OIKOS

Research article

Exploring reversibility and contrasting patterns in temperature–size relationships across spatial and temporal scales using subfossil chironomids

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Oikos
2025: e11370
doi: 10.1002/oik.11370

Subject Editor: Matthew Symonds
Editor-in-Chief: Pedro Peres-Neto
Accepted 09 June 2025

The extent to which different magnitudes and directions of temperature fluctuations explain long-term trends in aquatic invertebrate body size is largely unknown. Using elevational gradients and paleolimnological reconstructions, we tested the hypotheses that the size of subfossil chironomid (non-biting midges) head capsules (HCs) will covary with temperature changes, with opposite morphometric changes occurring during warming and cooling phases, and that body size variation can be modified by other environmental conditions unassociated with temperature variations. Results indicated that the effects of increasing temperatures on chironomid HC size were reversed when temperatures decreased, with both warming and cooling producing similar effect sizes, corresponding to a change in HC length of ~ 3% per 1°C. Additionally, our results showed that other environmental drivers can mask temperature effects on chironomid HC sizes. Specifically, we found that bottom water oxygen concentration was negatively associated with HC lengths of *Chironomus anthracinus*-type. We hypothesize that this pattern is driven by prolonged larval development in oxygen-depleted lakes and/or changes in basal food sources used by chironomid larvae, ultimately affecting their final body size. Future research should focus on disentangling the multiple drivers that control body size in aquatic insects, given their potential to either enhance or confound the temperature–size relationship, to improve our mechanistic understanding of aquatic insect size variation over long timescales.

Keywords: Body size variation, chironomidae, climate change, paleolimnology, phenotypic plasticity

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Introduction

Climate warming has been identified as one of the most important and immediate threats to freshwater biodiversity (Heino et al. 2009) which is dominated by ectothermic organisms that are especially sensitive to the effects of temperature changes (Paaijmans et al. 2013). Nevertheless, species might be able to persist under changed thermal conditions through, inter alia, phenotypic plasticity (Kroeker et al. 2020), as seen in the decreases in body size commonly observed for aquatic invertebrates along gradients of increasing temperatures (Merilä and Hendry 2014). Studies of temperature–size relationships in freshwater ectotherms have largely focused on the effects of warming on body size structures along present-day spatial (e.g. elevational) and temporal (e.g. seasonal) gradients (Kobayashi 1998, Daufresne et al. 2009). In contrast, studies over longer (multi-generational) time frames are scarce (Botsch et al. 2024), hindering our understanding of long-term trends in aquatic invertebrate body size.

Climate change has resulted in shifts in the magnitude and direction of temperature fluctuations (Seppä et al. 2009), with the Earth experiencing periods of both cooling and warming, which are likely to have induced long-term variations in the body size of aquatic invertebrates. Furthermore, high magnitudes of temperature changes are expected to exceed the thermal tolerance limits of many species (Troia 2023), potentially restricting their phenotypic plasticity. However, the potential for limitations in the phenotypic plasticity of individual species, including in their range of possible body size variations, to impose absolute limits on their ability to adjust to large magnitudes of climate change is poorly understood. The long-term history of alternating periods of cooling and warming raises important questions about the reversibility of phenotypic plasticity of aquatic invertebrates. Specifically, body sizes are expected to covary with temperature variation, exhibiting opposite morphometric changes during warming and cooling phases, with size changes observed during a transition from cool to warm conditions being reversible during a warm-to-cool transition. However, such reversibility in body size trends cannot be easily assessed using traditional approaches which are typically based on field-based observations or experiments.

Lake sediments form a valuable environmental archive of past climate changes, and sediment cores provide a time-integrated signal, as each sediment layer typically represents several years. This integration smooths short-term weather fluctuations, allowing us to focus on the effects of broader, longer-term climate patterns on species' phenotypes. Studying lake sediments also appears as a promising approach to unraveling long-term body size variations in aquatic invertebrates. In particular, head capsules (HC) of Chironomidae (Arthropoda, Diptera, Nematocera) are morphologically well-preserved in lake sediments (Smol et al. 2001). As Chironomidae are one of the most diverse and abundant insect families in lakes (Ferrington 2008), their remains can serve as surrogates for wider biodiversity in aquatic ecosystems. As with other aquatic invertebrates, temperature plays a key role in regulating both chironomid larval size (Frouz et al. 2002, Baek et al. 2012) and final adult size (McKie et al. 2004, Wonglersak et al. 2021). Applying the morphometric approach to subfossil chironomids should introduce a leap forward in phenotypic plasticity studies and allow us to make better predictions about climate-related future changes in the body size of aquatic biota.

Empirical observations of the temperature–size relationship from experiments and field studies have also uncovered highly variable responses among populations and species (Gardner et al. 2011, Wonglersak et al. 2021). Observed deviations from the typical pattern of reduced body size under warming conditions (Hayden et al. 2017, Bonacina et al. 2023) may result from additional environmental drivers that modify or confound the direct effects of temperature on aquatic invertebrate body size (Davidowitz et al. 2004). For example, the rate and duration of larval growth driven by in-lake properties (e.g. occurrence of longer larval development durations under oxygen depletion; Mackey 1977, Verberk et al. 2021) or variations in food quantity and quality (e.g. the association between lipid-rich food and larger individuals; Vos et al. 2000) are known to affect the body size of aquatic ectotherms. Changes in these environmental conditions can be amplified by climate warming (Jane et al. 2023, Jansen et al. 2024) or unassociated with temperature fluctuations (e.g. eutrophication-induced oxygen depletion; Millet et al. 2010, Foley et al. 2012). Disentangling the influence of various environmental factors that contribute to body size regulation is, therefore, crucial for understanding how ectotherm body size changes over the long term.

In this study, we employed two approaches for investigating temperature–size variation in subfossil chironomids. First, we investigated the size variation of subfossil chironomids over a current-day spatial gradient, capturing broad variations in temperature and dissolved oxygen, to disentangle the effects of temperature and an important additional environmental driver in regulating chironomid body size. We predicted (hypothesis 1) that variation in dissolved oxygen would reduce/modify the effects of temperature on chironomid size variation. We also investigated climate-induced changes in subfossil chironomid sizes over an extended temporal period covering the last 13 500 years to evaluate the potential range of body size variation within well-defined chironomid morphotypes, over warming and cooling climate phases. We predicted that (hypothesis 2) morphotype body size would covary with temperature changes, with opposite morphometric changes occurring during warming and cooling phases (i.e. size changes occurring during a cool-to-warm transition are reversible). However, given that thermal tolerance limits are likely to constrain the range of body sizes possible within morphotypes, we further predicted that (hypothesis 3) absolute limits in morphotype body size would be apparent, with similar maximum and minimum body sizes regardless of the magnitude of thermal change.

Material and methods

Study sites and climate data

First, we investigated recent changes in subfossil chironomid HC size along a present-day elevational and latitudinal gradient. Seventeen small (area range 5–505 ha) and relatively deep (maximum water depth range 6.6–32.0 m) lakes marginally disturbed by local activities were studied in Sweden (Supporting information and Fig. 1). In July 2017 and June–July 2020, surface sediment cores were retrieved from the deepest point of the lakes using a gravity corer (9 cm in diameter; UWITEC). The uppermost 1 cm of each sediment core was collected and used for subsequent analyses. When available, late summer water quality data covering the 5 years preceding the sampling year (from 2012 to 2017 or 2015 to 2020) were retrieved from the Swedish National Monitoring Program database (https://miljodata.slu.se/mvm/) including total organic carbon, total nitrogen, total phosphorus, calcium and oxygen concentrations, and water conductivity and Secchi depths (Supporting information). Unfortunately, oxygen concentration data were only available for low-elevation lakes (Supporting information). Temperature data covering the 5 years preceding the sampling year were also extracted from the HCLIM database (https://www.smhi.se), and mean July air temperature (MJAT; °C) was calculated and summarized in Table 1.

Second, we studied longer-term responses of chironomid HC size to different climate phases using seven sediment records covering the Late-Glacial and Holocene periods as summarized in Table 1. We used two Late-Glacial records to quantify morphometric changes induced by the ~ 3.5°C cooling observed between ca 13 500 cal. BP and ca 12 000 cal. BP, and the subsequent rapid ~ 3 °C warming observed between ca 12 000 cal. BP and ca 10 000 cal. BP (Veski et al. 2012). Ages given are indicative, and the sediment layers used for HC measurements usually spanned larger age ranges (corresponding to the ranges of depths in the core of the sieved sediment samples to find sufficient suitable HC). We also quantified the chironomid size response to long-term cooling of ~ 1.5°C reported between ca 5000 cal. BP and present (ca 1920–2017 AD) climates (Sjögren 2021, Wastegård 2022). Finally, we compared chironomid size between the end of the cold Little Ice Age period (at ca 1900 AD) and the present-day (ca 1990–2020 AD) warmer climate (Table 1) for which a temperature increase of ~ 1.8°C was observed for the studied lakes (https://www.smhi.se). The difference between the ages of the present-day climates (ca 1920–2017 AD and ca 1990–2020 AD) is caused by variations in the sedimentation rates in the studied lakes, thus inducing variation in the temporal resolution of sediment records.

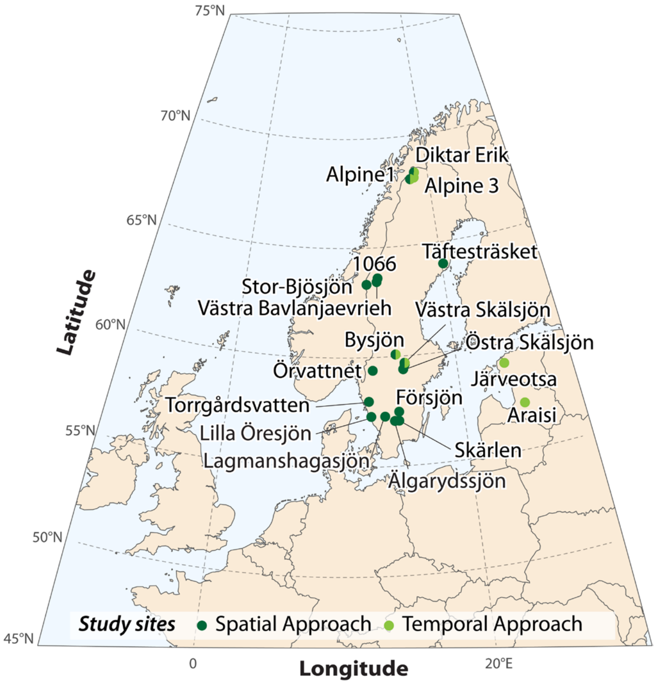

Figure 1. Map showing the location of the study sites. Colours refer to lakes included in the spatial approach (elevation gradient; dark green), the temporal approach (paleolimnological studies; light green) or both approaches (dark and light green circles).

Chironomid HC measurements

Depending on the taxonomic composition and the concentrations of HCs in sediment samples, morphometric measurements were alternatively conducted on *Micropsectra insignilobus*-type (Tanytarsini tribe), *Sergentia coracina*-type and *Chironomus anthracinus*-type (Chironomini tribe). These morphotypes typically inhabit the profundal zones of lakes and their HCs are usually found well-preserved (i.e. entire and not broken) and in high abundances in lake sediments. These sediment-dwelling chironomid larvae also belong to the functional feeding group of deposit feeders (Serra et al. 2016), thus forming an important link between basal resources and higher trophic levels in lake food webs (Wagner et al. 2012). Changes in their size structure have, therefore, the potential to have wider implications for the lake food web. In the present day, these morphotypes are broadly distributed in northern Europe along latitudinal and elevational gradients (revealing large ranges of thermal tolerance limits), and a gradient of thermal optima ranging from colder-water morphotypes (*Micropsectra insignilobus*-type) to warmer-water taxa (*Chironomus anthracinus*-type) with *Sergentia coracina*-type occurring at intermediate temperature conditions (Larocque et al. 2001, Luoto 2009, Heiri et al. 2011). *Chironomus anthracinus*-type can also persist under periods of lower oxygen concentration than the other two studied morphotypes (Little et al. 2000), and *Chironomus anthracinus*-type is, therefore, well suited for evaluation of the extent to which present-day changes in oxygen concentration might modulate the temperature–size relationship.

Sediment samples were washed with NaOH (10%) at room temperature and sieved through a 100 µm mesh sieve following Belle (2025). Previous studies (Frouz et al. 2002) reported that while higher temperatures lead to smaller final body sizes, they also promote faster larval growth rates. As a result, the temperature–size relationship may not hold during

Table 1. Elevation (m a.s.l.) and mean July air temperature (MJAT; oC) of the study sites. For the sites in the spatial gradient, temperature data covering the five years preceding the sampling date were extracted from the HCLIM database (https://www.smhi.se), and MJAT was calculated for each lake. For the paleolimnological sites, the periods used for temporal comparisons are highlighted with blue colour indicating cooling trends, while red colour denotes warming phases.

Lake	Elevation (m a.s.l.)	Spatial gradient	MJAT	Paleolimnological approach	Temporal comparisons (blue = cooling trend; red = warming trend)			
Forsjön	269	X	15.9					
Skärlen	213	X	16.1					
Torrgårdsvatten	132	X	16.3					
Västra Bavlanjaevrieh	696	X	12.5					
Sjö 1066	1066	X	9.6					
Alpine 1	999	X	9.3	X	**5000 cal. BP**	**1920-2017AD**		
Alpine 3	999	X	9.3	X	**5000 cal. BP**	**1920-2017AD**		
Diktar Erik	385	X	13.1	X	**5000 cal. BP**	**1920-2017AD**		
Älgarydssjön	199	X	16.1					
Täftesträsket	379	X	13.1					
Lilla Öresjön	155	X	16.8					
Lagmanshagsjön	182	X	16.1					
Örvattnet	283	X	15.7					
Stor-Bjösjön	568	X	12					
Östra Skälsjön	220	X	15.8					
Bysjön	106	X	16.4	X	**1900AD**	**1990-2020AD**		
Västra Skälsjön	236	X	15.5	X	**1900AD**	**1990-2020AD**		
Araisi	121			X	**13500 cal. BP**	**12000 cal. BP**	**12000 cal. BP**	**10000 cal. BP**
Järveotsa	42			X	**13500 cal. BP**	**12000 cal. BP**	**12000 cal. BP**	**10000 cal. BP**

early larval stages, and a warming-induced size reduction may not be evident in the initial instars. As a result, only the HC belonging to the fourth larval instar was used for morphometric analyses. HCs were picked out from the sieving residue under a stereo microscope, and mounted with Aquatex between microscope slides following the guidelines for phenotypic reconstruction studies outlined by Belle (2025). HCs were then identified under a microscope using Brooks et al. (2007), and measurements were performed using a microscope camera coupled to the Nikon Digital Sight 1000 software (Belle 2025). The measurements comprised quantification of the length between the top of the median tooth of the mentum (for *Micropsectra insignilobus*-type and *Chironomus anthracinus*-type) or the center of the median teeth (for *Sergentia coracina*-type), and the base of the HC (for *Sergentia coracina*-type and *Chironomus anthracinus*-type) or the inner margin of the post-occipital plate (for *Micropsectra insignilobus*-type) as shown in the Supporting information.

Data analysis

Principal component (PC) analysis was applied to lake morphometry, present-day water chemistry and temperature datasets to assess the potential covariation of additional environmental drivers with temperature along the elevation gradient. HC lengths of *Micropsectra insignilobus*-type, *Sergentia coracina*-type and *Chironomus anthracinus*-type were then added to each PC correlation circle as a supplementary (passive) variable to identify factors potentially associated with length variability. Linear regressions were used to quantify changes in HC lengths of *Micropsectra insignilobus*-type, *Sergentia coracina*-type and *Chironomus anthracinus*-type along the elevation and environmental gradients.

To visually show temporal changes in chironomid HC lengths of *Chironomus anthracinus*-type, *Micropsectra insignilobus*-type and *Sergentia coracina*-type between the different climate periods, boxplots were used, and Kruskal–Wallis tests were applied to quantify the level of significance. When comparing temporal trends in HC lengths, their variation was expressed relative to the average HC lengths reported during the older periods, thus taking positive values if HC lengths increased over time and negative values if HC lengths decreased. Correlations between relative changes in HC lengths and magnitudes of temperature changes were then tested for non-linear relationships with generalized additive models, but as no non-linear patterns were found, we proceeded with linear regression. All statistical analyses and plots were performed using the R ver. 4.5.0 software (www.r-project.org).

Results

In total, 76 individual measurements were taken on HCs of *Micropsectra insignilobus*-type from eight lakes across the current-day spatial gradient. The smallest HCs of *Micropsectra insignilobus*-type were found in Lake Torrgårdsvatten (with average HC lengths of 172.8 ± 3.6 µm) and the largest ones in Lake 1066 (with average lengths of 198.6 ± 2.8 µm; Table 2). The first two axes of the PC analysis of lake

Table 2. Means and standard deviations of head capsules lengths of *Micropsectra insignilobus*-type, *Sergentia coracina*-type and *Chironomus anthracinus*-type used for the spatial approach.

Lake	*Chironomus anthracinus*-type	*Micropsectra insignilobus*-type	*Sergentia coracina*-type
Forsjön	–	172.9 ± 6.4	233.2 ± 4.6
Skärlen	–	181.4 ± 1.4	–
Torrgårdsvatten	–	172.8 ± 3.6	–
Västra Bavlanjaevrieh	–	186.5 ± 4	–
Sjö 1066	–	198.6 ± 2.8	–
Alpine 1	–	196.7 ± 7.3	250 ± 9
Alpine 3	–	192.4 ± 6	252.1 ± 6.4
Diktar Erik	–	183.9 ± 4.6	–
Älgarydssjön	434.7 ± 6.9	–	–
Täftesträsket	411.7 ± 20	–	–
Lilla Öresjön	410.7 ± 16	–	–
Lagmanshagsjön	425.9 ± 7.8	–	–
Örvattnet	382.9 ± 13	–	243.1 ± 11
Stor-Bjösjön	–	–	242.4 ± 6
Östra Skälsjön	–	–	234.3 ± 5.8
Bysjön	392.1 ± 14.4	–	220.6 ± 4
Västra Skälsjön	–	–	238.5 ± 1.4

characteristics accounted for 55.8% and 18.3% of the total variance (Fig. 2A). PC1-axis captured the large-scale gradient in mean July air temperature (MJAT) that covaried with elevation and latitude, whereas the PC2-axis captured more local-scale variation in lake water total phosphorus and total nitrogen concentrations. Additional projection of the HC lengths of *Micropsectra insignilobus*-type in the PC correlation circle suggested a positive association with lake elevation and negative associations with site latitude and MJAT (Fig. 2A). HC lengths showed a significant positive relationship with lake elevation, corresponding to an increase of the HC length of ~ 3 μm for every 100 m elevation gain ($R^2 = 0.66$; p-value < 0.001; Fig. 2B).

Measurements (n = 59) on HCs of *Sergentia coracina*-type were performed from eight lakes across the current-day spatial gradient, and HC lengths ranged from 220.6 ± 4 μm in Lake Bysjön to 252.1 ± 6.4 μm in Lake Alpine 3 (Table 2). The first two PC axes of lake characteristics accounted for 47% and 23.3% of the total variance, respectively (Fig. 2C). PC1-axis captured the large-scale gradient in MJAT that covaried with elevation, whereas the PC2-axis captured more local-scale variation in lake water total phosphorus and nitrogen concentrations and lake water conductivity. Additional projection of the HC length of *Sergentia coracina*-type in the PC correlation circle suggested a positive association with lake elevation and negative association with MJAT (Fig. 2C). HC lengths showed a significant positive relationship with lake elevation, corresponding to an increase of the HC length of ~ 2 μm for every 100 m elevation gain ($R^2 = 0.46$; p-value < 0.001; Fig. 2D).

HCs of *Chironomus anthracinus*-type were found in sufficient numbers to be measured in six lakes (n = 39), and HC lengths ranged from 382.9 ± 13 μm in Lake Örvattnet to 434.7 ± 6.9 μm in Lake Älgarydssjön (Table 2). The first two PC axes of lake characteristics accounted for 54.9% and 26.5% of the total variance, respectively (Fig. 2E). PC1-axis captured a large-scale gradient in water colour (TOC and Secchi depth) whereas the PC2-axis captured variation in bottom water oxygen concentration. Additional projection of the HC length of *Chironomus anthracinus*-type in the PC correlation circle suggested a negative association with bottom oxygen concentration (Fig. 2E). HC lengths showed a significant negative relationship with bottom water oxygen concentration ($R^2 = 0.36$; p-value < 0.001).

The drastic cooling between ca 13 500 cal. BP and ca 12 000 cal. BP was associated with a significant increase in HC lengths of *Chironomus anthracinus*-type in both Lake Araisi and Lake Järveotsa records, from 409.9 ± 12.4 μm to 427.2 ± 7.5 μm and from 340.1 ± 9.7 μm to 416.7 ± 12.6 μm, respectively (Fig. 3C; Kruskal–Wallis test, p-value $<$ 0.05). The marked warming observed ca 12 000 cal. BP and ca 10 000 cal. BP was associated with a significant decrease in HC lengths of *Chironomus anthracinus*-type in Lake Järveotsa (from 416.7 ± 12.6 μm to 351.7 ± 11.5 μm; Fig. 3C) and of *Sergentia coracina*-type in Lake Araisi (from 277.8 ± 4.1 μm to 268.9 ± 4.5 μm; Fig. 3B). The long-term cooling between ca 5000 cal. BP and modern climates induced significant increases in HC lengths of *Micropsectra insignilobus*-type in both Lakes Alpine 1 and Alpine 3, from 183.9 ± 6.1 μm to 196.6 ± 6.9 μm and from 180.2 ± 6.1 μm to 192.3 ± 5.9 μm, respectively (Fig. 3A), but large overlaps between the two periods were observed in Lake Diktar Erik record (Fig. 3A, left panel). The warming reported between the end of the Little Ice Age and present-day climate was characterized by a significant decrease in HC lengths of *Sergentia coracina*-type in Lake Västra Skäljön (from 243.4 ± 1.9 μm to 239.1 ± 1 μm), and in Lake Bysjön (from 231 ± 6 μm to 220.6 ± 4.1 μm; Fig. 4C; p-value < 0.05; Fig. 3B). No significant difference was found in HC lengths of *Chironomus anthracinus*-type in Lake Bysjön during the same period (Fig. 3C).

A linear negative correlation was found between relative changes in HC lengths and magnitudes of temperature changes. Associations between temperature and HC length during warming and cooling climate phases were comparable in effect size (Fig. 4; $R^2 = 0.67$; p-value < 0.001),

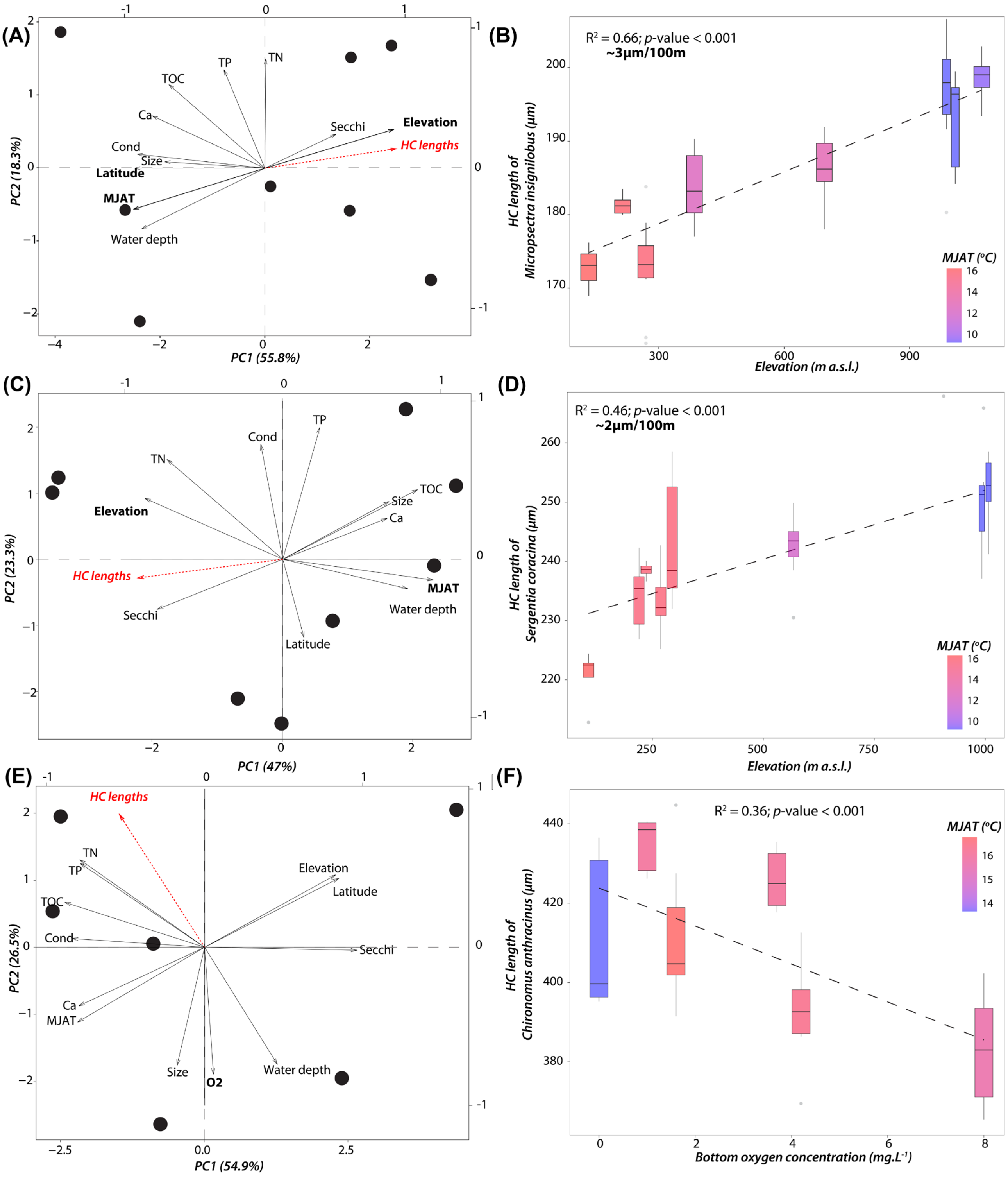

Figure 2. Left panels show factorial maps of the principal component analysis (PC1 versus PC2) performed on lake characteristics data (black dots). The black arrows display variable contributions to the first two PC-axes, and the variables contributing the most to PC1 are highlighted with bold labels. HC lengths were also added to the PCA correlation circles as passive variables and represented by dotted red arrows. The right panels show the relationship between elevation (B, D) or bottom oxygen concentrations (F) and HC lengths expressed as absolute change (in µm). The coefficient of determination (R^2) and statistical significance (p-value) of correlations between the variables are included at the top of the plot. Colours were used to reflect the mean July air temperature (MJAT; °C) of the studied sites. (A and B) refer to *Micropsectra insignilobus*-type, (C and D) to *Sergentia coracina*-type, and (E and F) to *Chironomus anthracinus*-type.

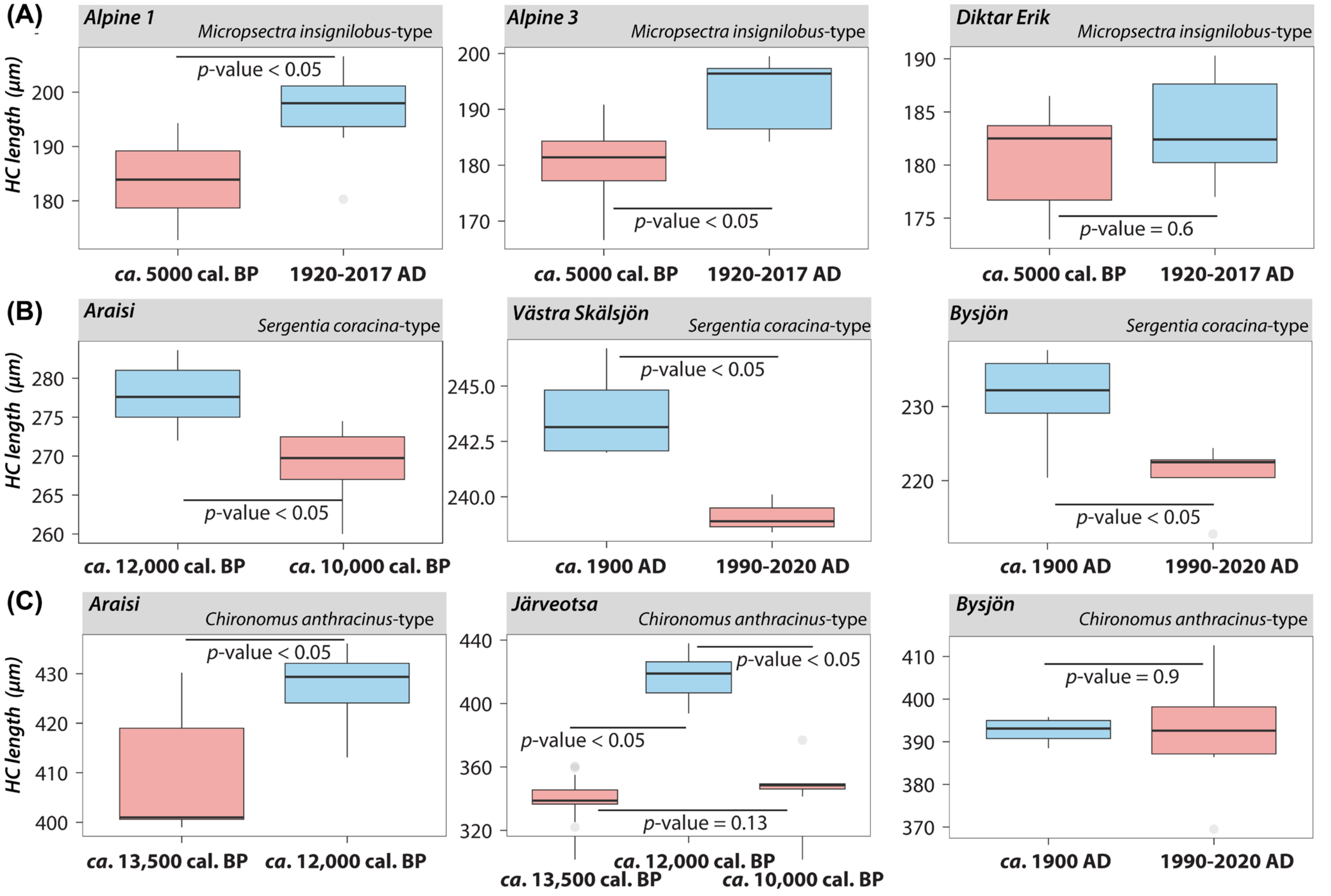

Figure 3. Response of head capsule (HC) lengths of *Chironomus anthracinus*-type (A), *Micropsectra insignilobus*-type (B) and *Sergentia coracina*-type (C) to the different climate phases. Colours were used to illustrate warm (red) and cold (blue) periods. The p-values of the Krukal–Wallis test are reported in each panel.

corresponding to a change in HC lengths of ~ 3% per 1°C. However, large differences in changes in HC lengths in response to similar temperature changes were observed among lakes. For example, the HC lengths of *Chironomus anthracinus*-type increased by 22.5% between ca 13 500 and 12 000 cal. BP in Lake Järveotsa, whereas an increase of only 4.2% was reported during the same period in Lake Araisi. Similarly, no change in HC lengths of *Micropsectra insignilobus*-type was found during the cooling observed between ca 5000 cal. BP and present (ca 1920 AD to present) in Lake Diktar Erik, whereas HC lengths of *Micropsectra insignilobus*-type were drastically increased during the same period in Lakes Alpine 1 and Alpine 3 (Fig. 4A).

Finally, strong variability in HC lengths was found for all studied taxa across both temporal and spatial gradients (Table 3). We found relative changes of HC lengths up to 22.5% for *Chironomus anthracinus*-type (with HC lengths ranging from 340 ± 9.9 µm to 416.7 ± 13), 17.4% for *Micropsectra insignilobus*-type (with HC lengths ranging from 172.8 ± 3.6 to 198.6 ± 2.8 µm) and 21.8% for *Sergentia coracina*-type (with HC lengths ranging from 220.6 ± 4 to 268.9 ± 4.8 µm), revealing comparable levels of size variation possible for the studied morphotypes.

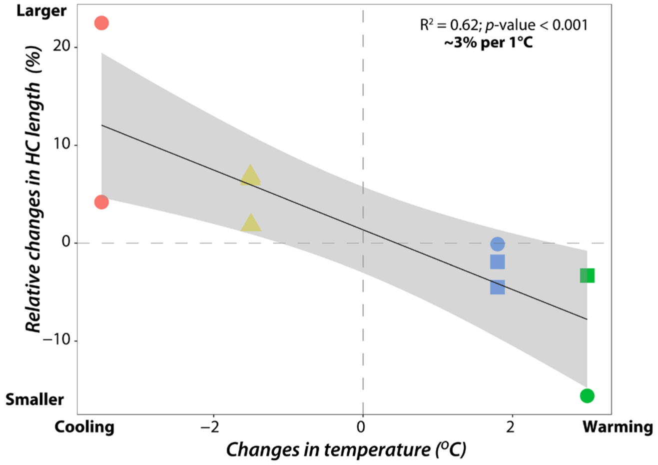

Figure 4. Relationship between changes in temperature and relative changes in head capsule (HC) lengths. Colours illustrate the comparison between ca 13 500 cal. BP – ca 12 000 cal. BP (red), ca 12 000 cal. BP – ca 10 000 cal. BP (green), ca 5000 cal. BP – 1920/2017 AD (yellow) and ca 1900–1990/2020 AD (blue) periods. Circles represent *Chironomus anthracinus*-type; triangles *Micropsectra insignilobus*-type and squares *Sergentia coracina*-type.

Table 3. Summary of the minimum, maximum and relative change in head capsule (HC) lengths of *Micropsectra insignilobus*-type, *Sergentia coracina*-type and *Chironomus anthracinus*-type.

Morphotype	Min HC length (μm)	Max HC length (μm)	Relative change (%)
Micropsectra insignilobus-type	172.8 ± 3.6	198.6 ± 2.8	17.40
Sergentia coracina-type	220.6 ± 4	268.9 ± 4.8	21.80
Chironomus anthracinus-type	340 ± 9.9	416.7 ± 13	22.50

Discussion

Among our most striking and novel results was evidence of the potential for the multigenerational effects of increasing temperature on the body sizes of freshwater ectotherms to be reversed. Specifically, in support of our second hypothesis, we found that during periods of warming the HC length of our focal chironomid morphotypes decreased as temperature increased, whereas the opposite was observed during periods of cooling. In both cases, the effect size was comparable with a change in HC length of 3% for every 1°C change in temperature. These reported changes are concordant with those previously reported in the literature for other freshwater taxonomic groups and geographical areas (reaching up ~ 9% per 1°C of warming; Baek et al. 2012, Forster et al. 2012, Audzijonyte et al. 2019, Wonglersak et al. 2021, Junker et al. 2024). Our results have implications for understanding changes in food webs under climate change. For example, a smaller mean size among chironomids, an important food source for many consumers in the freshwater food webs, might increase the amount of time their predators need to spend feeding to meet their energetic requirements, with negative consequences for predator fitness (Perry and Pianka 1997). Furthermore, deposit-feeding chironomids typically consume fine particulate organic matter and, therefore, can contribute substantially to carbon cycling in freshwater habitats, with variation in particle size preference among species partially dependent on their gape width (Bundschuh and McKie 2016). Warming-driven reduction in HC size has the potential to alter the particle sizes a species can process. Our understanding of the implications of temperature-related shifts in HC sizes for fine particulate organic matter processing rates and lake carbon cycling is a significant knowledge gap.

Changes in HC lengths responded linearly to temperature changes, with no upper or lower thresholds in the capacity of chironomids to acclimate to novel thermal conditions apparent. Accordingly, and contrary to the predictions of our third hypothesis, there is no evidence for the limitations in the extent of size variation in the studied morphotypes across the range of thermal conditions associated with the climate transitions studied. We postulate that these findings reflect the broad thermal tolerance limits of the studied morphotypes (see also Larocque et al. 2001, Luoto 2009, Heiri et al. 2011), with their capacity for body size adjustment favouring persistence under novel climate conditions. Future research should thus investigate changes in HC size along broad temperature gradients of morphotypes with more stenothermic requirements, such as the cold-water taxon *Micropsectra radialis*-type and warm-water taxon *Chironomus plumosus*-type (Larocque et al. 2001). It is also possible that longer-term climate change might drive adaptive changes in the degree of phenotypic plasticity exhibited by a species and modify its thermal niche shift (Geerts et al. 2015), potentially resulting in phenotypes with a narrower range of temperature tolerances but with a greater ability to persist under warmer conditions. Future research should combine investigations of subfossil records with multigenerational studies of present-day biota to understand how rapidly the phenotypic plasticity of chironomid taxa and their broader thermal niche respond to temperature changes over both short and long timescales (Antell et al. 2021). These findings have implications for paleoclimate reconstructions using subfossil chironomids, as a vast majority of these studies rely on the 'reasoning by analogy' paradigm consisting of applying contemporary features to ancient organisms, thus assuming that species have similar climatic and ecological requirements nowadays and in the past. Improving the understanding of species–temperature relationships across temporal scales will support formulation of more realistic predictions about climate-related changes in aquatic biodiversity and improve the reliability of paleoecological inferences.

Finally, our findings indicate that other environmental drivers can suppress temperature effects on chironomid size variations, as predicted by our first hypothesis. In contrast with our paleolimnological results, which showed that *Chironomus anthracinus*-type HC size was responsive to longer-term, temporal temperature fluctuations, temperature was a poor predictor of among-lake variations in HC size of *Chironomus anthracinus*-type in our spatial analysis. Rather, we found that bottom water oxygen concentration was negatively associated with HC lengths of *Chironomus anthracinus*-type in the spatial analysis. We hypothesize that this observed pattern is driven by prolonged larval development in oxygen-depleted lakes and/or changes in basal food sources used by chironomid larvae. On the one hand, *Chironomus anthracinus*-type can typically grow in the oxygen-depleted profundal zone (Little et al. 2000), and under such conditions, prolonged larval development may lead to larger chironomid sizes. On the other hand, Vos et al. (2000) found a positive association between chironomid larval length and food quality (e.g. lipid-rich food induces larger chironomid larvae). Oxygen-induced changes in basal food sources used by chironomid larvae have also been widely evidenced (Grey 2016, Belle et al. 2018, 2024) and shifts in basal resources across temporal and spatial scales have potential for driving large variations in HC sizes. Overall, our findings highlight the potential for additional environmental drivers to explain substantial variation in HC length and possibly alter (weaken or amplify) responses to temperature variation. Furthermore, several additional environmental drivers are likely to covary along the elevation gradient (e.g. water chemistry, geology, land cover), all potentially confounding or increasing the complexity of the relationships between temperature on chironomid size variation. Future research should, therefore, aim to unravel the environmental drivers other than temperature that contribute to regulating chironomid body size. Specifically, combining carbon stable isotope (Belle et al. 2018) and morphometric measurements of subfossil chironomid HC could be a promising approach to disentangle the respective influence of food source and temperature variations on body size. Understanding how body size was affected by climate change in the past will also help us to predict how it may be affected in the future.

A potential complication in interpreting our results is that the finest taxonomic unit at which subfossil chironomids can be reliably identified is the morphotype level, which often includes several species sharing the same morphological characteristics (Anderson et al. 2013, Mrozińska and Obolewski 2024). As such, a chironomid size decrease over time observed at the morphotype level could be due to two underlying mechanisms: higher proportions of small-sized species within the morphotype and/or a decrease in individual body sizes within species. Temperature–size relationships of ectotherms have been previously described using three generalizations that capture the influences of temperature on individuals, populations and species. Bergmann's (Bergmann 1847) and James' (James 1970) rules, focusing largely on community and population-level variation, respectively, postulate that longer-term evolutionary and ecological processes result in the dominance of small-sized species and/or individuals in warmer regions, and vice versa in cold regions. In contrast, the temperature–size rule focuses on phenotypic plasticity during development as a driver of decreasing individual body sizes in response to increasing temperature (Atkinson 1994). Increases in the proportion of small-sized species within the assemblage in response to warming as predicted by Bergmann´s rule is, however, not proven to be a common response for chironomid assemblages over large temporal scales (Stivrins et al. 2021, Belle et al. 2022). Instead, some studies of spatial patterns in chironomid assemblages report larger taxa at the warmest end of the temperature gradient (Heiri et al. 2011, Belle and Goedkoop 2021). Nevertheless, taxonomic turnover favouring the development of smaller species within each morphotype could theoretically be a valid but unverifiable hypothesis. Although these mechanisms cannot be disentangled using specimens from sediment cores, investigation of variation in subfossil chironomid sizes nevertheless has the potential to give novel insights into climate-induced size variations of aquatic invertebrates over substantially longer time scales than is possible in experiment- or field-based observations.

Acknowledgements – We thank Jenny L. Nilsson and Tobias Vrede for their assistance during fieldwork of some of the Swedish sites.

Funding – This study is part of the CENTURION (Cumulative effects of climate change and eutrophication on Swedish lakes; grant n NV-802-0047-19) project funded by the Swedish Environmental Protection Agency to SB (SLU). Additional funding was used for coring and dating and came from the Oscar and Lilli Lamm Foundation, and FORMAS (grant no. 2016-861 awarded to Tobias Vrede, SLU). Investigations were also supported by the Estonian Council of Research with grant no. PRG 1993 and no. TK-215.

Author contributions

Simon Belle: Conceptualization (lead); Formal analysis (lead); Funding acquisition (lead); Investigation (lead); Visualization (lead); Writing – original draft (lead). **Tymoteusz Matera**: Writing – review and editing (equal). **Merlin Liiv**: Funding acquisition (equal); Investigation (equal); Writing – review and editing (equal). **Normunds Stivrins**: Funding acquisition (equal); Investigation (equal); Writing – review and editing (equal). **Anneli Poska**: Funding acquisition (equal); Investigation (equal); Writing – review and editing (equal). **Atko Heinsalu**: Funding acquisition (equal); Investigation (equal); Writing – review and editing (equal). **Brendan G. McKie**: Writing – review and editing (equal).

Data availability statement

Data are available from the Dryad Digital Repository: https://doi.org/10.5061/dryad.2280gb64w (Belle et al. 2025).

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