrying out of an otherwise lawful activity" by means of review and approval of a conservation submitted to the IDNR under Section 5.5(b) of the IESPA and its regulations 17 Ill. Adm. Code I 080." Id. at 5. "IDNR therefore recommends that Marathon submit a conservation plan to the IDNR in pursuit of an Incidental Take Authorization (ITA) for review and approval by the IDNR, as provided for under Section 5 .5. of the IESPA and its regulation I 7 Ill. Adm. Code I 080.

Marathon responds,

"IDNR offers no support and fails to include any statutory or regulatory basis in its Response for its assertion that avoidance behavior constitutes harassment under the Illinois ESA. IDNR also cites to no case law or guidance to support its assertion. IDNR's position that avoidance constitutes a take in the form of harassment is unsupported by Illinois law, including IDNR's own regulations." 3/15/19 Marathon Resp. at 14.

a. *Please clarify whether responses from Marathon (3/15/19) and/or IEPA (4/12/19) to IDNR's Reply to IEPA's Recommendation changes IDNR's position regarding requiring Marathon to seek an Incidental Take Authorization (ITA) under the IESPA.*

IDNR Response:

An ITA is not required pursuant to any statute or regulation under the IESPA; it is a recommendation based on the likelihood of potential take. Marathon has communicated to IDNR that Marathon will not seek an ITA for its potential thermal impacts. DNR states that neither Marathon nor IEPA has provided any information in their responses, dated 3/15/19 and 4/12/19 respectively, that would change or alter IDNR's recommendation that Marathon submit a Conservation Plan as its application for an ITA based on the potential for taking an Illinois listed species incidental to performing an otherwise legal action

b. *If so, please explain the reasons why IDNR now believes that Marathon does not require an ITA.*

IDNR Response: See Response below at Question 1(c)

c. *If not, please elaborate on the ITA process and comment on whether Marathon must seek an ITA approval from IDNR before the Board rules on Marathon's ATEL request or should a potential grant of the requested ATEL be conditioned upon Marathon seeking an ITA approval.*

IDNR Response:

An ITA is not required pursuant to any statute or regulation under the IESPA; it is a recommendation based on the likelihood of potential take. Marathon has communicated to IDNR that Marathon will not seek an ITA for its potential thermal impacts. However, IDNR recommended that Marathon seek an ITA to avoid potential violation of the IESPA through the take of a State-listed endangered Bigeye Chub at their Robinson plant outfall without prior authorization.

An ITA would ensure that Marathon assess current habitat conditions and improves such conditions to minimize impact to the species, or if impossible, brings conservation benefit to the species elsewhere, or some combination of these elements. The ITA process allows the State of Illinois, and the public through review, to consider the potential loss of individual aquatic species due to Marathon's actions and to determine whether or not the taking will reduce the likelihood of survival or recovery of the species in the wild in Illinois (per IESPA).

The Conservation Plan is the ITA application as provided under 17 Ill Adm Code 1080. Within 30 days of receipt, IDNR will provide comments on the document identifying any deficits in meeting the IESPA and its administrative rules requirements. Conservation Plans must contain consideration of the biological life history needs of the species and consideration of alternatives or efforts to minimize impact to the species, among other details. Plans must also provide conservation benefit to the species through mitigation actions. Once the application is deemed complete, a Conservation Plan enters a 46-day public notice period followed by a 10-day period for applicant response to comment, if any. Statutorily, IDNR has 120 days from the first day of Public Notice to provide a draft ITA for the applicant's review.

2. *Marathon states that the "upper incipient avoidance temperature" derived by UIUC is not consistent with more established avoidance testing procedures since UIUC's procedure did not provide a gradient of thermal conditions. 3/15/19 Marathon resp. at 4 citing Chery, D.S., et al. Please comment on whether the upper incipient avoidance temperature derived in the UIUC study would have been significantly different if the fish were exposed to a gradient of thermal conditions instead of steady increase in temperature.*

IDNR Response:

UIUC thermal testing methodology was solid research methodology, common in the literature where fish are subject to gradual temperature increases within a confined raceway and then observed behavior within that aquatic setting. The likely citation that Marathon references involve a methodology where fish are subjected to a gradient of temperatures with warm water at one end of a raceway and cool water at the other end, and medium temperatures in between. (Cherry, D.S., Dickson, K.L. and Cairns Jr, J., 1975. Temperatures selected and avoided by fish at various acclimation temperatures. Journal of the Fisheries Board of Canada, 32(4), pp.485-491). Using the Cherry methodology, fish can therefore select its acclimated temperature from within a range of possible temperatures varying by approximately 3° C across a raceway; temperatures in this raceway can be adjusted, if desired. Fish observed during the UIUC testing methodology do not have such a choice of avoidance to find a comfortable aquatic thermal setting within the raceway and were confined during gradual warming of the entire raceway.

The question asks if the upper avoidance temperature would have been different if the UIUC study had used a gradient tank rather than the gradual warming test tank. The answer is that the Marathon/Cherry and the UIUC methodology are different types of tests, which measure different fish behavior, thereby making direct comparisons rather speculative.

If the fish were simply put in a tank with a gradient of water temperatures, they would swim around until they found a temperature that they liked - likely preferring their acclimation temperature. In other words, a gradient tank is good at demonstrating "preference". Temperatures in this graded tank could have been adjusted to give an approximation of avoidance. In contrast, the UIUC test defines the maximum temperature fish can tolerate, and at what point they become uncomfortable, and/or ultimate mortality. [Note: UIUC test design did not include maximum temperatures in order to prevent fish mortality.]

So, the results from a gradient tank (Marathon/Cherry) and the UIUC test results would be different, similar to comparing apples and oranges. If UIUC had designed their study using a gradient of thermal conditions, the UIUC final temperature would likely have approximated the fish's acclimation temperature – fish acclimated to 21°C would have preferred 21°C, and fish acclimated to 26°C would have preferred to inhabit 26°C. The range of temperatures avoided from a raceway test would have been relatively large. However, the UIUC study design is different because the fish acclimated to 21°C became uncomfortable (ATmax) at 29.9°C, and the fish acclimated to 26°C became uncomfortable (ATmax) at 33.3°C. This point of discomfort was shown by fish behavior in the tank (i.e., agitated movements, twisting, circling, breeching surfaces, etc.); as temperatures were gradually increased, the agitated movement increased because the fish could not swim away into a more comfortable aquatic environment. The point, however, is that these are two very different tests which produce two different avoidance behaviors – the gradient tank identifies fish preference to leave warming waters when given a choice, but UIUC thermal test measures the temperature level where fish act erratic when not comfortable with surrounding warm water.

Nevertheless, both types of observed fish behavior represent avoidance of harassment by thermal effluent temperatures, or a "take" as defined by the IESPA and its regulations.

Response	Acclimation temperature (°C)	Mean	SD	Median
AT_{max}*	21[a]	29.9	1.3	29.9
	26[b]	33.3	1.4	33.6
CT_{max}	21[a]	32.8	0.4	32.8
	26[b]	36.4	0.9	36.6

AT_{max} (upper incipient avoidance temperature) for Bigeye Chub was to be 33.3°C (91.9°F) for fish acclimated to 26°C (78.8°F). This is the temperature where the Bigeye Chub show avoidance behavior.

CT_{max} (critical thermal maximum) for Bigeye Chub to be 36.4°C (97.5°F) for fish acclimated to 26°C (78.8°F). This is the temperature fish lost equilibrium.

3. *Marathon contends that the proposed alternative thermal effluent limitations are lower than the upper incipient avoidance temperature (91.4°F) and the critical thermal maximum temperature (96.8°F) derived in the UIUC study. 3/15/19 Marathon resp. at 4-5. Please comment on whether IDNR's concern is more to do with the temperature being higher than the UIUC study's avoidance/critical thermal maximum temperatures within the mixing zone (1.7-mile section of the Robinson Creek) without a zone of passage rather than the limits proposed at the edge of the mixing zone.*

IDNR Response:

	Jan.	Feb.	Mar.	Apr.	May	June	July	Aug.	Sept.	Oct.	Nov.	Dec.
Current °F	60	60	60	90	90	90	90	90	90	90	90	60
Proposed °F	65	65	74	82	88	90	90	90	90	87	85	74
Net Change °F	+5	+5	+14	-8	-2	0	0	0	0	-3	-5	+14

Yes. IDNR is more concerned about the temperatures being higher than the UIUC study's avoidance/critical thermal maximum temperatures within the mixing zone with no zone of passage, rather than the proposed temperature limits at the edges of the mixing zone. Marathon's proposed alternative temperature regime creates effluent discharges into Robinson Creek that exceed the upper incipient avoidance temperature of 91.9°F for Bigeye Chub up to 1% of the time at a point 1.7 miles downstream (i.e., the maximum length of the proposed mixing zone) for four months of each year (90°F + 3°F allowance = 93°F).

Using MBI's temperature calibration studies, summer temperatures averaged 5.44 degrees warmer 0.1 miles downstream of the point of discharge start in 4/1/2016 continuing through 11/30/2016 (i.e. 8 months). This average causes the "mixing zone" temperature to be well above the upper incipient avoidance temperature of 91.9°F for Bigeye Chub for up to 6 months of each year; however, this average would still comply with the proposed discharge temperature at the 1.7 miles downstream point. The proposed MBI alternative temperature regime indicates occasional periods where discharge temperatures are above the critical thermal maximum of 97.5°F within the "mixing zone".

4. *Marathon also notes that the UIUC study has not been peer reviewed. 3/15/19 Marathon resp. at 4. Please comment on whether the results of the UIUC bioassay of Bigeye Chub been submitted for peer review since the filing of the report with the Board in December 2018. Also, given lack of research on thermal tolerance of the bigeye chub, please clarify whether the UIUC study based on a larger sample from a regional watershed provides more reliable information for making decisions regarding the protection of the endangered fish population.*

IDNR Response:

Yes. The UIUC study has been peer reviewed. The 2018 UIUC study was recently published in **Aquatic Biology**, October, 2019, as "Effects of acclimation temperature on critical thermal limits and swimming performance of the state-endangered bigeye chub *Hybopsis amblops* ." By Qihong Dai, Cory D. Suski. Department of Natural Resources and

Environmental Sciences, University of Illinois, Urbana-Champaign, Urbana, IL 61801. **Aquatic Biology 28; 137-147, 2019.** (See Attachment A.)

Yes. Testing samples obtained from a local water basin would be more reliable, if samples are available in sufficient numbers. The experiments conducted by Dai and Suski (2019) required 40 Bigeye Chub specimens, a number which was deemed difficult to obtain from Robinson Creek, endangering the local population of Bigeye Chub in Robinson Creek (i.e., MBI found two in Robinson Creek during their surveys), and prohibited under 17 Ill. Admin. Code 1070.30. The most reliable estimate of thermal tolerance for Bigeye Chub in Robinson Creek would presumably result from a well-controlled experiment using Bigeye Chub obtained from the closest appropriate water basin. Bigeye Chub from the Vermilion River basin were selected because this population is within the same regional water basin as Robinson Creek (i.e., Wabash River basin). Because both populations exist in a similar climatic, geologic, and landscape context, estimating thermal tolerance of the Bigeye Chub from the Vermilion River would represent similar tolerances to the Bigeye Chub in Robinson Creek. Using specimen from outside the Wabash River basin could reduce the reliability of the results by increasing the variability in conditions under which such specimen were to adapt.

5. *Regarding the requested mixing zone, IDNR states "Marathon's request for mixing zone on Robinson Creek fails to provide for a "zone of passage for aquatic life", as required, and further substantiating the likelihood of "take" of the Bigeye Chub" because the entire volume of Robinson Creek from Marathon's outfall to 1.7 miles downstream is utilized for mixing.*

a. *Please clarify whether providing a zone of passage within the requested mixing zone would address IDNR's concerns regarding protection of big eye chub in lieu of Marathon seeking an ITA approval.*

<u>IDNR Response:</u>

Providing a zone of passage within the mixing zone may or may not address IDNR's concerns regarding protection of the Bigeye Chub. Providing a zone of passage for aquatic life could potentially result in compliance with the requirements of 35 Ill. Adm. Code 302.102(b)(8). However, this option remains subject to the state water quality requirements that prohibit mixing in waters containing "endangered species habitat (i.e., 35 Ill. Adm. Code 302.102 (b)(4)).

b. *If so, comment on whether a zone of passage less than 50% of the volume stream flow afford adequate protection to bigeye chub and other aquatic species in Robinson Creek.*

<u>IDNR Response:</u>

A zone of passage less than 50% of the volume stream flow may or may not afford adequate protections to Bigeye Chub and other aquatic species in Robinson Creek. A "zone of passage less than 50% of the volume stream flow" could still allow the use of the majority of Robinson Creek as a mixing zone for Marathon's discharge outfall downstream up to the 1.7 mile compliance point, creating an inhospitable thermal habitat for the Bigeye Chub.

6. *IDNR's March 2018 letter to IEPA recommends that "a bioassay of representative fish species is warranted to identify the character and likely causes of observed DELTs [deformities, eroded fins, lesions, tumors] and to determine whether granting the Alternative Thermal Effluent Limits is likely to increase the incidence and/or severity of DELTs on fish in the receiving waters." 4/12/18 IEPA Mot., Attach. A at 4. Marathon, relying on its consultants' (MBI and EA Engineering) responses (Exhibits 1 and 2), responds that DELTs in Robinson Creek "are the result of non-thermal pollution influences and the thermal regime of Robinson Creek does not play a direct or synergistic role in the observed biological assemblage impairments." 8/15/18 Marathon Resp. at 11, and Resp. Exh. 2 at 3.*

a. *Please comment on whether the additional review of both literature and the stream/river databases by MBI (Marathon's consultant) addresses IDNR's concerns regarding the incidence of DELTs in Robinson Creek. See 8/15/18 Marathon Resp. Exh. 2 at 10-15.*

<u>IDNR Response:</u>

The literature and stream/river databases provided by MBI did not address IDNR's concerns regarding the incidence of DELTs in the Bigeye Chubs found in Robinson Creek.

In MBI's 8/15/18 response, MBI rejects IDNR's concern that temperature stress exacerbates prevalence of DELTs. MBI asserted that *only one study* (emphasis added) evaluated the relationship between temperature and disease. However, a brief search for relevant literature yielded several studies that address this topic both in concept and by directly measuring this relationship. The review by Lafferty and Holt (2003) presents the conceptual framework wherein stressed animals have less energy to devote to immune response and are therefore more susceptible to disease. This pattern has been confirmed by other reviews, including Moller (1987), Sandland and Minchella (2003), and Sindermann (1979), and numerous other studies focusing on individual stressors. This extensive body of research supports IDNR's concern that stress results in greater frequency or intensity of DELTs. Other studies that specifically address deformity, disease prevalence, or parasite load at high temperature or under conditions of thermal stress include Chang et al. (2010), Esch et al. (1976), and Sylvester (1972). These studies do not comprise an exhaustive list. However, a particularly pertinent quote comes from Sylvester (1972): "In the presence of domestic and industrial wastes, a slight increase in sublethal temperature could cause fish mortalities through synergism." IDNR finds MBI's evaluation of relevant literature insufficient and that Marathon's thermal discharge causes "harm" to Bigeye Chub (520 ILCS 10/2). (See Attachment B for full citations to the above referenced papers.)

In their cursory literature review, MBI compares the prevalence of DELTs to point-measures of temperature recorded during fish surveys conducted in Illinois and Ohio. MBI's attempts to make the conclusion that high temperatures do not correspond with relatively high frequency of DELTs. However, this comparison is irrelevant because IDNR asserts that the synergistic interaction of Marathon's thermal discharge and non-thermal pollutants produces DELTs in Robinson Creek fishes. MBI's analysis does not consider non-thermal stressors as well as IDNR's concerns regarding the incidence of DELTs in Robinson Creek remains.

b. *If not, please clarify the specific methodology that must be used to conduct the bioassay to identify the character and likely causes of DELTs in Robinson Creek. In this regard, comment on EA Engineering's (Marathon's consultant) assertion that no bioassay methodologies exist to address to identify the character and likely causes of DELTs. IDNR. 8/15/18 Marathon Resp. Exh. 2 at 3.*

IDNR Response:

IDNR notes several observational and experimental study designs are available to evaluate the character and likely causes of DELTs in Robinson Creek. (See Attachment C for discussion of study designs that evaluate the relationship between thermal pollution, non-thermal pollution and DELTs in Robinson Creek – "Bioassay Study of Aquatic Thermal Impacts (3), dated 4/1/2020".)

* * * * * * * * * * * * * * * * * * *

WHEREFORE, Illinois Department of Natural Resources respectfully submits the above stated Responses and Notes to questions from the Hearing Officer pursuant to the Order by the Illinois Pollution Control Board, as dated March 5, 2020, requesting additional information, and the Order by the Hearing Officer in this matter, dated March 10, 2020.

Illinois Department of Natural Resources

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DATED: July 7, 2020

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CERTIFICATE OF SERVICE

I, Virginia I. Yang, Legal Counsel for the Illinois Department of Natural Resources, herein certify that I have served a copy of the foregoing ANSWERS OF THE ILLINOIS DEPARTMENT OF NATURAL RESOURCES TO THE ORDER OF THE HEARING OFFICER FOR THE ILLINOIS POLLUTION CONTROL BOARD, dated March 10, 2020 via electronic mailing upon:

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Respectfully submitted,

DATED: July 7, 2020

ILLINOIS DEPARTMENT OF NATURAL RESOURCES

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Attachment A

Effects of acclimation temperature on Critical thermal limits and swimming performance Of the state-endangered bigeye chub *Hybopsis amblops*

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Effects of acclimation temperature on critical thermal limits and swimming performance of the state-endangered bigeye chub *Hybopsis amblops*

Qihong Dai*, Cory D. Suski

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ABSTRACT: Thermal stress can directly affect the survival of fishes and indirectly impact fish populations through several processes, including impaired swimming performance. Bigeye chub *Hybopsis amblops* is a state-endangered species in Illinois and is disappearing in the northern portion of its native range in North America. Limited temperature tolerance information exists on this species. The aim of this study was to define the impacts of 2 acclimation temperatures on the performance and behavior of bigeye chub. To accomplish this, we conducted 2 assays: critical thermal maximum (CT_{max}) testing for upper thermal tolerance limits, and swimming performance testing for critical swimming speed (U_{crit}) and burst swimming ability. With a 5°C acclimation temperature increase from 21 to 26°C, the CT_{max} of bigeye chub increased from 32.8 ± 0.4°C to 36.4 ± 0.9°C. U_{crit} was not different across acclimation temperatures, and fish from both acclimation groups could swim up to over 10 body lengths (BL) s^{-1}. Burst swimming duration also did not differ statistically across groups, but bigeye chub from the 26°C group swam 27 % longer in duration relative to fish from the 21°C group. Results from this study can help guide the protection and restoration of bigeye chub populations from thermal stressors.

KEY WORDS: CT_{max} · AT_{max} · Thermal tolerance · U_{crit} · Burst swimming · Global warming · Range distribution · Endangered

1. INTRODUCTION

For ectothermic organisms including fish, temperature is one of the most critical abiotic factors, and is recognized as an important ecological resource (Magnuson et al. 1979). Although acclimation to higher temperature can increase the upper thermal tolerance of fish, the scope of this enhanced tolerance decreases at higher tolerable acclimation temperatures (Beitinger et al. 2000). As a result, with exposure to sustained elevated temperatures or more intermittent heat waves, fish can suffer negative consequences including increased energy use, impaired swimming performance, reductions in fitness, altered range limits, or even death (Huey 1991, Beitinger et al. 2000, Xia et al. 2017, Morgan et al. 2018). To protect and restore populations of various fish species, it is therefore important to be able to quantify thermal tolerance and predict the possible impacts of thermal challenges.

Bigeye chub *Hybopsis amblops* is a member of the Leuciscinae subfamily (Page & Burr 2011), and these fish are commonly known as small minnows (Avise & Ayala 1976). The species once had a widespread distribution in North America, from the drainages of Lakes Ontario and Erie in the north to the Tennessee River drainage in the south (Page & Burr 2011). It is typically found in clear, gravel-bottomed streams with permanent flow and little silt, preferring to reside at the base of riffles or in quiet pools (Pfleiger 1997). The presence of bigeye chub has been viewed as an indicator of excellent water quality (Boschung

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& Mayden 2004). Bigeye chub distributions have been greatly reduced, particularly in the northern portion of their native range (Tiemann et al. 2004). At present, the species is believed to have been extirpated from Michigan and Virginia and is listed as an endangered species in Illinois (Warren & Burr 1988, Angermeier 1995, Berendzen et al. 2008, Illinois Endangered Species Protection Board 2015). The extirpation of bigeye chub in parts of its range has been attributed to bank siltation and release of fertilizers and pesticides from poor agricultural practices (Page & Retzer 2002); thermal stressors could also be contributing to its decline. For example, for stream fish, loss of riparian habitats is known to exacerbate the impacts of thermal challenges (Naiman & Décamps 1997) under more frequent heat waves and elevated temperatures (IPCC 2018). At present, however, there is one study on thermal tolerance of bigeye chub, using only one fish acclimated to a single temperature (Lutterschmidt & Hutchison 1997). Additional data on the thermal tolerance of bigeye chub are therefore essential to better understand and protect this rare species in the face of thermal stressors.

To quantify the direct and indirect impacts of thermal stressors on fishes, critical thermal maximum (CT_{max}) (Lutterschmidt & Hutchison 1997, Beitinger & Lutterschmidt 2011) and swimming performance (Xia et al. 2017) testing, respectively, are commonly used. CT_{max} is a laboratory-based procedure commonly used to define upper thermal tolerance limits of aquatic ectothermic animals and determine species' distributions (Sears et al. 2011). Compared to other dynamic or static assays, CT_{max} has emerged as the mostly widely used procedure, with the number of thermal tolerance studies using CT_{max} increasing 500% from 1990–2000 to 2010–2017 (Morgan et al. 2018). The procedure to define tolerance limits for fish using CT_{max} consists of increasing water temperature at a constant rate until a sublethal endpoint, such as the loss of equilibrium or the onset of spasms, is reached (Lutterschmidt & Hutchison 1997). Compared to other methods, such as incipient upper lethal temperature, CT_{max} has 2 main advantages: (1) it is a nonlethal method that requires relatively small sample sizes, which makes it ideally suited to the study of threatened species; and (2) it is very effective when evaluating the impacts of biotic (e.g. competition) and abiotic factors (e.g. pollution) on thermal tolerance (Becker & Genoway 1979, Beitinger & Lutterschmidt 2011). To date, different abiotic factors have been incorporated in thermal tolerance tests, and among these acclimation temperature has shown a positive relationship with CT_{max} (Bennett & Beitinger 1997, Beitinger & Lutterschmidt 2011), suggesting that the physiological plasticity of fish could help reduce the impacts of thermal challenges (Underwood et al. 2012).

In addition to directly quantifying thermal limits using CT_{max} tests, quantifying temperature-regulated swimming is an efficient way to define how thermal acclimation can impact performance and survival of fish in a laboratory setting (Plaut 2001) because swimming ability is critical for activities such as prey capture, predator avoidance, and reproduction in natural populations (Killen et al. 2010). Fish typically have a thermal optimum for swimming, and temperatures that exceed this optimum result in decreased swimming performance that can have negative consequences for survival and fitness (Lee et al. 2003). Thus, better understanding of the thermal tolerance of bigeye chub, as well as an improved ability to predict the response of bigeye chub to thermal challenges, can be achieved using CT_{max} and swimming performance testing across a range of acclimation temperatures.

To better protect and restore bigeye chub populations, the objectives of this study were to (1) quantify the upper critical thermal limits, (2) define the influence of acclimation temperatures on swimming performance, and (3) compare the thermal tolerance and swimming performance of bigeye chub to other Leuciscinae species. These 3 objectives will combine to improve our ability to quantify how thermal challenges can influence bigeye chub populations.

2. MATERIALS AND METHODS

2.1. Fish sampling and release

On 25 October 2018, an initial group of bigeye chub (n = 12) were collected from the Middle Fork Vermilion River (40° 12' N, 87° 44' W) at Kennekuk Cove County Park near Danville, IL, USA. Fish were sampled using a seine net, placed in coolers with aerators, and brought back to the University of Illinois Aquatic Research Facility in Urbana-Champaign. These 12 individuals were held in a single aerated aquarium to confirm their transition to consuming dry fish flakes (Freshwater Flakes; Omega One) in the laboratory. Following successful transition to flaked food within 1 d, an additional 28 bigeye chub were then sampled at the same site on 31 October 2018 using the same sampling techniques described above. Species identification of individual fish was confirmed by biologists working for the Illinois Department of Natural Resources.

After all experiments described below, all live fish were released back to the original sampling site after cooling acclimation temperature to match environmental temperatures.

2.2. Fish holding and acclimation

Thermal acclimation occurred in 2 identical 110 l glass aquaria (20 bigeye chub aquarium^{-1}). Each aquarium was filled with dechlorinated, conditioned tap water (AquaSafe Plus; Tetra), covered with gravel on the bottom, and outfitted with a power filter to maintain water quality (Aqua Clear Power Filter; Marineland). Lights for the aquaria were automatically turned on at 06:00 h and off at 18:00 h every day by timers, and fish were fed to satiation daily with dry fish flakes. Dissolved oxygen was measured daily (Pro Plus Multiparameter Instrument; YSI) and remained above 90% saturation. Every week, 10% of the water in each tank was replaced with fresh dechlorinated tap water, and excess food and feces in the bottom of the tanks were regularly removed using a siphon. Water temperature was set to 11°C (i.e. field temperature) for both aquaria at the beginning of the experiment (TK-500; TECO); 2 d later, water temperature was increased at a rate of 1°C d^{-1} (Xia et al. 2017) until 21 and 26°C were reached, respectively representing mean water temperature in May and August 2016 at our sampling site, based on records from the Illinois Environmental Protection Agency monitoring station (https://www2.illinois.gov/epa/topics/water-quality/monitoring/Pages/river-and-stream.aspx). Once target acclimation temperatures were reached, 10 bigeye chub from each temperature group were randomly selected and gently moved to a second, identical aquarium with the same water quality parameters. Thus, altogether, there were 4 acclimation aquaria used (2 aquaria at 21°C and 2 at 26°C), each holding 10 individuals. Fish were then held for 21 d at target acclimation temperatures to ensure thermal acclimation, and harmful ammonia-N was quickly reduced to 0 ppm by nitrifying bacteria (Currie et al. 1998, Carveth et al. 2006, Xia et al. 2017) (Table 1). During holding, there was no sign of any fungus on the fish, and all animals appeared to be robust, healthy, and vigorous. Neither total length (TL), total weight (TW), nor condition score (Fulton's condition factor, K, calculated as: TW / TL^3 × 10^5) (Neumann et al. 2012) differed across temperature groups (t-tests, t_{38} < 1.7, $p > 0.05$) (Table 1).

2.3. Critical thermal limit testing

Critical thermal limit tests occurred after the 21 d acclimation period, and all fish were fasted for 24 h prior to testing to reduce the impact of feeding on any behavioral response. Critical thermal limit testing was carried out in a 75 l testing tank containing 55 l of dechlorinated tap water. The tank contained a 1000 W electric immersion heater for temperature increase (SmartOne EasyPlug Axial Bottom Heater; Integrated Aqua Systems), 2 small aquarium pumps to mix water in the tank (Universal 600; Eheim), and an air stone attached to a small compressor (Tetra Whisper; Tetra) for aeration. A total of 6 individually numbered plastic compartments (20 × 10 × 10 cm) were attached to the sides of the tank and used to hold fish during testing. These compartments were perforated with holes to allow the circulation of water, but kept fish confined to minimize the likelihood of fish disturbing each other, and to make it easier to monitor individuals during the trial (Amundsen & Forsgren 2001). Either 4 or 6 fish were introduced into the compartments during each trial, and fish were given 1 h of acclimation time with nearly 100% saturation of dissolved oxygen (>7.5 mg l^{-1}) and water temperature identical to acclimation temperature (i.e. 21 or 26°C).

After 1 h acclimation, the air stone was removed from the tank, and water temperature was increased at a rate of 0.3°C min^{-1} (Beitinger et al. 2000, Beitinger

Table 1. Mean (±SD) water quality parameters and fish sizes for bigeye chub held at either 21 or 26°C during a 3 wk acclimation period (n = 20 fish treatment^{-1}). Dissolved oxygen, total length (TL), total weight (TW) and condition factor (K) did not differ across temperature treatments (t-tests, t_{38} < 1.7, $p > 0.05$). K, a metric to compare fish weight relative to its length, was calculated as: TW / TL^3 × 10^5

Target acclimation temperature (°C)	Water temperature during holding (°C)	Dissolved oxygen (% saturation)	Total length (TL) (mm)	Total weight (TW) (g)	Condition factor (K)
21	21.1 ± 0.2	99.0 ± 1.7	68.5 ± 7.2	2.9 ± 0.9	0.87 ± 0.06
26	26.0 ± 0.2	98.5 ± 1.6	68.2 ± 5.7	2.7 ± 0.7	0.83 ± 0.10

& Lutterschmidt 2011) (Fig. A1 in the Appendix). Every fish was closely observed for 2 different behavioral responses as temperature increased. First, the temperature at which fish displayed erratic behaviors, defined by burst swimming or attempts to jump out of their compartments, was recorded as the upper incipient avoidance temperature (AT_{max}) (Xia et al. 2017). Second, the temperature at which fish started to lose body equilibrium, defined by disorganization of locomotion and failure to maintain dorso-ventral orientation, was recorded as CT_{max} (Beitinger et al. 2000, Xia et al. 2017, Morgan et al. 2018). Once a fish lost equilibrium, it was quickly removed from its compartment, measured for TL and TW, and placed in a nearby holding tank with water at the acclimation temperature for recovery. During the trial, temperature was recorded every 1 min with the same YSI handheld meter described above. Dissolved oxygen was monitored regularly and did not fall below 98% saturation (>7.5 mg l^{-1}) despite the lack of aeration during observations. Altogether, a total of 8 trials were run, with sample size of n = 20 bigeye chub for each acclimation temperature. Trials for each temperature treatment were run on a single day to minimize the impacts of holding duration on any response to thermal challenges. After the conclusion of all trials, fish were returned to their acclimation aquaria and continued to be fed daily for 72 h and monitored for potential delayed mortality.

2.4. Swimming performance testing

After 1 wk of critical thermal tolerance testing, tests of critical swimming speed (U_{crit}) and burst swimming duration were performed in a 5 l (30 × 7.5 × 7.5 cm) flow-controlled swim tunnel respirometer (Loligo; www.loligosystems.com). The swim tunnel was calibrated using a flow meter (HFA; Höntzsch) to convert motor speed to water velocity (cm s^{-1}). Bigeye chub were fasted for 24 h prior to swimming tests to reduce the impact of feeding on any behavioral response. For U_{crit} tests, at each acclimation temperature, a single fish was randomly selected at one time and gently transferred to the swim tunnel, flowing at 5 cm s^{-1} (approximately 0.7 body lengths [BL] s^{-1}) for 30 min acclimation (Underwood et al. 2014, Kern et al. 2018). Water temperature in the tunnel was held close to acclimation temperature (±0.5°C) using a submersible 100 W aquarium heater (Top Fin). Following the 30 min acclimation, water velocity was increased by 5 cm s^{-1} every 5 min (Kern et al. 2018) until the fish became exhausted, determined when the fish failed to move off the rear screen of the chamber for >5 s. Once exhaustion was reached, the fish was gently removed from the swim tunnel, measured for TL and TW, and returned to its holding aquarium. Individuals were only tested in one swimming challenge. Trials for each temperature treatment were run on a single day to minimize the impacts of holding duration on any response to thermal challenges.

U_{crit} was calculated as:

$$U_{crit} = U + (t/T) \times \Delta U$$

where U (cm s^{-1}) is the highest sustained water velocity fish achieved for full 5 min, ΔU is the velocity increment (i.e. 5 cm s^{-1}), t (min) is the time fish swam during the final increment, and T is the time increment (i.e. 5 min) (Brett 1964). A correction for blocking was not performed because the maximal cross-sectional area of bigeye chub was <10% of the cross section in the swim tunnel (Bell & Terhune 1970), with measurements verified using calipers for each fish. Sample sizes for these tests were 5 fish from each acclimation temperature.

For burst swimming testing, at each acclimation temperature, a single bigeye chub was randomly selected at one time among remaining individuals in aquaria and acclimated to the swim tunnel for 5 min at 0.5 BL s^{-1} (approximately 0.35 cm s^{-1}) (Underwood et al. 2014, Kern et al. 2018). Following this acclimation, water velocity was increased to 12 BL s^{-1} in 5 s and swim duration then was recorded by a timer (Hasler et al. 2009). This increase in water velocity was chosen because the converted mean U_{crit} from the previous tests was larger than 10 BL s^{-1} but smaller than 12 BL s^{-1}, and a high velocity was necessary to ensure that fish were not swimming aerobically. Following this rapid increase in water velocity, fish swam until they could not move off the rear screen of the chamber for >3 s. Once swimming ceased, the fish was gently removed from the swim tunnel and measured for TL and TW. Sample sizes were 6 fish from each acclimation temperature. Trials for each temperature treatment were run on a single day to minimize the impacts of holding duration on any response to thermal challenges.

2.5. Statistical analyses

For thermal tolerance testing, comparisons of both CT_{max} and AT_{max} for each acclimation temperature were conducted using a single 2-way ANOVA. The main effects were acclimation temperature (21 or 26°C), response (AT_{max} and CT_{max}), and their interaction. If a significant difference was found for any term in the model, post hoc analyses to determine differences across factors were performed using a Tukey's HSD test. Following the completion of this 2-way ANOVA, an additional analysis was conducted using a fully parameterized model to quantify the impacts of *K*, trial number, compartment number, and holding aquarium on both AT_{max} and CT_{max}. The model that contained only acclimation temperature, response, and their interaction was compared to the fully parameterized model using a 1-way ANOVA (Crawley 2013).

For swimming performance testing, both U_{crit} and burst swimming duration were compared across acclimation temperatures using separate 1-way ANOVAs. An additional analysis was conducted to quantify the impacts of *K* and holding aquarium for both U_{crit} and burst swimming duration, using a 1-way ANOVA comparing the model containing only temperature with the full parameterized model (Crawley 2013).

All statistical analyses were conducted in R v.3.5.1 (R Core Team 2019) with $\alpha = 0.05$, and all data are reported ±SD where appropriate. Fit of all models to the data, as well as assumptions of normality and equal variances, were verified with inspection of residuals and quantile–quantile plots (Crawley 2013).

3. RESULTS

3.1. Critical thermal limits

Bigeye chub acclimated to 26°C began to show avoidance behaviors (i.e. AT_{max}) and lost equilibrium (i.e. CT_{max}) at 3.4 and 3.6°C higher, respectively, than fish acclimated to 21°C (Tukey's HSD, $p < 0.05$) (Table 2). The temperature causing equilibrium loss was significantly higher than the temperature causing avoidance behaviors (Table 2 and Table A1 in the Appendix).

Table 2. Temperature at which bigeye chub showed either avoidance behaviors (upper incipient avoidance temperature [AT_{max}]) or lost equilibrium (critical thermal maximum [CT_{max}]) acclimated to either 21 or 26°C. Results from statistical tests are shown in Table A1. (*) indicates a significant difference between CT_{max} and AT_{max}; superscript letters denote differences across acclimation temperatures. Sample sizes: n = 20 per acclimation temperature

Response	Acclimation temperature (°C)	Mean	SD	Median
AT_{max}*	21^{a}	29.9	1.3	29.9
	26^{b}	33.3	1.4	33.6
CT_{max}	21^{a}	32.8	0.4	32.8
	26^{b}	36.4	0.9	36.6

The behavioral responses of bigeye chub during the thermal testing were not influenced by compartment number ($F_{1,72} = 0.108$, $p = 0.744$) or holding aquarium ($F_{1,72} = 1.117$, $p = 0.294$). However, behavioral responses were influenced by trial number ($F_{1,72} = 7.598$, $p = 0.007$) and *K* ($F_{1,72} = 4.519$, $p = 0.037$). Inspection of CT_{max} and AT_{max} data across trials showed that changes in responses across trials were small (≤1.8°C on average across replicates), and no consistent or predictable changes in behavioral responses occurred over time (Fig. A2).

During the monitoring period that followed thermal testing, 1 bigeye chub from the 26°C group died. The *K* for this individual was 0.61, which is considerably below average in the study (approximately $K = 0.85$; Table 1). After excluding this individual from analyses, *K* no longer significantly influenced either CT_{max} or AT_{max} ($F_{1,70} = 2.620$, $p = 0.110$), indicating that the significant impact of *K* on behavioral responses was driven by this single fish. Despite this effect, the individual was retained in the analyses of CT_{max} and AT_{max} because excluding this data point did not impact CT_{max} or AT_{max}, verified by unpaired, 2 sample *t*-tests (CT_{max}: $t_{77} = -0.11$, $p = 0.91$; AT_{max}: $t_{77} = 0.03$, $p = 0.98$). In addition, this individual did not demonstrate any stress-like or abnormal behavior prior to the monitoring period.

3.2. Swimming performance

Neither U_{crit} ($F_{1,8} = 0.537$, $p = 0.485$) nor burst swimming duration ($F_{1,10} = 0.815$, $p = 0.388$) differed statistically between the 21 and 26°C groups, even though the mean burst swimming duration of fish from the 26°C group was 27% higher than fish from the 21°C group (Table 3). The swimming performance of bigeye chub for U_{crit} ($F_{8,6} = 0.104$, $p = 0.903$) or burst swimming duration ($F_{10,8} = 0.788$, $p = 0.487$) was not influenced by *K* or holding aquarium.

4. DISCUSSION

This is the first comprehensive study to define the thermal tolerance of bigeye chub. The CT_{max} of bigeye chub acclimated to 21°C was 32.8 ± 0.4°C; a 5°C increase in acclimation temperature increased CT_{max} to 36.4 ± 0.9°C. Standardized thermal tolerance measurements, such as CT_{max}, have been commonly

Table 3. Effects of acclimation temperature on critical swimming speed (U_{crit}) shown in absolute swimming velocity (cm s^{-1}) or relative swimming speed in body lengths (BL) (BL s^{-1}), along with burst swimming duration (s) for bigeye chub acclimated to either 21 or 26°C. Sample sizes: 5 per acclimation temperature for U_{crit}; 6 per acclimation temperature for burst swimming duration. U_{crit} were presented in both absolute and relative velocity to facilitate comparisons with previously published studies. Differences across temperatures within a swimming test were not significantly different; outputs from statistical testing are provided in the results

	Acclimation temperature (°C)	Mean	SD	Median
U_{crit} (cm s^{-1})	21	71.1	3.7	70.5
	26	76.6	10.8	76.0
U_{crit} (BL s^{-1})	21	10.8	1.5	10.7
	26	11.3	0.9	11.6
Burst swimming duration (s)	21	9.3	4.0	9.5
	26	11.8	5.5	10.5

used to quantify the impacts of thermal challenges on aquatic organisms (Terblanche et al. 2011). Despite various rates of temperature changes (i.e. from 1°C h^{-1} to 1°C min^{-1}) used for CT_{max} over the past few decades, 0.3°C min^{-1} has been widely used recently for thermal testing as it ensures the core temperatures of small fish species change consistently and closely with water temperature changes, while also eliminating the possibility of rapid acclimation that can occur at slower temperature increase (Beitinger et al. 2000). In this way, CT_{max} provides a consistent, repeatable, and nonlethal approach to define thermal tolerance limits for free-swimming fishes. Lutterschmidt & Hutchison (1997) reported the CT_{max} of a single bigeye chub acclimated to 10°C to be 31.7°C (Table 4). The lack of replication in that study prevents any general conclusions. Also, they used a relatively fast temperature increase (i.e. 1°C min^{-1}) during thermal tolerance measurements that could have generated higher CT_{max} values compared to the 0.3°C min^{-1} used here, due to the lag of core temperature increase (Beitinger et al. 2000).

Bigeye chub is a member of the subfamily Leuciscinae (i.e. minnows), and comparisons of thermal tolerance data from this study with other Leuciscinae species, which minimize the influence of phylogeny in thermal sensitivity (Hasnain et al. 2013), show that bigeye chub have moderate thermal tolerance (Table 4). For example, sand shiner *Notropis strami-*

Table 4. Comparisons of the critical thermal maximum (CT_{max}) (±SD when available) of bigeye chub with other small Leuciscinae species (i.e. small minnows) found in the United States. ΔT: the rate at which water was heated during the thermal trial

Species	Acclimation temperature (°C)	ΔT (°C min^{-1})	CT_{max} (°C)	Sources
Bigeye chub *Hybopsis amblops*	21	0.3	32.8 ± 0.4	This study (2019)
	26	0.3	36.4 ± 0.9	
	10	1	31.7	Lutterschmidt & Hutchison (1997)
Fathead minnow *Pimephales promelas*	23	0.3	34.4	Heath et al. (1994)
	32	0.3	40.4 ± 0.3	Richards & Beitinger (1995)
Loach minnow *Rhinichthys cobitis*	25	0.3	35.3	Bonar et al. (2005)
	30	0.3	36.1	
Spikedace *Meda fulgida*	25	0.3	34.7 ± 0.9	Bonar et al. (2005)
	30	0.3	36.9 ± 1.1	
Red shiner *Cyprinella lutrensis*	25	0.3	37.4	King et al. (1985)
	30	0.3	39.6 ± 0.2	Rutledge & Beitinger (1989)
Mohave tui chub *Gila bicolor mohavensis*	18	0.14	33.5	McClanahan et al. (1986)
	24	0.14	34.9	
	30	0.14	36.2	
Roundtail chub *Gila robusta seminuda*	10	0.24	27.9 ± 0.2	Deacon et al. (1987)
	15	0.24	32.3 ± 1.4	
	25	0.24	36.4 ± 0.7	
Speckled dace *Rhinichthys osculus*	10	0.24	30.5 ± 1.6	Deacon et al. (1987)
	15	0.24	32.6 ± 0.5	
	25	0.24	36.8 ± 0.6	
Woundfin *Plagopterus argentissimus*	10	0.24	30.7 ± 0.2	Deacon et al. (1987)
	15	0.24	33.6 ± 1.0	
	25	0.24	39.5 ± 0.2	
Sand shiner *Notropis stramineus*	21	0.3	33.0 ± 2.0	Q. Dai et al. (unpubl. data)
	26	0.3	36.8 ± 2.0	

neus, which were sampled at the same site and acclimated at the same temperatures as bigeye chub (Q. Dai et al. unpubl. data), had similar CT_{max} values (Table 4). The CT_{max} of bigeye chub with acclimation temperatures at 21 and 26°C were both around the median of CT_{max} range distributions, compared to similar acclimation temperatures (i.e. ±2°C) for other species (Table 4). In streams, thermal tolerance could play an important role in determining competitive advantages when different species have highly overlapping niches. For example, Mojave tui chub *Gila bicolor mohavensis*, which are native to the Mohave River, California, were partly displaced by the introduced Arroyo chub *Gila orcutti* because of Arroyo chub's better tolerance of fluctuating temperature conditions, which provided them with a competitive advantage over Mojave tui chub (Castleberry & Cech 1986). In the future, climate change will generate more frequent and extreme heat waves (Seneviratne et al. 2014, IPCC 2018). Therefore, it would be advantageous for future studies to conduct similar work using acclimation temperatures higher than those in the current experiment to better compare thermal tolerance of bigeye chub with other sympatric species, and to provide improved estimations for future habitat occupancy of streams and creeks.

The U_{crit} of bigeye chub was over 10 BL s^{-1} for both acclimation temperatures, and the 5°C difference in acclimation temperature did not impair or improve swimming performance. U_{crit} is commonly used to estimate maximum aerobic swimming ability (Brauner et al. 1994) and is assumed to represent maximum cardiac performance (Farrell & Steffensen 1987). In this way, U_{crit} has been used to quantify the effects of different factors, such as temperature, on swimming performance, and to predict the ecological effects of these factors on fishes (Plaut 2001). Compared to several other small Leuciscinae fishes (Table 5), bigeye chub are strong swimmers, with higher U_{crit} (cm s^{-1} and BL s^{-1}) (Boyd & Parsons 1998, Kolok et al. 1998, Webb 1998, Tritico & Cotel 2010, Nichols et al. 2018) despite different acclimation temperatures that could limit direct U_{crit} comparisons across species. Additionally, although the U_{crit} of bigeye chub is relatively high, it is possible that the swimming performance of the individuals generated in our swim tunnel is an underestimation of true swimming ability in the wild. For example, Boyd & Parsons (1998) showed that the U_{crit} of schooling fish was higher than individuals swimming alone, meaning that wild bigeye chub may exceed values shown here if they aggregate into shoals. Also, Castro-Santos (2011) argued that the small chambers of swim tunnels under controlled conditions in the laboratory prevented fish from exhibiting free-swimming behaviors, thus causing underestimation of swimming performance. As a species most often found in streams and creeks (Tiemann et al. 2004), good swimming performance is likely critical for bigeye chub to flourish under conditions of variable discharge rates that can occur in streams. Because the 5°C acclimation temperature increase from 21 to 26°C did not impair the swimming ability of bigeye chub, future studies with higher acclimation temperatures could better inform the threshold of its upper thermal limits for aerobic swimming performance. In addition, future work that combines U_{crit} testing at different temperatures with metabolic rate data (oxy-

Table 5. Comparisons of the critical swimming speed (U_{crit}) of bigeye chub with other small Leuciscinae fishes. BL: body length. Data are shown as mean ± SD when possible

Species	Acclimation temperature (°C)	BL (mm)	U_{crit} (cm s^{-1})	U_{crit} (BL s^{-1})	Source
Bigeye chub *Hybopsis amblops*	21	66.8 ± 7.8	71.1 ± 3.7	10.8 ± 1.5	This study
	26	67.4 ± 5.7	76.6 ± 10.8	11.3 ± 0.9	
Creek chub *Semotilus atromaculatus*	20.5	122 ± 12	53.2 ± 2.4	4.4[a]	Tritico & Cotel (2010)
River chub *Nocomis micropogon*	13	105 ± 31	59 ± 16	5.6[a]	Webb (1998)
	18	107 ± 49	59 ± 13	5.5[a]	
	23	106 ± 33	63 ± 17	5.9[a]	
Golden shiner *Notemigonus crysoleucas*	21–23	61 ± 3.5	25.6 ± 5.5	4.2[a]	Boyd & Parsons (1998)
Fathead minnow *Pimephales promelas*	24	61 ± 4	44.7 ± 4.1	7.3[a]	Kolok et al. (1998)
Spotfin shiner *Cyprinella spiloptera*	20	27-95	60.8 ± 11.3		Nichols et al. (2018)
Bluntnose minnow *Pimephales notatus*	20	49-83	63.0 ± 22.7		Nichols et al. (2018

[a]Calculated based on mean body length (cm) and mean U_{crit} (cm s^{-1})

gen consumption data) will provide a comprehensive understanding of the responses of bigeye chub to future climatic stressors.

Bigeye chub did not show a difference in burst swimming durations with a 5°C difference in acclimation. For fish, anaerobic (burst) swimming is used for short-duration, high-intensity swimming to avoid predation, capture food, and overcome abrupt transitions through difficult flow conditions (Plaut 2001, Hasler et al. 2009). For example, Taylor & McPhail (1985) found newly emerged coho salmon *Oncorhynchus kisutch* with better burst swimming performance were less susceptible to predation compared to conspecifics. For burst swimming duration of bigeye chub, although there was no statistically significant difference between the 21 and 26°C groups, it is notable that there was an approximate 27% increase in duration that burst swimming ability improved at the higher acclimation temperature. Considering the modest sample sizes (6 in each group) and relatively large inter-individual variation of duration within each temperature group, future trials at higher acclimation temperatures could be performed to better explore the threshold of upper thermal limits for burst swimming performance in this species. Regardless, our study shows that a 5°C increase in acclimation temperature from 21 to 26°C did not impair or improve the burst swimming duration of bigeye chub.

Comparing the results of behavioral tests with available environmental data will provide information that assists in the estimation of bigeye chub distribution. The Illinois Environmental Protection Agency maintained a stream temperature monitoring station at our sampling location in 2016. Records from this station showed mean water temperatures in June and August were 21.4°C and 26.7°C, respectively, which approximate the acclimation temperatures used in this study (https://www2.illinois.gov/epa/topics/water-quality/monitoring/Pages/river-and-stream.aspx). In both months, daily variation in temperature was approximately 5°C, with maximum temperatures reaching 31.8°C in the afternoon in August. In addition, the United States Geological Survey maintains a separate stream monitoring station (Site 03339000; https://waterdata.usgs.gov/il/nwis/rt) 25 km downstream from our collection site. Data from this station indicate that mean water temperatures in August (the hottest month of the year) averaged 26.9°C from 2015 to 2018, and average daily temperature once reached 29.1°C in August. Together, data from these 2 sites suggests that the daily maximum temperature at the site where fish were collected does not exceed either the CT_{max} or AT_{max} observed in this study. These thermal data likely partially explain why we were able to sample a large number (40) of state-endangered bigeye chub at this site, which is near the middle of its geographical distribution (Page & Burr 2011). Sunday et al. (2012) found that, under climate warming, ectotherms were predicted to shift their distribution ranges northward when considered globally. However, the greatest declines in bigeye chub populations appear to have occurred near the northern edge of their distribution (Tiemann et al. 2004). To better define whether temperature could be a factor influencing the range distribution of bigeye chub, we recommend that future studies consider not only elevated temperature, but also other forms of thermal stress such as heat waves. Meehl & Tebaldi (2004), for example, predicted more frequent and longer lasting heat waves in the northern portion of the midwestern region of North America compared to the southern portion. Also, in addition to considering thermal conditions alone, there are a number of other stressors that should be considered as part of habitat evaluations, particularly in terms of synergistic interactions with temperature changes (Folt et al. 1999). For example, both elevated water temperatures and eutrophication can result in an unavoidable decline in dissolved oxygen (Wetzel 2001), and this reduction in oxygen levels could exacerbate the sensitivity of bigeye chub to a range of environmental stressors. Holmstrup et al. (2010) reviewed the synergistic effects of a number of stressors, including low oxygen, on a number of pollutants in aquatic ecosystems, while Wajsbrot et al. (1991) showed that juvenile gilthead seabream *Sparus aurata* were more sensitive to ammonia toxicity at low dissolved oxygen levels relative to fish in normoxia. Future work should therefore be conducted on bigeye chub collected from a greater range of latitudes, coupled with more information on environmental components (e.g. dissolved oxygen and nutrient levels) and a more specific regional climate projection, to better predict thermal refugia and habitat suitability for bigeye chub across their range (Sunday et al. 2014, Pinsky et al. 2019).

Our study with bigeye chub from the Vermillion River, IL, USA, quantified both its thermal limits and thermal impacts on swimming performance, and demonstrated that natural thermal variation at this site likely did not exceed the thermal capacity of bigeye chub in the summer. In the future, results from our laboratory work should be better verified in the field; for example, either by sampling habitats with known thermal properties or through biotelemetry (Schrank et al. 2003). Overall, results from this study can help incorporate temperature into predicting range distribution of bigeye chub under conditions of climate change and point source thermal pollution (e.g. power plant discharge), and guide the protection and restoration of bigeye chub and other endangered species.

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Appendix

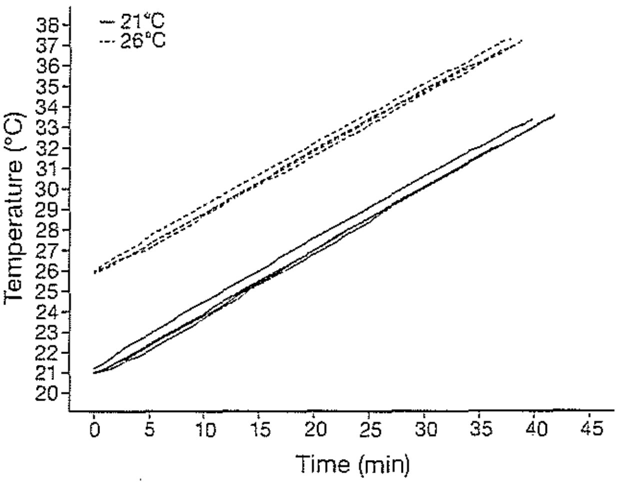

Fig. A1. Change in water temperature over time during thermal tests for bigeye chub acclimated to 21°C (solid lines) and 26°C (dashed lines). Water temperature was recorded every 1 min from the test tank using a handheld meter; 4 trials were run at each acclimation temperature. Equations for mean temperature increase: Temperature = 0.31 × Time + 21.0, R^2 = 0.995 (for 21°C groups); Temperature = 0.30 × Time + 25.9, R^2 = 0.996 (for 26°C groups)

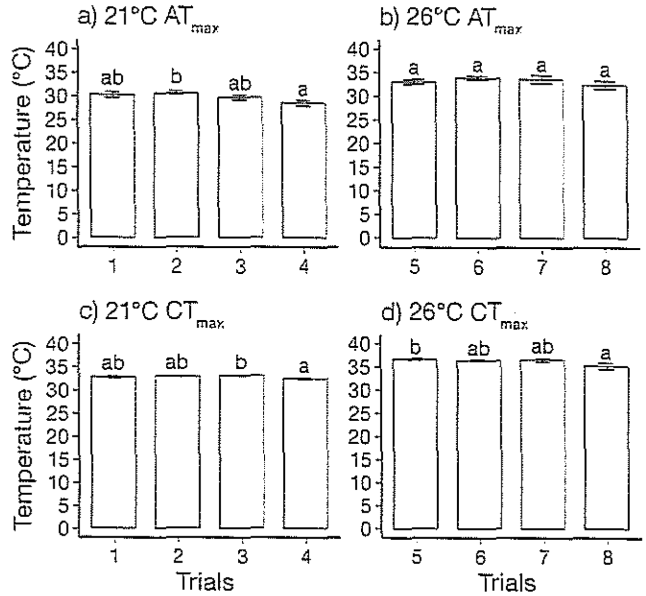

Fig. A2. Temperature at which bigeye chub acclimated to either 21 or 26°C showed either (a,b) avoidance behaviors (upper incipient avoidance temperature, AT_{max}) or (c,d) lost equilibrium (critical thermal maximum, CT_{max}). For each temperature/response combination, data were generated across 4 replicate trials (groups); each bar corresponds to 1 trial. Sample sizes for each trial were n = 4 or 6. Statistical differences across trials are denoted by dissimilar letters above bars (Tukey's post hoc test, $p < 0.05$)

Table A1. Results of a 2-way ANOVA comparing the effect of acclimation (either 21 or 26°C), behavioral response (either avoidance behaviors [upper incipient avoidance temperature, AT_{max}] or lost equilibrium [critical thermal maximum, CT_{max}]), and the interaction of acclimation and behavioral response on the temperature at which bigeye chub displayed behavioral changes. Data are shown in Table 2; significant factors are shown in **bold**

	df	Sum of squares	*F*	p-value
Response	**1**	**180.00**	**162.445**	**<0.001**
Acclimation	**1**	**244.30**	**220.475**	**<0.001**
Response × acclimation	1	0.00	0.004	0.949
Residuals	76	84.21		

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Attachment B

THERMAL IMPACTS AND DISEASE IN FISH

Chang, C.-W., Y.-T. Wang and W.-N. Tzeng. 2010. Morphological study on vertebral deformity of the thornfish *Terapon jarbua* in the thermal effluent outlet of a nuclear power plant in Taiwan." Journal of the fisheries Society of Taiwan 37, no. 1 (2010): 1-11.

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Attachment C

Bioassay Study Design (3)- Aquatic Thermal Impacts

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Background

The impacts of thermal discharge on both individual fish, as well as fish populations, has been a topic for researchers for decades. As such, the protocols, techniques and methods used to define many aspects of thermal discharge on fish are well-established and common. In addition, for many assays, technology has been advancing over the past few years, allowing significant improvements in accuracy and precision, as well as an ability to better understand how fish respond to stressors. Below is a brief scope of work that outlines a series of bioassays that would (1) identify the character and causes of deformities, eroded fins, lesions and tumors (DELTs) for fish in Robinson Creek, (2) quantify how acclimation to different temperatures would impact avoidance of different water temperatures, and (3) define how thermal discharge could impact reproductive output in bigeye chub.

DELTs (deformities, eroded fins, lesions and tumors)

Fish occasionally experience 'stress' (e.g., presence of a predator, low oxygen), and mechanisms exist for fish to overcome these short-duration stressors. However, if stress becomes prolonged and chronic, it can have negative consequences for fish, including a suppression of the immune system, resulting in susceptibility to pathogens. It is therefore reasonable to assume that chronic 'stress' caused by prolonged exposure to elevated temperatures in Robinson Creek has suppressed the immune system of fish, thereby leading to the presence of DELTs.

The relationship between thermal discharge and DELTS has been quantified in a number of past studies. For example, Chang et al. (2010) quantified the impacts of thermal effluent on vertebral deformities in fish near a nuclear power plant in Taiwan. Esch et al. (1976) quantified the role of thermal discharge from a cooling reservoir in North Carolina on the presence of bacterial infections that can lead to lesions ("red sore disease") in largemouth bass. Fin erosion, and red sores are commonly seen in fishes residing in areas of degraded habitat (Sindermann 1978) and deformities in healthy wild fish are typically rare.

Work to quantify the impact of thermal discharge in Robinson Creek on the presence of DELTs in the fish community would consist of a combination of field and laboratory experiments. Field studies would consist of sampling wild fish along a gradient both upstream and downstream of the discharge across several seasons. Fish would be inspected in the field for the presence of DELTS, and then lethally sampled. Lethal samples would allow for an assessment of a number of indices of health and condition including (1) plasma cortisol to quantify stress, (2) white blood cell counts (leukocytes) to quantify infection levels and immune function, (3) collection of tissues to quantify oxidative stress, and (4) indices of nutrition such as cholesterol or triglycerides. Fish that displayed lesions or tumors would have the lesion swabbed so that local bacteria could be quantified. Water samples would also be collected to quantify levels of pollutants or contaminants, along an identical gradient, moving both upstream and downstream of the discharge.

Lab-based studies would (1) hold fish for extended periods at temperatures that mimic conditions in Robinson Creek, and (2) extended holding in water that contains pollutants or contaminants identified from field sampling. After this extended holding period, fish would be (a) inspected for the presence of lesions, tumors or deformities, and (2) sampled for stress/health metrics as described above.

Because this study would involve lethal sampling to harvest tissues, the focus would be on surrogate species other than Bigeye Chub, prioritizing species that are commonly found across a range of habitat types (e.g., minnows closely related to Bigeye Chub such as Bluntnose Minnows or Sand Shiners). The use of surrogate species will adequately identify the relationship between temperature and frequency of DELTs while limiting take of Bigeye Chub to comply with the Illinois Endangered Species Act. Any Bigeye Chub incidentally captured during field studies would be closely examined, and likely swabbed for analyses, and then quickly released; the intentional use of Bigeye Chub would require IDNR approval.

Thermal avoidance

Temperature is an ecological resource for fish, and fish will relocate to avoid unfavorable water temperatures. In the past, work to quantify thermal avoidance has relied on thermally graded tanks or similar devices that provide fish with a range of water temperatures and allowing them to move to different parts of the tank. Unfortunately, these testing tanks are somewhat crude and blunt, and also lack accuracy and precision. As such, it is difficult to identify temperature preferences because temperature in these graded tanks cannot be defined at a fine spatial scale. Also, these graded tanks cannot identify avoidance thresholds (i.e., the temperature that induces movement or causes fish to seek out other habitat), and it is also difficult to achieve replication within an individual using these graded tanks. Recent developments in computer systems and animal monitoring has led to a number of improvements in thermal choice studies. These improvements are possible using a 'shuttle box' testing apparatus: an automated system that allow fish to actively 'choose' their preferred temperature, providing a realistic simulation of avoidance behavior in the field, and precisely identifying avoidance thresholds.

Proposed work on the topic of thermal avoidance would be conducted in a laboratory, and would consist of collecting fish from Robinson Creek, and acclimating groups of individuals across a range of temperatures. An automated 'shuttle box' system would then be used to identify both the temperature that these fish would avoid, but also the temperature they would prefer to inhabit. Measurements could be repeated both within an individual (i.e., testing the same individual multiple times), and also across individuals (i.e., testing many fish of the same species).

The focus of this study would again be on surrogate species other than Bigeye Chub, prioritizing species that are commonly found across a range of habitat types (e.g., minnows closely related to Bigeye Chub such as Bluntnose Minnows or Sand Shiners). However, discussions with DNR could occur to receive permission to use a small number of Bigeye Chub in this study that does not involve lethal sampling (i.e., a small number of Bigeye Chub could theoretically be collected from the wild, used in this study, and then returned to the wild at the conclusion of the study).

Reproduction

Successful reproduction is a key aspect of sustaining a healthy population of fish. Recently, Tarver and Stallsmith (2019) quantified the reproductive schedule for Bigeye Chub in Alabama based on field examinations, quantifying ovary maturation and oocyte stages. At present, the reproductive schedule, as well as the reproductive output, of Bigeye Chub from Illinois is not known, complicating projections for population trends and/or the impacts of thermal conditions on reproduction.

Proposed work on the topic of reproduction would consist of both field and laboratory activities. For laboratory work, groups of Bigeye Chub would be sampled from a population in Illinois other than Robinson Creek and brought to the aquatic research facility in Champaign. In Champaign, groups of Bigeye Chub would be held in aquaria at different thermal regimes and effort would be made to induce spawning (i.e., changes in photoperiod, changes in water temperature). Some thermal conditions would represent those seen close to the discharge in Robinson Creek, while other aquaria would be held at conditions farther from the discharge. Fish would be non-lethally sampled for reproductive hormones to identify when spawning might occur, and then would be sampled for the quantity of eggs/oocytes. In this way, efforts would be made to relate thermal conditions to reproductive output, allowing a determination of how thermal discharge does (or does not) influence egg output.

Field activities would consist of sampling Bigeye Chub in Robinson Creek to determine: (1) the quantity and level of reproductive hormones, and (2) the quantity of eggs/oocytes that could potentially be allocated to spawning. Samples would be collected in proximity to the discharge, as well as sites upstream and/or downstream, thereby allowing an assessment of how reproductive output does (or does not) change due to the presence of the discharge.

Please note that, because these two studies involve lethal sampling, approval would need to be granted from DNR for the work to commence.

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