



Effect of thallium on phototactic behaviour in *Daphnia magna*

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Abstract

Thallium (Tl) is a trace metal enriched in wastewaters associated with mining and smelting of base metals. The toxicity of Tl to aquatic biota is poorly understood, particularly with respect to its sublethal effects. In this study, phototactic behavioural responses of naïve (i.e. no previous exposure to Tl) *Daphnia magna*, a key regulatory freshwater crustacean species, were examined in waters containing Tl. Fed and fasted neonate daphnids (< 24 h old) and fed adults (10–15 days old) showed no significant response at any tested water Tl concentration. However, in fasted adults, an increase in the positive phototactic response (measured as a greater number of daphnids closer to the light source after a 5-min exposure) was seen at Tl concentrations of 917 and 2099 $\mu\text{g L}^{-1}$, values representative of extreme environmental Tl concentrations. The presence of Tl also decreased the swimming speed of adult *Daphnia* towards a light source. In the presence of cimetidine, a histamine receptor blocker, the increase in positive phototaxis induced by Tl disappeared, suggesting that Tl acts to perturb the phototaxis response through sensory inhibition. Conversely, although there was a trend towards enhanced activity, Tl had no significant effect on acetylcholinesterase, a marker of locomotor capacity.

Keywords Acetylcholinesterase · Histamine · Locomotion · Predation · Sensory impairment · Trace metal

Introduction

The freshwater invertebrate, *Daphnia magna*, is a key species for assessing the effects of toxicants on aquatic ecosystems from the perspective of both their ecological and regulatory importance. Daphnids occupy a critical niche in freshwater food chains, feeding on phytoplankton and acting as a food source for higher trophic levels (e.g. Miner et al. 2012). Furthermore, because of their relative ease of culture and general sensitivity to toxicants (Baudo

1987), daphnids are regulatory species recommended by multiple organisations, including the US Environmental Protection Agency (USEPA), the Canadian Council of Ministers of the Environment (CCME), and the Organisation for Economic Cooperation and Development (OECD) (CCME 2007; OECD 2004; USEPA 2002). In addition, *D. magna* is considered a sensitive bioindicator of potential ecological problems caused by mining and smelting activities (Tomasik and Warren 1996).

One contaminant of concern associated with mining and smelting activities is the trace metal thallium (Tl). Although found at low concentrations in most natural settings, in rivers adjacent to base metal mining activities in the USA and Canada, Tl concentrations up to $\sim 100 \mu\text{g L}^{-1}$ have been regularly recorded (USEPA 1980; Zitko and Carson 1975). Indeed, one report has even noted Tl concentrations in excess of 15 mg L^{-1} (Williams-Beam and Twidwell 2003). These concentrations exceed the CCME guidelines for Tl in surface waters, which recommend that surface water Tl concentrations not exceed $0.8 \mu\text{g L}^{-1}$ (CCME 2007). This CCME guideline is one of the few regulatory values that exist for Tl in aquatic environments, but is based only on the effects of Tl on growth of an aquatic plant (Brown and Rattigan 1979; CCME 2007, Kwan and Smith 1988).

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The sensitivity of aquatic animals exposed to waterborne TI has been previously characterised, but only for a limited number of endpoints. For example, acute median lethal concentrations (48 h LC₅₀) for TI toxicity to daphnids range between 1.66 and 1.86 mg L⁻¹ (Lin et al. 2005; Nagel et al. 2019). Lowest observed effect concentrations (LOEC) for chronic TI toxicity have been documented at environmentally relevant levels of 8.8 µg L⁻¹ for *Daphnia magna*, using the endpoints of growth and reproduction (Nagel et al. 2021). Similarly, first quartile effect concentrations (EC₂₅) have been calculated for the aquatic invertebrate species *Hyalella azteca* for survival (4-week LC₂₅: 48 nmol L⁻¹ = 9.8 µg L⁻¹), growth (6-week EC₂₅: 35 nmol L⁻¹ = 7.2 µg L⁻¹), and reproduction (10-week EC₂₅: 10 nmol L⁻¹ = 2.0 µg L⁻¹) (Borgmann et al. 1998). While these data indicate TI concentrations at which risks to aquatic biota may occur, there remains a large gap in our knowledge of the underlying mechanisms of TI toxicity to aquatic organisms.

In mammals, TI is a neurotoxicant (e.g. Bramanti et al. 2019). The mechanisms by which TI induces neurotoxicity are yet to be confirmed, but evidence exists for effects related to the promotion of oxidative damage, and inhibition of the basolateral Na⁺/K⁺-ATPase (Maya-López et al. 2018), the enzyme that drives cellular ionoregulation. This latter effect may relate to the known role of TI in perturbation of potassium homeostasis (e.g. Hassler et al. 2007). However, a neurological mode of TI toxicity has not been specifically examined in any freshwater organism.

One approach to investigating the neurotoxicity of TI to *Daphnia magna* is to assess effects on phototactic behaviour. Phototaxis is a behavioural endpoint whereby the animal moves towards or away from a directed light stimulus. In daphnids, this endpoint has been used to assess the toxicity of hydraulic fracturing flowback and produced waters (Delompré et al. 2019), impairments induced by trace metals (e.g. Kolkmeier and Brooks 2013; Michels et al. 1999; Wu et al. 2008), and the effects of food scarcity (e.g. Johnsen and Jakobsen 1987; Michels and De Meester, 1998; Van Gool and Ringleberg 2003). Negative phototactic behaviour reduces potential ultraviolet (UV) damage and predation risk by avoiding the top of the water column during daylight hours (e.g. McCoole et al. 2011), while positive phototaxis may facilitate feeding under predator-free conditions (Michels and De Meester 1998). Positive and negative phototaxis behaviours both exist in natural daphnid populations (e.g. De Meester 1993; Ringleberg 1999), and both responses have been used as indicators of potential adverse impacts of pollutants (e.g. Kolkmeier and Brooks 2013; Michels et al., 1999).

Changes in phototactic behaviour result from either impairment in the capacity of the organism to detect the stimulus (i.e. a sensory effect) or from a failure to respond appropriately to the stimulus (i.e. a motor effect). In

daphnids, phototactic responses are driven by a number of different neurotransmitter systems, including dopaminergic, GABAergic, and histaminergic signalling pathways (Barrozo et al. 2015; Bedrossiantz et al. 2021; McCoole et al. 2011). For example, McCoole and colleagues (2011) inhibited a negative phototactic response by exposing daphnids to the histamine receptor 2 (H2) antagonist cimetidine, which blocked the sensory stimulus. The motor responses of daphnids are linked to the activity of the enzyme acetylcholinesterase (AChE; Printes and Callaghan 2004). Acetylcholine is a critical signalling molecule in transmitting sensory information to nerves and muscles, and its activity is regulated by AChE. Consequently, changes in AChE activity can be informative of a toxicant effect on motor activity. Indeed, studies have shown that changes in swimming behaviour correlate with changes in AChE activity in daphnids (Ren et al. 2017) that these effects can be observed at sublethal toxicant concentrations (Ren et al. 2017) and are toxicant-specific (Guilhermino et al. 1996). A previous study has noted that AChE is a target of TI toxicity in mouse neuroblastoma cells in vitro (Repetto et al. 1994).

This investigation aims to determine the effects of TI on *D. magna* phototactic behaviour. These studies were conducted using naïve animals (i.e. not previously exposed to TI) only subjected to TI in the assay water. This experimental design was used to minimise any effect of TI on energy metabolism (e.g. Li et al. 2020), which could affect responses to light stimuli through mechanisms other than via the nervous system. We hypothesised that neurological effects of TI are conserved between mammalian and aquatic species, and that TI would therefore affect the capacity of daphnids to respond to light. This was assessed using behavioural assays to measure phototactic responses, coupled with biochemical assays to determine whether the effects of TI on daphnid behaviour were mediated by sensory or motor impairment.

Materials and methods

Daphnia magna

Daphnia magna were initially obtained from Aquatic Research Organisms (Hampton, NH, USA), and thereafter maintained in the Department of Biological Sciences at the University of Alberta. *Daphnia* were reared in International Organisation for Standardisation (ISO) test water 1 (hereafter referred to as ISO water): CaCl₂·2H₂O (2 mM), MgSO₄·7H₂O (0.5 mM), NaHCO₃ (0.77 mM), and KCl (0.08 mM), reconstituted in ultrapure water (OECD 2004). Feeding consisted of a combination of YCT (a yeast, cereal leaf, trout chow mix; ~0.5 mL L⁻¹), algae (*Raphidocelis subcapitata*; ~500 000 cells L⁻¹), and Roti-Rich liquid invertebrate food (~3 drops L⁻¹) once daily. Neonates (< 24 h old)

used for experiments were removed from culture beakers and transferred immediately into treatment waters for testing. Adult daphnids were separated after birth and maintained in individual 50-mL beakers for 15 days, under the feeding regime described above for the source culture. All *Daphnia* were maintained at room temperature (22 ± 1 °C) under a 16:8-h dark:light cycle.

Vertical phototaxis assay

Phototaxis was initially assessed via a vertical chamber method, modified from that described by De Meester (1989). A 9 cm² hole was cut in the top of a cardboard box (30 × 15 × 30 cm) which housed the test chamber. The test chamber (100 mL graduated glass cylinder; 25 cm height) was marked in 20-mL increments (upper, U; medial, M₁, M₂, M₃; lower L), to allow for quantification of phototactic response. Test solutions consisted of ISO test water 1 with TI added from a TiNO₃ (Sigma-Aldrich, Oakville, ON, Canada) stock solution to achieve nominal concentrations of 0, 0.8, 700, 1860, and 8000 µg TI L⁻¹. These concentrations represent a TI-free control; the CCME regulatory limit (CCME 2007); an elevated environmental concentration; the 48-h LC₅₀ value in this water chemistry (Nagel et al. 2019), and a 48-h LC₁₀₀, respectively. Ten naïve daphnids (i.e. not previously exposed to TI) were transferred into the chamber, which was then placed into the cardboard box. The box was closed, and a light source was placed over the opening at the top of the box (5.5 cm from the waterline). The light source only generated visible light. Daphnids were not exposed to UV light during the experiment. After 5 min, the box was opened, the chamber removed, and the number of daphnids in each zone was counted. After the data were collected, the following equation was used to calculate I_p , the “Phototaxis Index” (De Meester 1993):

$$\text{Equation 1.1 : } I_p = \frac{U - L}{U + M_1 + M_2 + M_3 + L}$$

where the letters represent the number of daphnids in each section of the test chamber, as defined above. The I_p value always lies between -1 and +1.

The vertical phototaxis behavioural assay was used to test naïve neonate (<24 h old) and naïve adult (10–15 days old) *Daphnia* in both fed and fasted states. For fed exposures, daphnids were given YCT, at a concentration approximating a normal daily YCT ration, 10 min prior to the test. For fasted exposures, food was withheld from daphnids for 24 h prior to experimentation. To account for the possibility of behavioural variation over the course of a day, all vertical chamber behavioural trials were performed by testing a new group of daphnids every hour between 8 am and 8 pm. This was repeated over 8 experimental days (i.e. $n=8$ for each

time of day). For all vertical assay assessments, a “no light” control was conducted to verify that positive phototaxis behaviour occurred in response to the light stimulus rather than a consequence of disturbance associated with the movement of animals to the test chamber. No measures of daphnid TI accumulation were conducted, owing to the short nature of the actual exposure to TI (5 min.).

Horizontal phototaxis assay

This assay was used to assess the speed at which daphnids moved along a horizontally placed tube towards a light source, providing an additional measure of the phototactic response. The system used was identical to that described in Delompré et al. (2019). Individual fasted naïve adults (10–15 days old) were placed in the test apparatus and exposed to one of 5 TI treatments (nominal concentrations: 0, 0.8, 700, 1860, and 8000 µg TI L⁻¹). A positive response was one where the daphnids appeared in the clear, illuminated portion of the tube. Speed was calculated as the length of distance travelled (from entry point to lit end), divided by the time to traverse that distance (cm s⁻¹). If the daphnid did not appear after 5 min, then it was assigned a score of 300 s. Again, due to the short duration of the actual TI exposure, no measures of daphnid TI accumulation were taken.

Histamine receptor inhibitor assay

A histamine receptor inhibitor assay was used to determine the effects of TI on phototactic behaviour and was conducted as described by McCooles et al. (2011). Prior to exposures, a freshly made 1 M cimetidine (an inhibitor of the H₂ receptor; Sigma-Aldrich, Oakville, ON, Canada) stock solution was prepared by dissolving cimetidine salt in DMSO. Test conditions included a control group without either cimetidine or TI, a vehicle control (DMSO only, at 0.2%), a TI control (nominally, 1860 µg L⁻¹), a cimetidine control (2 mM), and a TI + cimetidine treatment (1860 µg TI L⁻¹ + 2 mM cimetidine). The concentration of cimetidine used has been previously shown not to impair swimming behaviour (see Fig. 5 in McCooles et al. 2011). Each replicate represented ten naïve juvenile *Daphnia magna* (7–8 days old) that were placed in each test solution and observed in the vertical phototactic chamber as described above.

Acetylcholinesterase assay

A colorimetric assay (Ellman et al. 1961) was used to assess AChE activity. Briefly, naïve adult daphnids (10–15 days old) were exposed to control conditions (0 µg L⁻¹ TI) or the median acute lethal concentration (nominally, 1860 µg L⁻¹ TI) in ISO water. Following a 5-min exposure, daphnids (three per replicate) were removed, gently blotted dry

and immediately homogenised using a motorised mortar and pestle in 500 μL of ice-cold phosphate buffer (pH 7.4), before being stored at $-80\text{ }^{\circ}\text{C}$ until analysis. Using a 96 well plate, 50 μL of standard (i.e. AChE as a positive control) or sample was pipetted into the well followed by 100 μL of 5,5'-dithiobis-(2-nitrobenzoic acid) and 20 μL of acetylcholine iodide. The plate was then read at a wavelength of 412 nm once per minute for 6 min using a microplate reader (VersaMax Plate Reader). Bradford assays were conducted to quantify protein content within samples (Bradford 1976). Acetylcholinesterase activity is presented as moles of substrate hydrolysed per minute per μg protein.

Tl analysis

Immediately following the 5-min behavioural assay, water samples (10 mL) were collected and passed through a 0.45 μm syringe filter and acidified with 1% trace metal grade nitric acid. Inductively coupled plasma mass spectrometry (ICPMS RQ, Thermos Scientific) was used to analyse samples for dissolved Tl concentration. Multi Element Standard-1 (MES-1; Spex CertiPrep, Metuchen, NJ, USA), surface water standard reference materials SPS-SW2 (diluted 10—and 500-fold; Spectapure Standards, Oslo, Norway), and NIST 1640a (diluted 10- and 100-fold; National Institute of Standards and Technology, Gaithersburg, MD, USA) were used for quality assurance. Blank values for the analysis were determined by calculating the average of three 2% nitric acid replicates. To account for drift in ICPMS sensitivity, an internal standard (10% nitric acid solution spiked with 5 $\mu\text{g L}^{-1}$ indium and bismuth) was used. Limit of detection (LOD) and limit of quantification (LOQ) for the analysis were 0.11 and 0.76 ng L^{-1} , respectively. Any concentrations measured below the LOQ were assigned a value halfway between the LOQ and zero.

Calculations and statistics

Prior to statistical analysis, Grubb's test was employed for detection and subsequent removal of outliers. One outlier was subsequently removed from the adult swimming speed assessment at the highest Tl test concentration. For all analyses, assumptions of parametric analysis were tested using the Shapiro–Wilk test for normality and the Brown-Forsythe test for homogeneity of variance. Initially, a two-way ANOVA (with time of day and Tl concentration as the two factors) was conducted on assays conducted at distinct times of the day and showed that time of day did not have a significant effect on response. This allowed data to be pooled across the different test times. Subsequently, the phototactic responses of daphnids in the vertical assay were analysed using a two-way ANOVA (where the two factors were Tl concentration and the presence/absence of light), followed by a post hoc

Tukey's test. Swim speed data were not normally distributed, and therefore were analysed using a non-parametric Kruskal–Wallis test. Effects of the H2 receptor antagonist cimetidine on phototaxis were analysed by one-way ANOVA with a post hoc Tukey's test. The activity of AChE was analysed using an unpaired *t*-test. All reported values represent the mean \pm standard deviation. All statistical analyses were conducted using Prism GraphPad.

Results

Tl exposure concentrations

Measured mean Tl concentrations ranged from 103 to 131% of nominal values (Table 1). Background concentrations of Tl in ISO water were below the LOQ (i.e. $<0.76\text{ ng L}^{-1}$). From this point forward, measured concentrations will be referred to in the text.

Behavioural assay

Daphnids in the vertical test chamber displayed a positive phototaxis response. Significant differences were noted between the “light” and “no light” treatments for all test conditions (two-way ANOVA, *p*-value <0.05 ; Fig. 1). No significant effect of test water Tl concentration on daphnid behaviour was determined in any of the “no light” treatments. There were no differences in phototactic responses as a function of time of day (data not shown; two-way ANOVA, *p*-value range 0.11–0.28). Because of this lack of effect, data examining the effect of test water Tl on phototaxis were pooled independent of the time of day the tests were conducted. Subsequently, no significant differences in phototactic behaviour (i.e. in the presence of light in the test chamber) were observed in neonates (both fed and fasted) and fed adult *Daphnia magna* as a function of test water Tl concentration (Fig. 1a, b, and d). However, adult fasted daphnids tested in 917 or 2099 $\mu\text{g Tl L}^{-1}$ exhibited positive phototactic responses that were significantly greater relative

Table 1 Nominal and measured dissolved Tl concentrations

Nominal concentration ($\mu\text{g L}^{-1}$)	Measured concentration ($\mu\text{g L}^{-1}$)
0	$<\text{LOQ}$
0.8	0.82 ± 0.07
700	917 ± 34
1860	2099 ± 54
8000	8395 ± 59

Values represent mean \pm standard deviation, $n=6$, as determined by ICPMS. LOQ limit of quantification (0.76 ng L^{-1})

Fig. 1 Effect of waterborne Tl exposure on phototactic behaviour of fed (A) and fasted (B) neonate (<24 h); and fed (C) and fasted (D) adult (10–15 day) *Daphnia magna*. Plotted points represent means (\pm standard error) of 96 replicates. Letters represent data points collected in light exposures. Plotted values not sharing letters are statistically significantly different ($\alpha=0.05$)

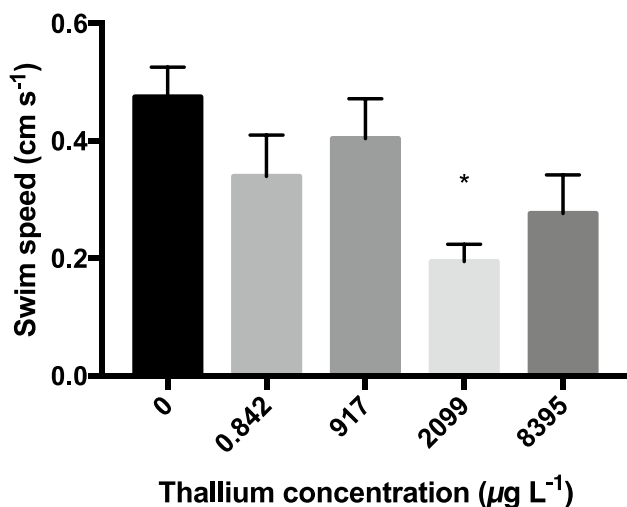
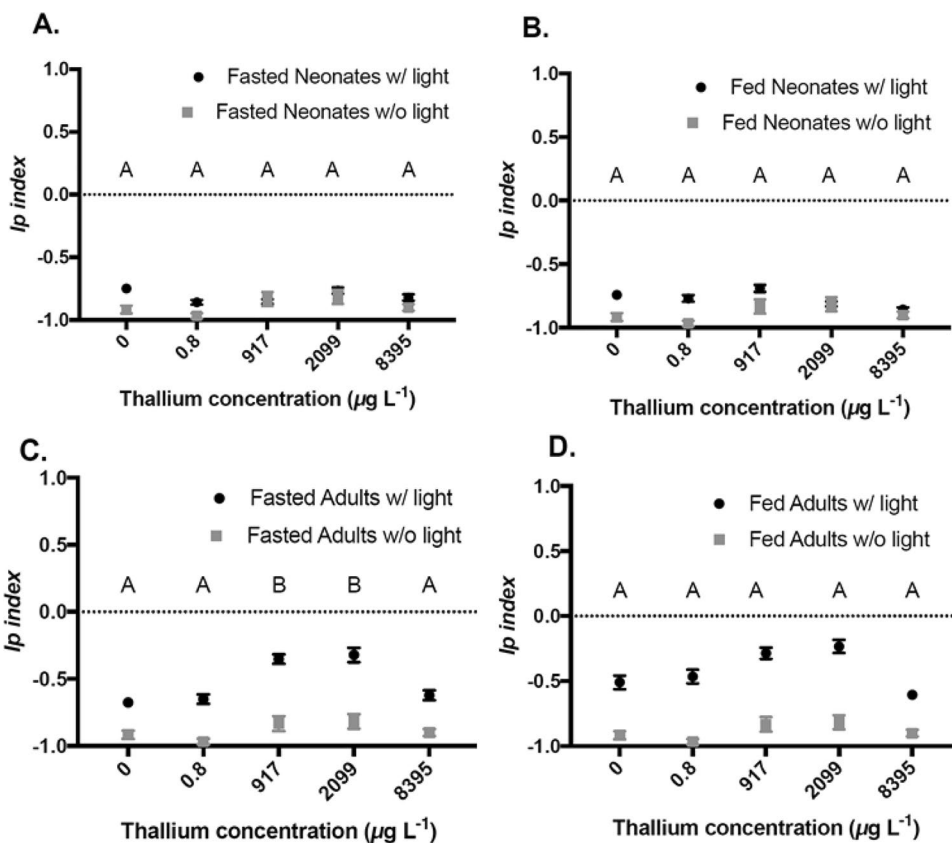


Fig. 2 Effect of waterborne Tl exposure on fasted adult (10–15 day) *Daphnia magna* swim speed. Plotted points represent means (\pm standard error) of 8 replicates. Asterisks (*) indicate a significant difference relative to the control (0 $\mu\text{g L}^{-1}$ Tl)

to the phototactic responses observed at all other test Tl concentrations (Fig. 1c).

Effects on adult swim speed in the horizontal test chamber were observed at a test concentration of 2099 $\mu\text{g Tl L}^{-1}$ (p -value = 0.01; Fig. 2). Adult daphnids exposed

to this concentration of Tl exhibited a swimming speed approximately 40% of that recorded in the control group daphnids. No significant differences in swim speed relative to the Tl-free control were observed in any of the other treatment groups.

Acetylcholinesterase assay

There was a trend towards an increase in AChE activity in adult daphnids exposed to 2099 $\mu\text{g Tl L}^{-1}$ (Fig. 3). However, this effect was not statistically significant (t -test, $p=0.10$).

Histamine receptor inhibition assay

The effects of the H2 receptor antagonist, cimetidine, on daphnid phototactic behaviour in a vertical test chamber are exhibited in Fig. 4. Cimetidine exposure resulted in a significantly more positive phototactic response than the Tl-free control ($p=0.001$), an effect was statistically identical to the response induced in the presence of Tl alone. When Tl and cimetidine were both present, the stimulatory effect of each component was abolished, and the phototactic response observed was not significantly different from the control ($p=0.99$).

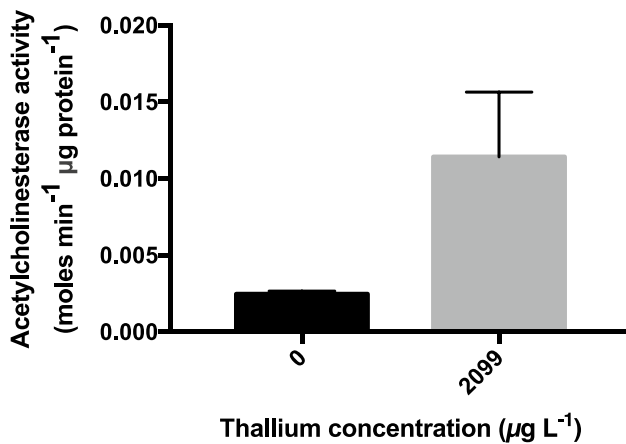


Fig. 3 Acetylcholinesterase activity ($\text{moles min}^{-1} \mu\text{g protein}^{-1}$) in fasted adult (10–15 days) *Daphnia magna* exposed to 2099 $\mu\text{g L}^{-1}$ Tl. Plotted points represent the mean (\pm standard error) of 6 replicates

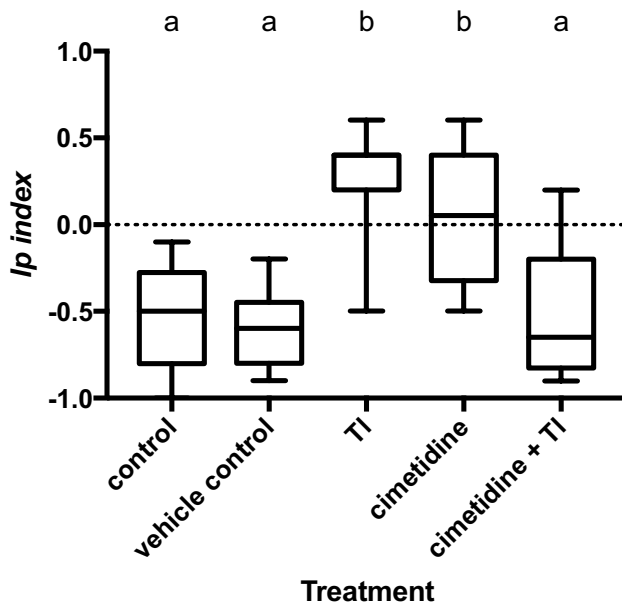


Fig. 4 I_p following 5-min exposure of fasted adult (7–8 day) *Daphnia magna* to 2099 $\mu\text{g L}^{-1}$ Tl in the absence or presence of 2 mM cimetidine. Plotted points represent the median, lower, and upper quartiles (\pm minimum and maximum values) of ten replicates. Plots sharing letters are not statistically significantly different ($\alpha=0.05$)

Discussion

The presence of Tl in the test water resulted in effects on daphnid phototactic behaviour. These effects were observed only for fasted adult daphnids, which exhibited a significantly more positive phototactic response in the presence of 917 and 2099 $\mu\text{g Tl L}^{-1}$ than in the Tl-free control. The phototactic responses of daphnids are hypothesised to mediate diel vertical migrations. As such, the

predicted response to light exposure would be a negative phototaxis, avoiding potentially harmful effects of UV irradiation and reducing exposure to visual predators (Ringelberg 1999). In the current study, however, the basal response of daphnids to light was a movement towards the light source. In natural settings, the negative phototactic response is likely reinforced through the presence of fish kairomones. Under laboratory culture conditions, where daphnids are raised for multiple generations in the absence of predatory cues and the absence of other negative stimuli such as UV exposure, then a positive phototaxis is not unexpected (De Meester 1993). Indeed, even in natural environments, clones of *D. magna* occur that display positive phototaxis (De Meester 1991). A positive phototactic response has been observed previously for the daphnid culture that was used in the current experiments (Delompré et al. 2019). In the current study, Tl enhanced this phototactic response, suggesting a disruption of the mechanisms that regulate daphnid responses to light.

It was notable that effects of Tl on the phototactic response were observed at 917 and 2099 $\mu\text{g Tl L}^{-1}$, but not the highest test concentration of 8395 $\mu\text{g Tl L}^{-1}$. This test concentration is approximately four times the LC_{50} value (Lin et al. 2005; Nagel et al. 2019) and thus represents an extreme scenario, if even only for the 5 min of Tl exposure. Therefore, it is likely that the lack of response at the highest test concentration is a consequence of daphnids being functionally immobilised. There is some evidence to support this from the horizontal phototaxis assay. In that experiment, the presence of 2099 $\mu\text{g Tl L}^{-1}$ reduced swimming speed (Fig. 2). In the vertical phototaxis assay, this reduced speed was insufficient to affect the capacity of the 2099 $\mu\text{g Tl L}^{-1}$ daphnids to respond towards the light (i.e. although swimming speed was reduced, over the 5-min exposure interval of the vertical behavioural assay, daphnids were still capable of exhibiting an enhanced capacity to move towards the light source). Swimming speed in the 8395 $\mu\text{g Tl L}^{-1}$ group trended towards inhibition but was not significant (Fig. 2). This suggests that daphnids tested at 8395 $\mu\text{g Tl L}^{-1}$ can reach the light source in the horizontal assay before the effects of immobilisation are induced, whereas the 5-min exposure in the vertical assay is sufficiently long to induce an incapacity to enact a Tl-mediated response to the light stimulus. A non-monotonic pattern of toxicity, where effects are seen at lower concentrations and not at higher concentrations, is not uncommon in studies of neurotoxicant effects in crustaceans (Fong and Ford 2014).

An impairment in phototactic behaviour could be mediated by an effect on the capacity of the animal to sense the stimulus or through disruption of the appropriate motor response enacted in response to the stimulus. These possibilities were examined in fasted adult daphnids tested in waters with 2099 $\mu\text{g Tl L}^{-1}$, the concentration where an

increase in the positive phototactic response was observed. Phototactic behaviour in daphnids is controlled in part by the histaminergic signalling system (McCoole et al. 2011). Cimetidine, an H₂ receptor antagonist, blocks histamine signalling pathways and subsequently inhibits negative phototactic behaviour (McCoole et al. 2011). In our study, the increase in positive phototaxis upon cimetidine exposure is consistent with this previous work and closely matched the response induced by Tl (Fig. 5). Intriguingly, in tests where Tl and cimetidine were both presented to daphnids, the significant effect of each component on positive phototaxis was abolished. This suggests that Tl and cimetidine act on different components of the histaminergic signalling pathway (i.e. the effect of Tl is not mediated by blockade of the H₂ receptor), and it also indicates that the presence of cimetidine inhibits the mechanism of Tl effect on this pathway. On this basis, the most obvious explanation for the Tl effect is that it involves inhibition of nerve function. Thallium is well characterised as a mimic of potassium and can interfere with potassium transporter conductance (e.g. Matteson and Swenson 1986). We therefore hypothesise that Tl interferes with histaminergic signalling by impeding potassium conductance in daphnid sensory neurons. In the absence of H₂ receptor activation (i.e. in the presence of cimetidine), this effect is ameliorated (i.e. there is no conductance to inhibit). In general, Tl has a very low binding affinity for organic ligands (Nagel et al. 2019), so it is doubtful that the lack of effect in mixed Tl/cimetidine test waters is due to complexation and consequent nullification of Tl reactivity.

As noted above, altered responses to a stimulus could be a consequence of an effect on the motor system. In the current study, we examined whether AChE activity was altered when daphnids were tested in the presence of Tl and whether this could therefore explain the effect of Tl on positive phototaxis in adult daphnids. In general, exposures to trace metals cause inhibition of AChE activity, an effect that correlates with impaired locomotion (Xuereb et al. 2009). This is also true specifically for insecticide-exposed *D. magna*, where increased immobility and swimming behaviour in general are directly related to reduced AChE activity (Printes and Callaghan 2004; Ren et al. 2017). In the current study, at Tl concentrations where a more positive phototaxis was recorded, a non-significant trend towards elevated AChE activity was observed, which is generally consistent with a role for this enzyme in daphnid locomotion. There is, however, insufficient statistical support to attribute the change in daphnid phototactic behaviour in response to Tl to a change in the capacity of the animal to enact a motor response to the stimulus. Given the lack of effect of Tl on AChE, and concomitantly the significant effect of the cimetidine exposure, it can be concluded that the Tl-induced change in phototaxis is mediated through a sensory mechanism of action.

There were no significant effects of Tl on positive phototaxis in neonates. Relative to adults, neonate daphnids have a less well-developed phototaxis response, a pattern that is obvious in Fig. 1, where the *Ip* value is lower in neonate tests than in adult tests. This is consistent with previous studies. For example, De Meester (1992) studied *D. magna* clones that display positive phototaxis and showed that juveniles (1–4 days old) had a reduced positive phototactic response compared to older individuals of that clonal group. Other authors have noted that relative to adults, daphnid neonates display higher variability in their responses to light (Delompré et al. 2019; Whitman and Miller 1982), which is a factor that might also contribute to their lesser response, and thus a reduced scope to delineate an effect of Tl.

In the current study, effects of Tl on positive phototaxis were only observed in fasted adults. One explanation for the lack of effect in fed animals is that Tl complexation to food may reduce its bioavailability and thus minimise its effects. However, as noted above, Tl does not have a high affinity for waterborne ligands (Nagel et al. 2019), and thus this is unlikely to explain the lack of effect of Tl on fed animals. Instead, the response is likely a consequence of the fed state of the animal. For example, previous studies have shown that culture conditions can influence phototactic behaviour. In contrast to the effects we observed (i.e. a more pronounced positive phototaxis in response to a lack of food), De Meester and Dumont (1989) tested the influence of different culture food concentrations on photobehaviour in *D. magna* and showed a reduced positive phototaxis in response to long-term food restriction. However, because of the long-term nature of the food restriction in that study, an effect due to behaviour or one due to a lack of physiological capacity to respond could not be distinguished. Similarly, reports of a reduced positive phototaxis in *Daphnia* in response to short-term fasting exist (Van Gool and Ringelberg 1995; 1998). Conversely, some authors have found results similar to ours, where short-term food deprivation increased positive phototaxis in daphnids (Clarke 1932). Differences between studies are likely due to factors such as the nature of the phototactic stimulus (Pearre 2003), food quality (Michels and De Meester 1998), and the ingrained response (i.e. positive or negative) of a specific daphnid clone to a light stimulus (De Meester 1993). We hypothesise that the lack of effect of Tl on fed adults is due to the availability of dietarily sourced potassium, which could be mobilised to offset the Tl-induced effects on potassium handling that may contribute to the sensory impairment.

The modification of daphnid phototactic behaviour by toxicants has important utility in environmental monitoring. For example, automated systems have been developed that facilitate the continuous recording of daphnid behaviour following a toxicant exposure (Gerhardt et al. 2006; Lechelt et al. 2000). The most refined approaches are capable of

deployment in field settings, where they can provide continuous, real-time data that reflect the extent of water pollution (Dyomin et al. 2020). Such techniques offer several advantages over traditional laboratory-based biomonitoring including high sensitivity, rapid identification of a hazardous pollution event, environmental relevance, and the avoidance of issues associated with the ethical use of vertebrates in research (Dyomin et al. 2020; Gerhardt et al. 2006; Lechelt et al. 2000). The current study suggests that daphnids are responsive to elevated water Tl concentrations, and as such behaviour-based biomonitoring may be applicable to receiving waters at risk of Tl contamination.

Conclusions

The current study shows that the presence of a very high concentration of waterborne Tl disrupts the phototactic response of fasted adult *D. magna*. It appears that this response is mediated through an effect on the histaminergic signalling pathway, thus representing the first mechanistic demonstration of an effect of Tl on the nervous system of a freshwater organism. It is important to note that all significant changes in phototaxis were observed at Tl concentrations of 917 or 2099 $\mu\text{g L}^{-1}$, representing very high environmental scenarios. No significant effects were observed at the regulatory threshold (0.8 $\mu\text{g L}^{-1}$: CCME 2007). In the current work, the effects of water Tl were tested in a *D. magna* clone that exhibits a positive phototactic response, in contrast to most natural *Daphnia* populations, which display negative phototaxis. The effects of Tl on daphnids that naturally exhibit a negative phototactic response remain unexplored.

As noted above, behavioural responses to toxicants by invertebrate species such as *D. magna* have potential value as a biomonitoring tool. Such approaches allow for continuous evaluation of an at-risk watershed, can be sensitive and rapid, and avoid ethical issues associated with more invasive methods of assessment (e.g. Dyomin et al. 2020; Gerhardt et al. 2006; Lechelt et al. 2000). Our data provide preliminary support for the implementation of daphnid photobehaviour as an endpoint for monitoring waterborne Tl toxicity.

Author contribution AHN: conceptualisation, investigation, supervision, writing — original draft, and writing — review and editing; ASRR: conceptualisation, investigation, writing — original draft, and writing — review and editing; GGG: supervision, resources, and writing — review and editing; CNG: supervision, resources, and writing — review and editing.

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Data availability Data is available from the corresponding author on request.

Declarations

Ethics approval This work did not involve research on human subjects or animals covered by ethics regulations.

Consent to participate This work did not involve human subjects.

Consent for publication All authors have consented to the submission of this manuscript for publication.

Competing interests The authors declare no competing interests.

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