Nematodes infest winter-active chironomids in Minnesota trout streams

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Abstract

We present preliminary findings of the effects of nematode infestation on winter-active chironomid biology, and how short-term temperature spikes could affect host-parasite interactions. Results are limited but indicate nematodes may infest winter-active chironomid communities and significantly affect host chironomid biology. Further research on winter-active insects should include investigations into nematode parasitism to better understand how climate change will affect chironomid survival at the population and community level.

Introduction

Winter-active chironomids are abundant in temperate regions, and are especially common in groundwater-fed streams in the Midwestern US (Bouchard and Ferrington 2009). These chironomids are key members of winter food webs in aquatic and terrestrial ecosystems, and are important food sources for brown trout in Minnesota trout streams (Anderson et al. 2016). Winter warming from climate change will likely have detrimental effects on winter-active chironomids because of their extreme cold-adaptation (Anderson et al. 2022).

Many nematodes are obligate parasites of arthropods (Kiontke and Fitch 2013). Research on parasitic nematodes has focused on the biological control of pestiferous insects (e.g., Edmunds et al. 2017) or the developmental and morphological effects on the host insect (e.g., Bhattacharya et al. 2014). However, little research has explored the ecological consequences of nematode parasitism in chironomid populations.

We collected cold-adapted chironomids for analyses of longevity and reproduction in the winter of 2021. Incidentally, we discovered many chironomids were parasitized by nematodes. This note reports our limited findings on how nematodes affect winter-active chironomids to encourage further work on this topic.

Materials and Methods

Chironomidae collections and temperature treatments

Chironomidae were collected from two groundwater-fed streams in 2021: Ike's Creek in Bloomington, MN (21 February 2021) and Pickwick Creek in Winona County, MN (4 January 2021) following protocol from Ferrington et al. (2010). The adult midges were placed in a 6°C incubator, a temperature consistent with previous studies on chironomid longevity (e.g., Anderson et al. 2022). The chironomids were split into three equal-sized treatment groups to investigate potential impacts of winter temperature spikes on longevity and reproduction. The control group was kept at constant 6°C and treatment groups were exposed to 22°C for 24 or 48hrs before returning to 6°C. One group of midges from Ike's Creek was also exposed to constant 22°C due to larger sample sizes than Pickwick Creek. Individual chironomids were inspected daily to record longevity, reproduction, and nematode emergence. Dead midges were preserved in >70% ethanol for taxonomic identification. *Diamesa* Meigen, 1835 identifications were made using Hansen and Cook (1976). Male Orthocladiinae were identified using Oliver and Dillon (1989) and female Orthocladiinae were identified using Sæther (1977).

Statistical analysis

All statistical analyses were conducted with RStudio (v.1.4.1717, R Core Team 2021), and figures were produced with packages *ggplot2* (Wickham 2016), *survival* (Therneau 2020), and *survminer* (Kassambara et al. 2021). Only Ike's Creek Orthocladiinae females were used in statistical analyses because of small numbers of Orthocladiinae males and parasitized chironomids from Pickwick Creek. Differences in survivorship due to temperature treatments and nematode parasitism were assessed with boxplots and Kaplan-Meier analyses. Non-parametric tests were performed to test differences in boxplots because of small sample sizes. Log-rank tests were used to analyze Kaplan-Meier curves.

Results and Discussion

Chironomidae collections summary

The composition of chironomids varied by stream (Table 1). The majority of chironomids collected from Pickwick Creek were *Diamesa* sp. with few other species present, whereas the majority of chironomids collected from Ike's Creek were in the subfamily Orthocladiinae (Table 1). The longevities of Orthocladiinae and Diamesinae were significantly different across all temperature treatments and both streams (Wilcoxon, p=0.019). The longevities of male and female *Diamesa* sp. were not significantly different across streams (Wilcoxon, p=0.081). Conversely, the longevities of male and female Orthocladiinae were significantly different (Wilcoxon, p=0.002), with females living longer than males.

Table 1. Summary of total collected chironomid taxa, including number of chironomids and number and percent of parasitized chironomids in each taxon.

	Stream					
	Ike's Creek			Pickwick Creek		
Taxon	N°	N° Parasitized	% Parasitized	N°	N° Parasitized	% Parasitized
Total Orthocladiinae	179	22	12.3%	2	0	0
Males	67	2	3.0%	2	0	0
Females	112	20	17.9%	0	0	0
Total *Diamesa*	36	0	0	295	8	2.7%
Total males	22	0	0	221	0	0
D. mendotae	11	0	0	218	0	0
D. nivoriunda	11	0	0	3	0	0
Females	14	0	0	74	8	10.8%
Total	215	22	10.2%	297	8	2.7%

NOTE.– *Diamesa* females could only be identified to genus using available keys. Two additional chironomids were collected from Pickwick Creek: one Chironominae specimen and one chironomid specimen too decomposed to identify.

Nematode parasitism summary

In Pickwick Creek, there were 20 nematodes found only in female *Diamesa* (Table 1). Most parasitized *Diamesa* sp. were parasitized by two nematodes, with up to four nematodes in a single midge. Conversely, in Ike's Creek, there were 43 nematodes found only in Orthocladiinae, with the majority of parasitized Orthocladiinae being female (Table 1). The parasitism rate was significantly different between male and female Orthocladiinae from Ike's Creek (Fisher's Test, p=0.004). The majority of parasitized Orthocladiinae were parasitized by one nematode, with a few having two or three nematodes, and one midge with eight nematodes.

Nematode parasitism altered chironomid biology

Surprisingly, the presence of nematodes significantly increased female Orthocladiinae longevity from Ike's Creek in our constant 6°C, 22°C/24hr, and 22°C/48hr treatment groups (Fig. 1a). A small increase in longevity was observed in parasitized females in the constant 22°C group, but this difference was not significant (Fig. 1a). We found similar increases in longevity in parasitized females compared to non-parasitized females using a log-rank test and Kaplan-Meier survival analysis, but this increase was only significant in constant 6°C individuals (Fig. 1b). Parasitized female Orthocladiinae appeared to have greater survivorship early in life, but maximum survivorship did not surpass that of non-parasitized females (Fig. 1b).

We did not statistically analyze parasitized *Diamesa* sp. longevity from Pickwick Creek because sample sizes were small. There were no nematodes that emerged from *Diamesa* sp. at constant 6°C. However, we found mean longevities of parasitized *Diamesa* were 9.2 and 5.7 days in the 22°C/24hr and 22°C/48hr groups, compared to 12.3 and 10.7 days of non-parasitized *Diamesa* sp., respectively. Future studies could determine whether this decrease in longevity with parasitism is significant, as it contradicts our findings from parasitized Ike's Creek Orthocladiinae.

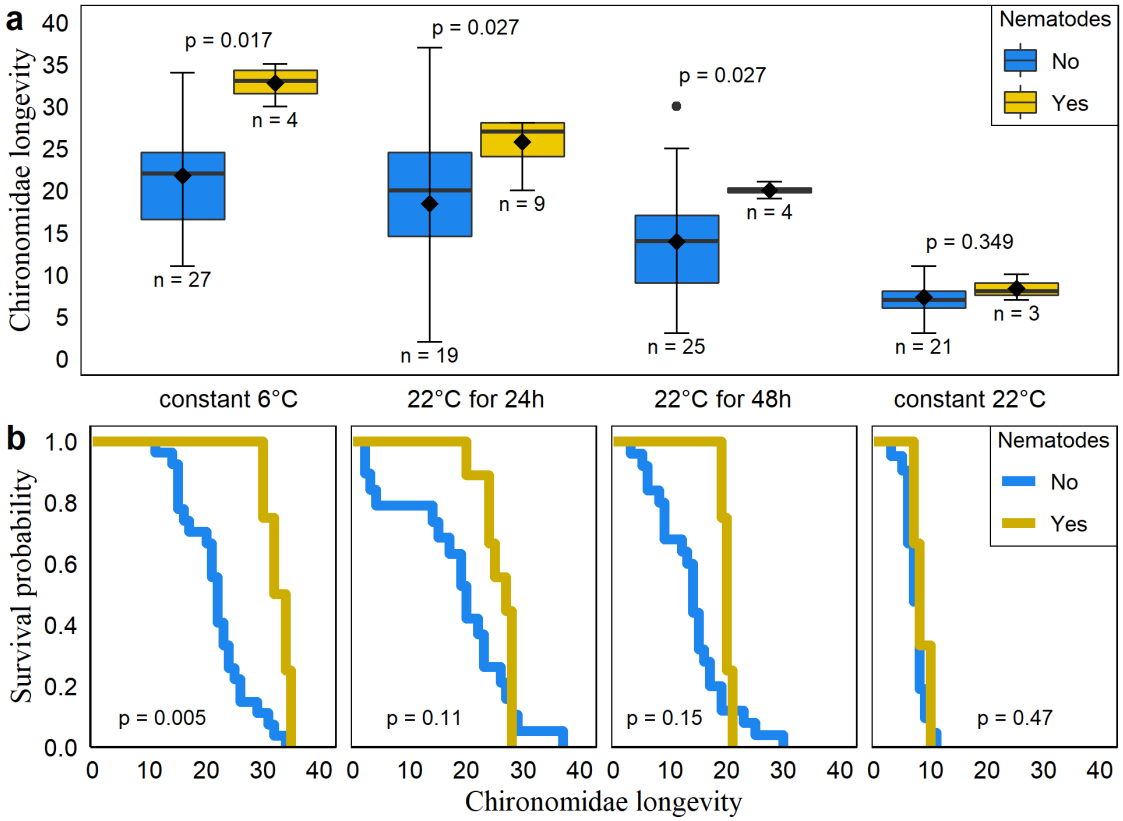

Figure 1. Female Orthocladiinae longevity and survival probability with and without nematode parasitism in Ike's Creek. Longevity defined as days lived post collection. (a) Box and whisker boundaries signify the maximum, 75th percentile, median, 25th percentile, and minimum longevity values. Black diamonds (◆) indicate mean longevity and dots (•) indicate outliers. Wilcoxon tests with Benjamini & Hochberg multiple test corrections were used to determine statistical differences between parasitized and non-parasitized individuals. (b) Kaplan-Meier survivorship curves indicate the proportion of individuals alive on a given day. Log-Rank tests were used to determine statistical differences between parasitized and non-parasitized individuals.

Conclusion

Our findings are preliminary due to small sample sizes and a lack of nematode identifications. However, we demonstrate nematodes are present in winter-active chironomid populations in Minnesota and nematode parasitism affects chironomid longevity. Further research is needed to determine how nematodes affect winter-active chironomid populations, and in turn, how climate change may disrupt these community dynamics.

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