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How does pollution change the tolerance of warming conditions in *Daphnia galeata*?

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Abstract:

Climate warming and eutrophication are major stressors for freshwater habitats. To understand how these two factors influence the primary consumer, five *Daphnia galeata* lineages were retrieved from the sediments of Swiss Lake Greifensee using resurrection ecology. Those five lineages were raised in low and high phosphorus conditions and exposed to six static temperatures to assess how the phosphorus condition impacted their temperature tolerance. The lineages were also compared with each other to see if their tolerance was impacted by historical conditions. Temperature tolerance was assessed using the Thermal-Death-Time (TDT) modelling, which allows for modeling the temperature-exposure duration relationship and avoids the limitations of Critical Thermal maximum (CT_{max}). In addition, several parameters can be obtained from the TDT, such as the thermal sensitivity (z), dynamic Critical Thermal maximums, and predicted critical temperature for a given long-exposure duration (T_c). We observed a strong impact of temperature on knockdown time, while phosphorus treatments had no significant effect on the heat tolerance of the five lineages. Lineages from the cooler historical period showed a lower heat tolerance, which was confirmed by the calculated dCT_{max} and T_c. Our results highlight how *D. galeata* may adapt to slow changes in temperature but may be at risk with longer extreme thermal events, such as heatwaves.

Introduction:

Climate change is a major concern for the 21st century, and its impacts are diverse. Earth's temperature is expected to rise by an average of 1,5-4°C, depending on the scenario (Dahal et al., 2025). Freshwater habitats and their inhabitants are especially at risk, with at least one-fourth of freshwater species being threatened by extinction (Sayer et al., 2025). In addition, those habitats offer numerous services to humanity, from their cultural importance to ecosystem services such as climate regulation and nutrient cycling (Lynch et al.,

2023). Those habitats are also often the sites of aquaculture or next to agricultural zones. This may lead to eutrophication and water pollution due to the increase in phosphorus and nitrogen locally originating from fish farms or from runoff of fertilizers from nearby crops (Potharaju et al., 2025). In Europe, poor waste management, intensified agricultural practices, and the use of phosphorus-rich detergents significantly contributed to nutrient inputs and eutrophication of water bodies, particularly in the 1960s and 1970s (Chorus et al., 2020). This can also lead to a reduction in water

quality and the occurrence of harmful algae blooms, with adverse effects for the local biodiversity (Akinawo, 2023). Many lakes in Europe were eutrophic in the 1960s and 1970s. However, after the 1970s, policy regulations helped lower the input of nutrients in freshwater habitats, leading to an oligotrophication process. This state usually implies higher oxygen concentrations, clear water, and a lower primary production due to lower nutrient concentration (Chorus et al., 2020).

This is what happened in Lake Greifensee. This lake is a Swiss lake that was overloaded with nutrients and became hypertrophic in the 1970s. The eutrophication process spurred the invasion and dominance of *D. galeata*, overcoming the indigenous *Daphnia longispinna* population (Rellstab et al., 2011). Yet, the population did decline when the lake started to become less eutrophic due to new regulations (Spaak et al., 2012). This decline could be linked with the lower phosphorus concentration in the lake. This lower concentration impacts *Daphnia* indirectly by lowering the food quality. The carbon:phosphorus concentrations of algae tend to increase due to the oligotrophication process. *Daphnia* are P-demanding grazers and thus are negatively impacted by this lower food quality (Van Donk et al., 2008). Understanding how *Daphnia* species can be affected by an

increase in nutrients in their freshwater habitat is of prime importance to predict potential impact on their populations and on the trophic chain. Higher temperatures can also impact freshwater communities, favouring small-bodied zooplankton (Cremona et al., 2020). This may result in smaller cladoceran and possibly alter the food web (Havens et al., 2015). In addition to food web alterations and shifts to smaller sizes, some species might gain a competitive advantage due to the higher temperatures, possibly outcompeting other species in the ecosystem and further altering the community (Rasconi et al., 2015). Temperature changes may also favor species migration, for example, towards higher latitudes, to seek thermal refuge. Some freshwater species may also face local extinctions, as they don't possess the means to move towards more favourable habitats (Dziuba et al., 2020). An indirect impact of climate warming is the changes in precipitation and run-off patterns. This may lead to a higher input of nitrogen and phosphorus into freshwater habitats (Mack et al., 2019). Thus, understanding how rising temperatures and eutrophication potentially interact and impact freshwater communities is of prime importance.

This work aims at understanding how a freshwater cladoceran species, *Daphnia galeata*, reacts to temperature stress and

eutrophication. *Daphnia galeata*, an herbivorous cladoceran, serves as the basis of the food chains in the lakes and ponds it inhabits throughout the Northern Hemisphere (Cui et al., 2018). Because of this, it is an interesting model to test the impacts of temperature changes and eutrophication, to understand how those factors can influence other aquatic invertebrates and broader communities. In addition, *D. galeata* possesses a lifecycle with two distinct reproductive cycles and a population dominated by female individuals. The first is parthenogenetic reproduction, which occurs most of the time. However, when the individuals are stressed, sexual reproduction happens, and sexual eggs are produced (Fig. 1) (Ebert, 2022).

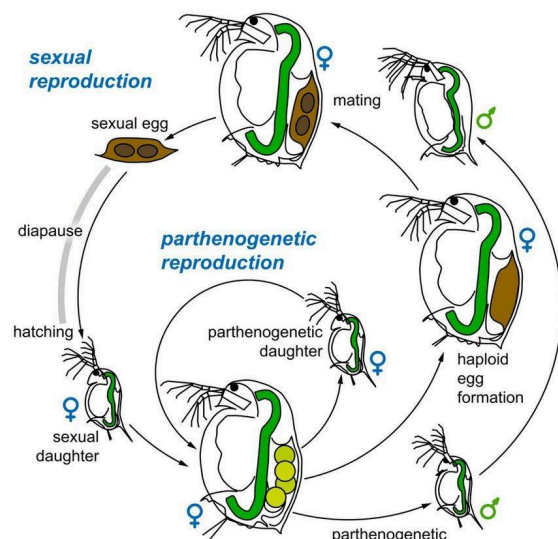


Figure 1: Lifecycle of *Daphnia galeata*, showing both sexual reproduction and parthenogenetic reproduction (Ebert, 2022). Stress-induced sexual reproduction is a strategy aimed at surviving stressful

conditions (low/high temperatures, drought, limited food, or predation). In temperate lakes, sexual eggs are also produced before winter. Eggs will then hatch when conditions become more favorable (Gerber et al., 2018). However, some may sink to the bottom of the lake or pond and stay stuck in the sediment. This allows for the later collection of resting eggs, which can be dated based on the layer of sediment they were found in (Fig. 2) (Brendonck & De Meester, 2003). Thus, different lineages can be raised in laboratory conditions to compare their performances and tolerance to stress, representative of different temperatures and pollution that their native lake experienced over time.

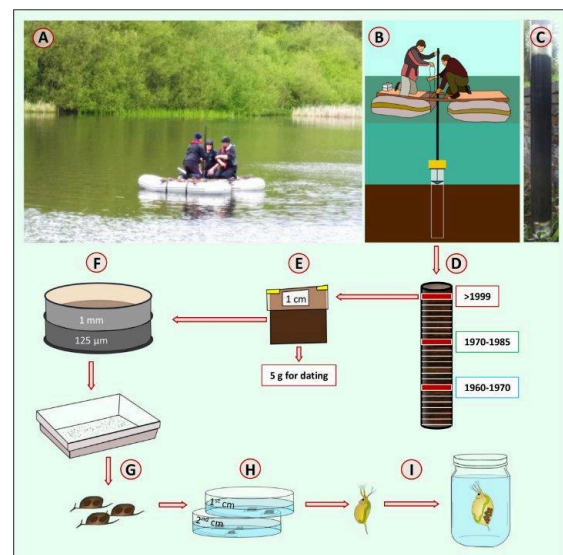


Figure 2: Description of the collect of *Daphnia* resting eggs: A) A boat is sent to the lake of interest, B) Sediment is dug up from the bottom of the lake, C) The tube of sediment, D) The different strata of sediment are dated, E) Each layer of sediment is then

put in a specific bag for dating and eggs isolation, F) Using fine mesh, the Daphnia resting eggs are separated from the sediments, G) The eggs are decapsulated, H) The eggs are exposed to favorable conditions to induce hatching, I) Hatchlings are separated in different lines corresponding to the sediment strata (Cambronero & Orsini, 2018).

To assess the heat tolerance of *D. galeata*, we use the Thermal-Death-Time (TDT) modeling approach. In ectotherms, heat-related organismal failure is driven by both temperature intensity and the duration of exposure. The TDT model explores this relationship through a gradual range of progressively more stressful temperatures, allowing us to understand the impact of temperature intensity and duration of exposure on the survival of organisms. The results obtained can then be used to infer how natural thermal fluctuation may impact populations in their habitats. This contrasts with other methods that can be used to assess the temperature-induced mortality, such as the critical thermal maximum (dCT_{max}), where individuals are exposed to a ramping temperature until heat-induced knockdown (Terblanche et al., 2025). The added value of TDT (Fig.3) compared to a dynamic temperature test is the various endpoints of survival, which allow for assessing how mild and acute stressful temperatures can lead to

mortality. In addition, dynamic tests can differ in terms of heating rates or exposure duration, with faster ramping rates ($>1^{\circ}\text{C}/\text{min}$) overestimating CT_{max} values by a few degrees (Jumbam et al., 2008). In addition, in natural conditions, heat stress is experienced by organisms as microclimatic events that vary in frequency, duration, and intensity. They also possess the capacity to acclimate and repair heat-induced damage between stressful events. Thus, relying only on survival endpoints like CT_{max} is not sufficient to frame how natural heat stress might impact organisms in their environment (Terblanche et al., 2025). The TDT method circumvents this use and also allows us to calculate different values, such as the critical temperature (lowest temperature inducing slow mortality, T_c), the coefficient of thermal sensitivity (z, how quickly heat-induced mortality progresses after T_c). These metrics allow us to derive the outcomes of theoretical dynamic ramping assays with great accuracy ($\pm 0,1^{\circ}\text{C}$) (Jørgensen et al., 2019; Willot et al., 2022). Thus, the TDT model can compute dCT_{max} assays, bypass limitations such as different ramping rates, and give a greater depth of information to link ecologically relevant thermal fluctuation to survival outcomes for ectotherms.

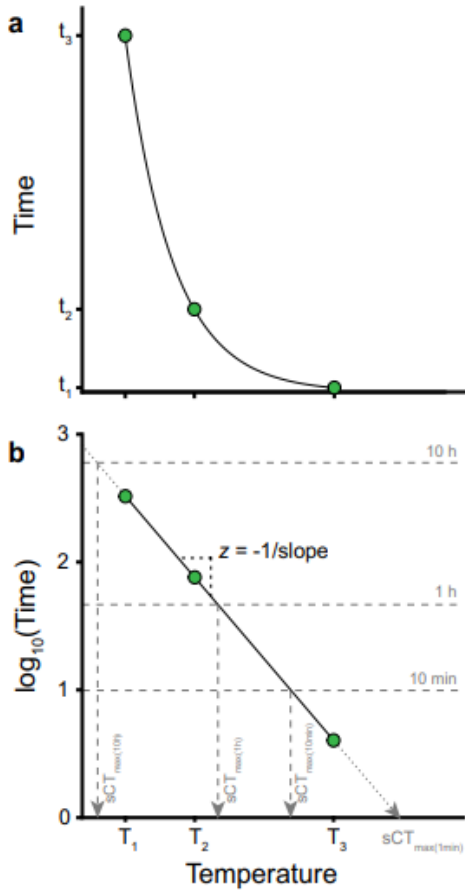


Figure 3: a) Graph of the time to heat-induced knockdown as a function of temperature, showing the exponential relationship elicited in ectotherms (Ørsted et al., 2022). b) The log transformation of the exponential relationship using several static experiments gives the TDT curve. We obtain the z value, which is relative to the slope and is a measure of thermal sensitivity, describing the time to heat knock down from mildly stressful (low temperature) to acute thermal (high temperature) exposure.

This z , the coefficient of thermal sensitivity, is directly linked to the slope of the TDT and represents the temperature sensitivity. A low z indicates that the organisms are more sensitive to changes in temperature, while a

high z indicates more tolerant organisms (Jørgensen et al., 2021).

Methods:

Maintenance of lineages

For the experiment, we chose five lineages of *D. galeata* obtained from a sediment core originating from Lake Greifensee. Each lineage was raised into a sufficiently large population to perform experiments. The individuals were placed in 100 mL glass jars filled with Artificial Daphnia Medium (ADaM). This medium aims to replicate as closely as possible the conditions of their lake of origin (Annex ADaM preparation). To create both low-phosphorus and high-phosphorus conditions, the individuals were divided equally into the glass jars to establish the two test populations. Those populations are kept in the same room, with conditions remaining similar throughout the year. The temperature is set at 20°C (+/- 1°C), while the light follows an 18-hour light and 6-hour dark cycle per day. The glass jars were kept in trays on shelves, directly under the neon tubes. They produce a luminosity of 67,5 $\mu\text{mol/s/m}^2$ and, given their position above the trays, ensure a good luminosity. When the conditions are favorable, asexual reproduction occurs through parthenogenesis. This may lead to excessively high density, which can be stressful for individuals. Thus, copies of each lineage were made every four weeks.

Those copies were made in the same 100 mL glass jars, with a starting population of five adult *Daphnia*. They allowed for a backup for each lineage and for both conditions. When the density in the 100 mL glass jar reaches a certain point (around 20 adult individuals), the *Daphnia* were transferred to a bigger, 400 mL glass jar, to allow for a higher population size.

Feeding regime

Daphnia were fed the algae *Tetradesmus obliquus*. It's a green microalgae grown in a homemade 3N-BBM+V medium (Annex Algae preparation) in a 1L glass container. They are grown in the same room where *Daphnia* were kept, and they thus benefit from the same conditions of temperature, luminosity, and light cycle. Additionally, a bubbler enables continuous oxygenation of the algae. *Daphnia* are fed thrice per week, on Mondays, Wednesdays, and Fridays. They were fed 0.75 µg/L of algae solution measured at an absorbance level of 0.750. To achieve this, the algae solution is diluted using the ADaM medium. The absorbance is then measured with a spectrophotometer until the desired value is achieved.

Phosphorus treatments

The two phosphorus conditions were obtained by modifying the medium and the algae solution. For the high-phosphorus condition, phosphorus was added to the

medium during its creation, and the individuals were fed using the regular algae solution. For the low-phosphorus condition, the regular medium was used, and the algae were grown in the 3N-BBM+V medium deprived of some phosphorus (Annex high-phosphorus ADaM preparation and low-phosphorus algae preparation).

Experiment setup

The TDT experiment was performed by connecting a circulating heat bath to an aquarium in which the individuals were immersed. In those aquariums, a metal rack was used to hold glass tubes. Those glass tubes were filled with the ADaM and pre-heated by putting them on the rack and setting the desired temperature on the heat bath. Adult *Daphnia* were then put individually in the tubes. The distinction between adults and juveniles was made on the observation of the presence/absence of eggs. We used six static temperature experiments ranging from 30 to 36°C to create the TDT curves for each lineage. Eight adult *Daphnia* were used for each temperature. A timer was set once all eight individuals were exposed. The time at which an individual ceased to move and was considered dead was then recorded. The time of death for each temperature was calculated as the median of the time of death over 8 individuals (Jørgensen et al., 2019). This gives us six endpoints per condition and thus

12 per lineage. From acute temperature leading to fast heat-knockdown in 10 minutes to low temperature inducing heat-knockdown in 4 to 7 hours.

Results:

TDT results

The impact of six static temperatures on survival was tested, leading to long knockdown time (30°C) and short knockdown time (36°C) (Annex TDT results). Lineage 2 displayed a different temperature at which it reached short knockdown as compared to other lineages, with 35°C leading to knockdown in around 10 minutes, while it was 36°C for the other groups. The most striking and unexpected information obtained from those graphs is the lack of significant differences between low phosphorus (triangles, dashed lines) and high phosphorus (circles, full lines)

conditions in each lineage. The time to knockdown remains consistent despite the difference in phosphorus treatment. Using a linear model and an ANOVA, we obtained the p-value for the temperature effect, the intercept, and the slope compared within groups. The temperature effect on the duration is highly significant ($p < 0,05$). The p-values for the intercept comparison are not significant, which means we don't have a difference in duration due to either low or high phosphorus conditions. The p-values for the slope comparison are also not significant. This means that individuals exposed to either low or high phosphorus conditions don't react differently and have a similar heat tolerance. Only in group 2, the p-value of slope comparison is close to the significant value with a value of 0,052 (Annex Statistical analysis of the TDT results).

Duration to coma log10-transformed in relation to temperature for each group

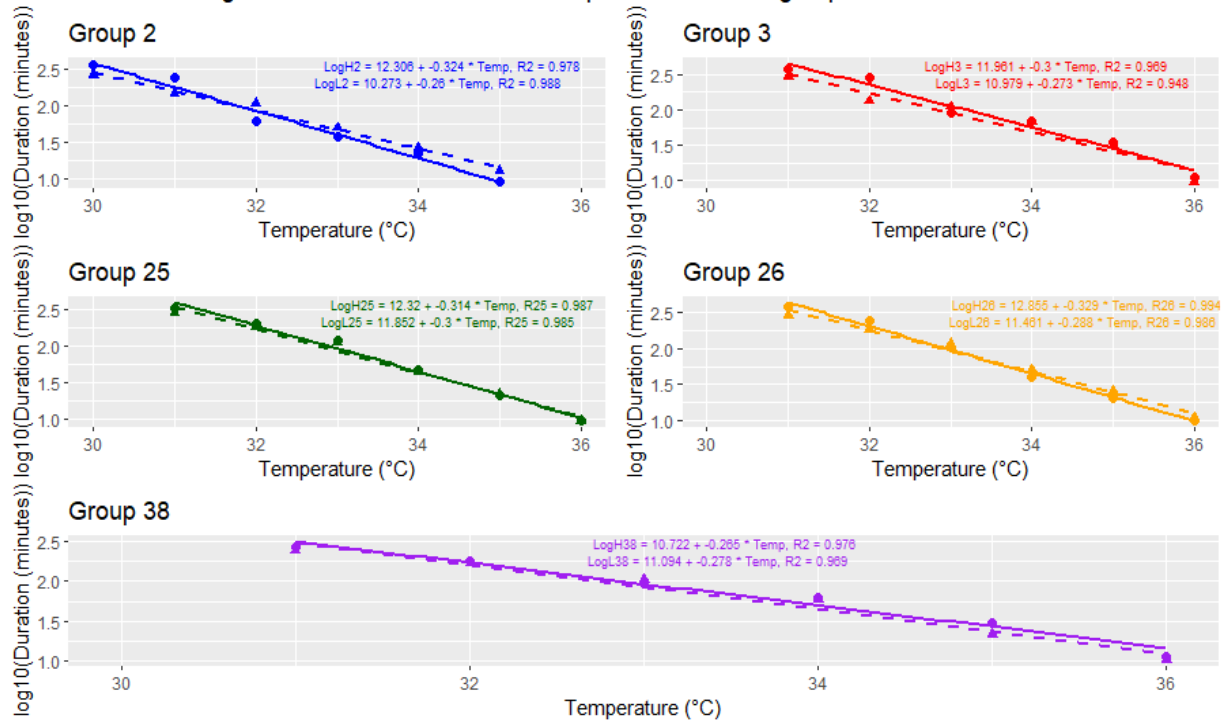


Figure 4: Graph of the TDT obtained for each group and their two conditions, Regression plot log₁₀-transformed median knockdown times against six static temperatures per species. This parameterization provides a model of time-to-knockdown in the full stressful range of temperatures, with the time log10 transformed in relation to temperature (in Celsius degrees). The low phosphorus conditions are shown by triangles and a dashed line, while the high phosphorus conditions are shown by circles and a full line. In addition, there are the equations of the lines, for both conditions (L2: Low phosphorus, group 2, H2: High phosphorus, group 2). The R² is also given for each line.

The lower heat-stress tolerance of group 2 as compared to the other groups is clearly visible when all the lines are grouped (Fig.5). There's a clear shift of the time for knockdown for group 2 (blue lines) towards lower temperature, as the 10-minute time was recorded at 35°C instead of 36°C like

the others. We can also observe that the different groups do slightly differ in terms of thermal sensitivity.

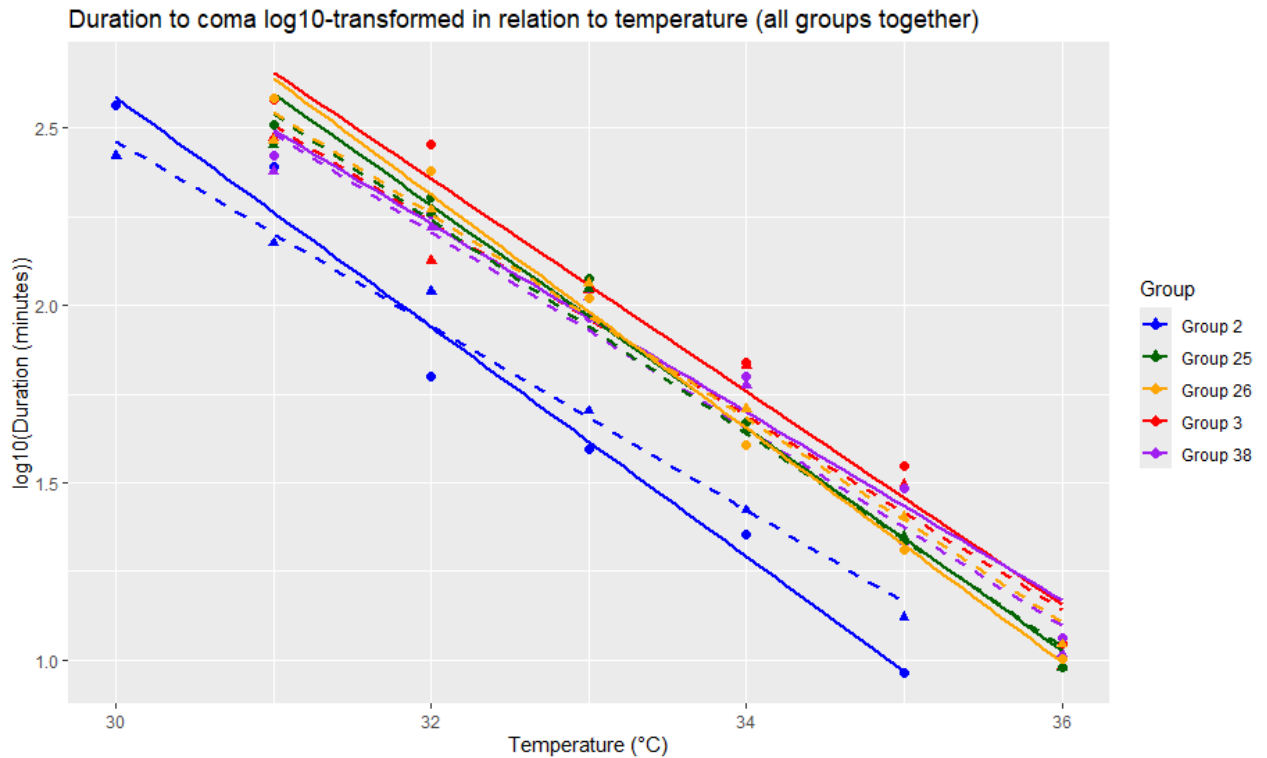


Figure 5: Graph of the TDT obtained for each group and their two conditions, with the time $\log_{10}$ transformed in relation to temperature (in Celsius degrees), all groups combined. The low phosphorus conditions are shown by triangles and a dashed line, while the high phosphorus conditions are shown by circles and a full line.

Parameters obtained from TDT results

The information obtained from the TDT curves covers all potential times of exposure in relation to temperature intensity combination, as well as their effect on survival. It can then be used to infer dCTmax with great accuracy ($\pm 0,1^{\circ}\text{C}$) (Willot et al., 2022). Our modelled dCTmax were obtained by using a ramp rate of $0,1^{\circ}\text{C}$ per minute and a starting temperature of 20°C . It was calculated for each group using an R script designed by Jørgensen et al. (2021). This R-script allows for calculating different values based on the results from

static and dynamic temperature tolerance assays (Jørgensen et al., 2021) (Annex R-script). Overall, the dCTmax values computed remain consistent with TDT results. Group 2 has the lowest dCTmax of all the groups, which is in line with the value obtained from the TDT model. Despite this, the z of the L2 is the highest one, indicating a good tolerance to changes in temperature. We can also observe a trend of the high phosphorus conditions (except H38) having a lower z than their group's lower phosphorus condition (Table 1).

Table 1: Table containing the predicted dCTmax for a 3°C/min ramp rate and the predicted dCTmax for a 0,1°C/min ramp rate, for each group and both of their conditions, using our TDT results.

Group	Ramp rate (°C/min)	dCTmax predicted (°C)	Ramp rate (°C/min)	dCTmax predicted (°C)
L2	3	40,28	0,1	35,01
H2	3	38,99	0,1	34,43
L3	3	41,06	0,1	36,06
H3	3	40,65	0,1	36,13
L25	3	40,22	0,1	35,3
H25	3	40,08	0,1	35,37
L26	3	40,61	0,1	35,37
H26	3	39,93	0,1	35,45
L38	3	40,58	0,1	35,26
H38	3	41,13	0,1	35,56

Additionally, to estimate how *Daphnia* would do during a week of heat stress, a time to coma of one week was calculated using the TDT results. We observe a trend with the high phosphorus condition (except H38) having a predicted higher temperature to reach coma in one week than their group's lower phosphorus conditions (Table 2).

Group	Time to coma	Temperature (°C)
L2	A week	24,08
H2	A week	26,03
L3	A week	25,53
H3	A week	26,51
L25	A week	26,13
H25	A week	26,51
L26	A week	26,33
H26	A week	27,26
L38	A week	25,53
H38	A week	25,32

Table 2: Predicted temperature at which the individuals will reach coma in a week of

constant exposure for each group and their two conditions.

Discussion:

The interesting aspect of the TDT experiment is that it permits the calculation of various values. The first one is the dCTmax. This is a measure of the highest temperature an organism can survive under a ramping rate of temperature (Jørgensen et al., 2021). On its own, this measure is easy to produce. Exposing organisms to a ramping rate and then measuring their time to arrive at coma is straightforward, but comparing results between studies is more arduous. If the starting temperature or the ramping rates differ, the dCTmax obtained is different. It then creates a collection of recorded dCTmax across the literature that

differ due to various methodologies used, of up to a few degrees (Jumbam et al., 2008). TDT can prevent such problems and can be used to infer other parameters like T_c , z , or to model long-term exposure and survival outcomes when individuals are exposed to mildly stressful temperatures (Table 2), without added methodological variations confounding biological effects.

For the dCTmax of our groups, we have a range of values between 34,43 (H2) and 36,13°C (H3) (Table 1). The dCTmax are quite similar within lineages, which may indicate that the phosphorus doesn't have a marked effect on the stress tolerance to temperature of these *Daphnia*. Group 2 seems to be the least heat-tolerant. This may be linked to the lower temperature it experienced back in 1958, which is the year with the lowest temperature among all the lineages. In comparison, group 3 shows the highest dCTmax, which could be the result of warmer conditions in 1964, as it's the warmest summer temperature recorded among the five groups (Table 3). Groups 25 and 26 have similar values. Those are expected, given they came from the same year. There's a lack of differences between low and high phosphorus conditions within the same lineage. This may mean that the low phosphorus condition is not harmful because the individuals can resist it or because the phosphorus concentration was

not low enough to have a visible effect. Alternatively, phosphorus concentration may have no impact at all on the heat tolerance of the *Daphnia*, at least at those concentrations. It can also imply that the food given is nutritious enough even when the algae are raised with less phosphorus, and that the excess phosphorus doesn't bother the individuals. Our phosphorus levels for high phosphorus condition (0,5 mg/L in the medium, algae raised with 0,163 mg/L of PO_4^{3-}) and for low phosphorus condition (no PO_4^{3-} in the medium, algae raised with 0,128 mg/L of PO_4^{3-}) are quite different from the concentration found in Lake Greifensee (Table 3). The bioavailable phosphorus, PO_4^{3-} , is relatively rare, especially after 1964, when the concentration is roughly three times higher than that of 1988 and 2000. The difference in depth is due to the higher activity of algae during the summer time, leading to a depletion of phosphorus in the upper, well-lit layer of the lake (Guy et al., 2011). Our algae don't have such low values because we need them to grow efficiently to allow us to feed our *Daphnia*. However, when we look at the total phosphorus concentration, we have higher values, especially before 1988. This is in line with the eutrophied state of the lake before the regulations lowered the amount of nutrient input in the lake. Despite this higher concentration, it's still at least five times lower than the one we used in our

experiment. Yet, we see no difference in terms of heat tolerance between the two conditions. We may conclude that the

phosphorus concentration has no visible effect on heat tolerance.

Table 3: Table containing the temperature and phosphorus concentration for each group. The temperature and phosphorus were measured at 0 and 2,5m depth. Their values are the mean values of the observations of June, July, and August from Lake Greifensee (Spaak et al., unpublished).

Lineage number	Year of the substrate layer	Temperature 0m (°C)	Temperature 2,5m (°C)	PO ₄ ³⁻ concentration, 0m,(mg/L)	PO ₄ ³⁻ concentration, 2,5m,(mg/L)
Group 2	1958	22,9	21,3	0,02391	0,0587
Group 3	1964	24,03	20,46	0	0,00326
Group 25	1988	22,53	21,96	0	0,00033
Group 26	1988	22,53	21,96	0	0,00033
Group 38	2000	20,7	20,26	0,00066	0,00066
Lineage number	Year of the substrate layer	Total phosphorus concentration, 0m,(mg/L)	Total phosphorus concentration, 2,5m,(mg/L)		
Group 2	1958	0,064	0,145		
Group 3	1964	0,111	0,111		
Group 25	1988	0,041	0,049		
Group 26	1988	0,041	0,049		
Group 38	2000	0,025	0,027		

The study from Vanvelk et al. (2021) aimed to compare the CTmax of three *Daphnia* species: *D. galeata*, *D. magna*, and *Daphnia pulicaria*. They followed the same methodology with the three species, all obtained from resting eggs from the same Belgian lake. They mention using the eggs from the 2cm upper layer, collected in 2018. We can thus assume that the eggs were laid recently, but before 2018. It would mean that those *Daphnia* were likely laid after our most recent clone, group 38. After hatching them, they put the clones of each species in three separate mesocosms in June 2018 in Belgium. The organisms then experienced a

natural heatwave, resulting in 22 days during which the average daily temperature of the water in the mesocosms exceeded 25°C, from June until August 2018. After this, they raised some of the lineages of the mesocosm in a common garden experiment, at either 20°C or 26°C, using individuals hatched from the sediments in laboratory conditions (without heatwave) as control. They then calculated the CTmax of those three species. To obtain CTmax, they exposed a single individual to a constant heating of 1°C per 20s and recorded CTmax when the animal lost all motor function. That ramp rate is very high, with 3°C per minute (Vanvelk et

al., 2021). Using the same script as we used to obtain our own dCTmax and our own TDT results, we replicated a dCTmax using a 3°C ramp rate instead of the 0,1°C we used (Table 1). It would make sense that their CTmax is higher than our own, simply because of this difference in methodology. Using this method, they found for *D. galeata* a CTmax of ~38.2 (control) and ~38.8 (exposed to heatwave) when raised at 20°C. This is superior to our own findings and computed CTmax, but it's likely due to the difference in ramp rate and possibly their lineages being more recent. The CTmax when *D. galeata* is raised at 26°C is higher, with ~39.5 (control) and ~39.6 (exposed) (Vanvelk et al., 2021). In both 20 °C and 26°C, we can see that exposure to higher temperatures led to offspring with better heat tolerance via acclimatization. That could be an explanation for our own group 3, which has the highest CTmax and the highest temperature recorded. Temperature acclimatization could also be the reason why they manage to raise *Daphnia* at 26°C, while our Tc for one week shows a temperature of around 26°C for our *Daphnia* raised at 20°C. We would certainly have better CTmax and higher temperature for Tc if our own *Daphnia* were raised at more than 20°C. The other species, *D. pulicaria*, showed similar results to *D. galeata*, with higher CTmax after exposure at 26°C. However, at 20°C, the CTmax was lower after exposition. For

the last species, *D. magna*, the CTmax at 20°C is higher compared to the other two species, with ~40,5 (control) and 41,5 (exposed), but still follows the same rise after exposure as *D. galeata*. Yet, at 26°C, the CTmax stabilizes at 41,1 (control) and 41,2 (exposed). Overall, they still observe the same plasticity in *D. galeata* with a higher CTmax value obtained when animals were raised at a high temperature (Vanvelk et al., 2021). This is in line with our own findings, yet the other two species follow a different trend of change in CTmax.

This relationship between heat tolerance and the temperature experienced by *D. galeata* during their lifetime is also noted in other *Daphnia* species by Geerts et al. (2015). They focused on comparing the CTmax of *D. magna* individuals hatched from resting eggs from 1955-1965 and 1995-2005 from the Felbrigg Hall Lake in Norfolk, UK. They observed that the most recent *Daphnia* had a CTmax (~37,4°C) compared to the older *Daphnia* CTmax (~36,8°C). They also note that the Felbrigg Hall Lake has experienced an increase in temperature of 1,15°C between 1960 and 2000, as well as three times more heatwaves (Geerts et al., 2015). The increase in heat tolerance following the increase in temperature and longer heat waves is confirmed from previous *Daphnia* populations through the comparison of CTmax. These changes in CTmax are likely

caused by changes in maximum temperatures rather than changes in average temperature, since CT_{max} measures tolerance to extreme temperature. Despite those evolutionary changes, populations are still at risk of climate change. Simply, their response was modified due to a slightly higher temperature, and the degree to which they may adapt to climate change is unknown (Geerts et al., 2015).

This is also what De Meester et al. (2011) found when comparing *D. magna* raised at 20°C and 24°C. The 24°C raised *Daphnia* showed an overall higher thermal tolerance than the 20°C raised ones and better performance, such as a higher growth rate (De Meester et al., 2011). This is confirmed by another study, with *D. magna* raised at 24°C showing higher CT_{max} than those raised at 20°C (Brans et al., 2017).

The second measure we can obtain is *z*. For this value, we have clearer differences between the conditions. For group 2, the low phosphorus condition shows a higher *z*, which is in line with the higher dCT_{max} compared to the high phosphorus conditions. For groups 3, 25, and 26, we have a similar higher heat tolerance in the low phosphorus conditions, while it's the high phosphorus conditions in group 38 that have a higher *z*. For the first four groups, low phosphorus conditions may be beneficial for the

Daphnia heat tolerance. Low phosphorus conditions negatively impact food quality (Van Donk et al., 2008). This may cause constant stress in the individuals, with the production of heat-shock proteins (HSP). Those proteins also help with heat tolerance (Demertzioglou et al., 2025). We can then hypothesize that the already produced HSP allows low-phosphorus *Daphnia* to have higher heat tolerance than the high-phosphorus condition *Daphnia*, as they don't need to produce them when stress occurs. This is still only an hypothesis and is quite surprising. Lower phosphorus is supposed to lower the food quality of *D. galeata*, leading to reduced growth and overall lower performance (Adamczuk, 2024).

Laboratory experiments often lack some ecological relevance as their conditions only partly represent what wild animals may experience. For our experiment, the individuals were exposed to a temperature that didn't vary. This is very unlikely to happen in freshwater habitats. Thus, to have an idea of the impact that increased temperature may have on *D. galeata*, a critical temperature was estimated. A temperature was obtained at which the individuals will reach coma in a week (Table 2). This prediction is still different from natural conditions, but it gives an insight into the impact a week-long heatwave may have

on a *Daphnia* population. All high phosphorus groups (except H38) show a higher heat tolerance than the low phosphorus groups, with a time to coma for one week ranging from 25,32 (H38) to 27,26 °C (H26). The low phosphorus groups' time to coma for one week ranges from 24,08 (L2) to 26,33 °C (L26). This could be linked to the H groups' increased food quality, allowing them to tolerate stress longer. It's interesting to note that the temperatures observed are only a few degrees above the mean temperature the *Daphnia* experienced in the wild. *D. galeata* is an epilimnetic species (inhabiting the upper layers in freshwater habitats). The upper layers of water are the most affected by the temperature increase and usually the warmest (Seda et al., 2007). Despite this, *D. galeata* can potentially negate some of the impacts of prolonged high temperatures, through behaviour, as the species can migrate through the upper layers of the water column (Henning-Lucass et al., 2016). Even if this study is focused on heat tolerance in lab conditions, *Daphnia* populations in the wild can respond to increased temperatures via acclimatization. The climate changes will make life history traits plasticity very relevant for ectotherms like *Daphnia*, as their metabolism depends on environmental temperature. *Daphnia* possesses this plasticity in response to temperatures. As temperatures get higher, *Daphnia* species

tend to grow faster, reproducing earlier and more often (Bowman et al., 2023). This is confirmed by other studies for *D. galeata* (Geerts et al., 2015) and on other *Daphnia* species (Brans et al., 2017; De Meester et al., 2011; Vanvelk et al., 2021). We may thus expect that if the temperature increases in the coming decades, *D. galeata* populations may adapt, the offspring becoming more heat-tolerant than their progenitors. However, if a gradual increase in temperature may be less impactful, longer periods with harmful temperatures may lead to a more negative impact on the populations. Thus, understanding how *Daphnia* can adapt to temperature changes may be a good opportunity to anticipate how their populations might respond under future environmental conditions.

Conclusion:

Temperature is the dominant stressor in our heat tolerance experiments, with phosphorus levels not significantly impacting their heat tolerance. The lineages' thermal tolerance differs from each other and is likely caused by long-term thermal adaptation and plasticity. This plasticity seems to be limited, with dCTmax and Tc values highlighting how *D. galeata* can be negatively impacted by a sudden rise in temperature. This was assessed using the TDT modelling, allowing for more parameters to be calculated than CTmax alone. However, this method is still

only an approximation of fluctuating natural temperatures, and caution must be applied when analysing the results. Our results highlight how evolutionary history shapes *Daphnia galeata*'s heat tolerance and potential response to climate change.

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AI statement:

Grammarly's AI was used for sentence-level and word-level correction. Microsoft's AI was also used to provide insights and suggestions to enhance the R script for graphs.

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Annex:

ADaM preparation:

This medium is an artificial medium designed for the culture of zooplankton. It's made of Milli-Q® water, sea salt (from an

aquarium supply company), and three different solutions. Solution A (SA) is composed of $\text{CaCl}_2 \times 2\text{H}_2\text{O}$, at a concentration of 117.6 g/L. Solution B (SB) is composed of NaHCO_3 at a concentration of 25.2 g/L. Solution C (SC) is composed of SeO_2 at a concentration of 0.07 g/L. All of these are added to the container containing Milli-Q® water. The different solutions are kept in different containers to avoid the formation of precipitates. In addition, to avoid any contamination, the measurements are done under a hood. We usually create 20L of this medium, since our main containers are 20L jerrycans. They are kept in the same room as the *Daphnia* and are shaken before use.

High phosphorus ADaM preparation:

This medium, specific to the high phosphorus condition, is prepared the same way as the regular ADaM. However, phosphorus is added to achieve a concentration of 0.5 mg/L of PO_4^{3-} . This is done by adding 2.19 mg/L of KH_2PO_4 during the medium preparation.

Algae preparation:

The macro and micronutrient bottles are kept at a 1000 times concentration. This means that to create the medium, a container is filled with some Milli-Q® water, then 1ml from each bottle is added. The container is then filled again with Milli-Q® water until 1 L is reached. After preparation, the medium contains a total of 0,163 mg/L of PO_4^{3-} .

3N-BBM+V					
For Tetradesmus BCCM DCG					
		mg/L	mg/200 mL	g/200 mL	
macro nutrients	CaCl ₂ .2H ₂ O	25	5000	5	Each macro nutrient in 6 separated 200 ml bottles
	K ₂ HPO ₄	75	15000	15	
	KH ₂ PO ₄	175	35000	35	
	MgSO ₄ .7H ₂ O	75	15000	15	
	NaCl	25	5000	5	
	NaNO ₃	750	150000	150	
		micro nutrients in g/L			All micro nutrients can be mixed in one 1L bottle. Respect the order, start with Na2.EDTA.
micro nutrients	Na ₂ .EDTA	4,500		4,500	
	FeCl ₃ .6H ₂ O	0,582		0,582	
	MnCl ₂ .4H ₂ O	0,246		0,246	
	ZnCl ₂	0,030		0,030	
	CoCl ₂ .6H ₂ O	0,012		0,012	
	Na ₂ MoO ₄ .2H ₂ O	0,024		0,024	
					stored à 4°C

Figure 6. Various macro nutrients and micro nutrients, with their concentrations, are used to create the 3N-BBM+V medium.

Low phosphorus algae preparation:

The algae medium is created following the same protocol as the regular algae medium.

However, the algae medium was depleted of KH_2PO_4 by lowering its amount by 50 mg, leading to a concentration of 125 mg/L

before the 1000 dilution to create the medium (0,125 mg/L of KH₂PO₄ in the low phosphorus algae medium instead of 0,175 mg/L in the regular algae medium after the

dilution). After preparation, we thus obtain a medium that contains a total of 0,128 mg/L PO₄³⁻.

Statistical analysis of the TDT results:

Table 4: Table comparing within the group (between Low phosphorus and high phosphorus conditions) the p-value of the temperature effect on the log transformed time to coma, the p-value of the difference of intercept, and the p-value of the difference of slope.

Group	Temperature effect (p-value)	Intercept difference (p-value)	Slope difference (p-value)
2	2,78E-08	0,477	0,052
3	7,67E-07	0,284	0,538
25	9,55E-09	0,615	0,617
26	2,04E-09	0,824	0,083
38	1,63E-07	0,474	0,713

TDT results:

Table 5: Table with the recorded time to coma (in minutes) in relation to the temperature (in Celsius) per group and their two conditions.

Group	Time to coma (min)	Temperature (°C)	Group	Time to coma (min)	Temperature (°C)	Group	Time to coma (min)	Temperature (°C)	Group	Time to coma (min)	Temperature (°C)
L2	13,14	35	L3	9,59	36	L25	9,5	36	L26	11,03	36
L2	26,41	34	L3	31,3	35	L25	22,25	35	L26	25,39	35
L2	50,35	33	L3	67,53	34	L25	44,57	34	L26	51,05	34
L2	109,13	32	L3	110,54	33	L25	111,5	33	L26	115,5	33
L2	149,54	31	L3	133,26	32	L25	182,44	32	L26	186,23	32
L2	264,11	30	L3	298,03	31	L25	283,44	31	L26	292,24	31
H2	9,15	35	H3	11,17	36	H25	9,53	36	H26	10,02	36
H2	22,49	34	H3	35,39	35	H25	21,54	35	H26	20,49	35
H2	39,25	33	H3	69,13	34	H25	46,59	34	H26	40,5	34
H2	63,26	32	H3	93,52	33	H25	119,42	33	H26	105,05	33
H2	246,54	31	H3	285,02	32	H25	201,12	32	H26	238,54	32
H2	366,13	30	H3	379,29	31	H25	324,26	31	H26	384,5	31
L38	10,36	36	H38	11,53	36						
L38	21,52	35	H38	30,51	35						
L38	59,49	34	H38	63,31	34						
L38	105,36	33	H38	92,06	33						
L38	165,29	32	H38	180,17	32						
L38	239,11	31	H38	265,53	31						

R-script:

The R script used was the one provided by Jørgensen et al. (2021), using the

following

link:

<https://github.com/MOersted/Thermal-tolerances>

Synthesis and state of the art:

Species:

Daphniidae are a family of herbivorous cladocerans that process food particles by filter-feeding them in the water column (Becker et al., 2004). The species of interest for this thesis is *Daphnia galeata*. It is distributed in the Northern Hemisphere and is small, measuring between 1 and 2.5 mm in size. This water flea serves as the basis of the food chain in the aquatic ecosystem, functioning as a primary consumer that filter-feeds in the water column (Cui et al., 2018). Living in an aquatic environment, the populations are known to react to climatic shifts; they rise and peak in spring and then decline towards summer. This summer decline is called a midsummer decline (MDS). The density of the population and the MDS duration are impacted by several factors, such as water temperature and food quantity. A higher temperature in summer often leads to a higher MDS due to thermal stress. However, a milder winter might lead to an early MDS due to the old individuals dying early in summer, in May or June. On the contrary, a colder winter may delay the reproduction and induce a later die-off of the individuals and thus a postponed MDS around July (Benndorf et al., 2001; Wagner & Benndorf, 2007).

Reproduction:

Concerning reproduction, females usually reproduce asexually, producing parthenogenetic offspring. This reproduction strategy leads to populations of clonal lineages, with multiple copies of the same genotype. However, this parthenogenetic means of reproduction only occurs when conditions are favorable for the species (Henning-Lucass et al., 2016; Tsugeki et al., 2009). Under unfavourable conditions like high temperatures, lack of available food, crowding, or eutrophication, sexual reproduction is favored by the females. Thus, they produce encapsulated dormant eggs that are resistant to stressful conditions. These eggs usually sink to the bottom of the aquatic environment and can be covered by sediment. They then form a resting egg bank. In that state, they can remain viable for decades, even centuries. With this reproduction technique, the species can survive adverse conditions and persist in its habitat (Frisch et al., 2014; Tsugeki et al., 2009). A branch of biology called resurrection ecology takes advantage of these dormant eggs in dated sediment layers. By rehydrating and hatching them, it is possible to bring back lines of *Daphnia* that have been extinct for decades and date them with the dated sediment layers. These resurrected lines

allow us to perform genetic or life-history experiments by comparing the actual species and genotypes living in the aquatic environment and the clones from those dormant eggs. With those experiments, it is then possible to test the evolutionary responses to changes in environmental parameters during the lifetimes of the clones (Henning-Lucass et al., 2016; Tsugeki et al., 2021).

Phosphorus pollution:

Freshwater habitats around the world can be affected by eutrophication, deteriorating water quality, inducing species shifts in the trophic levels, and cyanobacterial blooms. One of the ways a lake becomes eutrophic is through the supply of nutrients from the surrounding land, such as phosphorus. These nutrients are then caught in the internal recycling of the lake. The nutrients are fixed in the sediments when water circulates, and are then released when water stagnates, usually during summer (Förster et al., 2021). In the case of oligotrophication, especially through a decrease in phosphorus concentration, a decrease in phytoplankton can be observed. This leads to a limitation for herbivores like *D. galeata*, which require a high phosphorus supply to grow. In a study analysing Lake Bodensee (Switzerland) oligotrophication process, the *D. galeata* population declined in abundance

compared to the population before the oligotrophication process took place (Hartwich et al., 2012). Eutrophication favored *D. galeata* when the lakes Bodensee and Greifensee (Switzerland) started to become more eutrophic, allowing *D. galeata* to invade the habitat and hybridize with the local populations of *D. longispinna*. This eutrophication process was correlated with an increased concentration of phosphorus due to human activities. Overall, the analysis of 43 Swiss lakes showed a trend of *D. galeata* inhabiting warm and productive (eutrophic) habitats (Rellstab et al., 2011). However, around the 1980s, stricter environmental management helped lower the lakes' phosphorus concentrations, inducing an oligotrophication process. This process led to a decrease in the population of *D. galeata*. It highlights the need for the species for a productive, eutrophic lake and, indirectly, a high phosphorus concentration (Spaak et al., 2012; Stich & Brinker, 2010).

The lower phosphorus level also impacted the phytoplankton because phosphorus is a limiting nutrient. Being the food source of *D. galeata*, a decrease in phytoplankton further induced a lower abundance of the species in places such as Lake Bodensee (Stich & Brinker, 2010). Algae grown in phosphorus-limited conditions also impact

Daphnia. The digestibility is altered, which can lead to reduced growth and reproduction even when algae are given in high density (Adamczuk, 2024). Limited phosphorus in the water may also impact *D. galeata* directly, with later reproduction and fewer eggs for the brood. In experimental settings, this led to an overall lower population growth rate. Some deformations also occurred, with black spots on the head as well as facial deformations on the organisms, in addition to some moulting problems. Those results come from a study using *D. galeata* clones from Lake Gerifensee reared under low (2 µg/L) and high (50µg/l) phosphorus conditions. These concentrations mirrored the conditions in two Swiss lakes in which *Daphnia* lives, to reflect realistic conditions in both eutrophic and oligotrophic lakes. Oligotrophic conditions lead to changes in life history traits, with the changes mentioned above (Zanetti, unpublished). In contrast, higher phosphorus concentration can help the *Daphnia* by enhancing the food resources in the habitat. The nutrient addition can help boost primary productivity and induce higher growth rates for grazers species like *D. galeata*. The increase in phosphorus concentration didn't seem to impact the populations of cladocerans in the experiment of Adamczuk (2024), and they showed high resistance to phosphorus

enrichment (Adamczuk, 2024). Since *D. galeata* is a part of the cladocerans, we can expect them to react in the same way. This was confirmed by the experiment from Frost et al. (2025), which showed a higher abundance for the population when phosphorus concentration was high and a lower population when phosphorus concentration was low. This population change was mediated by the algae, which concentrated the phosphorus present in the habitat (Frost et al., 2025).

Temperature:

Given the predicted rise of temperature in the coming years and decades of around 1.5°C (Calvin et al., 2023), it is reasonable and needed to try to understand how climate change may impact species. Depending on their physiological limitations and evolutionary potential, species distribution could change. If they're limited to a specific habitat or geographic area, local extinction or local persistence might take place. In this case, we could observe an evolutionary response from populations to global warming by mechanisms of phenotypic plasticity, micro-evolutionary adaptations, or genetic changes (Kim et al., 2024; McGaughan et al., 2021). A common trend seen in ectotherms subjected to an increase in temperature is a reduction in size following the temperature-size rule.

Higher temperatures lead to accelerated development, inducing earlier reproduction and a smaller adult body size. This is due to a trade-off in energy allocation: The rapid development leads to less time and resources available for somatic growth, reducing the time to reach adulthood but leading to a smaller size in adults (Kozłowski et al., 2004). In *Daphnia* species, smaller individuals usually produce fewer eggs and are less efficient at feeding. However, fish predation is generally lower on smaller individuals, which benefits them (Brucet et al., 2010). Despite this assumption, work by Dziuba et al. (2020) showed an increase in size for *D. galeata* clones in warmer lakes compared to control (colder) lakes. Following the principle of allocation, metabolic rate increases with body size and temperature, while eventually resources become limiting when temperature increases. The organism thus faces a trade-off and uses its resources to increase fitness. In the case of an increase in temperature, the organism becomes smaller to allow more resources for reproduction. The observed increase seems to be out of the ordinary, but it could be correlated with more efficient grazing due to the larger size. It also makes the organism more resistant to starvation and allows *D. galeata* to overwinter if the conditions are mild enough, which gives it

an edge over other *Daphnia* species that can't overwinter. The lack of fish predation (the lakes are devoid of any fish species) may also help the organisms to reach a larger size since there's no predation pressure favoring smaller individuals (Dziuba et al., 2020).

Another potential impact of climate change on freshwater communities is the risk of invasion by other species. In temperate lakes, environmental constraints such as temperature can modify the community structure of *Daphnia*. For example, *D. galeata* inhabits warmer lakes than *D. longispina*, another *Daphnia* species similar in size to *D. galeata*. The two species are separated in the Alps, with *D. galeata* inhabiting the warmer lakes. Temperature was identified to be the factor explaining the dominance of the species in those lakes (Keller et al., 2008). In addition to this, better tolerance to warmer climates compared to some other species, *D. galeata* can overwinter if the winter temperatures are mild. Zeis et al (2010) showed that this capacity to overwinter by using parthenogenetic reproduction instead of diapause could give *D. galeata* a competitive advantage over other species of *Daphnia* sharing the same German reservoir (Zeis et al., 2010). The same distribution can be seen outside Europe, with a clear geographic distribution in

Chinese lakes between *D. galeata* and *D. dentifera*. *D. galeata* inhabits lower altitude, warmer lakes than the other species, which prefer colder conditions. Interestingly, their hybrids inhabit the intermediate area (Ma et al., 2019). Ectotherms like *D. galeata*'s metabolic rates are directly linked to environmental temperatures, making freshwater ecosystems especially vulnerable. Henning et al. (2016) used a resurrection ecology experiment on *D. galeata* inhabiting a lake in Germany. Using dormant eggs taken from the lake's sediment, they brought back lines from 1965-1974 and 2000-2009 to compare their adaptive response to an increase in temperature. They expected a better fitness under higher temperatures for the 2000-2009 lineages, given that the lake's mean summer temperature rose by 2 °C between the 20th and 21st centuries. They exposed individuals to higher temperatures, which led to smaller individuals growing faster and reproducing earlier, as expected by the temperature-size rule (individuals in warmer environments tend to grow faster but mature at a lower body size). In addition, a gradual performance increase was noted, with an optimum temperature for performance at 25°C. However, as soon as the upper thermal limit was reached, a rapid decline was observed (Henning-Lucass et al., 2016).

Another impact highlighted by a study from Tsugeki et al. (2009) is that warmer winter conditions could lead the populations to not produce resting eggs because they are not necessary for overwintering. The work also highlighted differences in overwintering strategy between different lakes in Europe, with some populations relying on resting eggs while others simply don't. This shows that the species can overwinter without resting eggs, avoiding the need for sexual reproduction. This would mean that a climate favorable for the species all year round would favor asexual reproduction because more eggs are produced that way. This could lead to a potential loss of genetic variation and thus could increase the potential risk of extinction due to unexpected changes in environmental conditions (Tsugeki et al., 2009). The study from Vanvelk et al. (2021) compared different *Daphnia* species, including *D. galeata*. Their results of the experimental rearing of *Daphnia* at higher temperatures show similar results to the two studies mentioned above, with a smaller size and maturity reached faster. However, the population went extinct during the heatwave (More than 25°C for more than 5 days), which could be a potential problem in case of increasing heat waves in the future (Vanvelk et al., 2021).

An increase in temperature in the freshwater habitat shared by *Daphnia* species could also modify competition between species. Dziuba et al. (2020) monitored the populations of *D. galeata* and two other *Daphnia* species in five natural lakes that were heated for 5 decades, with an increase in temperature of 3 to 4°C. They noted a separation from *D. galeata* and *D. longispina*, the first thriving in warmer conditions while the second preferred colder waters, and suggested that *D. galeata* could invade and outcompete the other species amid climate change. *D. galeata* was also noted to rely more on parthenogenetic reproduction, which could lead to a loss of genetic diversity, lessening the capacity to face environmental stress in the future. They conclude that climate warming might alter overwintering strategies, promote invasion, and even competitive exclusion of species not adapting fast enough (Dziuba et al., 2020).

Interactions between stressors:

Given *Daphnia magna*'s use as a model organism in ecotoxicology and lower research effort on *D. galeata*, it is easier to find experiments on the combined effects of temperature and phosphorus on *D. magna*. From these experiments, we can extrapolate, with caution, the impact of

those combined effects on our species of interest, *D. galeata*. Adamczuk (2024) highlighted the combined effects of increased temperature and increased phosphorus concentration on *Daphnia*. The temperature increase showed the same effects as in other experiments, with a lower size; however, the increased phosphorus concentration didn't significantly impact the population. In the habitat, higher temperatures might lessen the concentration of phosphorus in the sediment and increase its concentration in the water column (Adamczuk, 2024).

The lack of phosphorus in the environment could also lead to a lower growth rate due to a lower efficiency of assimilation of carbon assimilation. This effect was observed on *D. magna* reared at high temperature with a phosphorus limitation. The level of phosphorus in the organism was lower at higher temperatures because of a higher efficiency of the use of phosphorus in biochemical processes, which lowers the content. This higher efficiency comes with a higher respiration rate to release excess carbon taken from the food poor in phosphorus (McFeeters & Frost, 2011). The study of Wojewodzic et al. (2011) corroborates the impact of the lack of phosphorus, which is more pronounced when the temperature is high (Wojewodzic et al., 2011).

Thermal death time method:

Given the likely increase in temperature in the coming years and decades, investigating the impact of temperature changes on *D. galeata* is of great interest. Even if the impact of increased temperature is understood to be harmful for the ectotherms, no single physiological disruption can be pinpointed as the cause of heat failure (Ørsted et al., 2022). Hypotheses to explain ectothermic heat failure vary, but most of them relate to several biological processes (Bowler 2018): Protein denaturation, thermal inactivation of enzymes, or membrane dysfunction. These processes could operate together to ultimately lead to heat failure in the individual. In poikilotherms, such as *Daphnia galeata*, membrane impairment linked to high temperature might be the cause of heat failure. The study of Bowler (2018) highlighted this to be a cause of heat death. Acclimation to higher temperatures leads to a change in the composition of the membrane's lipids, which could indicate that membrane composition plays an important role in dealing with an increase in temperature. Heat damage or death might thus occur when the membranes are too much perturbed, which leads to a cascade of cellular dysfunction (leakage of ions, failure of membrane proteins, impairment

of cell functions), possibly leading to cell death (Bowler, 2018).

Multiple metrics can be used to assess thermal tolerance in ectotherms, most commonly CT_{max} (critical thermal maximum, the temperature at which locomotor function is lost (Jørgensen et al., 2021)), either through static or dynamic assays (exposing individuals to either constant or ramping temperature until heat-induced knockdown, respectively). This variety of techniques can make it difficult to compare metrics of heat tolerance and results across methodologies. In addition, laboratory protocols measuring heat tolerance in ectotherms through static or dynamic assays have only a limited ecological relevance. This is due to a simplification of the complex and unpredictable changes in the microclimatic conditions that organisms face in nature. Heat knockdown results from a combination of temperature intensity and duration of exposure. In other words, an organism can survive longer at lower temperatures but only briefly at higher temperatures, highlighting an interactive effect between temperature and time. In a dynamic CT_{max} assay, where both temperature and time increase simultaneously, it's difficult to disentangle their individual contributions to knockdown. Was the insect exposed to

temperatures that were too high, for too long, or both? CT_{max} cannot answer this question, as it conflates the effects of temperature intensity and exposure duration. In contrast, static assays allow us to isolate the effect of exposure time at each temperature. When multiple static assays are combined, they end up forming a thermal death time (TDT) curve, which captures the full relationship between temperature and duration leading to knockdown. This approach is more informative, and this is why it's superior, but also more labor-intensive (Jørgensen et al., 2019, 2021; Rezende et al., 2014; Willot et al., 2022).

A possible answer to these limitations is to use the thermal death time (TDT) model to deconstruct the exponential relation between temperature intensity and time of exposure. To achieve this, thermal death time (TDT) will be used to evaluate temperature response (Jørgensen et al., 2021). TDT represents the combination of several static assays, deconstructing the entire range of temperature intensity and time of exposure on survival. As shown in Figure 7, we can observe on the TDT graph “a” an exponential relationship between temperature and the time to coma (used as a mortality indicator) which is conserved in all ectotherms studied so far

(Jørgensen et al., 2019, 2021; Rezende et al., 2014; Willot et al., 2022).

On the graph “b”, the TDT curve is shown as a line due to the log transformation of the time to coma. This linear TDT relationship provides the time needed for knockdown in studied organisms at each temperature, and vice versa.

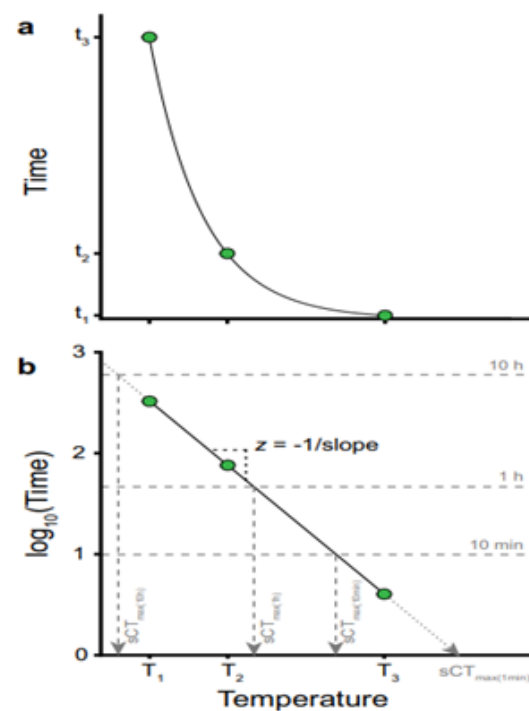


Figure 7. Graph “a”: Exponential relationship between time and temperature and coma. Shows the three times (t₁, t₂, and t₃) at which coma was observed. A curve crosses the three points, giving the TDT curve.

Graph “b”: Linearized relationship between log(Time) and temperature, giving a linear TDT curve. The arrows show the sCT_{max} for the three different dashed lines, corresponding to three different durations. $z = -1/\text{slope}$ of this line

describes the thermal sensitivity of heat failure (Jørgensen et al., 2021).

To explore the impact of temperature on the survival of *Daphnia*, thermal death time (TDT) will thus be used. The main advantage is to understand the entire effect of time of exposure with the temperature intensity on survival. In addition, the use of the TDT curve highlights the exponential decrease of survival time as temperature and thus stress increase. Heat injury accumulates exponentially with temperature, hence the exponential relationship seen in Figure 1. A great advantage of using TDT is the possibility to design experiments to test heat tolerance between different populations across time of exposure and temperature intensity. In addition, this method also allows for examining how temperature acclimation and adaptation shape heat tolerance across this relationship and how different factors might affect the heat stress response (Ørsted et al., 2022).

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