

THE EFFECTS OF THREE ORGANIC CHEMICALS ON THE UPPER THERMAL TOLERANCES OF FOUR FRESHWATER FISHES

RONALD W. PATRA,*†‡§ JOHN C. CHAPMAN,†§ RICHARD P. LIM,‡§ and PETER C. GEHRKE||

†Department of Environment and Conservation, Lidcombe 1825, New South Wales, Australia

‡Department of Environmental Sciences, University of Technology, Sydney, New South Wales 2007, Australia

§Department of Environment and Conservation & University of Technology, Sydney Centre for Ecotoxicology, PO Box 29, Lidcombe 1825, New South Wales, Australia

||Commonwealth Scientific and Industrial Research Organisation, Division of Land and Water, Indooroopilly, Queensland 4068, Australia

(Received 30 March 2006; Accepted 26 January 2007)

Abstract—The upper temperature tolerance limits of four freshwater fish species, silver perch *Bidyanus bidyanus*, eastern rainbowfish *Melanotaenia duboulayi*, western carp gudgeon *Hypseleotris klunzingeri*, and rainbow trout *Oncorhynchus mykiss*, were determined using the critical thermal maximum (CTMaximum) method. The CTMaximum tests were carried out with unexposed fish and fish exposed to sublethal concentrations of endosulfan, chlorpyrifos, and phenol to determine whether or not the CTMaximum was affected. The CTMaximum temperature of *B. bidyanus* decreased by 2.8, 3.8, and 0.3°C on exposure to endosulfan, chlorpyrifos, and phenol, respectively. Similarly, in *M. duboulayi*, the CTMaximum was decreased by 4.1, 2.5, and 0°C, while in *H. klunzingeri* it decreased by 3.1, 4.3, and 0.1°C, respectively, and in *O. mykiss* by 4.8, 5.9, and 0.7°C, respectively. Exposure to sublethal test concentrations of endosulfan and chlorpyrifos caused significant ($p \leq 0.0001$) reductions in CTMaximum values for all fish species compared to that of unexposed fish. However, exposure to phenol did not cause any significant ($p \geq 0.05$) change of CTMaximum temperatures.

Keywords—Critical thermal tolerance Fish Endosulfan Chlorpyrifos Phenol

INTRODUCTION

The toxic effects of chemicals can be influenced by various physicochemical factors including temperature [1,2]. Increase in use and production of toxic chemicals, and the contemporary issue of global warming become subjects of concern for ecologists in obtaining relevant knowledge on the tolerance of organisms to abiotic factors such as temperature. Not only do the chemicals affect temperature tolerance of fishes, but temperature also influences the sensitivity of fish to toxic chemicals [3]. A reciprocal influence of temperature on copper toxicity and the influence of copper on temperature tolerance in fathead minnows were determined by Richards and Beitinger [4]. Exposure to sublethal concentrations of chemicals can cause stresses, which limit an organism's ability to survive or ability to tolerate changes in various environmental factors, such as temperature [5]. Beitinger and McCauley [6] provided a minireview of the effects of toxic chemicals on temperature tolerance, which described the environmental factors that could serve as stressors to organisms. Toxic chemicals can affect the temperature responses of fish in different ways; for example, fish may exhibit a preference for or avoidance of a particular temperature [7] or they may undergo changes in thermal tolerance [8,9]. This study used the critical thermal maximum (CTM) method [10] to determine if the dynamic elevation in temperature changes the thermal tolerances of fish pre-exposed to chemicals.

The CTM test method has been recognized as a measure of thermal tolerance and an indicator of thermal stress in ectothermal animals [11,12]. The term CTM represents both a parameter and a method, and often has been used to define

the upper temperature tolerance limit for various amphibians and reptiles [13–17]. The concept of the CTM method was introduced and defined by Cowles and Bogert [13] was later redefined by Lowe and Vance [14] and amended by Hutchison [15]. Considering all these modifications a more comprehensive definition of CTM was advanced by Cox [10], who states that, “The Critical Thermal Maximum or Minimum is the arithmetic mean of the collective thermal points at which locomotory activity becomes disorganized and the animal loses its ability to escape from conditions that will promptly lead to its death when heated from a previous acclimation temperature at a constant rate just fast enough to allow deep body temperatures to follow environmental temperatures without a significant time lag.” However, Lutterschmidt and Hutchison [18] and Beitinger et al. [19] reported two major reviews of CTM. In the latter review, the authors departed from Becker and Genoway [20] and have chosen to use the designation CTM to refer to the general method (critical thermal method), i.e., exposing animals to dynamic changes in temperature from a pretest acclimation temperature, and the specific terms CTminimum and CTmaximum as the measured sublethal but near lethal endpoints. This was done because the original definitions [10,13] of CTM referred only to heating, and CTM referred to critical thermal maximum. In other words, one cannot use the critical thermal maximum as an estimate of lower temperature tolerance.

Critical thermal maximum has many potential applications, particularly in assessing the interaction of temperature stress and other stressors in the environment. For example, the CTM value is appropriate for determining the relative temperatures for loss of equilibrium and death of fish exposed to various industrial wastes, pesticides, diseases, gas supersaturation, ex-

* To whom correspondence may be addressed
(ronald.patra@environment.nsw.gov.au).

Table 1. Experimental parameters of the critical thermal maximum tests using four fish species and three chemicals. Values in brackets indicate the holding time in days (d) in the treatments and their corresponding controls; * = concentrations are nominal

Particulars	<i>Bidyanus bidyanus</i>	<i>Melanotaenia duboulayi</i>	<i>Hypseleotris klunzingeri</i>	<i>Oncorhynchus mykiss</i>
Fish length (mm) mean \pm standard deviation (SD)	46.3 \pm 8.2	70.2 \pm 9.0	35.5 \pm 3.1	67.2 \pm 7.6
Fish weight (g) mean \pm SD	1.4 \pm 0.7	4.4 \pm 1.4	0.4 \pm 0.1	3.1 \pm 1.0
No. of fish used	50	50	50	50
Acclimation temperature	20°C	20°C	20°C	10°C
Endosulfan concn. ($\mu\text{g/L}^*$)	0.3 [12 d]	1.0 [10 d]	0.8 [10 d]	0.5 [10 d]
Chlorpyrifos concn. ($\mu\text{g/L}^*$)	5.0 [14 d]	5.0 [14 d]	3.5 [14 d]	5.0 [14 d]
Phenol concn. (mg/L*)	5.0 [14 d]	5.0 [14 d]	5.0 [14 d]	5.0 [14 d]

treme pH values, or other suspected sublethal stressors [20]. The CTM method also has an ethical advantage over conventional lethal temperature tests in that the endpoint of the test does not require killing the test animals. The method is economical in terms of test animals, equipment, and the time required to complete sufficient tests to permit statistical treatment and validation [12]. Although the CTM method has not been yet established as a protocol, this method is a useful way of studying the thermal physiology of animals.

The chemicals investigated in this study were two widely used agricultural pesticides, endosulfan and chlorpyrifos, as well as phenol, a common industrial chemical and a component in plant extracts. Endosulfan, an organochlorine pesticide, is a central nervous system poison. Chlorpyrifos, an organophosphorus compound, acts as an acetylcholinesterase inhibitor, altering the behavior of organisms and leading to death [21]. Four fish species dwelling in different habitats in Australia were selected for the tests.

The present study focussed on whether the effects of progressive changes in temperature using the CTMaximum method influenced the upper temperature tolerance limits of fish pre-exposed to sublethal concentrations of the nominated chemicals. The aims of the study were to determine (1) the upper limits of temperature tolerance for four freshwater fish species using the CTMaximum method and (2) whether or not prior exposure to sublethal concentrations of nominated chemicals affects the CTMaximum values of the four species of fish.

MATERIALS AND METHODS

Three of the test fish species are native to Australia, these being the silver perch *B. bidyanus* (Mitchell), the eastern rainbowfish *M. duboulayi* (Castelnau), and the western carp gudgeon *H. klunzingeri* (Ogilby), though the other species, rainbow trout *O. mykiss* (Walbaum), is an introduced species. All test species were juveniles; their mean lengths and weights are given in Table 1. *Bidyanus bidyanus* and *H. klunzingeri* were obtained from the Inland Fisheries Research Station, Narrandera, New South Wales, Australia. *Melanotaenia duboulayi* were cultured at the Centre for Ecotoxicology, University of Technology Sydney, New South Wales, Australia. *Oncorhynchus mykiss* were supplied from Gaden Trout Hatchery, New South Wales Fisheries, Jindabyne, Australia. The chemicals used in this study were technical-grade endosulfan and chlorpyrifos, and analytical reagent-grade phenol. Endosulfan, chlorpyrifos, and phenol were supplied by Hoechst Australia, Dow Elanco Australia, and Rhone Poulinc Laboratory Products, Australia, respectively. Endosulfan and chlorpyrifos are widely used agricultural pesticides, and phenol is a naturally found component in urban and country rainwater in Australia

as a result of leachate from vegetation [22]. Fish maintenance, acclimatization, and CTM tests were carried out in dechlorinated bore water, passed through two sets of filters including an activated carbon filter prior to use. The physicochemical profile of the water for acclimatization and tests was measured regularly and was within the ranges that did not cause any adverse effects to the fish (dissolved oxygen 90–95% saturation, conductivity 600–700 $\mu\text{S cm}^{-1}$, pH 7.5–8.0, hardness 115 mg L^{-1} as CaCO_3 , and ammonia $<1,000 \mu\text{g N L}^{-1}$). The upper temperature tolerance tests were carried out both in the absence and presence of each of the chemicals.

Chemical concentrations used in the present study are presented in Table 1. Measured values or recovery rates of test chemicals can be estimated on the basis of the results obtained from acute tests, conducted simultaneously in glass vessels using the same stock solutions of these chemicals, with the fish species as part of the other aspect of the project [23,24]. Recovery rates after 24 h for endosulfan, chlorpyrifos, and phenol were 73 to 77%, 10 to 15%, and 78 to 85%, respectively [24]. However, the nominal concentrations of the tests chemicals were presented in the result for this paper because each CTM test lasted for <31 min only.

Acclimatization

Before conducting the CTM tests, *B. bidyanus*, *M. duboulayi*, and *H. klunzingeri* were held at 20°C, although *O. mykiss* were held at 10°C and maintained in the dilution water for 10 to 14 d in 20-L glass aquaria (Table 1) as required by the protocol [25,26]. The fish also were held in dilution water in 20-L glass aquaria containing sublethal concentrations of endosulfan, chlorpyrifos, or phenol at the same temperature for a period of 10 to 14 d for the CTM tests. Corresponding controls for each chemical also were maintained at the same temperature for the same period of time (Table 1). Holding temperatures were chosen to reflect their average habitat temperatures [27]. Only one acclimation temperature was used for each species, because the present study was designed to determine whether or not the CTMaximum temperature of fish species not exposed to chemicals differed from that of fish exposed to chemicals. Tank water was renewed daily. Fish during holding and tests were in healthy conditions with regard to food and water quality such as pH, dissolved oxygen, and conductivity [23]. Concentrations of chemicals used in the tests (Table 1) were based on the lethal concentration at 50% values obtained by conducting acute tests over a period of 96 h at various temperatures using *B. bidyanus* [23]. The 96-h lethal concentration at 50% values for endosulfan, chlorpyrifos, and phenol for this fish were $1.3 \pm 0.25 \mu\text{g L}^{-1}$, $17 \pm 6 \mu\text{g L}^{-1}$, and $14 \pm 4 \text{mg L}^{-1}$, respectively. Water quality of the dilution water for the lethal concentration at 50% test was pH 7.7 to 7.9,

conductivity was 792 to 830 $\mu\text{S cm}^{-1}$, and hardness was 115 mg L^{-1} as CaCO_3 .

Test equipment

Twenty-liter glass aquaria, similar to those used for acclimatizing the fish, were used for conducting the CTMaximum tests. The fish were selected randomly and transferred from the acclimation aquarium to the test aquarium using small dip nets. A 220-V, 1000-W Thermomix heater (Paratherm II, Juchheim Labortechnik, Schwarzwald, Germany) was used to elevate the water temperature. The temperature of water in each aquarium was monitored using a digital thermometer (0.01°C scale), which was calibrated against a mercury thermometer and a single channel graphical readout thermometer. A 5-mm mesh plastic screen was placed across the test aquarium to protect the fish from coming into direct contact with heating coils.

Test procedure

The upper temperature tolerances of fish in the absence and presence of chemicals were measured individually using the CTMaximum test method. The methodology for conducting the tests for this study was designed on the basis of the CTM definition suggested by Hutchison [15] and Beitingger et al. [19].

For this study, the CTM endpoint was defined as the temperature at which the fish showed final loss of equilibrium and failed to keep itself in the dorso-ventrally upright position on gentle prodding [28–32]. During the CTM tests, distinctive behaviors in fish in responses to changes in temperature were noted. The transition from behavioral stages of loss of equilibrium to loss of ability to keep itself dorso-ventrally upright was used as the indicator that the CTMaximum had been reached [6].

All the thermal tolerance experiments were conducted by randomly taking 10 batches of five (total 50 individuals) appropriately exposed fish from the selected acclimation aquaria and transferring one fish at a time to the test aquaria after its water had stabilized at the acclimation temperature (i.e., $20 \pm 1^\circ\text{C}$ or $10 \pm 1^\circ\text{C}$). The water in the control aquaria contained no toxicants, although the chlorpyrifos, endosulfan, and phenol treatments contained the same concentration of toxicants used in the acclimation phase (Table 1). The temperature of the water in the test tank then was elevated gradually at a constant rate ($0.8 \pm 0.02^\circ\text{C min}^{-1}$) to determine the critical thermal maximum (CTMax) [10,11]. This rate of temperature change during heating is within the rates ($0.01\text{--}2.0^\circ\text{C min}^{-1}$) used by several other authors [33–35]. The tests were conducted until all the fish in the group reached the test endpoint.

Tests were conducted for each species and at each chemical and replicated over 10 consecutive days at approximately the same time each day in order to minimize the effects of diurnal fluctuations [36]. The lengths and weights of fish were measured after completion of each CTMaximum test. Each fish was tested once only. After reaching the test endpoint, fish were removed immediately from the test aquaria and returned to their acclimation temperature to record subsequent survival. Only CTMaximum data for those batches of fish that had 100% survival after the CTMaximum determination were analyzed statistically. Experimental parameters for the CTMaximum tests are given in Table 1.

The mean CTMaximum temperatures for control and treatments were calculated from the untransformed data of 50 in-

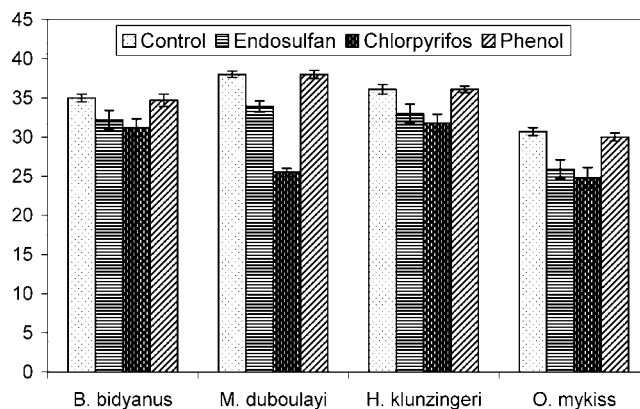


Fig. 1. The critical thermal maximum temperatures of four fish species to control and three chemicals (Sample size = 50; the error bars indicate the \pm standard deviation).

dividual fish tested for each species in each treatment. Statistical significance was tested at $p \leq 0.05$ by one-way analysis of variance (ANOVA) using SYSTAT® [37].

RESULTS

As the temperature increased, the test fish generally went through several behavioral responses as classified by other authors [20,38,39]: Increased opercular movement and swimming activity; rapid erratic swimming followed by quiet periods; continual uncoordinated movement with body quivering, rolling over on the sides or back, and the commencement of gulping; loss of ability to remain dorso-ventrally upright; and floating or resting on its side or upside down with very feeble opercular movement. Early stages in this process are more likely to be effects of heating rather than physiological effects.

These behavioral reactions were demonstrated by three species, but the gudgeons *H. klunzingeri* did not exhibit the second behavioral response and, due to their smaller size, their opercular movements could not be observed clearly. However, other behaviors were prominent in this species.

The highest CTMax in the absence of chemicals was exhibited by *M. duboulayi* ($38.0 \pm 0.4^\circ\text{C}$), followed by *H. klunzingeri* ($36.0 \pm 0.6^\circ\text{C}$) and *B. bidyanus* ($35.0 \pm 0.5^\circ\text{C}$), and then *O. mykiss* ($30.7 \pm 0.5^\circ\text{C}$). Intraspecies variations in CTMax values were small (i.e., \pm standard deviation $\leq 0.7^\circ\text{C}$) in all the species and lowest in the rainbowfish (Fig.1). The mean CTMax for the three native warm water fishes *B. bidyanus*, *M. duboulayi*, and *H. klunzingeri* acclimatized at 20°C and decreased between 2.5°C (6.1%) and 4.2°C (11.7%) when treated with endosulfan and chlorpyrifos (Table 2). One-way ANOVA tests indicated that the mean difference in CTMax between control and treatment for these fishes were statistically significant ($p \leq 0.0001$). Similarly, the mean CTMax for *O. mykiss*, an introduced cold water fish, acclimatized at 10°C and, treated with endosulfan and chlorpyrifos, decreased between 4.8°C (15.6%) and 5.8°C (19.2%; Table 2). One-way ANOVA tests determined that the mean CTMax temperatures were significantly different from their control CTMax values for *O. mykiss* ($p \leq 0.0001$). However, one-way ANOVA tests indicated that in all four fishes the difference in the mean CTMax temperatures between control and phenol treated fish were not statistically significantly different ($p \geq 0.5$; Fig.1 and Table 2).

Table 2. Mean critical thermal maximum (CTMaximum) temperatures of four fish in the absence and presence of chemicals

Treatments	Particulars	<i>Bidyanus bidyanus</i>	<i>Melanotaenia duboulayi</i>	<i>Hypseleotris klunzingeri</i>	<i>Oncorhynchus mykiss</i>
Control	CTMaximum (°C)	35.0	38.0	36.1	30.7
Endosulfan	CTMaximum temperature (°C)	32.2	33.9	33.0	25.9
	Decrease in CTMaximum temperature (°C)	2.8	4.1	3.0	4.8
	% Decrease in CTMaximum temperature (°C)	8	10.8	8.3	15.6
	<i>p</i> =	<0.001	<0.0001	<0.0001	<0.0001
Chlorpyrifos	CTMaximum temperature (°C)	31.2	35.5	31.8	24.8
	Decrease in CTMaximum temperature (°C)	3.8	2.5	4.2	5.8
	% Decrease in CTMaximum temperature (°C)	10.9	6.1	11.7	19.2
	<i>p</i> =	<0.0001	<0.0001	<0.0001	<0.0001
Phenol	CTMaximum temperature (°C)	34.7	38.0	36.1	30.0
	Decrease in CTMaximum temperature (°C)	0.3	0	0	0.7
	% Decrease in CTMaximum temperature (°C)	0.9	0	0	2.3
	<i>p</i> =	>0.5	>0.5	>0.5	>0.5

DISCUSSION

According to the definition of the CTM [7,12], the rate of temperature change must be constant, implying that a progressive linear relationship exists between CTMaximum temperature and resistance time until the loss of equilibrium has occurred. The heating rates in the present study were constant and any deviations were for short periods. Such deviations from linearity have little effect upon the loss of equilibrium endpoint [20].

The behavior of the test species at the CTMaximum were similar to those described by Cheetham et al. [38] for immature channel catfish (*Ictalurus punctatus*), Wattenpaugh and Beiting [39] for fathead minnows (*Pimephales promelas*), Becker and Genoway [20] for coho salmon (*O. kisutch*) and pumpkinseed sunfish (*Lepomis gibbosus*), and Rodriguez et al. [40] for the prawn *Macrobrachium tenellum*.

It is important that all treated test animals survive to determine whether the response of endpoint criteria corresponds to the CTMaximum of the test animals. Almost all fish (99%) survived in the current study. Any data that had deaths were not included in the analyses. In contrast, Rodriguez et al. [40] reported that 53 and 60% of the prawn *M. tenellum* survived CTM determinations when acclimatized at 22 and 25°C, respectively.

Three of the four fish species tested in the current study (*B. bidyanus*, *M. duboulayi*, and *H. klunzingeri*) are native to Australia and live in warm water habitats [27], although *O. mykiss* is a cold water fish introduced to Australia. Results of CTM tests without a toxicant suggest that *M. duboulayi* was most tolerant to higher temperatures, and *H. klunzingeri* and *B. bidyanus* were slightly less tolerant to high temperatures, whereas *O. mykiss* did not tolerate temperatures above 31.0°C. The observed upper thermal tolerance for *B. bidyanus* (35.0 ± 0.5°C) was close to those reported in the literature for this species [41]. The upper CTMaximum of 30.7°C for *O. mykiss* was similar to those reported by various authors [41–43].

However, all aquatic organisms possess their own range of temperature tolerances. These limits of tolerance in the thermal spectrum may be influenced by temperature acclimation but ultimate limitations are fixed genetically [44]. It is apparent from the present study that exposure to toxicants when the organism is near the upper end of its tolerance zone may impose significant additional stress. In CTM, when a fish was acclimated at a particular temperature for a period of time, any change in temperature (within tolerance zone) can lead to a major change in metabolism, cardiovascular respiratory rate,

fluid electrolyte balance, and acid base relationship [45]. However, ectotherms possess some interacting homeostatic systems that act to minimize the deleterious effects of rapid temperature change [45]. Water-breathing animals also act against disruptions of osmotic and ionic balance following moderate or large temperature change [46]. The stress of exposure to a toxicant decreases the ability of a fish to withstand the additional stress of increasing ambient temperature [47].

The results obtained from the present laboratory tests are relevant to many Australian aquatic environments. Many inland rivers in Australia do not flow permanently and consist of a series of pools or billabongs where temperatures can reach up to 40°C in summer [48]. The effects of the intensive use of pesticides on Australia's aquatic ecosystems are of particular concern to water managers and the general public. Intensive agricultural enterprises, such as the cotton industry and fruit production, rely heavily on various chemicals, insecticides, herbicides, conditioners, and defoliants [49]. Concentrations up to 4 µg L⁻¹ of endosulfan [50] and 0.24 µg L⁻¹ of chlorpyrifos and its derivatives [51] have been reported from Australian rivers. This concentration of endosulfan in river waters exceeds the 96-h median lethal concentration values to *B. bidyanus* [24,52]. Endosulfan and chlorpyrifos are commonly used in summer in the cotton growing areas in northern New South Wales and Queensland, Australia, where water temperature often reaches 30°C during the spraying season and 35°C in enclosed waterholes. Therefore, the observed decrease in CTMaximum values of 2 to 5°C caused by sublethal concentrations of some organic chemicals may reduce the ability of fish to survive natural temperature fluctuations. Exposure of wild fish to sublethal concentrations of chemicals in these areas also may limit their ability to survive in high water temperatures.

Results clearly demonstrated that exposure of all four test species to concentrations of endosulfan and chlorpyrifos that did not cause mortality over 10 to 14 d caused significant (*p* < 0.0001) reductions in CTMaximum values, compared to the control values. A fish stressed by sublethal levels of toxicant may have a much lower temperature tolerance. For example, Paladino et al. [12] reported that sublethal doses of arsenic reduced the temperature tolerance of muskellunge larva (*Esox masquinongy*). Similarly, exposure to sublethal concentrations of selenate significantly (*p* < 0.05) decreased the CTMax of *P. promelas* by 5.9°C [39] compared with that of the control. Sublethal copper exposure significantly decreased the thermal tolerance of fantail (*Etheostoma flabellare*) and johnny darters

(*E. nigrum*) [53]. Similar results were reported for bluegill (*L. macrochirus*) exposed to sublethal concentrations of zinc [54] and for juvenile coho salmon (*O. kisutch*) and *O. mykiss* exposed to sublethal levels of nickel [55]. The present study clearly reflects these findings that organic chemicals also could reduce the temperature tolerance of fish.

It has been suggested that the toxic effects of chemicals that act on cellular enzymes involved in energy metabolism, or that cause a change in the rate of uptake of chemicals, likely are increased by temperature rises [56]. At higher temperatures, organisms may be forced to physiologically deal with greater amounts of toxicant because of increased diffusion or more active uptake. This increase in diffusion or uptake, in turn, would induce increased rates of movement of water and solutes across the gill or other cellular membranes [2]. This means that, as metabolism increases, so does chemical uptake. The elevated temperatures, which increased the metabolic rate of fish, also enhance the demand by tissue for oxygen [57]. The reduction in CTM of test fish induced by endosulfan and chlorpyrifos may be explained by a combination of the increasing demand for oxygen and sublethal toxic effects caused by the chemicals. The reduction of CTM temperatures in chemically exposed fish suggests that the rising temperature probably caused an additional alteration in the response mechanisms of the chemically pre-exposed fish, causing it to reach loss of equilibrium (total disorientation) at a significantly lower CTM temperature compared to that of control fish.

Sublethal exposure to phenol had no effect on CTMaximum for the four species because the CTMaximums were not significantly ($p > 0.05$) reduced. Studies using the same four species of similar sizes indicated a trend of decreasing acute toxicity of phenol with increasing temperature up to 30°C [23]. Similar relationships between temperature and toxicity of phenols for *M. duboulayi* [41] and *O. mykiss* [58] have been reported. The rapid temperature increase used in this study for the CTM experiments might have reduced the availability of highly volatile phenol. However, this finding for phenol contrasts with Changon and Hlohowskyj [59], who reported that phenol decreased CTMax in the eastern stoneroller, *Camposotoma anomalum*.

CONCLUSION

Temperature tolerance of fishes is limited by a combination of biotic and abiotic factors [60], including various toxicants [4,6]. The reduction in thermal tolerance of fish in the presence of endosulfan and chlorpyrifos suggest that, not only does temperature influence the sensitivity of fish to a toxic chemical [24,52], but chemical exposure also affects the temperature tolerance of fishes. However, the relationship between temperature and lethality is complex, difficult to predict, and has not been the focus of many studies [4].

Acknowledgement—Funding for this research project was provided by the Australian and New Zealand Environment Conservation Council Trust Fund and the New South Wales Environment Protection Authority (now Department of Environment and Conservation). This work also was supported by the University of Technology, Sydney. The New South Wales Fisheries, Narrandera, provided the facilities for conducting this study. Thanks to R.I.M. Sunderam for comments on a version of this manuscript.

REFERENCES

- Howe GE, Marking LL, Bills TD, Boogaard MA, Mayer FL. 1994. Effects of water temperature on toxicity of 4-nitrophenol and 2,4-dinitrophenol to developing rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 13:79–84.
- Mayer FL, Marking GE, Brecken JA, Linton TK, Bills TD. 1991. Physicochemical factors affecting toxicity: pH, salinity, and temperature. Part 1 Literature Review. EPA 600/X-89/033. U.S. Environmental Protection Agency, Gulf Breeze, FL.
- Heath S, Bennett WA, Kennedy J, Beitinger TL. 1994. Heat and cold tolerance of the fathead minnow, *Pimephales promelas*, exposed to the synthetic pyrethroid cyfluthrin. *Can J Fish Aquat Sci* 51:437–440.
- Richards VL, Beitinger TL. 1995. Reciprocal influences of temperature and copper on survival of fathead minnows, *Pimephales promelas*. *Bull Environ Contam Toxicol* 55:230–236.
- Carrier R, Beitinger TL. 1988. Reduction in thermal tolerance of *Notropis lutrensis* and *Pimephales promelas* exposed to cadmium. *Water Res* 22:511–515.
- Beitinger TL, McCauley RW. 1990. Whole animal physiological process for the assessment of stress in fishes. *J Gt Lakes Res* 16: 542–575.
- Cherry DS, Dickson KL, Cairns Jr J. 1975. Temperatures selected and avoided by fish at various acclimation temperatures. *J Fish Res Board Can* 32:485–491.
- Silbergeld ED. 1973. Dieldrin. Effects of chronic sublethal exposure on adaption to thermal stress in freshwater fish. *Environ Sci Technol* 7:846–849.
- Baroudy E, Elliot JM. 1994. The critical thermal limits for juvenile arctic charr *Salvelinus alpinus*. *J Fish Biol* 45:1041–1053.
- Cox DK. 1974. Effects of three heating rates on the critical thermal maximum of bluegill. In Gibbons JW, Sharitz RR, eds. *Thermal Ecology*. CONF-730505. National Technical Information Service, Springfield, VA, USA, pp 158–163.
- Otto RG, Gerking SD. 1973. Heat tolerance of a Death Valley pupfish (*Genus Cyprinodon*). *Physiol Zool* 46:43–49.
- Paladino FV, Spotila JR, Schubaur JP, Kowalski KT. 1980. The critical thermal maximum: A technique used to elucidate physiological stress and adaptation in fishes. *Rev Can Biol* 39:115–122.
- Cowles RB, Bogert CM. 1944. Preliminary study of the thermal requirements of desert reptiles. *Bull Am Mus Nat Hist* 83:261–296.
- Lowe Jr CH, Vance VJ. 1955. Acclimation of the critical thermal maximum of the reptile *Urosaurus ornatus*. *Science* 122:73–74.
- Hutchison VH. 1961. Critical thermal maximum in salamanders. *Physiol Zool* 34:92–125.
- Sealander JA, West BW. 1969. Critical thermal maxima of some Arkansas salamanders in relation to thermal acclimation. *Herpetologia* 25:122–124.
- Seibel RV. 1970. Variables affecting the critical thermal maximum of the leopard frog, *Rana pipiens* Schreber. *Herpetologia* 26:208–213.
- Lutterschmidt W, Hutchison VH. 1997. The critical thermal maximum: History and critique. *Can J Zool* 75:1561–1574.
- Beitinger TL, Bennett WA, McCauley RW. 2000. Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ Biol Fish* 58:237–275.
- Becker CD, Genoway RG. 1979. Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Environ Biol Fish* 4:245–256.
- Ware GW. 1986. *Pesticides. Theory and Application*. W. H. Freeman, New York, NY, USA.
- Gehrke PC, Revell MB, Philbey AW. 1993. Effects of river red gum *Eucalyptus camaldulensis* litter on golden perch *Macquaria ambigua*. *J Fish Biol* 43:265–279.
- Patra RW. 1999. Effects of temperature on the toxicity of chemicals to Australian fish and invertebrates. PhD thesis. University of Technology, Sydney, NSW, Australia.
- Patra RW, Chapman JC, Lim RP, Gehrke PC. 2002. Effects of temperature on acute toxicity of several organic chemicals to fish. *Abstracts, Interact 2002*, Sydney, NSW, Australia, July 22–25, p 343.
- American Society for Testing and Materials. 1980. Standard practice for conducting toxicity tests with fishes, macro invertebrates and amphibians. E 729–780, Philadelphia, PA.
- U.S. Environmental Protection Agency. 1975. Methods for acute toxicity tests with fish, macro invertebrates, and amphibians. Ecological Research Series. EPA 660/3-75-009. National Environmental Research Centre, Washington DC.

27. Merrick JR, Schmida GE. 1984. *Australian Freshwater Fishes: Biology and Management*. Griffin, Netley, South Australia.
28. Bonin JD, Spotila JR. 1978. Temperature tolerance of larval muskellunge (*Esox masquinongy* Mitchel) and F₁ hybrids reared under hatchery conditions. *Comp Biochem Physiol A* 59:245–248.
29. Kowalski T, Schubauer JP, Scott CL, Spotila JR. 1978. Interspecific and seasonal differences in the temperature tolerance of stream fish. *J Thermal Biology* 3:105–108.
30. Bonin JD. 1981. Measuring thermal limits of fish. *Trans Am Fish Soc* 110:662.
31. Smith MH, Scott SL. 1975. Thermal tolerance and biochemical polymorphism of immature largemouth bass *Microptera salmoides* Lacepede. *Bull Georgia Acad Sci* 34:180–184.
32. Cortemeglia C, Beitinger TL. 2005. Temperature tolerances of wild-type and red transgenic Zebra danios. *Trans Am Fish Soc* 134:1431–1437.
33. McFairlane RW, Moore BC, Williams SE. 1976. Thermal tolerance of stream cyprinid minnows. In Esch GW, McFairlane RW, eds, *Thermal Ecology II*. CONF. 750425. National Technical Information Service, Springfield, VA, USA, pp 404.
34. Hassan KC, Spotila JR. 1976. The effect of acclimation on the temperature tolerance of young muskellunge fry. In Esch GW, McFairlane RW, eds, *Thermal Ecology II*. CONF. 750425. National Technical Information Center, Springfield, VA, USA, pp 139–163.
35. Hickman GD, Dewey MR. 1973. Notes of the upper lethal temperature of the dusky stripe shiner, *Notropis pilsbryi*, and the bluegill, *Lepomis macrochirus*. *Trans Am Fish Soc* 102:838–840.
36. Wattenpaugh DE, Beitinger TL, Huey DW. 1985. Temperature tolerance of nitrite-exposed channel catfish. *Trans Am Fish Soc* 114:274–278.
37. SYSTAT. 1992. SYSTAT® for Windows. Statistics, Ver 5 ed. Evanston, IL, USA.
38. Cheetham JL, Garten Jr CT, King CL, Smith MH. 1976. Temperature tolerance and preference of immature channel catfish (*Ictalurus punctatus*). *Copeia* 3:609–613.
39. Wattenpaugh DE, Beitinger TL. 1985. Se exposure and temperature tolerance of fathead minnows, *Pimephales promelas*. *J Therm Biol* 10:83–86.
40. Rodriguez MH, Ramirez LFB, Herrera FD. 1996. Critical thermal maximum of *Macrobrachium tenellum*. *J Therm Biol* 21:139–143.
41. Cadwallader PL, Backhouse GN. 1983. *A Guide to the Freshwater Fish of Victoria*. Victorian Government Printing Office, Melbourne, Australia.
42. Currie RJ, Bennett WA, Beitinger TL. 1998. Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environ Biol Fish* 51:187–200.
43. Strange RJ, Petrie RB. 1993. Slight stress does not lower critical thermal maximums in hatchery-reared rainbow trout. *Folia Zoolologica* 42:251–256.
44. Fry FEJ. 1971. The effect of environmental factors on the physiology of fishes. In Hoar WS, Randall DJ, eds, *Fish Physiology*. Academic, New York, NY, USA, p 1098.
45. Crawshaw LI. 1977. Physiological and behavioral reactions of fishes to temperature change. *J Fish Res Board Can* 34:730–734.
46. Crawshaw LI. 1979. Responses to rapid temperature change in vertebrate ectotherms. *Am Zool* 19:225–237.
47. Takle JCC, Beitinger TL, Dickson KL. 1983. Effects of the aquatic herbicide endothal on the critical thermal maximum of the red shiner *Notropis lutrensis*. *Bull Environ Contam Toxicol* 31:512–517.
48. Glover CJM. 1982. Adaptations of fishes in arid Australia. In Barker WR, Greenslade PJM, eds, *Evolution of the Flora and Fauna of Arid Australia*. Peacock, South Australia, Australia, pp 241–246.
49. Barrett JWH, Peterson SM, Batley GE. 1991. The impacts of pesticides on the riverine environment with specific reference to cotton growing. CSIRO Division of Coal and Energy Technology Investigation Report, CET/IRO33. Menai, NSW, Australia, p 91.
50. Leonard AW, Hyne RV, Lim RP, Leigh KA, Le J, Beckett R. 2001. Fate and toxicity of endosulfan in Namoi River water and bottom sediment. *J Environ Qual* 30:750–759.
51. Hyne RV, Pablo F, Aistrophe M, Leonard AW, Ahmad N. 2004. Comparison of the integrated pesticide concentrations determined from field deployed passive samplers with daily river water extractions. *Environ Toxicol Chem* 23:2090–2098.
52. Sunderam RIM, Cheng DMH, Thompson GB. 1992. Toxicity of endosulfan to native and introduced fish in Australia. *Environ Toxicol Chem* 11:1469–1476.
53. Lydy MJ, Wissing TE. 1988. Effect of sublethal concentrations of copper on the critical thermal maxima (CTMax) of the fantail (*Etheostoma flabellare*) and johnny darters (*E. nigrum*). *Aquat Toxicol* 12:311–322.
54. Burton DT, Morgan EL, Cairns Jr J. 1972. Mortality curves of bluegills (*Lepomis macrochirus* Rafinesque) simultaneously exposed to temperature and zinc stress. *Trans Am Fish Soc* 101:435–441.
55. Becker CD, Wolford MG. 1980. Thermal resistance of juvenile Salmonids sublethally exposed to nickel determined by the critical thermal maximum method. *Environ Pollut* 21:181–189.
56. Cairns Jr J, Heath AG, Parker BC. 1975. The effects of temperature upon the toxicity of chemicals to aquatic organisms. *Hydrobiologia* 47:135–171.
57. Howe GE, Marking LL, Bills TD, Rach JJ, Mayer Jr FLR. 1994. Effects of water temperature and pH on toxicity of terbufos, trichlorfon, 4-nitrophenol, and 2,4-dinitrophenol to the amphipod *Gammarus pseudolimnaeus* and rainbow trout (*Oncorhynchus mykiss*). *Environ Toxicol Chem* 13:51–66.
58. Brown VM, Jordan KHM, Tiller BA. 1967. The effect of temperature on the acute toxicity of phenol to rainbow trout in hard water. *Water Res* 1:587–594.
59. Changon N, Hlohowskyj I. 1989. Effects of phenol exposure on the thermal tolerance ability of the central stoneroller minnow. *Bull Environ Contam Toxicol* 42:614–619.
60. Hutchison VH. 1976. Factors influencing thermal tolerances of individual organisms. In Esch GW, McFairlane RW, eds, *Thermal Ecology II*. CONF. 750425. National Technical Information Service, Springfield, VA, USA, pp 10–26.