



Contrasting responses of black fly species (Diptera: Simuliidae) to experimental whole-stream warming

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Abstract

1. As global temperatures continue to rise, assessment of how species within ecological communities respond to shifts in temperature has become increasingly important. However, such assessments require detailed long-term observations or ecosystem-level manipulations that allow for interactions among species and the potential for species dispersal and exchange with the regional species pool.
2. We examined the effects of experimental whole-stream warming on a larval black fly assemblage in southwest Iceland. We used a paired-catchment design, in which we studied the warmed stream and a nearby reference stream for 1 year prior to warming and 2 years during warming and estimated population abundance, biomass, secondary production, and growth rates for larvae of three black fly species.
3. Experimental warming by 3.8°C had contrasting effects on the three black fly species in the assemblage. The abundance, biomass, growth, and production of *Prosimulium ursinum* decreased in the experimental stream during the warming manipulation. Despite increasing in the reference stream, the abundance, biomass, and production of another species, *Simulium vernum*, decreased in the experimental stream during warming.
4. In contrast, warming had an overall positive effect on *Simulium vittatum*. While warming had little effect on the growth of overwintering cohorts of *S. vittatum*, warming led to an additional cohort during the summer months and increased its abundance, biomass, and production. Overall, family-level production was enhanced by warming, despite variation in species-level responses.
5. Our study illustrates that the effects of climate warming are likely to differ even among closely related species. Moreover, our study highlights the need for further investigation into the uneven effects of warming on individual species and how those variable effects influence food web dynamics and ecosystem function.

KEYWORDS

climate change, community shifts, Iceland, secondary production, temperature

1 | INTRODUCTION

Temperature regimes of freshwater ecosystems are shifting as a result of climate change (Kaushal et al., 2010; Lammers, Pundsack, & Shiklomanov, 2007; Liu, Yang, Ye, & Berezovskaya, 2005; Webb, 1996). Warming of freshwater ecosystems is of particular concern because these habitats contain disproportionately high species richness and endemism (Collen et al., 2014; Dudgeon et al., 2006). Moreover, a vast majority of species that make up this biodiversity are ectotherms, the abundance and distribution of which may be particularly responsive to warming because many aspects of their development and life history exhibit strong temperature dependence (Pritchard, Harder, & Mutch, 1996; Sweeney & Vannote, 1978; Vannote & Sweeney, 1980; Woodward, Perkins, & Brown, 2010). Climate warming can also induce phenological mismatches within freshwater food chains, resulting in changes in the strength of trophic interactions (Winder & Schindler, 2004). Thus, climate warming will cause complex changes in freshwater populations and community structure through direct and indirect effects on geographical distributions, biotic interactions, and physiology.

Species responses to warming can be idiosyncratic (Twomey et al., 2012). Empirical studies demonstrate that different species show contrasting responses to warming based on their thermal tolerance (Cuculescu, Hyde, & Bowler, 1998; Guo, Hao, Sun, & Lekang, 2009; Nelson et al., 2017a; Younger, van den Hoff, Wienecke, Hindell, & Miller, 2016), with some species occupying relatively narrow thermal windows (i.e. cold or warm stenotherms), while others are less sensitive (i.e. eurytherms). For species near or above their thermal optimum, warming reduces population abundance and biomass because of physiological stress (Somero, 2010). In contrast, warming can benefit species existing below their thermal optimum by facilitating growth and development, potentially resulting in additional cohorts within a year and higher rates of annual production. In some species, warming may also alleviate cold stress, which otherwise has sublethal effects and negatively affects population dynamics (Lencioni, 2004). Therefore, some species within communities are predicted to benefit from warming while others will incur net costs.

Aquatic insects have long been recognised as a model group for detecting organismal responses to environmental warming because of their short life spans and the strong effects of temperature on their life histories (Sweeney, Funk, Camp, Buchwalter, & Jackson, 2018; Sweeney, Jackson, Newbold, & Funk, 1992; Vannote & Sweeney, 1980; Ward & Stanford, 1982). In addition, populations of such short-lived species are more likely to show earlier responses to changes in temperature than those of longer-lived species, such as many fishes or crayfishes, which may accumulate more subtle changes over time. Nevertheless, aquatic insect species, even those in the same family or genus, are expected to differ in their responses to warming (Nelson et al., 2017a; Nilsson-Örtman, Stoks, De Block, & Johnson, 2012; Perry, Perry, & Stanford, 1987) and our ability to predict how different species will respond to climate warming remains fairly limited (Dawson, Jackson, House, Prentice, & Mace, 2011). Improving our understanding of the interspecific responses of aquatic insects to climate warming

is crucial, however, as the functioning of aquatic ecosystems is highly dependent on the biodiversity and structure of insect communities (Covich, Palmer, & Crowl, 1999; Wallace & Webster, 1996).

To investigate the potential for species-specific differences in response to warming, we quantified the effects of experimental whole-stream warming on a larval black fly assemblage in southwest Iceland. Larval black flies (Diptera: Simuliidae) were selected for this study because of their relatively high abundances in our study streams and their importance in the food webs in those streams (Nelson et al., 2020; O'Gorman et al., 2012; Woodward, Dybkjær, et al., 2010) and in streams and rivers worldwide (Adler, Currie, & Wood, 2004). Furthermore, cohorts could be easily defined, enabling the estimation of cohort growth rates and the calculation of secondary production using the instantaneous growth rate method (Benke & Huryn, 2017). Underscoring how black flies may vary in their response to climate warming, several studies have shown temperature to be an important factor influencing the development of black fly larvae (Gíslason & Gardarsson, 1988; Merritt, Ross, & Larson, 1982; Ross & Merritt, 1978) and the optimal temperatures for growth, development, and survival are known to differ among closely related species (Bernotienė & Bartkevičienė, 2013). Furthermore, the three black fly species found in our study streams vary in their abundance and production across a natural geothermal gradient (5–22°C), showing potential for interspecies variation in thermal preferences. While other studies have demonstrated the importance of temperature to black flies, these have been restricted to laboratory experiments (Becker, 1973; Ross & Merritt, 1978), studies using artificial streams or chambers (Hauer & Benke, 1987), or field surveys (Bernotienė & Bartkevičienė, 2011; Merritt et al., 1982). In contrast, we conducted a controlled warming experiment at a temporal and spatial scale that encompassed natural physical and chemical processes and biotic interactions.

We warmed a 35-m stream reach by approximately 3.8°C for 2 years and used a paired-catchment design, in which we studied larval black fly populations in the experimental stream (the warmed stream) and a nearby reference stream for 1 year prior to warming and 2 years during warming (Nelson et al., 2017a, 2017b). Specifically, we estimated larval population abundance, biomass, production, and growth rates for three black fly species that differ in their relative abundances across a natural thermal gradient at our study site. We hypothesised that black fly species would respond differently to experimental warming and that their responses would reflect differences in their thermal preferences. An additional goal was to assess the effects of warming on larval black flies at both the family and species level. Thus, we assessed whether or not results at the family level were in concordance with those for individual species.

2 | METHODS

2.1 | Study sites and experimental warming

This study was conducted in two first-order groundwater-fed streams in the Hengill geothermal region of southwest Iceland. This

region contains numerous small streams that are subject to varying degrees of geothermal influence (Friberg et al., 2009; Woodward, Dybkjær, et al., 2010). Our two focal streams have comparable mean annual temperatures, chemical characteristics (e.g. NO_3^- -N, NH_4^+ -N, and soluble reactive phosphorus), and similar invertebrate communities (Nelson et al., 2017a, 2017b). We sampled each stream during a 12-month pre-warming period (YR1) beginning in October 2010. We began warming a 35-m reach of the experimental stream using a gravity-fed geothermal heat exchanger (Nelson et al., 2017a; O’Gorman et al., 2014) in October 2011. The warming manipulation was possible because the cold experimental stream (mean annual temperature 5.5°C) runs parallel to a warm stream (mean annual temperature 22°C), with just 2 m separating the two streams at their closest point, before discharging into the Hengladalsá River. Water from the cold stream was diverted into the heat exchanger submerged in the warm stream, heated, and discharged back into the experimental stream, resulting in an average temperature increase of approximately 3.8°C during the 2 years of warming (YR2 and YR3, Figure S1, Nelson et al., 2017a, 2017b). This temperature increase is consistent with upper levels of warming predicted over the next century by current climate models (IPCC, 2018).

2.2 | Study organisms

Four species of black flies are known to occur in Iceland (Peterson, 1977). However, only three species were present in sufficient abundance in the study streams to estimate growth rates and production. *Prosimulium ursinum* Edwards, a Holarctic species, is known from Sweden, Norway, Bear Island (Norway), Iceland, Greenland and eastern North America (Peterson, 1977). *P. ursinum* is widespread throughout Iceland and inhabits small to moderate-sized, cold streams. *Simulium vernum* Macquart is distributed throughout North America and Europe (Peterson, 1977). In Iceland, *S. vernum* occurs predominantly in lowland streams (Peterson, 1977). The third species, *Simulium vittatum* Zetterstedt, is known to occur in North America, Greenland, Iceland, and the Faroe Islands; it is the most widespread and abundant black fly species in Iceland (Peterson, 1977). *Simulium vittatum* can be found in both cold and warm streams throughout Iceland, although in the Hengill region it attains higher densities in warmer streams (Hannesdóttir, Gíslason, Ólafsson, Ólafsson, & O’Gorman, 2013; Woodward, Dybkjær, et al., 2010). Together these three species comprised 7–25% of total annual invertebrate production in the two study streams over the 3-year study (Nelson et al., 2017b).

2.3 | Sample collection

We conducted monthly quantitative benthic sampling in the experimental and reference stream from October 2010 to September 2013 using a Surber sampler (0.0225 m², 250- μ m mesh). We collected five samples from each stream and preserved them in the

field with 5% formaldehyde. Samples were brought back to the laboratory and separated into fine (0.25–1 mm) and coarse (>1 mm) fractions using nested sieves. We removed invertebrates from both fractions using a dissecting microscope (10–15 \times magnification). Fine fractions were subsampled (1/2 to 1/8) when necessary using a Folsom plankton splitter. We identified black flies to species (Peterson, 1977) and measured the body length of each individual to the nearest mm. Biomass was estimated with the family-level (Simuliidae) length–mass regression from Benke, Huryn, Smock, and Wallace (1999).

2.4 | Production

We used bootstrap and Monte Carlo methods to generate mean and 95% confidence interval estimates for growth rates and production. All three black fly species exhibited well-defined cohorts. Thus, we used the instantaneous growth rate method (Benke & Huryn, 2006) to estimate production. Briefly, we resampled the size-specific abundance data 1,000 times with replacement to generate vectors of mean body size and biomass. Consecutive vectors of \ln -transformed mean body size were regressed against the number of elapsed days to generate 1,000 estimates of instantaneous growth rate. The vector of growth rate estimates was multiplied by the vectors of mean biomass for two consecutive dates to generate 1,000 estimates of daily production. We multiplied each vector of daily production by the number of days between sampling periods to generate vectors of interval production, which were then summed across months to generate 1,000 estimates of annual production. We generated confidence intervals (95% CIs) by discarding the upper and lower 2.5% of bootstrap values (Benke & Huryn, 2006). Unlike the univoltine *P. ursinum* and *S. vernum*, *S. vittatum* exhibited a bivoltine life cycle with a long winter generation and a short summer generation (Hannesdóttir et al., 2013; Figure 1). Thus, some estimates of annual production, mean annual abundance, and mean annual biomass for this species include two cohorts (Figure 1).

2.5 | Data analysis

The effect of warming on black fly abundance, biomass, and daily production was tested using generalised additive mixed models (GAMMs). The models were fitted using the *gamm4* function in the *GAMM4* package (Wood, 2006) in the R platform (R Core Team, 2014). Models included stream (experimental, reference), year (YR1, YR2, YR3) and the interaction term (stream \times year) as categorical fixed factors and month as a random factor. Coefficients for random effects were allowed to vary by stream. We used cubic regression smoothing to capture the seasonality in the data and the interaction (stream \times year) was fitted using the *by* command. The interaction (stream \times year) is of primary interest because it can be used to test the hypothesis that the temporal dynamics in the experimental stream diverged relative to those of the reference stream

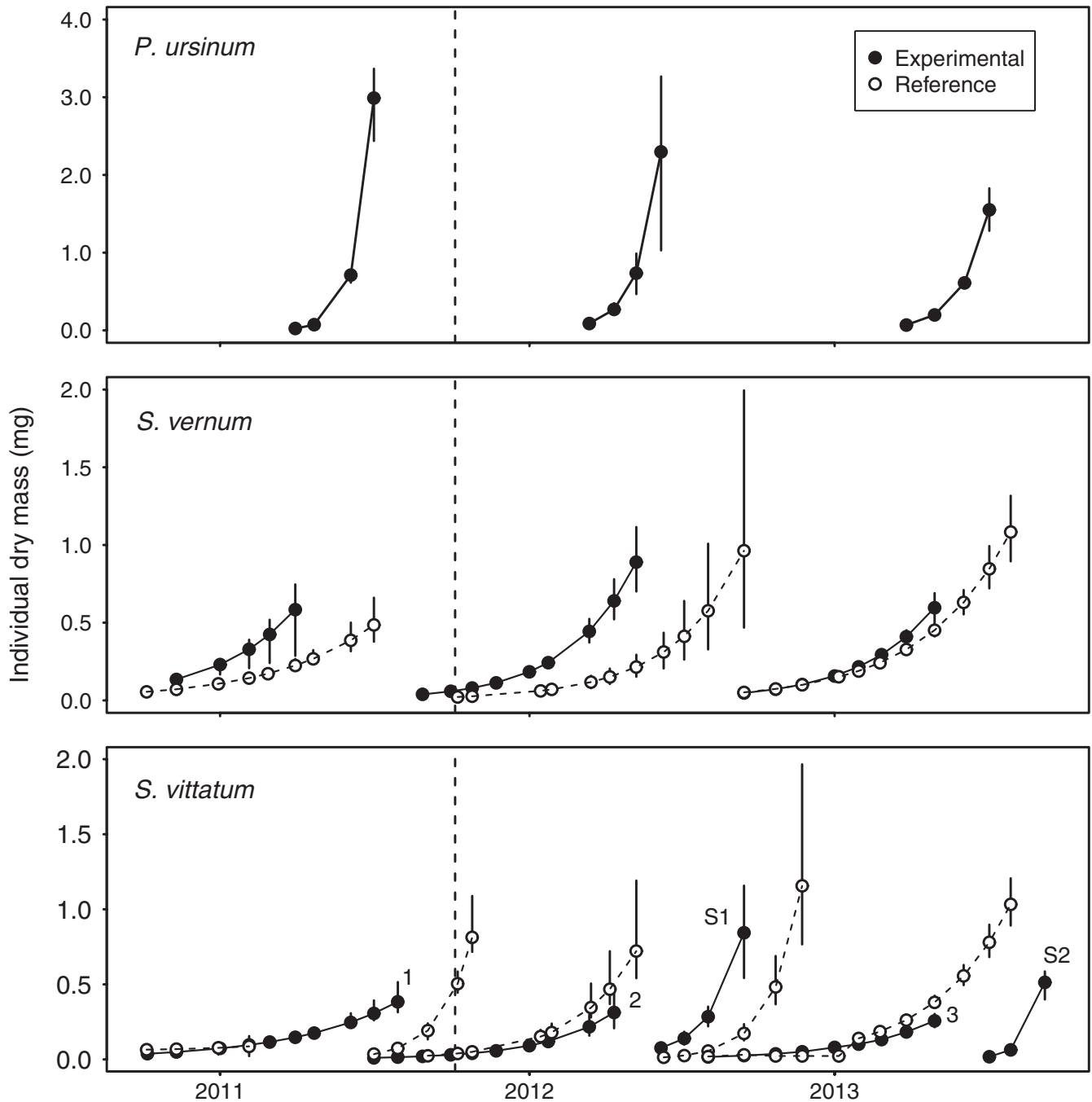


FIGURE 1 Time series of mean body mass (mg dry mass) for *Prosimulium ursinum*, *Simulium vernum*, and *Simulium vittatum* in the experimental (closed circles) and reference (open circles) stream over the course of the experiment. The dashed vertical line indicates the onset of warming in the experimental stream. Error bars represent 95% confidence intervals. Cohorts of *S. vittatum* in the experimental stream are labelled as follows: 1 = long winter cohort (October 2010–July 2011); 2 = long winter cohort (June 2011–April 2012); 3 = long winter cohort (July 2012–May 2012); S1 = short summer cohort (May 2012–September 2012); S2 = short summer cohort (June 2012–September 2013). Note the shift in voltinism of *S. vittatum* from univoltine before warming to bivoltine during warming

after warming. Data were \log_{10} -transformed prior to analysis to meet statistical assumptions when necessary. Note that *P. ursinum* only occurred in the experimental stream, precluding evaluation of stream \times year interactions for this species.

We also made intra-stream statistical comparisons among years for annual production and growth rates by inspecting the overlap of the 95% confidence intervals. Non-overlapping confidence

intervals indicated a significant difference. Finally, to assess the relationships between black fly production and temperature, we regressed daily production ($\text{mg dry mass m}^{-2} \text{ day}^{-1}$) against the mean stream temperature during the interval between sampling dates at the family level and for each species. We used least-squares regression to fit linear models and variables were \log_{10} -transformed prior to analysis.

3 | RESULTS

3.1 | Voltinism and growth rates

Both *P. ursinum* and *S. vernum* exhibited univoltine life cycles during all 3 years of the study (Figure 1). Before warming, *S. vittatum* was univoltine in the experimental stream with just one long overwintering cohort (Figure 1). However, during warming, *S. vittatum* became bivoltine with one long winter cohort and a short summer cohort (Figure 1). *Simulium vittatum* was bivoltine in the reference stream over the course of the experiment (Figure 1). Growth rates varied among the three black fly species (Table 1). In general, growth rates of *P. ursinum* were higher than those of *S. vernum* and *S. vittatum*. However, *P. ursinum* occurred only in the experimental stream and this prevented comparisons with the reference stream. Nevertheless, growth of *P. ursinum* declined in the experimental stream from YR1 (0.053 day⁻¹) to YR2 (0.039 day⁻¹) and from YR1 to YR3 (0.032 day⁻¹; Table 1). There were no differences in growth rates of *S. vernum* among years in either stream (reference range: 0.008–0.011 day⁻¹, experimental range: 0.010–0.013 day⁻¹; Table 1). Growth rates of *S. vittatum* winter cohorts were similar among years within each stream (Table 1). However, the growth rate of the YR3 summer cohort was nearly double that of the YR2 summer cohort in the experimental stream (Table 1).

3.2 | Abundance and biomass

The abundance of *P. ursinum* declined from YR1 to YR3 (Figure 2a, Table S1) in the experimental stream. Between YR1 and YR3, *P. ursinum* abundance decreased by 87% (mean YR1: 120 individuals/m²; mean YR3: 16 individuals/m²; Table 2). Similarly, the biomass of *P. ursinum* decreased from YR1 to YR3 (Figure 3a; Table S2). In the experimental stream, the abundance and biomass of *S. vernum* declined markedly during warming (Figures 2b and 3b; Table 2; Tables S1 and S2). In the experimental stream, the mean annual abundance of

S. vernum decreased 64% from YR1 to YR3, while mean annual biomass decreased 83% (Table 2). However, *S. vernum* abundance and biomass increased from YR1 to YR3 in the reference stream by 235 and 41%, respectively (Table 2). *Simulium vittatum* abundance and biomass increased significantly in the experimental stream from YR1 to YR3 (Figures 2c and 3c; Tables S1 and S2). From YR1 to YR3, *S. vittatum* abundance and biomass increased in the experimental stream by 726 and 1,378%, respectively (Table 2). At the family level, black fly abundance increased in the experimental stream relative to the reference stream from YR1 to YR3 (Figure 2d; Table S1). In the experimental stream, mean black fly abundance increased 199% from YR1 to YR3, while black fly abundance increased in the reference stream by only 26% over that same time frame (Table 1). There was no significant change in biomass in the experimental stream relative to the reference stream at the family level (Figure 3d; Table S2).

3.3 | Production and biomass turnover

As a result of reduced standing biomass and growth rate, annual production of *P. ursinum* decreased from 870 mg m⁻² year⁻¹ in YR1 to 43 mg m⁻² year⁻¹ in YR3 (Table 2), but there was no significant change in daily production (Figure 4a; Table S3). In the experimental stream, daily production generally declined with increasing temperature for *P. ursinum*, but this relationship was not significant (Figure 5a; Table S4). Annual biomass turnover of *P. ursinum* declined from 5.2 in YR1 to 3.2 and 2.9 in YR2 and YR3, respectively (Table 2). In the experimental stream, annual production of *S. vernum* decreased 72% from YR1 to YR3 (Table 2). In the reference stream, annual production of *S. vernum* increased significantly from YR1 to YR3 (Table 2). Daily production of *S. vernum* also decreased in the experimental stream relative to the reference stream (Figure 4b; Table S3). In the experimental stream, daily production of *S. vernum* declined significantly with increasing temperature (Figure 5b; Table S4). Annual biomass turnover increased from YR1 to YR2 in the experimental stream but remained relatively constant among

TABLE 1 Mean daily growth rates (day⁻¹) of black fly cohorts in the reference (R) and experimental (E) streams

Cohort	<i>Prosimulium ursinum</i>		<i>Simulium vernum</i>		<i>Simulium vittatum</i>	
	E	R	E	R	E	
1	0.053 (0.051–0.057)	0.008 (0.006–0.11)	0.010 (0.006–0.013)	0.002 (–0.01–0.009)	0.008 (0.007–0.010)	
2	0.039 (0.027–0.045)	0.011 (0.008–0.016)	0.013 (0.011–0.014)	0.014 (0.012–0.016)	0.012 (0.009–0.016)	
3	0.032 (0.030–0.034)	0.010 (0.008–0.011)	0.011 (0.010–0.014)	0.011 (0.010–0.012)	0.010 (0.009–0.011)	
S1	–	–	–	0.028 (0.023–0.040)	0.026 (0.019–0.033)	
S2	–	–	–	0.027 (0.022–0.036)	0.052 (0.045–0.058)	

Note: Numbers in parentheses represent 95% confidence intervals around the mean. *Simulium vittatum* exhibited a bivoltine life cycle with a long winter cohort and a short summer cohort. Cohorts of *S. vittatum* in the experimental stream are labelled as follows: Summer 1 = short summer cohort (May 2012–September 2012); Summer 2 = short summer cohort (June 2012–September 2013).

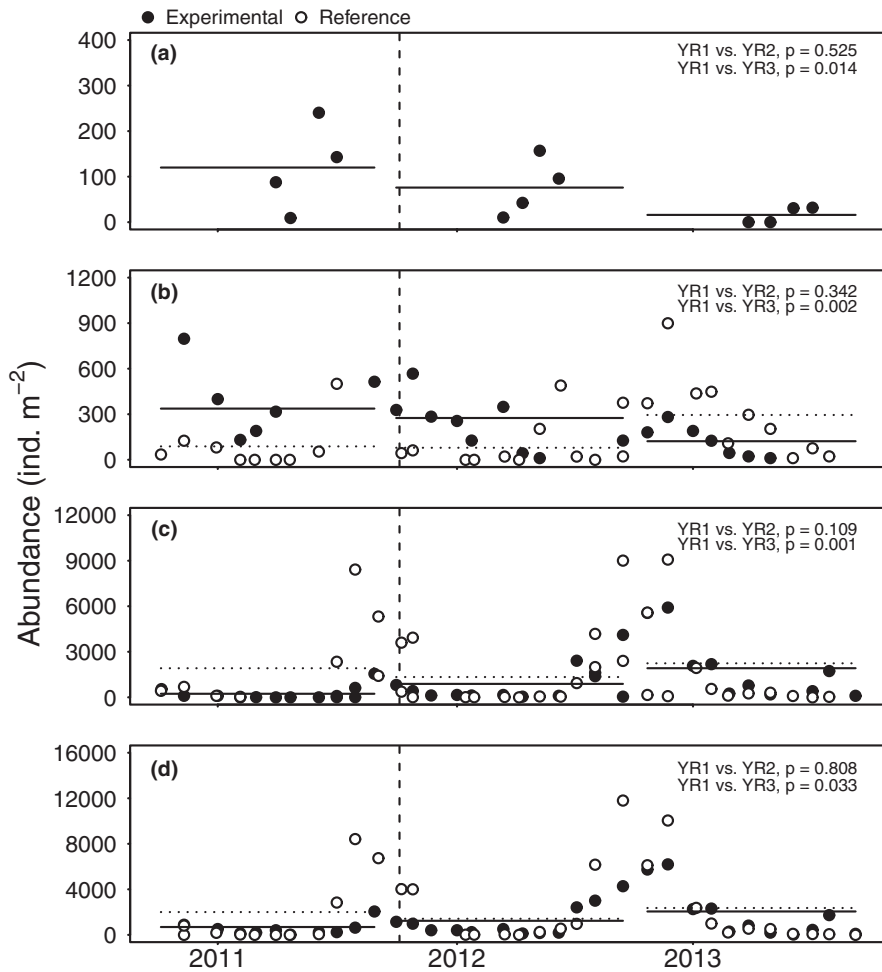


FIGURE 2 Time series of mean abundance (individuals/m²) for (a) *Prosimulium ursinum*, (b) *Simulium vernum*, (c) *Simulium vittatum*, and (d) all Simuliidae in the experimental (closed circles) and reference streams (open circles). Points represent the monthly means based on five samples. The dashed vertical lines indicate the onset of experimental warming. Horizontal lines indicate annual mean values (solid horizontal lines = experimental, dashed horizontal lines = reference). The p -values represent the results of the stream × year interactions tested in the GAMMs. *Prosimulium ursinum* only occurred in the experimental stream. Thus, p -values for that species represent apparent effects of temperature or differences between years only

years in the reference stream (Table 2). In the experimental stream, annual production of *S. vittatum* increased from 43 mg m⁻² year⁻¹ in YR1 to 906 mg m⁻² year⁻¹ in YR2 and 648 mg²/year in YR3 (Table 2). Daily production of *S. vittatum* also increased significantly from YR1 to YR3 in the experimental stream relative to the reference stream (Figure 4c; Table S3). Daily production of *S. vittatum* significantly increased with temperature in both the experimental and reference streams (Figure 5c; Table S4).

At the family level, annual production decreased significantly in the experimental stream by 49% from YR2 to YR3 (Table 2). However, experimental warming had no effect on daily production of black flies at the family level (Figure 4d; Table S3). At the family level, daily black fly production was positively related to temperature in both streams (Figure 5d; Table S4). In the experimental stream, the percent of total black fly production attributable to each species varied among years (Figure 6; Table S5). In YR1, approximately 79% of all black fly production was attributed to *P. ursinum*. The contribution by *P. ursinum* to black fly production declined to c. 6% in YR3 (Figure 6; Table S5). While the contribution of *P. ursinum* declined from YR1 to YR3, the percentage of total black fly production attributed to *S. vittatum* increased from 4% in YR1 to 87% in YR3 (Figure 6; Table S5). The contribution of *S. vernum* and *S. vittatum* to black fly production in the reference stream remained relatively constant (Figure 6; Table S5).

4 | DISCUSSION

Our results showed that the effects of warming varied among sympatric black fly species in both direction and magnitude. Although not present in the reference stream, the abundance, biomass, growth, and population production of *P. ursinum* decreased in the experimental stream during warming. The abundance, biomass, and production of *S. vernum* also decreased in the experimental stream during warming. However, the production of *S. vernum* increased in the reference stream from YR1 to YR3, indicating that factors other than temperature are important in the life history of that species. Experimental warming had an overall positive effect on the performance of *S. vittatum*, resulting in increases in population abundance, biomass, and production in the experimental stream. Overall, the findings of this study illustrate how the effects of climate warming may vary even among closely related species, and that such species-specific responses are important to consider when assessing the results of climate change studies.

Experimental warming had no effect on the growth rates of *S. vernum* or the long winter cohorts of *S. vittatum*. Moreover, the growth rate of *P. ursinum* declined in the experimental stream during warming. In addition, experimental warming had contrasting effects on the abundance, biomass, and production of the three black fly species in our study. While the abundance and biomass of *P. ursinum* and *S. vernum*

TABLE 2 Mean annual abundance (N; number of individuals/m²), biomass (B; mg dry mass/m²), production (P; mg dry mass m⁻² year⁻¹), and production and biomass turnover (P:B) of black fly species in the experimental and reference stream

Species	Stream	Year	N	B	P	P:B
<i>Prosimulium ursinum</i>	E	1	120 (65–187)	160 (67–282)	870 (341–1,515)	5.2 (4.6–5.7)
		2	76 (48–108)	97 (52–144)	352 (163–559)	3.2 (2.2–3.8)
		3	16 (3–34)	15 (4–32)	43 (8–96)	2.9 (2.6–3.3)
<i>Simulium venum</i>	E	1	337 (233–453)	106 (42–208)	193 (88–320)	2.1 (1.4–2.6)
		2	275 (204–352)	52 (38–69)	207 (148–274)	3.6 (3.2–4.0)
		3	122 (77–184)	18 (10–27)	54 (33–82)	2.8 (2.5–3.3)
	R	1	88 (36–153)	46 (7–106)	54 (13–121)	2.0 (1.6–2.9)
		2	79 (21–174)	25 (4–61)	93 (15–246)	3.6 (2.5–5.3)
		3	295 (210–383)	65 (44–89)	229 (149–314)	3.3 (2.8–3.6)
<i>Simulium vittatum</i>	E	1	232 (144–337)	9 (5–16)	43 (21–74)	4.6 (3.4–6.4)
		2	884 (702–1,104)	101 (65–156)	906 (568–1,400)	9.0 (7.1–11.3)
		3	1,917 (1,575–2,337)	133 (105–165)	648 (485–813)	4.9 (3.9–6.0)
	R	1	1,915 (654–3,680)	155 (57–280)	914 (317–1,722)	5.9 (4.5–9.0)
		2	1,336 (905–1,856)	150 (94–210)	1,690 (884–2,597)	11.1 (8.7–14.3)
		3	2,239 (1,602–2,899)	130 (98–164)	698 (513–899)	5.4 (5.0–5.8)
Simuliidae	E	1	688 (537–847)	276 (149–423)	1,106 (540–1,805)	
		2	1,235 (1,038–1,459)	250 (183–317)	1,465 (1,070–1,951)	
		3	2,055 (1,702–2,476)	166 (132–200)	745 (576–912)	
	R	1	2,004 (736–3,788)	200 (84–334)	967 (344–1,789)	
		2	1,415 (959–1,951)	175 (112–247)	1,782 (960–2,704)	
		3	2,534 (1,873–3,183)	195 (156–235)	927 (727–1,136)	

Note: Numbers in parentheses represent the 95% confidence intervals around the mean. E = experimental stream; R = reference stream; Year 1 = October 2010–September 2011; Year 2 = October 2011–September 2012; Year 3 = October 2012–September 2013.

declined during warming, experimental warming had a positive effect on the abundance and biomass of *S. vittatum*. Furthermore, population production declined for *P. ursinum* and *S. venum* but increased for *S. vittatum*. It is possible that these contrasting effects of warming were due to changes in food quantity or quality, or a result of altered trophic interactions within the food web. However, based on previous work on streams in the Hengill region (Hannesdóttir et al., 2013; Nelson et al., 2017a, 2017b; Woodward, Dybkjær, et al., 2010), these deviations may also be a result of variation in the thermal physiology and preferences of each species. Previous studies in Iceland have demonstrated that aquatic invertebrate taxa from the Hengill region are highly variable in their thermal preferences (Hannesdóttir et al., 2013; Nelson et al., 2017a, 2017b; Woodward, Dybkjær, et al., 2010). For example, *S. vittatum* may represent up to 83% of total invertebrate abundance in warm streams (mean summer temperatures > 20°C) near our study site, but comprise a much smaller percentage of the community in colder streams (Hannesdóttir et al., 2013; Woodward, Dybkjær, et al., 2010). Our study and others demonstrate that this variation in thermal physiology among species is an important factor to consider in studies examining the effects of climate warming on ecological communities.

Changes in the relative production of species within a community could have both direct and indirect effects on community interactions and the flow of energy between trophic levels (Benke & Huryn, 2010). For example, the three species of black flies in our

study fill different functional roles in the stream food web. While *S. vittatum* and *S. venum* both feed primarily on diatoms (c. 90% of the production of these two species was attributable to diatoms; Nelson et al., 2020), a large proportion (40–50%) of the diet of *P. ursinum* during YR1 and YR2 of the experiment was animal prey (early-instar chironomids and black flies; Nelson et al., 2020). Based on quantitative food web analysis, *P. ursinum* was responsible for 40 and 20% of total predation in the experimental stream during YR1 and YR2, respectively (Nelson et al., 2020). The decrease in production of *P. ursinum* during warming may have released first- or second-instar *S. vittatum* larvae from predation, allowing *S. vittatum* to increase in abundance and biomass during warming. In the same way, the high abundance of *S. vittatum* in the reference stream could be due to the absence of *P. ursinum*. Thus, the dramatic decrease in the abundance and production of this species in YR3 of the experiment potentially had significant consequences for biotic interactions, food web structure, and energy flow in the experimental stream.

Our study and others have shown that temperature is an important factor influencing the life history of black flies (Colbo & Porter, 1981; Ladle, Bass, & Jenkins, 1972; Merritt et al., 1982; Ross & Merritt, 1978). In particular, temperature is important in influencing the development of black fly larvae (Gíslason & Gardarsson, 1988; Merritt et al., 1982; Ross & Merritt, 1978) as it controls when and how quickly the eggs of black flies hatch, while also affecting larval

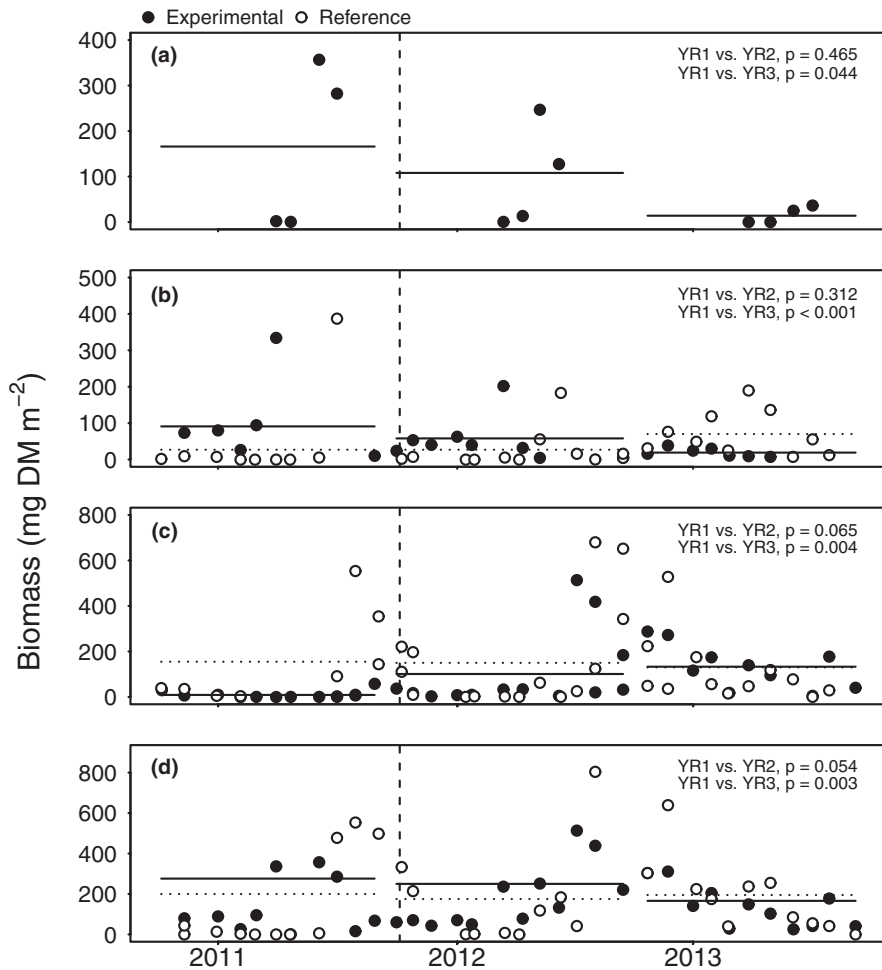


FIGURE 3 Time series of mean biomass (mg dry mass [DM]/m²) for (a) *Prosimulium ursinum*, (b) *Simulium venum*, (c) *Simulium vittatum*, and (d) all Simuliidae in the experimental (closed circles) and reference streams (open circles). Points represent the monthly means based on five samples. The dashed vertical lines indicate the onset of experimental warming. Horizontal lines indicate annual mean values (solid horizontal lines = experimental, dashed horizontal lines = reference). The *p*-values represent the results of the stream × year interactions tested in the GAMMs. *Prosimulium ursinum* only occurred in the experimental stream. Thus, *p*-values for that species represent apparent effects of temperature or differences between years only

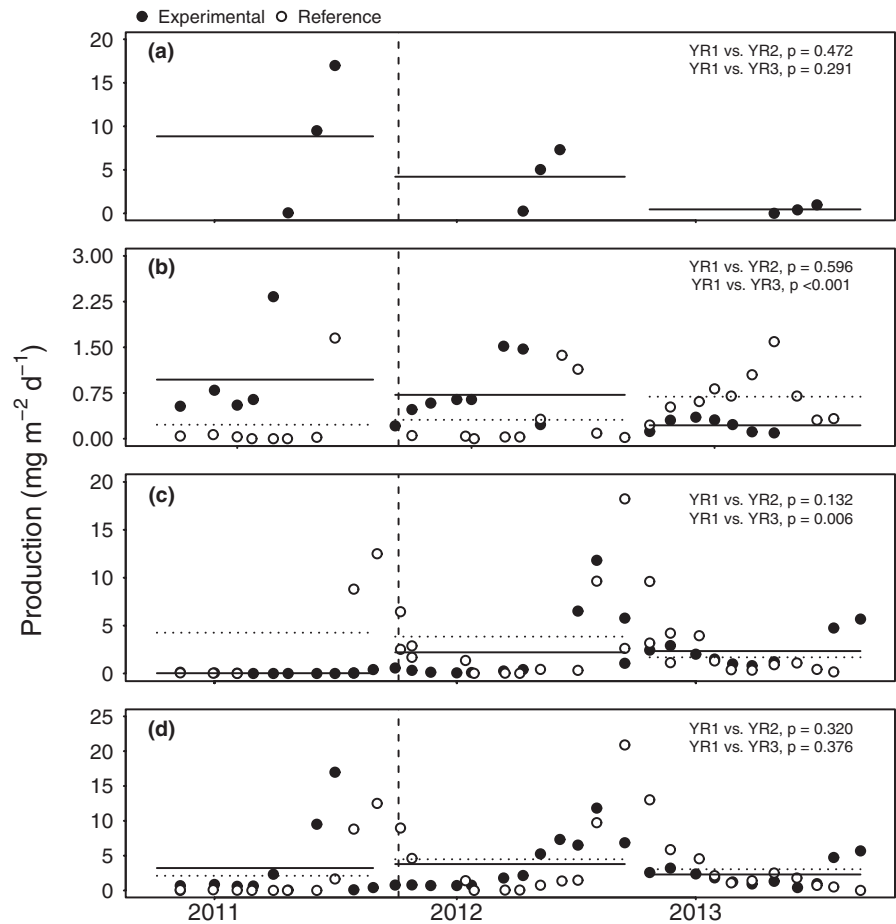
survival. The eggs of some black fly species hatch in four days at 25°C but can remain viable for up to 2 years when held at temperatures slightly above freezing (Freedon, 1959). Similar patterns hold for larval development. For example, the duration of development for *S. maculatum* is 42 days at 13.2°C but only 21 days at 18.8°C (Bernotienė & Bartkevičienė, 2011). Similarly, an increase in water temperature from 17 to 27°C has been shown to reduce the duration of the development period of *S. vittatum* by more than half (Becker, 1973). Temperature has also been shown to affect survival of black flies. Black flies in the *S. verecundum* species complex suffer mortality at temperatures below 10°C (McCreadie & Colbo, 1991), while *P. approximatum* does not suffer severe mortality until the temperature is below 4°C (Mansingh & Steele, 1973).

Although temperature is an important factor in larval development of black flies, other factors also affect black fly development and life history. Ross and Merritt (1978) found that spring floods caused eggs of some species to hatch, producing second cohorts of species that otherwise have only one cohort of overwintering larvae per generation. Additionally, Hauer and Benke (1987) found that black flies grew more rapidly during the initial stages of high river discharge, probably a result of increased seston quantity and quality during flooding. Black fly biomass and growth rates have also been shown to be dependent on food quantity and quality (Hauer & Benke, 1987; Morin & Peters, 1988). Experimental warming

increased gross primary production by c. 175% in the experimental stream (Hood et al., 2018). As diatoms made up a significant proportion of the diet of *S. vittatum* in the experimental stream during the experiment (0.85–0.93; Nelson et al., 2020), it is possible that an increase in resource supply led to its positive response to warming.

Population abundance, biomass, and production of *S. venum* generally increased over the course of the study in the reference stream, whereas those same variables declined for *S. venum* in the experimental stream. Given that the mean annual temperature and thermal regime in the reference stream remained relatively similar among years, it is likely that factors not measured or considered in this study were responsible for driving these changes. Previous results from this experiment show no significant differences in seston concentrations among years in the reference stream (Nelson et al., 2017b). However, seston quality (e.g. C:nutrient ratios, fatty acid composition, protein content) was not measured and has been shown to be an important factor influencing the reproduction and population dynamics of aquatic invertebrates, including black flies (Choi et al. 2016; Hessen, van Donk, & Gulati, 2005; Morin & Peters, 1988). While it is possible that factors other than temperature regulate the population dynamics of *S. venum*, temperature probably played a strong role in driving changes in the life history and population dynamics of *S. vittatum*. In the experimental stream, annual production of *S. vittatum* increased by >1,400% from YR1 to YR3. However, in the reference stream,

FIGURE 4 Time series of mean daily production ($\text{mg dry mass [DM] m}^{-2} \text{ day}^{-1}$) for (a) *Prosimulium ursinum*, (b) *Simulium vernum*, (c) *Simulium vittatum*, and (d) all Simuliidae in the experimental (closed circles) and reference streams (open circles). Points represent the monthly means based on five samples. The dashed vertical lines indicate the onset of experimental warming. Horizontal lines indicate annual mean values (solid horizontal lines = experimental, dashed horizontal lines = reference). The p -values represent the results of the stream \times year interactions tested in the GAMMs. *Prosimulium ursinum* only occurred in the experimental stream. Thus, p -values for that species represent apparent effects of temperature or differences between years only



annual production of *S. vittatum* was similar among years. In addition, experimental warming elicited a second cohort of *S. vittatum* in the experimental stream, whereas the species produced only one cohort in that stream prior to warming. Hannesdóttir et al. (2013) showed that *S. vittatum* was bivoltine in warmer streams in the Hengill region (mean annual temperature $c. 10^{\circ}\text{C}$ and above) but univoltine in colder streams (Hannesdóttir et al., 2013). Moreover, in Iceland, *S. vittatum* reaches its highest abundances at $c. 17^{\circ}\text{C}$ (Nelson et al., 2017b). Thus, our results corroborate the results from other studies suggesting that the life history and abundance of *S. vittatum* are strongly controlled by temperature.

Like all whole-ecosystem experiments, our experiment suffered from limitations. First, our experiment was unreplicated. While replication in whole-ecosystem experiments is desirable, costs and feasibility often make replication impossible (Carpenter, 1989). Nevertheless, if the experimental manipulation is realistic and the expected effects are large enough, unreplicated whole-system experiments can be informative despite statistical limitations (e.g. Carpenter et al., 1987; Wallace, Eggert, Meyer, & Webster, 1997). Second, the reference stream was not identical to the experimental stream in terms of physicochemical (e.g. thermal regime, hydrology) and biotic (e.g. assemblage structure) factors, even before the temperature manipulation began. The term *reference ecosystem* (instead of *control*) was introduced by Hasler, Brynildson, and Helm (1951) to acknowledge the fact

that no two ecosystems are identical (Carpenter, 1998). Reference ecosystems are used to assess the possibility that the responses observed in the manipulated ecosystem were a result of regional trends and not the manipulation itself (Carpenter, 1998). This is achieved with statistical methods that incorporate multiple observations of the manipulated and reference ecosystems before and after the manipulation. A significant interaction between time and ecosystem type (reference or manipulated) indicates temporal divergence in the manipulated ecosystem relative to the reference ecosystem. Hence, it is not essential for the manipulated ecosystem to be identical to its reference ecosystem prior to the manipulation. Third, our goal was to increase the mean temperature of the experimental stream by $3\text{--}4^{\circ}\text{C}$, an increase consistent with predictions for the next century (IPCC, 2018). While successful in achieving this increase, we inadvertently introduced variation into the diel and seasonal temperature cycles. Along with changes in mean temperature, changes in the diel and seasonal thermal regime of streams can have effects on organismal physiology and life history (Vannote & Sweeney, 1980). Partitioning the relative effects of temperature variability versus an increase in mean temperature was beyond the scope of this experiment. However, we acknowledge that a combination of factors could have had important influences on black fly life history in our experiment.

Our 3-year whole-stream warming experiment demonstrates that biological responses to changes in temperature can vary

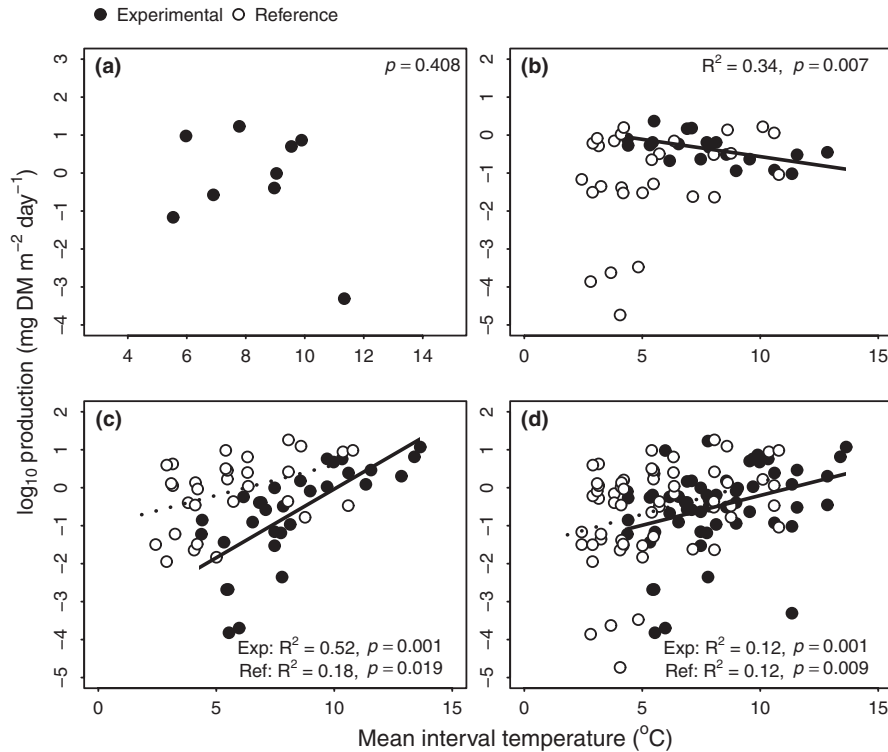


FIGURE 5 Relationship between daily production ($\text{mg dry mass m}^{-2} \text{ day}^{-1}$) and mean stream temperature during the interval between sampling dates for (a) *Prosimulium ursinum*, (b) *Simulium vernum*, (c) *Simulium vittatum*, and (d) all three species combined (Simuliidae) in the experimental stream (closed circles, solid regression line) and reference stream (open circles, dotted regression line)

greatly, even among closely related taxa. While experimental warming had an overall negative impact on the life histories and performance of two black fly species in our experiment, warming had the opposite effect on a third species. Although experimental warming did not illicit a positive response in growth of *S. vittatum*, warming did lead to an additional cohort of *S. vittatum* during the summer, which ultimately resulted in higher abundance, biomass, and annual production. Such changes would have been

difficult to observe using single-species laboratory experiments or even short-term mesocosm experiments. Furthermore, our study highlights the importance of factors other than temperature in influencing the life history of black flies, as evidenced by changes in the performance of *S. vernum* in the reference stream that were in contrast with changes in the experimental stream. Thus, our whole-ecosystem warming experiment, which encompassed natural physical and chemical processes and allowed for inherent biotic interactions, revealed important species-specific differences in response to warming that led to changes in assemblage structure. While the consequences of such changes in assemblage structure for the functioning of freshwater ecosystems (e.g. energy flux, nutrient cycling) remain largely unknown, the results of this study highlight the need for further investigation into the variable effects of climate change on individual species, and how species-level shifts are propagated to food web dynamics and ecosystem function.

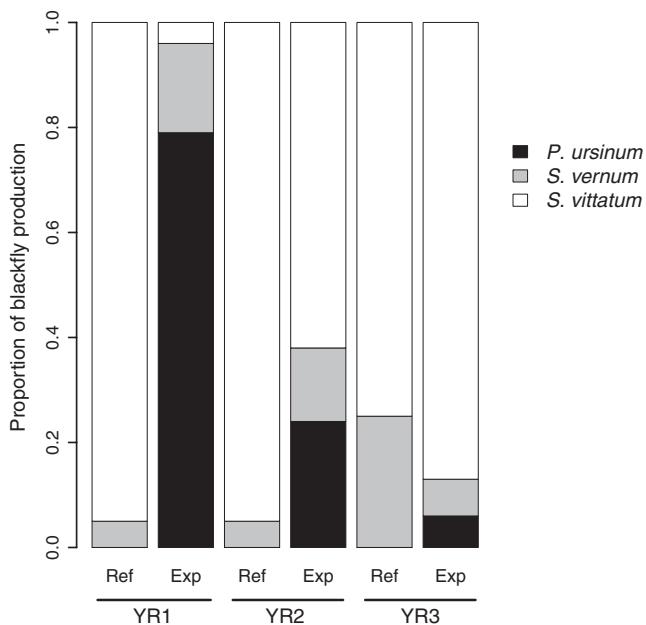


FIGURE 6 Proportion of total black fly production attributable to each species in the reference (R) and experimental (E) streams during all 3 years of the study

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AUTHORS' CONTRIBUTIONS

J.P.B., A.D.H., and W.F.C. designed the study; D.N., J.P.B., A.D.H., W.F.C., J.M.H., J.R.J., G.M.G., and J.S.O. conducted the fieldwork; P.W.J. designed and built the heat exchanger; D.N. analysed the data; all authors contributed to interpretation of results; D.N. led the writing with contributions from all authors.

DATA AVAILABILITY STATEMENT

Data will be deposited in the Dryad Digital Repository.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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