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ASSESSMENT OF CRITICAL THERMAL MAXIMA OF AQUATIC MACROINVERTEBRATES IN THE EERSTE RIVER, EASTERN CAPE, SOUTH AFRICA

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Abstract

The impact of temperature on the physiology, ecology, and evolution of both endothermic and ectothermic organisms is pivotal. Every species possesses a distinct optimum temperature range that is crucial for survivorship. Determining upper thermal limits provides insight into the relative sensitivity of organisms to elevated water temperatures and provides valuable insights when monitoring for environmental thermal alterations. The current study examined the upper (CTmax) thermal limits of ten (10) freshwater macroinvertebrate taxa in the Eerste River, south-western of South Africa. All test insects were acclimated at 17 °C and subsequently exposed to a steady rise in temperature of 0.34 °C per minute using the Critical Thermal Methods (CTM). Generally, we observed a narrow range in thermal tolerance among the test organisms, suggesting that a similar threshold increase in temperature is likely to hamper the population of these organisms. However, significant differences were found in the mean CTmax values among the test insect within the same environment. The most sensitive species were the Aphanicerca species (32.3±1.2) and Chimarra species (33.7.09±2.8). These species may be useful indicators of thermal alteration in their habitats, due to their relatively low tolerance to increasing temperatures and the ease with which behavioral responses can be detected. Consequently, their disappearance from a stream may indicate that thermal or chemical pollution has occurred. We recommend future studies to explore on the adaptation and mitigation measures for climate change to save the biodiversity of Chimarra and Aphanicerca.

Keywords: Eerste River; Critical Thermal Maxima (CTmax); Macroinvertebrates; Thermal Point of Discomfort (TPD)

1. Introduction

Temperature has been documented as one of the most important abiotic factors affecting insect development, reproduction, seasonal phenology, population dynamics, distribution, survival, and abundance [1-3].
Water temperature fluctuations can occur naturally (i.e. global warming) or due to anthropogenic perturbations (thermal pollution). These may be indirect or direct, such as water discharges at a temperature above or below ambient due to land–use changes, irrigation return–flows, flow modification, inter-basin water transfers, modification to riparian vegetation and global warming [4]. Activities that influence the upper limits of water temperature in South Africa are mostly water abstraction, removal of riparian vegetation and climate change [5].

Since environmental temperatures vary in space and time, organisms are continually challenged to maintain their body temperature following an increase in ambient temperature [5]. Thus, elevated water temperatures may negatively affect aquatic organisms, and if the temperatures are high enough, results can be lethal to aquatic life. Elevated temperatures can reduce the solubility of dissolved oxygen in water and subsequently result in significant stress to aquatic organisms. Thus, an understanding of an organism's thermal sensitivity or tolerance is therefore crucial in determining the likely effect of changes in water temperature on aquatic ecosystems and generating thermal criteria for protecting aquatic ecosystems. Responses to such environmental variation have been characterised by a relatively consistent relationship in macroinvertebrates, which relates performance and environmental temperature [4]. This relationship is a right-shifted function, where performance gradually increases with temperature until reaching an optimum value and then decreases abruptly towards higher temperatures. Insect performance is dependent on body temperature, and it varies with species and taxa [6]. Insects generally have a developmental thermal window and optimum temperature at which all activities are optimal. Hence any deviation from the optimal may have negative consequences on fitness and survival [6].

The Critical Thermal Method (CTM) is a nonlethal test determined by increasing the temperature at a constant rate of (0.34 °C per minute) while watching the behavioural responses of the test animals until an accepted critical endpoint is reached [5]. The method is experimentally effective and has been recommended by (Heiman and Knight 1975) [7], who argued that studies utilising death (i.e. LT50) as an endpoint have little ecological value because a slight deviation in the physical environment would probably induce chronic, subtle effects on the population. So, the critical thermal maximum is more ecologically meaningful than lethal methods such as LT50. The Critical Thermal Maximum (CTmax) is the arithmetic mean of the collective thermal points at which locomotors function of organisms will become disorganised, and they will lose their ability to escape from an increase in conditions that will promptly lead to their death. The test is considered successful if the test organism survives after it is returned to the pre-test acclimation temperature [5]. Rates of temperature change used to determine upper CTmax values can range from 0.1 to 1.0 °C.

The variables that affect the experimentally determined CTmax of an organism include length of time held in captivity before experimentation [8], body size [9], concentration of oxygen or chemical pollution within the experimental environment [10-12], rate of experimental temperature increase [8,9,11]. One of the fundamental criticisms of CTmax studies is that organisms are being tested experimentally at temperatures higher than will be encountered in the wild; thus, the relevance of CTmax to survival in natural thermal regimes is not clear [10, 13]. In view of the above, Dallas and Rivers-Moore (2012)[5] argue that the real value of CTmax studies is not in the "significance" of a particular result, but rather in the comparisons of the responses of organisms to those of other organisms and identify several particularly stenothermic families that may be useful as thermal bio-indicators.

Tolerance to changes in water temperature has been documented for several aquatic organisms [5, 14-19]. For instance, Chown, Duffy, and Sørensen (2015) [20] provides a synthesis of studies on aquatic insect thermal tolerance, highlighting the need for further experimental work and the lower average thermal tolerance of aquatic insects compared to terrestrial species. (Shah et al. (2017)[21] explores the relationship between climate variability and thermal limits of aquatic insects across elevation and latitude, comparing temperate and tropical streams. The most recent study in Southern Africa [5] documented upper temperature tolerances for 23 insect families across the different aquatic systems. Unfortunately, their study species were not identified to species level making it appealing to determine the extent to which upper thermal limits vary within a family. Our hypothesis was based on the assumption that, organisms from the same or similar habitat would exhibit similar thermal tolerances. Thus, the aim of this study was to examine the thermal tolerance of ten (10) aquatic macroinvertebrates and determine the most thermally sensitive species of the Eerste River as bio-indicators for thermal pollution or climate change. The specific objectives of this study were to (i) determine and compare the critical thermal end points of selected aquatic macroinvertebrates with the hypothesis that, organisms from the same or similar habitat would exhibit similar thermal tolerances, (ii) assess their behavioral responses to elevated water temperatures in order to identify species sensitivity and suitability as sentinel organisms for biomonitoring and (iii) rank the species for thermal sensitivity based on the South African Scoring System (SASS).

2. Material and Methods

2.1 Study site

The study was conducted on the upper reaches of the Eerste River (33° 59'.625" S, 18° 58'.533" E), from April to May 2017 (Figure 1). This first through second-order stream originates in the Jonkershoek Mountains in the Western Cape of South Africa and covers an estimated area of 420 km2, extending about 40 km long. The source of the river lies 60 km east of Cape Town at an altitude of 530 m, from where it flows in a north-westerly direction towards the town of Stellenbosch. At this juncture, the river takes an abrupt turn, flowing southwards towards the Atlantic Ocean in False Bay. Stream water temperature at the time of collection varied between 11 and 23°C, with an average temperature of 17°C. Annual rainfall within the study area can range from 3000 mm in the upper reaches to around 570 mm in the lower reaches of the stream [22]. About 80% of this precipitation falls in winter with only 7% of the annual rain occurring between December and March [23].

2.2 Test organisms

Data collection was restricted to a single site in the Hottentots Holland Nature Reserve, south-western cape (Figure 1). The selection of test organisms was based on their availability at the site and our ability to maintain them in the laboratory. For test purposes, we aimed for a single species within each family, with ten species used. Organisms were collected using a 30 × 30 cm d-net of 1 mm diameter mesh held below individual stones and the rocks, which were scuffed to dislodge organisms. The collected material was placed in collecting buckets stored on crushed ice to maintain low water temperatures and subsequently transported to a freshwater aquarium. Invertebrates were sorted in the laboratory and placed in PVC containers. Organisms were housed in separate aerated PVC holding buckets filled with stream water in a constant environment with room temperature set at 17 °C. This temperature was selected as it was the average ambient stream temperature in the study area. Large volumes (50 litters) of stream water were collected for indoor experiments.

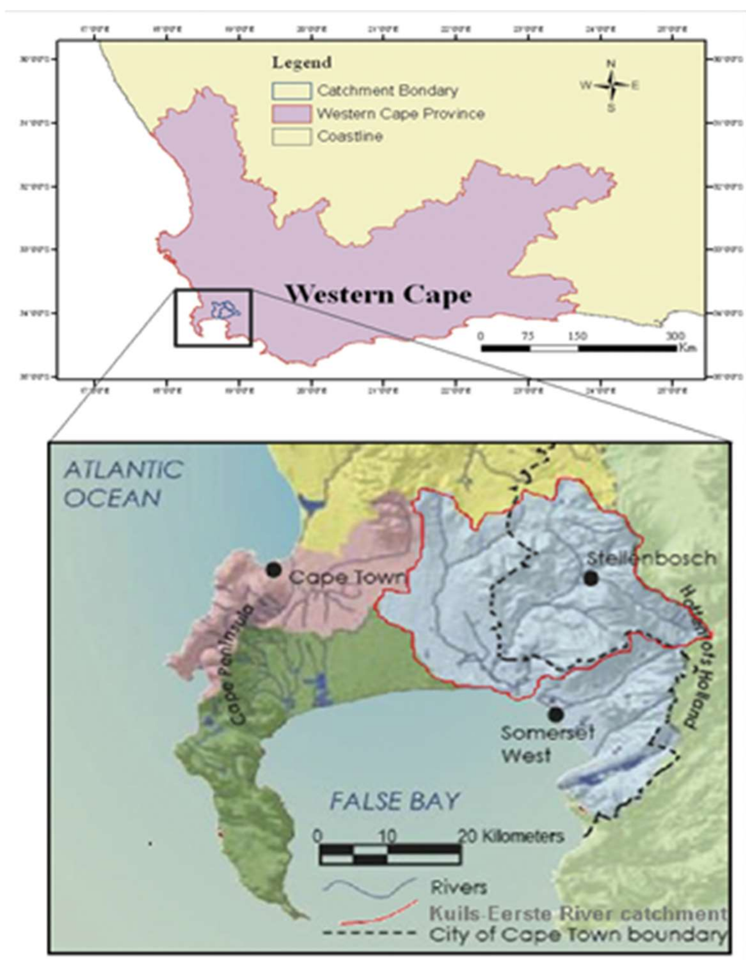

Figure 1: Map of the Western Cape depicting the Eerste River Catchment in the Southern Western Cape region. (Source: Thomas et al., 2010).

2.3 Experimental Procedure

Organisms were identified to family, genus or species level where possible. Note that "species" represents all test taxa, even though not all organisms were identified as species using the identification key guides. Individuals of similar size were used as younger and older instars might have different thermal tolerances. A total of 30 individuals were used for each experimental trial (except for Leptophlebiidae, which comprised two species and only seventeen (17) individuals of the Castanophlebiidae species were used in the analyses). For most cases, experiments were run over two trials, with most individuals collected during the first trip. However, where insufficient individuals were collected, they were combined with individuals collected during the second sampling trip. All tests were conducted in a circulating water bath (dimensions l × w × h: 790 × 345 × 260 mm), which was covered in meshwork to restrict the movement of test organisms whilst ensuring free flow with a homogenous mixing of water. An immersion circulator (Julabo Mc) maintained the water bath at a constant temperature, heating and continually mixing the water in the bath. During the experiment, water was heated at 0.34 °C per minute, as recommended by Ernst et al, 1984[24].

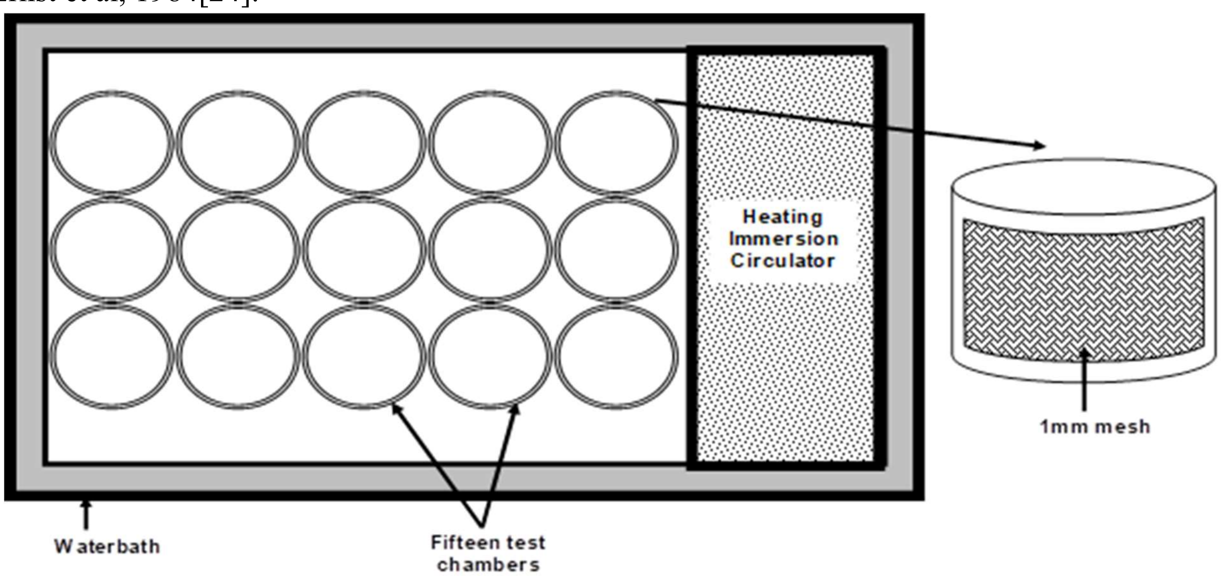

Figure 2: An experimental water bath was used during the CTM trial tests.

2.4 Critical Thermal Maxima Trials

The study deployed an internationally renowned protocol by (Helen F Dallas and Rivers-Moore 2012)[5] to determine their behavioural responses associated with Point of Thermal Discomfort (PTD) and Critical Thermal Maxima endpoints (CTmax). During each trial, thirty individuals' insects were placed in a honeycomb in order to restrict movement and to create more laminar flow. During each ramp, we monitored each test species continuously and recorded the onset of two events (i) Point of Thermal Discomfort (PTD) and (ii) Critical Thermal Maxima endpoints

(CTmax). The CTmax was measured over a minimum of 60 minutes at an increasing rate of (0.34 °C per minute). For the Point of Thermal Discomfort, we monitored and noted the physical behavioural responses in organisms and established behaviour indicator for thermal stress. These behaviours are listed in Table 1. For thermal tolerance, we generally used the loss of grasp, equilibrium, hold, and body bending to measure Critical Thermal maxima (CTmax); see the detailed report in (Table 1) on each taxon. This was used to identify the point of locomotory disorganisation for different taxa following an increase in water temperature.

Individual behavioural activity responses were observed and recorded in terms of occurrence after every two minutes. There were three distinctive experimental phases over a continuous increase in temperature. Phase one: Before increasing water temperature, test species were held at (17 °C for 30 minutes). This phase was a control that enabled us to assess behavioural changes without elevated water temperature. Phase two (2) and three (3): Temperature was increased at a rate of (0.34 °C per minute) until all organisms had reached the CTmax endpoint. It was between the two phases (2 & 3) where behavioural responses associated with Point of Thermal Discomfort (PTD) were observed and noted. Test species were removed when they exhibited the behaviour associated with their critical endpoint, and the CTmax and time were recorded using a separate data sheet, which allowed for listing of behavioural responses per taxon. Removed organisms were placed in aerated recovery containers filled with stream water at (17 °C). All recovered test organisms were later preserved in 70% ethanol for identification purposes.

Family	**Species/Genus**	**PTD Criteria**	**CTM Criteria**
1. Notonemouridae	*Aphanicerca sp.*	Abdominal lift, inactive swimming	Loss of grip, righting, and immobile for five seconds.
2. Baetidae	*Demoreptus capensis*	Rapid swimming and body lift (stilting).	Loss of grip, righting, immobile for five seconds.
3. Leptophlebiidae	*Castanophlebia sp.*	High swimming frequency, walking backwards, and gill movements	Loss of grip, hold and righting and immobile for five seconds.
4. Aeshnidae	*Aeschna minuscule*	Frequent walking and constant swimming, especially near the surface	Loss of grip, hold and immobile for five seconds.

5.	Corydalidae	Corydalidae	Rapid swimming, face cleaning, ventral spreading of gills, and bubble formation on their heads.	Loss of grip, hold and curl in for five seconds without moving.
6.	Hydropsychidae	*Cheumatopsyche sp.*	Frequent swimming, rapid walking.	Loss of grip, righting, and immobile for five seconds.
7.	Philopotamidae	*Chimarra sp.*	Frequent head lifts, constant swimming, and walking.	Loss of hold, grip and immobile for five seconds.
8.	Elmidae	*Stenelmis sp.*	Frequent walking	Loss of grip, hold and righting, and immobile for five seconds.
9.	Athericidae	Athericids	Rapid gliding.	Immobile for five seconds.
10.	Simuliidae	Simuliidae	Frequent flips of antennae, body bending and somersaulting.	Cease in antennae flips, loss of grip and lie on the side for five seconds.

Table 1: Different criteria used to determine the Point of Thermal Discomfort (PTD) and the Critical Thermal Maxima (CTM) for all test organisms.

2.5 Statistical Analysis

All CTmax data were tested for normality using the Kolmogorov–Sminorv test in Statistica Version 9 for Windows and found to be largely non-normally distributed. The non-parametric Kruskal-Wallis test by ranks method was thus used to test the hypothesis that CTmax values are the same among the test species, with the significance level set at p=0.05. In cases where differences in CTmax values reached significance ($p<0.05$), a post-hoc multiple comparison analysis (Dunn's test) was conducted for all groups to identify specific significant relationships between the test species.

3. Results

3.1 Behavioral Activities of all test organisms

A total of 287 aquatic macroinvertebrates species, representing 10 different families were examined for CTmax experiments. Their behavioral activities during the CTM trials are depicted in Table 2. The behavioural activities of each species are described in three phases of the experiment: (1) Initial phase (17–24 °C), (2) Point of Thermal Discomfort (PTD) (24–32 °C) and (3) Critical Thermal Endpoint (CTE) (>32 °C). Activities included walking, swimming, and sitting, as well as behaviors specific to a particular test species such as "Face cleaning" in Corydalidae, "Stilting" in Baetidae, “rapid gliding" in Athericids, somersaulting in Simuliidae species and gill movement in Leptophlebiidae species.

3.2 Suitability of test organisms as bio-indicator species

The thermal tolerance of all test species in this study was based on species whose behaviour responses associated with their median CTmax endpoints was easy to define (Table 2). The most suitable test organisms for CTM experiments were; Aeschina minuscule and Corydalidae sp. The most suitable species with limitations were; Aphanicerca sp, Castanophlebia, Cheumatopsyche sp and Chimarra sp. Aeschna while Demoreptus capensis, was found unsuitable for CTM experiments. However, the critical endpoint of two species in our study (i.e. Elmidae and Simuliidae) was very difficult to determine and they were therefore not considered to be suitable test organisms for thermal tolerance experiments. The third species (Athericidae) had a very wide range of CTMax.

Taxon	**Behavioral responses**	**TSR**	**SR**
Notonemouridae *(Aphanicerca sp)*	During the initial phase (control), most individuals were slightly swimming up and down or clung to the mesh of the testing chambers. At PTD, an increase in swimming and abdominal lift was observed. At CTE, most individuals lost their grip and sank to the bottom.	1	2
Baetidae: *Demoreptus capensis*	During the initial phase (control), most individuals were clung to the mesh of the test chambers. At PTD, a rapid increase in surface swimming and body stilting was observed. At CTE, most individuals lost their grip, sank to the bottom of the chambers and became inactive.	2	3
Leptophlebiidae (*Castanophlebia*)	During the initial phase (control), most individuals were either walking or swimming. At PTD, most individuals started to walk backwards while constantly flipping their gills. At CTE, individuals either sink to the bottom of the chamber or become inactive.	2	2
Aeshnidae (*Aeschna minuscule*)	During the initial phase (control), most individuals sat or walked at the bottom of the testing chambers. At PTD, a rapid increase in walking and swimming activities was observed. At CTE, individuals swam and sank to the bottom of the chamber.	3	1
Corydalidae	During the initial phase (control), most individuals were walking at the bottom of the testing chambers. At PTD, a	3	1

	peak in both walking and face-cleaning activities was observed. At CTE, individuals stopped moving and became inactive.		
Hydropsychidae (*Cheumatopsyche*) *sp:*	During the initial phase (control), most individuals were either swimming or clung to the mesh of the testing chambers. At PTD, there was an increase in swimming. At CTE, most individuals sank to the bottom or remained clinging to the mesh with little or no movement observed.	2	2
Philopotamidae (*Chimarra sp*)	During the initial phase (control), individuals were attached to the bottom or sides of the chamber or floating on the water's surface. At PTD, an increase in swimming individual and head lifting was observed. At CTE, most individuals lose their grip on the bottom or side of the chamber and float to the surface or remain immobile on the bottom.	1	2
Elmidae (*Stenelmis sp*)	During the initial phase (control), most individuals sat on the bottom or clinging to the chamber mesh. At PTD, rapid swimming and walking were observed. At CTE, most individuals lose their grip, turn upside down and become inactive.	NA	NA
Athericidae	During the first phase, most individuals were either sitting or walking. At PTD, rapid swimming and gliding activities were observed. At CTE, most individuals lose their grip and become immobile.	NA	NA
Simuliidae	During the first phase, individuals clung to the mesh sides of the chamber. At PTD, individuals began to swim around or somersault at the bottom of the testing chambers. At CTE, most individuals lost their grip and sank to the bottom of the chambers.	NA	NA

Table 2: Behavioral responses of a subset of families based on Suitability Rank (SR: 1 = highly suitable; 2 = suitable but with limitations; 3 = unsuitable) and Thermal Sensitivity Ranks according to the South African Scoring System (SASS). (TSR: 1 = highly thermally sensitive; 2 = moderately thermally sensitive; 3 = not sensitive). Experimental mental phases: (1) Initial phase (17 - 24 °C); (2) Point of Thermal Discomfort (PTD) (25 - 32°C); and (3) Critical Thermal Endpoint (CTE) (>33 °C).

3.3 Critical Thermal Maxima Tests

The Critical Thermal Maxima temperature values (CTMax) of all test species are summarised in (Table 3) and graphically displayed in (Figure 3). The species' thermal tolerance in this study was based on species whose behavioral responses associated with their median CTmax endpoints were easy to define. Critical thermal maxima values ranged between 31.9 ± 0.6 and 40.8 ± 1.1 °C (Table 2). The most thermally sensitive species based on their suitability as test organisms for Critical Thermal Maxima studies were the Notonemouridae; Aphanicerca sp (32.3±1.2) and Philopotamidae; Chimarra sp (33.7±2.8). Moderately tolerant test species were the Hydropsychidae; Cheumatopsyche sp (35.7±2.3), Leptophlebiidae; Castanophlebia (36.9 ± 1.5) and Baetidae; Demoreptus capensis (37.1± 1.3) while the most tolerant species were the Corydalidae (37.4±1.1) and Aeshnidae; Aeschna minuscule sp (39.6 ±1.0) respectively. The Kruskal Wallis test revealed significant differences in the median CTmax values among the test species as depicted in Table 4 ($x2 = 167.19$, $p < 0.01$, $df = 9$). The results of a multiple comparison of median ranks of all groups show significant relationships between corresponding tested organisms in Table 4. This shows specific species that were significantly different from each other.

Family	**Genus/species**	**n**	**Median**	**Mean**	**SD.**	**MinX**	**MaxX**
Simuliidae	Simuliidae	30	31.9	32	1	31	33.3
Notonemouridae	*Aphanicerca sp.*	30	32.3	32.2	1	29.6	34.2
Philopotamidae	*Chimarra sp.*	30	33.7	33.6	3	29.1	37.4
Hydropsychidae	*Cheumatopsyche sp.*	30	35.7	34.6	2	29.4	37
Baetidae	*Demoreptus capensis*	30	37.1	36.8	1	33.6	38.5
Leptophlebiidae	*Castanophlebia sp.*	17	36.9	36.8	2	33.5	38.9
Corydalidae	Corydalidae	30	37.4	37.3	1	34.6	39.7
Aeshnidae	*Aeschna minuscule*	30	39.6	39.6	1	36.9	39.7
Athericidae	Athericidae	30	40.5	40.5	5	28.6	44.8
Elmidae	*Stenelmis sp.*	30	40.8	41.2	1	38.1	42.2

Table 3: Descriptive statistics of tested species Critical Thermal Maxima (CTmax) ranked from the lowest to the highest.

	Notonemouridae	Baetidae	Leptophlebiidae	Aeshnidae	Corydalidae	Hydropsychidae	Philopotamidae	Elmidae	Athericidae	Simuliidae
		p < 0.01	p < 0.01	p < 0.01	p < 0.01	NS	NS	p < 0.01	p < 0.01	NS
Baetidae	p < 0.01		NS	NS	NS	NS	p < 0.01	p < 0.01	NS	p < 0.01
Leptophlebiidae	p < 0.01	NS		NS	NS	NS	NS	p < 0.01	NS	p < 0.01

Aeshnidae	p < 0.01	NS	NS		NS	P < 0.01	p < 0.01	NS	NS	p < 0.01
Corydalidae	p < 0.01	NS	NS	NS		NS	p < 0.01	p < 0.01	NS	p < 0.01
Hydropsychidae	NS	NS	NS	p < 0.01	NS		NS	p < 0.01	p < 0.01	NS
Philopotamidae	NS	p < 0.01	NS	p < 0.01	p < 0.01	NS		p < 0.01	p < 0.01	NS
Elmidae	p < 0.01	p < 0.01	p < 0.01	NS	p < 0.01	P < 0.01	p < 0.01		NS	p < 0.01
Athericidae	p < 0.01	NS	NS	NS	NS	P < 0.01	p < 0.01	NS		p < 0.01
Simuliidae	NS	p < 0.01	p < 0.01	p < 0.01	p < 0.01	NS	NS	p < 0.01	p < 0.01	

Table 4: Results from multiple comparisons of mean ranks for all groups test for significant relationship between tested insect species during the CTM tests. NS = denotes None Significant results.

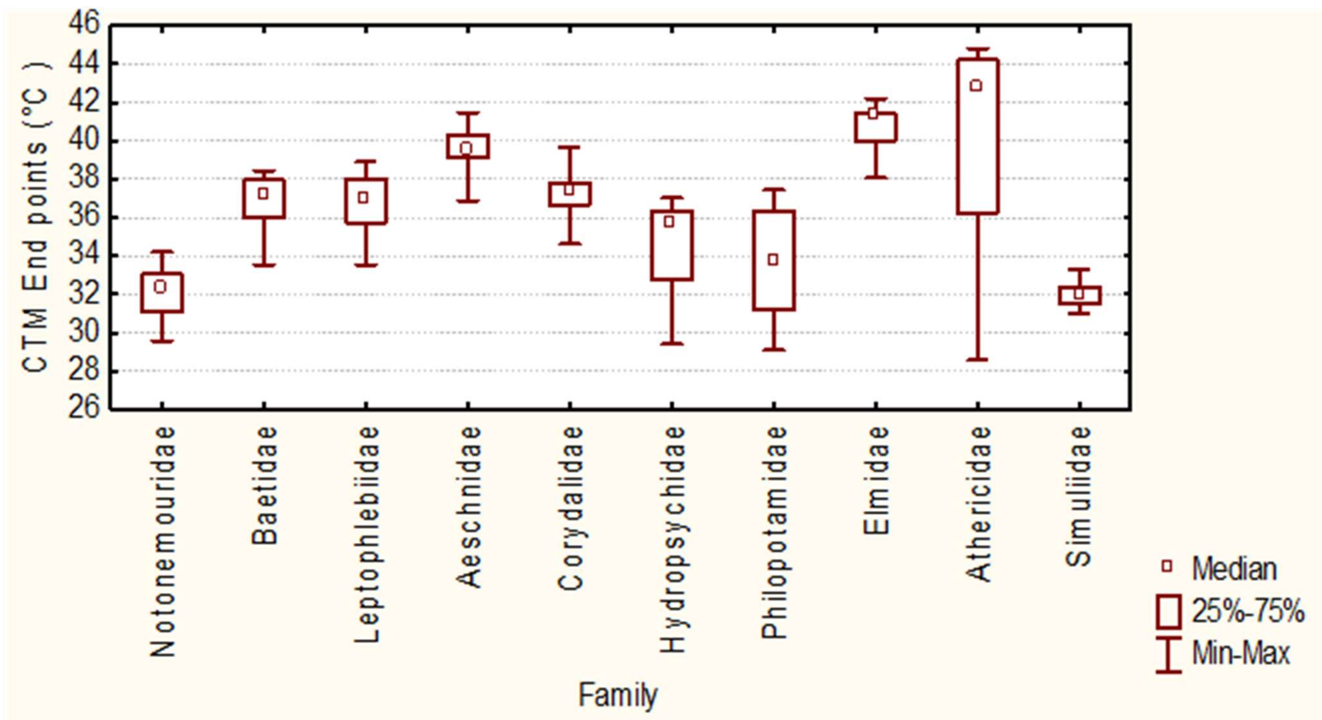

Figure 3: Median (25% - 75% interval, minimum and maximum) temperature for each invertebrate family during the CTM experiments

4. Discussions

The current study has facilitated the identification of potential bioindicators of thermal alteration, which are both thermally sensitive and experimentally suitable using the Critical Thermal Method. A 30-minute acclimation period during the Critical Thermal Maxima trials assumed that the physiological processes were adjusted to a common baseline for all tested species. Based on these assumptions, all observed responses to thermal increase in this study reflected specific genetic differences and not differences due to previous thermal experiences of individual organisms. Despite the paucity of regional studies on CTM for precise comparisons, a general comparison was compelled to similar studies that were conducted elsewhere in temperate regions [24-26].

4.1 Behavioral Activities of all test organisms

Behavioral activity frequencies of our test species tended to increase with an increase in temperature, with maximum performance being reached at the intermediate temperature range (Point of Thermal Discomfort; 24–32 °C) and then decreased at a higher temperature range (Critical Thermal Endpoint; >32 °C). Considerable reflex responses included the test organism's physical structure movements, such as abdominal lift, face cleaning, walking, and gliding, depending on the test species. As the water temperature approached the CTMax endpoint, all test species were initially super active. They dashed around, after which they calmed down on the bottom or remained in suspension, followed by losing their equilibrium. An exception to this pattern was observed in (Elmidae, Simuliidae and Athericidae) whose behavioural interpretation could not be achieved with confidence. Based on these observations, we emphasize the importance of specific criteria in choosing suitable species for future CTM studies. This requires organisms which are easy to observe and exhibit identifiable behavior patterns for easy ecological interpretations.

4.2 Critical Thermal Maxima (CTMax) endpoints by test species

Our experimental investigation assumed that organisms from the same or similar habitat would exhibit similar thermal tolerances. However, our CMax results revealed significant differences in the CTmax values among the test species and this substantially disapproves the hypothesis of this study. Three of our test species (i.e. Elmidae, Simuliidae and Athericidae) were neither considered as thermally sensitive, moderately nor tolerant species, due to lack of discernible behavioural responses during the CTM experiment. Nonetheless, a general discussion on their attained CTmax value is provided below with the rest of all other tested species. As a result, the most thermally sensitive test insects were the two Plecopterans; Aphanicerca and Chimarra species while the Demoreptus capensis, Castanophlebia and Cheumatopsyche were moderately sensitive. Corydalidae and Aeschna minuscule were ranked as thermally tolerant species. Similarly, (H.

Dallas 2008)[27] supports these findings, confirming that the most thermally sensitive groups are Plecopteran, Trichopteran and Ephemeropteran.

4.3 Thermally sensitive test species

The stonefly, Aphanicerca (Notonemouridae: Plecoptera), was found to be the most sensitive test insect species to heated waters by yielding a lower CTmax value of 32.3 °C. The defined critical endpoint of this species was the point at which most individuals lost grip on the meshwork and sank to the bottom of the water bath. The upper thermal limit of the Aphanicerca (32.3 °C) was comparable to 30.3°C that was reported by Dallas and Ketley (2011)[14] but slightly higher than the 29.3 °C rating for Aphanicercopsis tabularis reported by Dallas and Rivers-Moore (2012)[5]. We assume that the difference in CTmax value could be related to different test species under investigation since different species will differently acclimate to stream ambient temperature depending on their life histories [5]. Several studies [24, 25, 26, 28] have shown that acclimation temperature affects CTmax. Similarly, (Moulton et al. 1993)[26] noted that CTmax was highly significantly related to acclimation temperature among philopotamid caddisfly, Chiamarra obscura, and the hydropsychid caddisfly, Hydropsyche simulans. Alternatively, such differences in CTMax values can be associated to their longer, semivoltine life cycle and exposure to greater range of environmental temperature range. Hence, we propose that the relationship between semivoltine life cycles and CTmax needs to be closely examined to clarify these assumptions. According to the South African Scoring System (SASS), Notonemouridae is rated as a sensitive species [30], and this is consistent with the current ranking of 1 (sensitive) in this study. Thus, Aphanicerca are valuable indicators of water quality, and their disappearance from a stream may indicate that thermal or chemical pollution has occurred.

Chimarra species (Philopotamidae; Trichoptera) was the second most sensitive species in this study and attained a median CTmax value of 33.7 °C. The defined critical endpoint of this species was the point at which most individuals lost grip of the chamber and floated to the surface or remained immobile in the water column. Previous investigations had set the critical thermal maxima for various Trichopteran species at a range between 33.6°C and 36°C respectively [27]. The assigned CTmax values of 33.7°C for Chimarra species in this study falls within this threshold. Trichopteran are generally confined to cool rheophilic waters that contain high levels of dissolved oxygen [31] and has been reported that, the upper thermal limits of many aquatic ectotherms appear to be affected by oxygen limitation [16,32,33]. A rise in temperatures typically stimulate metabolic demand for oxygen more than they increase rates of oxygen supply from the environment. This may explain the sensitivity of Chimarra species to elevated water temperatures in this study. According to SASS, Chimarra species are thermally sensitive [30] and this affirms the assigned rank of 1 (sensitive species) in the present study.

4.4 Moderately sensitive test species

The Cheumatopsyche species (Trichoptera: Hydropsychidae) was among the moderately tolerant species in this study and was rated with a CTmax value of 35.7 °C. Hydropschid larvae taxonomy identification to species level can be challenging, and most thermal tolerance research on this species has been conducted on organisms identified to genus level [34]. The defined critical endpoint of this species was the point at which most individuals lost their grip on the testing chambers, and sank to the bottom or instead remained clinging to the mesh with little or no movement observed. As indicated earlier, previous reports have ranked the critical thermal maxima for various Trichopteran species at a range between 33.6 and 36.0 °C [27], and these values approximates the current CTmax value of 35.7 °C for Cheumatopsyche species in this study. However, the upper thermal limit of 35.7 °C for Cheumatopsyche species, acclimated to 17°C and heated at a rate of 0.34°C/min) is slightly higher than the CTmax of 34.5 °C for Hydropsyche Simulans that was acclimated to 13.5°C and heated at a rate of 2.9°C/min [35]. Evidently, one potential source of variation in thermal tolerance between this study and that of [35] may be related to the rate of change in temperature. For instance, Buchanan, Stewart, and Davies (1988)[36] recorded a CTmax value of 34.1°C for amphipods (Paramelita nigroculus) from Skeleton Gorge, south-western Cape, South Africa that was acclimated to 13.5°C and heated at a rate of 1.4°C/min, compared to the CTmax of 29.8°C when acclimated to 17°C, at a heating rate of 0.34°C/min (this study). Similarly, Terblanche et al. (2007)[37] demonstrated that the rates of temperature change, together with acclimation temperature, may affect the CTmax. Generally, it is clearly justified that, Hydropschid caddisflies are tolerant of many forms of pollution, including thermal pollution. According to SASS, the Philopotamidae species is rated as a moderately sensitive species [30] approximating the assigned rank of 2 (moderately sensitive) in this study.

Our Ctmax results for two mayflies, Castanophlebia (36.9 °C) and Demoreptus capensis (37.1 °C), were relatively similar and we assume that a shared physiological constraint may dictate their upper thermal boundary of their CTMax endpoints. The defined critical endpoint of these species was the point at which most individuals lost grip on the meshwork and remained immobile for at least five seconds. The current CTmax values for both species are comparably higher than the CTmax value of 32.5 °C for Castanophlebia and 32.2 ° C for Demoreptus capensis from the Skeleton Gorge River (Helen Fiona Dallas 2009)[38]. These variations may be linked to variable stream ambient temperatures. Several studies have established that CTmax values based on similar species from different aquatic ecosystems that are characterized with different stream ambient temperatures may affect the Ctmax results of test organisms. Buchanan, Stewart, and Davies (1988)[36] recorded a Ctmax value of 34.1 °C for amphipods (Paramelita nigroculus) from Skeleton Gorge that was acclimated to a stream ambient temperature of 13.5 °C, compared to the CTmax value of 29.8 °C for the same species of the Eerste River that was acclimated to 17°C (Dallas, 2008)[27]. Conversely, Dallas (2009)[38] reported a significant difference in CTmax of L. penicillata that was sampled from different sites, (i.e. 33.2°C for Window Gorge, compared to 30.8°C for Wolwekloof and 31.6°C for Rooielskloof, in South Africa). Thus, the disparity in CTmax values of Castanophlebia and Demoreptus capensis between this study and that of Dallas

(2009)[38] may be related to differences in stream ambient temperatures. According to SASS, Leptophlebiidae is rated as a moderately tolerant species [30] and this is in agreement with the current rating of 5 (moderately tolerant) in this study.

4.5 Thermally tolerant test species

The Aeschna minuscule (Odonata: Aeshnidae) was the most tolerant insect species to heated water in this study and showed higher mean CTmax values of 39.6 °C. The defined critical endpoint of this species was the point at which most individuals swam frequently and sank to the bottom of the testing chambers. The reported lethal temperature of 32.5 °C for Aeshnidae [38] is relatively lower than the current CTMax value of 39.6 °C in this study. Such variation may be aligned to differences in species investigated to the latter species, or it could result from different experimental techniques employed by this study to previous lethal studies. For instance, Dallas (2009)[38] used the lethal method (LT50) whereas the current study employed the non-lethal CTmax technique. The upper lethal method are longer term experiments, whereas the CTM are short-term acute experiments and this is likely to affect the CTmax values for test organisms. Heiman and Knight (1975)[7], argued that studies utilising death (i.e. LT50) as an endpoint have little ecological significance and that a slight variation in the physical environment would probably induce chronic effects on the population. According to SASS, Aeshnidae is rated as a tolerant species (Dickens and Graham 2002)[30], and this confirms the assigned ranking of 3 (tolerant) in this study.

Corydalids species (Megaloptera: Corydalidae) was also found to be among the most tolerant species to heated water and revealed an upper CTmax value of 37.4 °C. The defined critical endpoint of this species was the point at which most individuals stopped moving and became inactive. The most critical aspect of this species were its extensive observable features and efficiency in displaying distinctive responses in its behavioral responses during the CTM experiments, making it an exceptional test organism for CTM studies. Dallas and Rivers-Moore (2012)[5] conversely ranked this species as the least sensitive test organisms among the 17 groups of macroinvertebrates from several selected water bodies in South Africa. The larval stage of this species can last up to two years. It is assumed that, the longer the exposure of this species to variable stream water temperature, the more it becomes genetically adapted to these harsh conditions [38]. According to SASS, Corydalidae species is moderately tolerant to pollution [30] and this confirms the assigned ranking of 3 (tolerant) in this study.

4.6 Non-desirable species for Critical Thermal Methods

Simuliids species showed the lowest mean CTmax value of 31.9 °C, but no considerations were made regarding its thermal tolerances due to a lack of discernable behavioural responses. This species has limited locomotory powers and relies on flowing waters to obtain food and maintain its desired oxygen levels [31]. Thus, Simuliid is not a suitable test organism for CTM studies. In addition to these limitations, there are no previous thermal tolerance studies on the CTM of this species in South Africa prior to this investigation. Comparisons of these results are inferred to closely related species based on field observations. For instance, a closely related species; Samarium ruficorne, has been recorded in small water trickles with an estimated CTmax of 35 °C [31]. This value is higher than the reported CTmax value of 31.9 °C in this study and these variations could be subjective to different target species by this study and that of [31].

Stenelmis species (Coleoptera: Elmidae) showed a mean CTmax value of 41.2 °C. No thermal tolerance considerations were made on this species prior to this study. Among other reasons, their small-sized structures were a major constraint for precise physical observations. The lack of adequate species identification makes comparison of CTmax values difficult to closely related species, making it a non-desirable species for CTM studies. Nonetheless, the current CTmax value of 41.2 °C falls within the reported CTmax range between 34 and 45 °C for Coleopterans [39].

The Athericids species (Diptera: Athericidae) showed the highest CTMax value of 42.8 °C. However, there was no thermal tolerance considerations made on this species owing to a lack of discernible responsive behaviour to elevated water temperatures. The lack of adequate identification features of this species made comparison with other related species more difficult. Hence, Athericids are not suitable test species for CTM studies. In a broad sense, upper lethal limit for this species was rated at 29.09 °C by Dallas (2008)[27]. This is relatively lower than the current CTmax value of 42.8 °C in this study. These variations may be ascribed to different experimental approaches between studies [34]. Dallas (2008)[27] used the lethal method (LT50) whereas the current study employed the non-lethal CTmax as reported for Aeschna minuscule and this is likely to result in the disparity in the CTmax values for test organisms.

4.7 Implications for Climate change on aquatic organisms

The African continent is highly vulnerable to climate change and the general consensus emerging from modeling suggests an increase of 0.3-0.6° C per decade [40]. As a result, the winter rainfall in the Western Cape is correspondingly predicted to decrease by 25% [27]. In this context, the Eerste River Catchment is expected to experience a significant increase in evapotranspiration over the next century. The combination of rising air temperatures combined with decreasing rainfall is likely to lead to elevated water temperatures [27]. This is likely to affect the most thermally sensitive aquatic insect species such as Chimarra and Aphanicerca. This is exacerbated by a minor difference of 8 °C between the maximum stream temperature (24 °C) and the mean CTmax value (32.3 °C) of the most thermally sensitive test species in the study area. These observations suggests that, Aphanicerca species will be affected by a slight increase in water temperature and less likely to survive any rise in temperature above the CTmax value of 32.3 °C. Tolerant species such as A. minuscule will be far better and be able to persist such an increase in stream temperature and resist any slight increase in water temperature, which is likely to affect their counterparts in the same habitat. However, the observed narrow range of CTMax endpoints (31.9±0.6 - 40.8±1.1) among our test species implies that a similar threshold increase in temperature is likely to hamper the population of these organisms. This is quite risky in the sense that most species populations are likely to be exterminated once exposed to water temperatures above their CTmax limits.

5. Conclusions

Thermal adaptation is a key facet safeguarding organismal function among ectothermic organisms. Understanding macroinvertebrate communities' responses in a rapidly changing environment can serve as an early warning of thermal water pollution. The study revealed two thermally sensitive species (Chimarra and Aphanicerca) in the Eerste River. These species are likely to be exterminated due to thermal stress. Hence, Chimarra and Aphanicerca species should be declared as valuable indicators of water quality, and their disappearance from an aquatic system may indicate that thermal or chemical pollution has occurred. Thus, there is a need to monitor and regulate any human stream activities that are likely to compromise the community structure and function of aquatic invertebrates. Macroinvertebrates are ecologically important and their decline will negatively affect trophic structures. We therefore propose increased efforts and commitment to the longer-term assessment of climate change effects on stream water organisms. There is a need to explore research work focused on adaptation and mitigation measures of climate change to save the biodiversity of Chimarra and Aphanicerca. Proactive management efforts will halt any ecosystem risks and may be less costly than reactive efforts.

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