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ORIGINAL ARTICLE

Phenology of alpine zooplankton populations and the importance of lake ice-out

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The prolonged ice cover inherent to alpine lakes incurs unique challenges for aquatic life, which are compounded by recent shifts in the timing and duration of ice cover. To understand the responses of alpine zooplankton, we analyzed a decade (2009–2019) of open-water samples of *Daphnia pulicaria* and *Hesperodiaptomus shoshone* for growth, reproduction and ultraviolet radiation tolerance. Due to reproductive differences between taxa, we expected clonal cladocerans to exhibit a more rapid response to ice-cover changes relative to copepods dependent on sexual reproduction. For *D. pulicaria*, biomass and melanization were lowest after ice clearance and increased through summer, whereas fecundity was highest shortly after ice-off. For *H. shoshone*, biomass and fecundity peaked later but were generally less variable through time. Among years, ice clearance date varied by 49 days; years with earlier ice-out and a longer growing season supported higher *D. pulicaria* biomass and clutch sizes along with greater *H. shoshone* fecundity. While these large-bodied, stress tolerant zooplankton taxa were relatively resilient to phenological shifts during the observation period, continued losses of ice cover may create unfavorably warm conditions and facilitate invasion by montane species, emphasizing the value of long-term data in assessing future changes to these sensitive ecosystems.

KEYWORDS: high-elevation lakes; cladocera; copepod; life history; ice phenology

INTRODUCTION

Alpine lakes are challenging environments that often support communities of highly specialized organisms (Stoddard, 1987; Catalan *et al.*, 2009). Relative to lower elevation systems, these lakes experience prolonged ice cover (Preston *et al.*, 2016; Christianson *et al.*, 2020b), increased exposure to solar ultra violet radiation (UVR, 290–400 nm), rapid flushing, colder temperatures and lower resource availability (Blumthaler *et al.*, 1992; Williams *et al.*, 1996; Rose *et al.*, 2009; Williamson *et al.*, 2009). Importantly, these characteristics can be compounded by the extreme seasonal and interannual climatic variability typical of high-elevation catchments (Sadro *et al.*, 2018, 2019). For many alpine lake ecosystems, the open water season—in which lakes are considered the most biologically active—lasts <20% of a given year (Ruttner, 1973; Ohlendorf *et al.*, 2000; Caine, 2002). During the remaining 80%, ice and snow create a barrier that partially buffers aquatic organisms from the strong winds and large diurnal temperature gradients, but concurrently limits light penetration and oxygen exchange (Ohlendorf *et al.*, 2000; Thies *et al.*, 2000; Seastedt *et al.*, 2004). The combination of short growing seasons, limiting temperatures and low nutrient availability cause the inhabitants of alpine lakes to exist on the "razor's edge of tolerance" (Williams *et al.*, 2002). A key knowledge gap is thus to understand how aquatic communities are responding to recent and relatively rapid changes in alpine environments.

Recent findings that global ice cover is decreasing due to warming climate trends (Brown and Duguay, 2010; Sharma *et al.*, 2019; Christianson *et al.*, 2020b) highlight the importance of understanding how changes in ice-phenology will affect these specialized taxa. Ice cover for alpine lakes, for instance, is decreasing 50% faster than for other temperate lakes in the Northern Hemisphere (Christianson *et al.*, 2020b). Nonetheless, the remote nature of alpine systems poses a challenge for observing the seasonal life history strategies and developmental patterns of their inhabitants under natural conditions. The highly variable and restrictive conditions typical of alpine lakes are often associated with simplified food webs, comprised of few trophic levels (Ward *et al.*, 1904; McNaught *et al.*, 1999) and relatively low species richness (Fjellheim *et al.*, 2009; Loewen *et al.*, 2019; Loria *et al.*, 2020). Inhabitants of these lakes exhibit strategies for persisting through extended cold periods and maximizing growth and reproduction during short growing seasons. Specifically, zooplankton in alpine lakes are cold-adapted, UVR-tolerant, and often have dormant stages, such as resting eggs. Past studies have identified large-bodied cladocerans (e.g. *Daphnia melania*, *Daphnia middendorffiana* and *Daphnia pulicaria*) and calanoid copepods (e.g. *Hesperodiaptomus shoshone*) as among the most frequently observed and numerically abundant zooplankton taxa in natural mountain lakes (Forbes, 1893; Ward *et al.*, 1904; Shantz, 1907; Dodds, 1917; Loewen *et al.*, 2019). Analyses of zooplankton community composition for mountain lakes along the west coast of North America suggest these taxa are relatively unconstrained by dispersal limitation, but likely influenced by environmental heterogeneity in local climate and catchment characteristics (Loewen *et al.*, 2019).

Temperature is a critical factor for zooplankton feeding rates, growth, reproduction and emergence from resting eggs (McLaren, 1965; Gillooly, 2000), although fewer studies have examined tolerance to the extreme low temperatures characteristic of alpine systems and those with prolonged lake ice cover (Morales-Baquero *et al.*, 2006; Miller and McKnight, 2015). The short duration of the growing season highlights the importance of reproductive timing to coincide with optimal temperature and increased food availability (Gliwicz *et al.*, 2001), which is likely influenced by the timing and duration of ice cover (Pérez-Martínez *et al.*, 2013). For instance, adult artic copepods (*Leptodiaptomus minutus*) rely on pre-winter primary production to accumulate sufficient quantities of fatty acids and algal-derived carotenoids to sustain them through winter and facilitate reproduction immediately after lake ice clearance, thereby allowing their offspring to capitalize on spring phytoplankton blooms (Schneider *et al.*, 2016, 2017; Grosbois and Rautio, 2018). Concurrently, alpine-adapted zooplankton often have strategies for mediating intense UVR exposure (Sommaruga, 2001). Cladocerans produce melanin as a form of photoprotection in response to UV light (Weider *et al.*, 1987; Herbert and Emery, 1990), while copepods can develop exterior coloration through carotenoid pigmentation (Byron, 1982; Stoddard, 1987; Moeller *et al.*, 2005). For instance, experimental exposure to UVR-induced pigmentation expression for both *Daphnia* spp. and copepods (Hansson *et al.*, 2007), which decreased by up to 40% within 10 days or removing UV exposure (Hansson *et al.*, 2007). Although cladocerans and copepods are capable of repairing UV-damaged tissue (Mitchell and Karentz, 1993; Reid and Williamson, 2010), kinetic mechanisms of UV repair slow-down in cold environments (Hessen, 1996), such that the ability to tolerate the extreme conditions of alpine systems likely incurs an energetic tradeoff between survival and reproduction for their zooplankton inhabitants (Hessen, 1996; Gliwicz *et al.*, 2001; Hansson *et al.*, 2007; Williamson *et al.*, 2011).

Understanding patterns of reproduction and survival in alpine zooplankton can be informative for understanding how these populations may alter under continued climate change and decreased ice cover duration (Lotter and Bigler, 2000). Alpine and arctic ecosystems are among the most rapidly changing systems globally and baseline data are essential to illuminate the "invisible present" and provide a foundation for detecting future changes (Magnuson, 1990; Hobbie *et al.*, 2003). Here, we collected 10 years (2009–2019) of data on individual and population-level zooplankton characteristics during the open water period from one of the highest elevation lakes with long-term data (Green Lake 4, i.e. GL4, in the Southern Rocky Mountains, USA, at 3550 m above sea level; ASL; Fig. 1). As part of the Niwot Ridge Long-term Ecological Research Program, GL4 has been studied intensively since the 1960s for patterns of ice cover and since the 1980s for water quality metrics (total dissolved nitrogen, phosphorus, organic carbon and major ions). Our goal was to evaluate how two major species of high-elevation zooplankton—the daphnid *D. pulicaria* and the calanoid copepod *H. shoshone*—responded to this highly variable environment (Fig. 2). Specifically, we characterized zooplankton abundance, size and fecundity to understand potential metabolic tradeoffs among adult size, melanization expression (for *D. pulicaria* only) and reproductive output in relation to the timing of ice clearance interannually as well intra-annually. Due to differing reproductive strategies, UV tolerance and thermal responses between cladocerans and calanoid copepods (Holzapfel and Vinebrooke, 2005; Pérez-Martínez *et al.*, 2007; Schneider *et al.*, 2017; Preston *et al.*, 2020), we expected that the clonally reproducing *D. pulicaria* populations would exhibit greater sensitivity and more rapid responses to changes in the annual date of ice clearance and seasonal warming relative to *H. shoshone*, which is dependent on sexual reproduction. We further predicted that the frequency and intensity of melanization in *D. pulicaria* would correlate with days post-ice-off, helping to mitigate the influence of high UVR exposure.

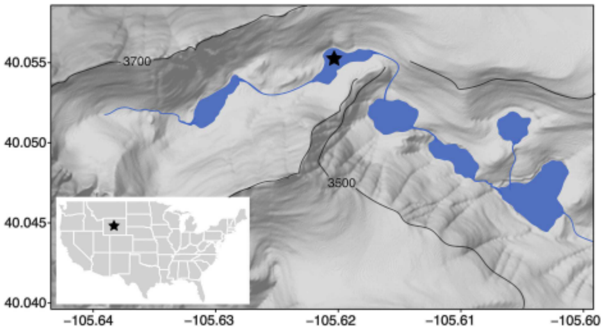

Fig. 1. Map of the GLV, CO USA with Green Lake 4 (3550 m ASL) indicated by a star. Contours provided by stamenmap terrain-background map layer.

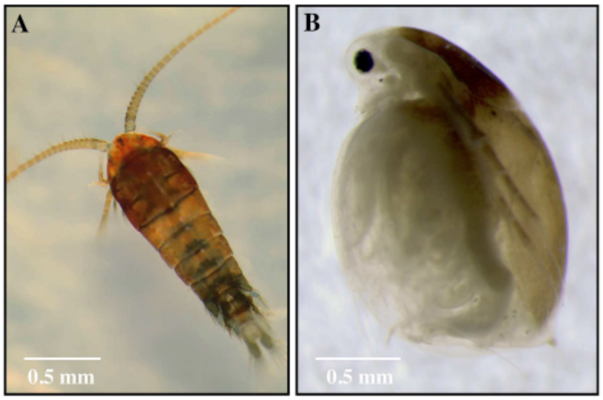

Fig. 2. Microscope photos of adult *H. shoshone* (**A**) and *D. pulicaria* (**B**) from Green Lake 4 with scale bar in mm.

MATERIALS AND METHOD

Field sampling

Green Lake 4 is located at 3550 m ASL within the Green Lakes Valley (GLV), a glaciated bedrock basin in north-central Colorado, USA, which serves as the headwaters of North Boulder Creek (Fig. 1). The lake has a surface area of 5.3 ha with a mean depth of 4.1 m, a maximum depth of 13.5 m, and remains unstocked and naturally fishless (Baron and Caine, 2000; Williams *et al.*, 2001). While records of ice cover and water quality for this lake date back to 1960s and 1980s, respectively, regular monitoring of the zooplankton community began in 2009. Since 2009, GL4 has been surveyed approximately six times during July and August using an inflatable raft at the deepest point in the lake as part of the Niwot Ridge Long Term Ecological Research program. During each visit, we measured temperature and pH at 1-m depth intervals using a YSI multi-probe meter (Preston *et al.*, 2016; Mcknight *et al.*, 2020), photosynthetically active radiation (PAR; using a Li-Cor meter at 1-m intervals with a quantum sensor probe) and water clarity with a Secchi disk (30 cm width) to 0.25 m resolution. Additionally, we collected water samples from the surface (0), 3 and 9 m, using a Van Dorn vertical sampler for dissolved organic carbon (DOC, mg L^{-1}), total dissolved nitrogen (TDN, μM N L^{-1}), total dissolved phosphorus (TDP, μM P L^{-1}) and chlorophyll-a concentrations (μg L^{-1}).

We filtered each water sample in preparation for nutrient (100 mL for TDN and TDP or 125 mL for DOC) and chlorophyll-a (500 mL) analysis through pre-rinsed Millipore 47 mm GF/F filters (0.7 µm pore size) into high-density polyethylene bottles for nutrient analysis, or through pre-combusted Whatman 47 mm GF/F filters (0.7 μm pore size) into new acid-washed, pre-combusted amber glass bottles for DOC analysis. We performed all DOC and chlorophyll-a filtration and analysis in a dark acid-free environment. For chlorophyll-a, we extracted residue from filters using a 90% buffered acetone reagent and analyzed for both un-acidified and acidified replicates with an ISA Jobin Yvon-SPEX Fluoromax-2 within 24 h of extraction. We stored all filtered and leftover raw water samples at 4 °C. The Arikaree Environmental Laboratory performed chemistry analyses (TDN, TDP, DOC) in accordance with standard methods (https://instaar.colorado.edu/research/labs-groups/arikaree-environmental-lab/free-play; Mcknight *et al.*, 2020).

We calculated the attenuation of diffuse PAR in the water column (K_{dPAR}, m^{-1}) (Buiteveld, 1995). K_{dPAR} is inversely related to water column transparency and is commonly used to parameterize the vertical partitioning of radiative energy in the water column (Read *et al.*, 2015). Lastly, we calculated the number of days post ice clearance (when the lake was 100% cleared of ice) from observations of ice phenology (Caine, 2019; Christianson *et al.*, 2020b).

Zooplankton community composition and trait analysis

During each survey, we collected zooplankton samples using a 80 μm mesh Wisconsin net (open sizes: 10 cm from 2009 to 2011 or 20 cm from 2012 to 2018) from the deepest point in the lake in two vertical tows and preserved the contents in ethanol. We analyzed these samples using an Olympus SZX10 stereo dissection microscope in accordance with standard methods (Johnson *et al.*, 2020; Loria *et al.*, 2020). We measured individual zooplankton size (mm) as the length of the carapace or chitinous body structure for the first 50 individuals of each taxonomic group (*D. pulicaria*, *H. shoshone*, as well as juvenile stages of each neonates and nauplii respectively) when present. For *D. pulicaria* we classified adults based on development of the brood chamber, which is undeveloped in neonates (Ebert, 2005), while we differentiated adult *H. shoshone* from nauplii based on developmental stage (Haney *et al.*, 2013). For measured adult zooplankton, we also evaluated individual sex (male or female), reproductive state (gravid or not), clutch size and for cladocerans the presence of melanin. We estimated zooplankton biomass and standardized it for sampling effort to account for net variation ($\mu\mathrm{g\ L}^{-1}$) using established regressions for individual size and biomass (Lafrançois, 2006) and dividing by the lake water volume (L) sampled across the two vertical tows and assuming 100% sampling efficiency of our net for analysis, (Loria *et al.*, 2020) while the capturing effort of nets used was likely between 60 and 90% efficient (Mack *et al.*, 2012).

For a subset of 5 years (2009, 2010, 2014, 2015 and 2016), we made additional observations on the degree of melanization for measured non-gravid adult female *D. pulicaria*. For this subset, we individually isolated the first encountered 20 *D. pulicaria* (when present), placed each individual on a white background, and photographed it three times via the microscope under constant light conditions in order to calculate grayscale-based melanization scores using the open-source software "GIMP" (GIMP Development Team, 2018). We then converted all three images to grayscale, cropped each image using the "free draw tool" to isolate the head and carapace of the individual *D. pulicaria*, and obtained a grayscale value score for the cropped image. As *D. pulicaria* are partially transparent, we also obtained grayscale values from the uppermost left corner of each pre-cropped image background. We subtracted the background value of a given image from the cropped grayscale *D. pulicaria* image to standardize the image background in order to remove any non-pigmentation pixels from the background. We then averaged the grayscale values of a given *D. pulicaria* across the three replicated photos for a final melanization score. Higher scores represented more melanization intensity for a given individual. Additionally, we used this grayscale analysis to create a qualitative rating for melanization from 0 (referring to grayscale values of 0–15, almost no pigment) to 5 (refering to grayscale values of 90–120 , extremely pigmented).

Statistical analysis

We used generalized linear mixed-effects models (GLMMs) to evaluate patterns of zooplankton abundance and trait expression during the open water seasons of each sample year. Our goal was to characterize seasonal variation within zooplankton biomass, fecundity (juvenile biomass, proportion gravid, clutch size and proportion of males for *H. shoshone*) as well as melanin expression for *D. pulicaria* (proportion and intensity) and within the lake habitat (Secchi depth, K_{dPAR}, water temperature, pH, DO, chlorophyll-a, DOC, TDN and TDP). Additionally, to estimate how this system might respond to earlier ice-out, we analyzed how the timing of ice clearance ($_{\mathrm{IClear}}$) was related to the same zooplankton metrics used in our initial characterization of seasonal dynamics.

All models were built with the lme4 package (Bates *et al.*, 2014), implemented in R version 3.0.1 (R Development Core Team, 2013), and fit iteratively using maximum-likelihood based estimation, which was able to accommodate a range of response variable distributions, handle unbalanced designs, and include a combination of fixed and random effects, thereby incorporating sources of non-independence (e.g. survey year) (Bolker *et al.*, 2009). Specifically, we used linear models (LMs) with Gaussian response distributions and a random intercept for year to characterize how zooplankton abundance and trait expression changed interannually in relation to ice clearance as well intra-annually throughout the open water season. For intra-annual (seasonal) characterizations, we included a term for days post ice-off ($_{\mathrm{IDay}}$) instead of Julian date due to the wide range in ice-off timing observed during data collection. As seasonal dynamics can be non-linear, we checked to see if model fit improved by adding a quadratic term for days post ice-off (IDay^2). For responses that related seasonal variation within the lake habitat that were observed on the same day at multiple depths (water temperature, pH, DO, chlorophyll-a, TDN, TDP and DOC), models included an additional random effect of collection depth (epilimnion: 0–3 m, metalimnion: 3–7 m and hypolimnion: >7 m). Certain responses were $\log_{10}$ transformed (with the addition of 1 for any transformed variables that included zeros) to account for skewed raw data distributions. For zooplankton fecundity, we used a binomial distribution with a logit-link function to model the number of fecund versus non-fecund adults (combined using the "cbind" function in R). For all models, we checked for variance inflation using VIF (implemented in the car package) (Fox and Weisberg, 2019) and estimated the significance ($P > 0.05$) of individual terms and used manual comparisons of model fit (residual distributions and AIC scores implemented in the lmerTest package) (Fox and Weisberg, 2011; Kuznetsova *et al.*, 2017). Approximate R^2 values for the final models were obtained using the function rsquaredglmm (Barton and Barton, 2015).

RESULTS

Interannual analysis: influence of ice phenology

Between 2009 and 2019, ice-out occurred as early as June 12 (day of year: 168) and as late as August 5 (day of year: 217; Fig. 3a). Typically, the open water season ranged from 82 to 134 days, with an average ice-free date of July 8 (SD = 14.49 days). The average onset of ice cover occurred on October 25, with a narrower SD of 4.97 days relative to ice-off. We observed negative correlations between ice clearance date and water clarity (i.e. Secchi depth [$_{\mathrm{IClear}}$: 0.556 0.185, $P = 0.005$, $R^{2c} = 0.181$, $n = 42$] and K_{PAR} [$_{\mathrm{IClear}}$: 0.039 0.013, $P = 0.007$, $R^{2c} = 0.266$, $n = 25$]; Fig. 3a), chl-a concentrations ($_{\mathrm{IClear}}$: 0.750 0.303, $P = 0.015$, $R^{2c} = 0.094$, $n = 136$; Fig. 3a), water temperature ($_{\mathrm{IClear}}$: 0.567 0.099, $P < 0.001$, $R^{2c} = 0.416$, $n = 155$; Fig. 3a) and TDN ($_{\mathrm{IClear}}$: 1.918 0.099, $P < 0.001$, $R^{2c} = 0.100$, $n = 125$; Fig. 3a). We also observed marginal correlations between ice-out date TDP ($_{\mathrm{IClear}}$: 0.018 0.010, $P = 0.073$, $R^{2c} = 0.028$, $n = 114$; Fig. 3a) and DOC ($_{\mathrm{IClear}}$: 0.109 0.046, $P = 0.019$, $R^{2c} = 0.076$, $n = 125$; Fig. 3a).

For *D. pulicaria*, earlier ice-out was associated with greater biomass ($_{\mathrm{IClear}}$: 46.89 16.20, $P = 0.0136$, $R^{2c} = 0.359$; Fig. 4). For example, when ice-out occurred before June 30, the first quartile of ice clearance dates, average adult *D. pulicaria* standardized biomass (mean SD; 151.52 156.92 g L^{1}) was 90% higher than for years in which ice-out occurred after July 14, the third quartile of ice clearance dates (14.61 10.17 g L^{1}). Additionally, earlier ice-out was associated with a marginal increase in clutch size ($_{\mathrm{IClear}}$: 1.541 0.681, $P = 0.070$, $R^{2c} = 0.175$; Fig. 4b), but we found non-significant or marginal relationships ($P > 0.070$) between the date of ice clearance and the proportion of gravid adults, neonate biomass, melanin intensity and the proportion of melanized adult *Daphnia*. Concurrently, earlier ice-out was not associated with a change in biomass for *H. shoshone*, clutch size or the proportion of males. Instead, earlier ice-out was related to greater proportions of gravid adults ($_{\mathrm{IClear}}$: 0.575 0.197, $P = 0.004$, $R^{2c} = 0.168$; Fig. 4b) as well as marginally lower nauplii biomass ($_{\mathrm{IClear}}$: 0.563 0.268, $P = 0.067$, $R^{2c} = 0.288$; Fig. 4b).

Intra-annual analysis: seasonal in-lake conditions

Generally, following ice clearance overall water temperature increased ($_{\mathrm{IDay}}$: 0.918 0.084, $P < 0.0001$, $R^{2c} = 0.752$, $n = 158$; Fig. 3b). Water temperatures near the surface (0–7 m) typically increased quickly then stabilized ($_{\mathrm{IDay}}$: 0.845 0.099, $P < 0.0001$, $R^{2c} = 0.629$), while deeper water (>7 m) temperature increases were more gradual and linear ($_{\mathrm{IDay}}$: 1.069 0.089, $P < 0.001$, $R^{2c} = 0.913$). Similarly, dissolved oxygen concentrations (mg L^{1}) decreased with time post ice clearance ($_{\mathrm{IDay}}$: 0.193 0.090, $P = 0.034$, $R^{2c} = 0.467$, $n = 147$; Fig. 3b), likely in association with warmer water temperatures. Alongside seasonal warming, chlorophyll-a concentrations at 9 m also increased with days post ice-off ($_{\mathrm{IDay}}$: 0.1005 0.042, $P = 0.017$, $R^{2c} = 0.575$, $n = 136$; Fig. 3b), helping account for the deep-water chlorophyll-a maxima in GL4, which are also common to other alpine lake systems (Rodhe *et al.*, 1966). No such seasonal increase in chlorophyll-a was detected for depths < 9 m. Concurrently, TDN concentrations and

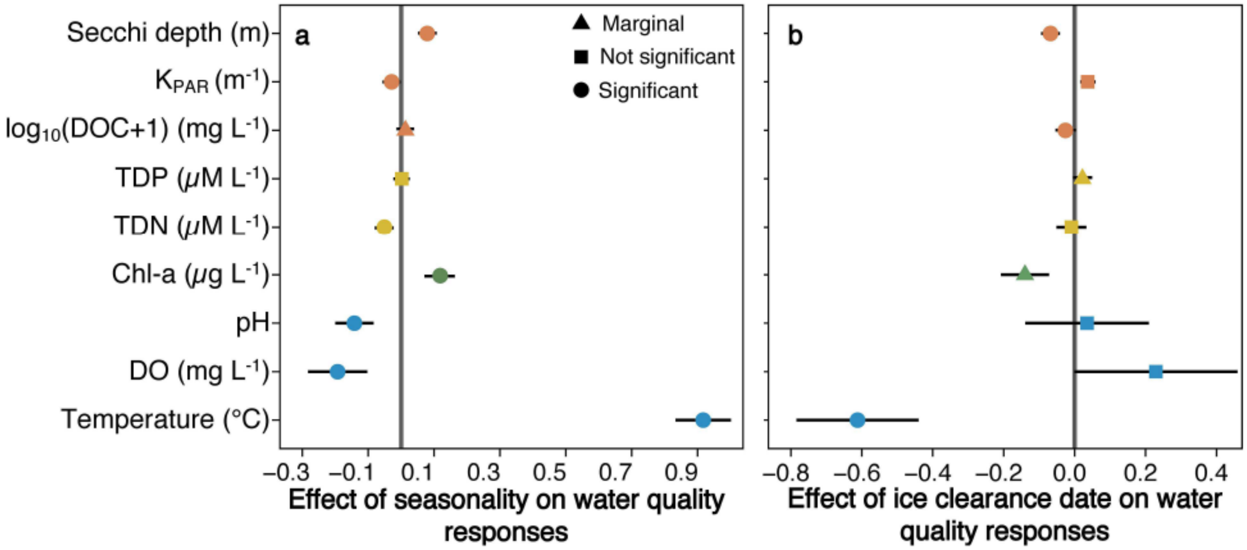

Fig. 3. Depicted in the plot is the effect of either (**a**) ice clearance date (scaled) or (**b**) seasonality (i.e. days post ice clearance, also scaled) on each of the following response variables: Secchi depth in m KPAR (m^{-1}), log10 (DOC + 1) (mg C L^{-1}), TDP and TDN (μM L^{-1}), chlorophyll-a (μg L^{-1}), pH, DO (mg L^{-1}), and temperature (°C), based on individual GLMMs in which either (a) ice clearance date or (b) days post ice clearance is a fixed numeric predictor. Points thus represent the beta coefficients, where shape refers to a significant relationship for each physiochemical response and the horizontal bars intersecting each point represent the standard error.

pH levels decreased throughout the open water season (β_{IDay}: -0.051 ± 0.010, $P < 0.0001$, $R^{2c} = 0.767$, $n = 158$; Fig. 3b) (β_{IDay}: -0.142 ± 0.059, $P = 0.017$, $R^{2c} = 0.622$, $n = 143$; Fig. 3b), accompanied by a marginal increase in DOC (β_{IDay}: $1.287 \times 10^{-02} \pm 6.73 \times 10^{-03}$, $P = 0.058$, $R^{2c} = 0.213$, $n = 158$; Fig. 3b). These relationships were strongest in depths deeper than 9 m, which could be evidence of heightened heterotrophic respiration. Lastly, water clarity increased throughout the open water season, as indicated by increasing Secchi depth (β_{IDay}: 0.079 ± 0.016, $P < 0.0001$, $R^{2c} = 0.630$, $n = 45$; Fig. 3b), and decreasing K_{PAR} (β_{IDay}: -0.029 ± 0.009, $P = 0.004$, $R^{2c} = 0.674$, $n = 26$; Fig. 3b).

Intra-annual analysis: seasonal zooplankton biomass

Across 51 seasonal samples, the biomass of adult *D. pulicaria* (μg L^{-1}) increased significantly throughout the open water season (β_{IDay}: 0.208 ± 0.073, $P = 0.007$, $R^{2c} = 0.647$; Fig. 5a) and correlated positively with water temperature (β_{Temp}: 0.144 ± 0.067, $P = 0.038$, $R^{2c} = 0.647$). On average, the standardized biomass of *D. pulicaria* was typically lowest (5.93 μg L^{-1}) immediately after ice-out—a trend that was primarily driven by density rather than average body size resulting in dense populations of smaller bodied adults. Similarly, the biomass of adult *H. shoshone* increased marginally with days post ice clearance (β_{IDay}: 0.076 ± 0.040, $P = 0.062$, $R^{2c} = 0.690$), and also correlated positively with water temperature (β_{Temp}: 0.126 ± 0.042, $P = 0.0004$, $R^{2c} = 0.711$). For *H. shoshone*, however, there was evidence that the seasonal pattern in biomass was non-linear: standardized biomass (103.98 μg L^{-1}) typically occurred around 30 days post ice-off before subsequently declining, such that including a quadratic term for days post ice-off ($P = 0.004$, $R^{2c} = 0.685$, quadratic model delta AIC = 4; Fig. 5b) improved model fit. On average, adult *D. pulicaria* standardized biomass (mean±SD, 66.61±100.39 μg L^{-1}) was 24% lower than average *H. shoshone* standardized biomass (87.16±67.30 μg L^{-1}). Additionally, the biomass of *D. pulicaria* and *H. shoshone* were not significantly correlated with K_{PAR}, pH, chlorophyll-a or DOC.

Intra-annual analysis: seasonal zooplankton reproduction

Patterns of zooplankton reproduction linked closely with ice clearance for both *D. pulicaria* and *H. shoshone*. The peak in the proportion of gravid *D. pulicaria* typically occurred shortly after ice-off, which then decreased non-linearly throughout the season (β_{IDay}: -0.265 ± 0.069, $P < 0.0001$; Fig. 5e). For example, on average, 25% (SD = 22%) of adults were fecund within 20 days of ice clearance, which then dropped to 14% (SD = 11%) between 20 and 40 days, and slightly increased to 16% (SD = 18%) after 40 days. Thus, inclusion of a quadratic term improved the model relative to a strictly LM

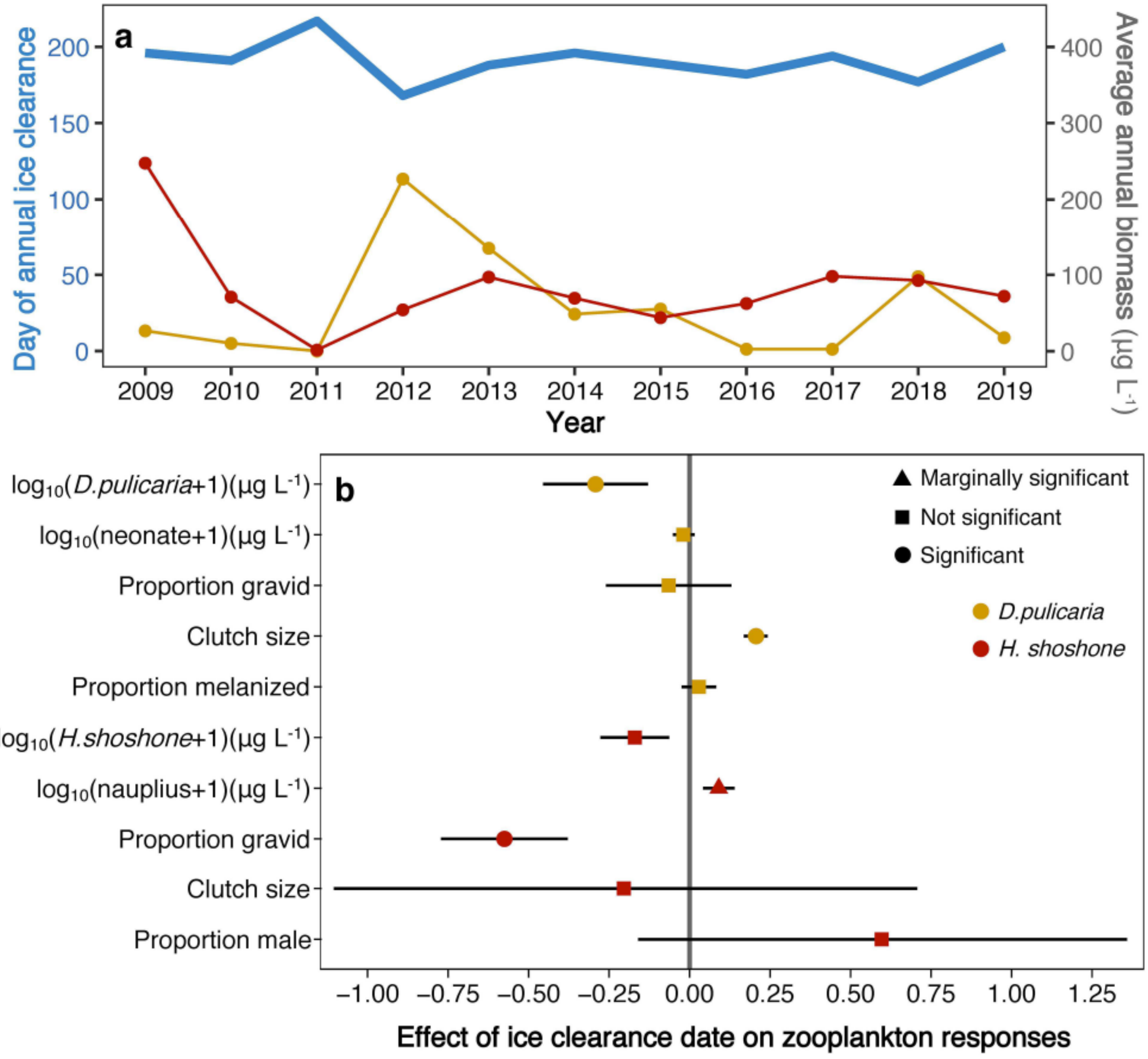

Fig. 4. (**a**) The interannual variation in ice phenology and average zooplankton biomass for *D. pulicaria* and *H. shoshone*. The day of ice clearance ranged from 168 to 217 (the left *y*-axis) and is depicted in blue. Biomass for *D. pulicaria* and *H. shoshone* (the right *y*-axis) ranged from 0 to 226.93 ($\mu g\ L^{-1}$) and 1.37 to 284.27 ($\mu g\ L^{-1}$), respectively. (**b**) Depicted in the plot is the effect of ice clearance date on each of the following response variables: standardized biomass ($\mu g\ L^{-1}$) of log10 (*D. pulicaria* + 1), log10 (neonate + 1), log10 (*H. shoshone* + 1), and log10 (nauplius + 1); the proportion of gravid adult zooplankton; clutch size for gravid individuals; and for *D. pulicaria*, the proportion melanized; and for the proportion of male *H. shoshone* based on individual GLMMs for ice clearance date occurred (scaled). Points thus represent the beta coefficients, where shape refers to a significant relationship for each zooplankton response and the horizontal bars intersecting each point represent the standard error. Responses associated with *D. pulicaria* are colored in yellow (lighter), and responses associated with *H. shoshone* are colored in red (darker). These data indicate significant effect of ice clearance date on *D. pulicaria* biomass, clutch size and the proportion of gravid *H. shoshone*; thus for years with later ice clearance dates *D. pulicaria* biomass is lower and their clutch sizes are higher, and the proportion of gravid *H. shoshone* is lower.

($P < 0.0001$, quadratic model delta AIC = 30; Fig. 5e). Similarly, for gravid adult *D. pulicaria* ($n = 357$), clutch size or the average number of eggs per egg-bearing females (mean±SD, 7.14±5.22 eggs) tended to decrease throughout the open water period, with an average clutch of 4.18 (SD = 1.92) eggs after 40 days post ice-off. This relationship also tended to be non-linear and adding a quadratic term improved model fit ($P = 0.03$, $R^{2c} = 0.203$, quadratic model delta AIC = 5; Fig. 5g). Clutch size was also was positively correlated with water temperature (β_{Temp}: 1.142 ± 0.382, $P = 0.003$, $R^{2c} = 0.360$). Interestingly, the occurrence of male *D. pulicaria* and resting eggs was exceptionally rare; only one male was found on 10 July 2018, and individual adult females with resting eggs were observed 21 July 2009 and 7 August 2012.

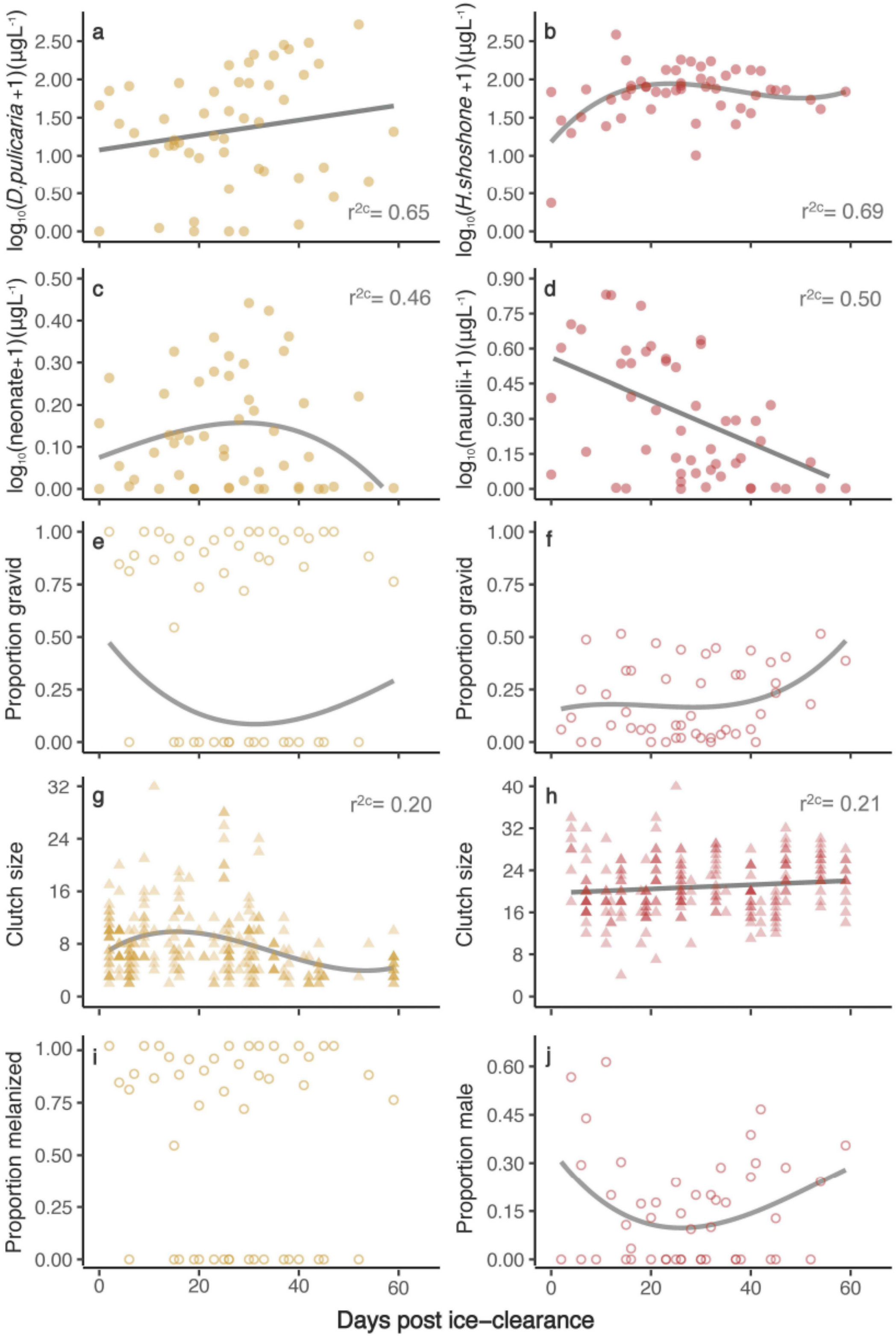

Fig. 5. Panel of seasonal or intra-annual zooplankton community dynamics. *Daphnia pulicaria* population traits are shown in yellow (left; Fig. 5a, Fig. 5c, Fig. 5e, Fig. 5g and Fig. 5i), while *Hesperodiaptomus shoshone* population traits are in red (right; Fig. 5b, Fig. 5d, Fig. 5f, Fig. 5h and Fig. 5j). Generalized linear mixed-effects models for β_{IDay} are included when significant, and the polynomial trend is displayed when significant. Point shape corresponds to zooplankton sex status either non-gravid (closed circle), gravid (triangle) or proportion (open circle). Conditional r squared values are reported for each model when significant, and not reported for proportional data due to the binomial distribution (Fig. 5e, Fig. 5f, Fig. 5i and Fig. 5j).

For adult *H. shoshone*, the proportion of gravid individuals also increased non-linearly with days post ice clearance and was positively associated with water temperature ($_{\text{Temp}}$: 0.288 0.082, $P = 0.0004$). Fecundity was relatively stable early in the open water season, then increased monotonically from around 17% to around 28% between 20 and 40 days post ice clearance ($P < 0.0001$, quadratic model delta AIC = 20; Fig. 5f). Similarly, for fecund *H. shoshone* ($n = 226$), average clutch size (20.9, SD = 5.48 eggs) marginally increased with days post ice clearance ($_{\text{IDay}}$: 0.683 0.370, $P = 0.066$, $R^2_c = 0.210$; Figs 4h and 5h). The proportion of male *H. shoshone* was high (20%, SD = 22%) after ice-off, deceased between 20 and 40 days (9%, SD = 10%), and increased toward the end of the season (20%, SD = 17%); again this relationship was not linear and adding quadratic term improved model fit ($P < 0.0001$, quadratic model delta AIC = 480; Fig. 5j).

Incorporating the abundance of juvenile plankton for both of these groups, we found that the biomass of juvenile *D. pulicaria,* or neonates, was marginally correlated with days post ice-off (Fig. 5c), such that peak biomass (1.765 g L ^{1}) occurred approximately 30 days post ice-off. This relationship improved model fit with the addition of a quadratic term ($P = 0.038$, $R^2_c = 0.459$, quadratic model delta AIC = 2; Fig. 5c). Conversely, *H. shoshone* nauplii biomass decreased linearly throughout the open water season ($_{\text{IDay}}$: 0.112 0.029, $P = 0.0003$, $R^2_c = 0.500$, Fig. 5d) and was also negatively correlated with water temperature ($_{\text{Temp}}$: 0.780 0.206, $P = 0.0004$, $R^2_c = 0.356$).

Intra-annual analysis: additional zooplankton traits

After controlling for ordinal date, fecund individual *D. pulicaria* were approximately 73% larger than non-fecund adults and fecund individual *H. shoshone* were 26% larger than non-fecund individuals. Individual clutch size was positively correlated with both *D. pulicaria* ($_{\text{biomass}}$: 1.44 0.25, $P < 0.0001$, $R^2_c = 0.310$) and *H. shoshone* ($_{\text{biomass}}$: 2.30 0.38, $P < 0.0001$, $R^2_c = 0.281$) biomass, respectively.

For a subset of observations using grayscale to quantify melanization intensity in non-fecund adult *D. pulicaria* ($n = 577$), melanization intensity ranged from 1.17 (almost no pigment) to 127.67 (extremely pigmented) (mean SD; 53.18 23.04). Melanization intensity correlated positively with individual biomass ($_{\text{BM}}$: 10.42 0.86, $P < 0.0001$, $R^2_c = 0.373$) and the more qualitative melanization scores (0–5) ($_{\text{BM}}$: 0.466 0.045, $P < 0.0001$, $R^2_c = 0.293$). Although the proportion of melanized individuals was fairly stable during the open water season (Fig. 5i), the average melanization intensity increased seasonally ($_{\text{IDay}}$: 2.654 1.323, $P = 0.046$, $R^2_c = 0.203$). This relationship improved with a quadratic term for days post ice clearance ($P < 0.0001$, $R^2_c = 0.619$; quadratic model delta AIC = 50), as average melanin intensity was lowest (48.44 22.49) between 10 and 20 days after ice clearance and was highest (63.01 19.15) between 30 and 40 days after ice clearance. In regards to within lake conditions, melanization intensity correlated positively with Secchi depth ($_{\text{Secchi}}$: 4.333 1.329, $P = 0.0013$, $R^2_c = 0.110$), chlorophyll-a concentrations ($_{\text{Chl-a}}$: 12.150 1.931, $P < 0.001$, $R^2_c = 0.405$), TDP ($_{\text{TDP}}$: 5.466 1.845, $P = 0.0035$, $R^2_c = 0.296$), DO ($_{\text{DO}}$: 2.836 1.521, $P = 0.064$, $R^2_c = 0.097$) and negatively with DOC concentrations ($_{\text{DOC}}$: 2.052 1.141, $P = 0.073$, $R^2_c = 0.081$), and TDN ($_{\text{TDN}}$: 3.068 1.544, $P = 0.048$, $R^2_c = 0.172$), with no significant relationship to water temperature, or K_{PAR}. Lastly, the proportion of melanized individuals was fairly stable throughout the open water season (Fig. 5i).

DISCUSSION

Based on a decade of measurements of two commonly observed alpine zooplankton taxa, we found that years with earlier ice-out dates were associated with increases in *D. pulicaria* biomass, likely due to warmer water temperatures and an extension of the growing season duration. However, years with earlier ice-out were associated with relatively few other changes in zooplankton response metrics, with marginal or non-significant effects for the proportion of gravid and melanized *D. pulicaria*, neonate and *H. shoshone* biomass, *H. shoshone* clutch size and the proportion of male *H. shoshone* (Fig. 4). Within years, analyses of the intra-annual or seasonal zooplankton dynamics indicated that *D. pulicaria* biomass and melanization tended to be lowest immediately following ice clearance, whereas fecundity was relatively high. As the summer progressed, *D. pulicaria* biomass and melanization increased linearly while fecundity decreased. In contrast, demographic patterns for *H. shoshone* tended to be more non-linear, with peaks in biomass and fecundity around 30–40 days after ice-off, respectively, and overall less variability throughout the open water season relative to *D. pulicaria*. These results indicate that the two dominant zooplankton groups likely diverge in their reproductive strategies, with the majority of *D. pulicaria* reproduction likely occurring in one event immediately after ice clearance, while *H. shoshone* reproduction occurs more evenly throughout the open water season.

Results of our physical and chemical measurements from Green Lake 4 (GL4) emphasize the extreme nature

Table I: Summary statistics for individual environmental variables from GL4 (2009–2019) collected throughout the summer

Variable	Mean	Min	Max	SD
Surface water temperature (°C)	9.84	6.45	11.72	1.37
Deep water temperature (°C)	8.15	4.92	10.34	1.36
Surface water pH	7.21	5.32	8.75	0.62
Deep water pH	6.11	3.73	7.60	0.80
Surface chlorophyll-a (μg L^{-1})	3.16	0.00	12.01	2.61
Deep chlorophyll-a (μg L^{-1})	4.42	0.40	15.91	3.36
Surface TDN (μM L^{-1})	13.63	1.35	25.50	5.78
Deep TDN (μM L^{-1})	12.79	2.27	30.19	6.84
Surface TDP (μM L^{-1})	0.11	0.03	0.38	0.08
Deep TDP (μM L^{-1})	0.13	0.01	0.70	0.13
Surface DOC (mg L^{-1})	0.68	0.31	1.18	0.16
Deep DOC (mg L^{-1})	0.71	0.35	2.52	0.33
Secchi depth (m)	4.24	2.00	8.25	1.27
K_{PAR} (m^{-1})	0.36	0.28	0.56	0.06

Note: SD denotes standard deviation and surface water here refers to depths less 3 m and deep water refers to depths deeper 9 m.

of alpine lake environments for aquatic taxa. The brevity of the open water season (typically <4 months long), combined with the low temperatures, weak thermal stability and low concentration of nutrients creates a challenging and highly dynamic environment. Throughout the open water season GL4 rarely stratified, a quality unique to cold lake environments, and on average experienced a <2°C difference from the surface to the hypolimnion (Table I). This unstable physical environment was compounded by extremely low concentrations of TDP (max = 0.70 μM L^{-1}), as well as low TDN and chlorophyll-a concentrations, relative to lower elevation lakes (Carlson, 1977; Table I). Interestingly, zooplankton dynamics in GL4 were somewhat decoupled from chlorophyll-a concentrations. While chlorophyll-a and zooplankton biomass both increased with days post ice clearance, neither *D. pulicaria*, neonate, *H. shoshone*, nor nauplii biomass were directly correlated with chlorophyll-a concentrations. The only potential influence of chlorophyll-a on zooplankton dynamics we detected was a positive correlation between chlorophyll-a and *D. pulicaria* melanization intensity. The unpredictable timing of fall ice formation (Kolesar *et al.*, 2002) poses a challenge in capturing late fall dynamics, which could have lag effects on zooplankton resources related to strategies for reproduction and growth (Bayer *et al.*, 2016).

Our analysis supported the expectation that the extreme physicochemical conditions of alpine lakes limited biological growth (e.g Williamson *et al.*, 2010; Preston *et al.*, 2016). Specifically, water temperature (which was correlated with ice clearance date, $R^{dc} = 0.752$, Fig. 3) correlated strongly with zooplankton population traits. Water temperature was positively associated with *D. pulicaria* and *H. shoshone* biomass, *D. pulicaria* clutch size and the proportion fecund *H. shoshone*, and negatively correlated with nauplii biomass. Ecologically, water temperature plays a central role in lake stratification dynamics (Kirillin, 2010; Christianson *et al.*, 2020a) and can alter biogeochemical rates (Adrian *et al.*, 2009), which interactively control lake metabolism. Past research has shown zooplankton abundance and grazing rates in high-elevation systems are driven by water temperature, rather than by food or nutrient availability (Williamson *et al.*, 2010; Fischer *et al.*, 2011; Bayer *et al.*, 2016). Experimental studies indicate that the rate of molting (instar duration) in species such as *D. pulex* depends more on the temperature than food availability, for which instar duration and egg development time are inversely proportional to temperature (Dodson *et al.*, 2010). Zooplankton vulnerability to threats such as intense UVR exposure is further exacerbated by low temperatures (Doyle *et al.*, 2005). For zooplankton, the kinetic processes of repair from UVR damage are slower in cold environments (Hessen, 1996; Hansson and Hylander, 2009). Here, we observed minimum temperatures just above freezing—even during the open water season and especially at depths > 9 m (Table I). Because *D. pulicaria* migrate diurnally to deeper and colder waters both to seek refuge from UVR exposure and access the chlorophyll-a maxima, they may be more negatively impacted from cold temperatures relative to *H. shoshone* (Herbert and Emery, 1990; Hessen, 1996; Williamson *et al.*, 2010; Williamson *et al.*, 2011). Previous work has demonstrated substantial differences in the life history strategies of boreal calanoid copepods (*L. minutus*) and *Daphnia* spp., where copepods were more impacted by food availability rather than temperature (Grosbois *et al.*, 2017; Schneider *et al.*, 2017).

Increases in zooplankton pigmentation and body size also appeared to benefit *D. pulicaria* and *H. shoshone* in their responses to the challenging environmental conditions observed in GL4. Although we did not directly measure pigmentation in *H. shoshone,* we did find an average 33% seasonal increase in melanin intensity for *D. pulicaria* throughout the open water season. Melanin intensity also positively correlated with Secchi depth, likely reflecting the adaptive value of melanization against changing UVR exposure among *D. pulicaria*. Previous experimental research has shown that melanization in *Daphnia* spp. is inducible in response to UV light exposure (Hessen, 1996; Hansson *et al.,* 2007); however, melanized individuals also exhibit slower growth rates relative to transparent conspecifics, suggesting potential trade-offs between UV protections and other dimensions of organismal fitness, such as growth and reproductive output (Hansson *et al.,* 2007; Hansson and Hylander, 2009). Zooplankton size is thought to be tied to reproductive potential (Allan, 1976), and here we found larger fecund individuals (for both *D. pulicaria* and *H. shoshone*) tended to produce more eggs. Among all adult *D. pulicaria,* individual size varied more widely relative to *H. shoshone* throughout our observation with individual carapace length ranging from 0.660 to 5.704 mm and from 0.394 to 3.737 mm, respectively. Past research has hypothesized that melanized *Daphnia* spp. tend to use a reproductive strategy that allows them to grow slower and produce fewer offspring, mostly via asexual production of polyploid clones (Hessen, 1996; Gliwicz *et al.,* 2001). Correspondingly, male *D. pulicaria* and females with sexually produced ephippia were almost never observed, which could provide further support as to the importance of polyploidy. However, our results are correlational and require additional genetic exploration to definitively determine the actual mechanism of *D. pulicaria* reproduction in GL4.

Our results suggest that the annual timing of ice clearance is an important cue for reproduction by both *D. pulicaria* and *H. shoshone*. The timing of ice clearance correlated was negatively with water temperature ($R^{2c} = 0.416$, Fig. 3a), and the annual date of ice-off among years was an influential predictor of both zooplankton density and fecundity. For the Rocky Mountains of USA, variation in annual seasonal phenomena has been linked to Southern Oscillation forcing, which has implications for the timing and duration of both watershed snowpack and lake ice cover (Hauer *et al.,* 1997; Christianson *et al.,* 2020b). The variation in snowmelt for the GLV in particular was three times greater than a comparable watershed in the southern Sierra Nevada, California with a maritime snowpack (Jepsen *et al.,* 2012). In the Sierra Nevada, the duration of snow and ice cover can regulate the timing of light availability, the length of the growing season and amount phytoplankton biomass in alpine lakes (Sadro *et al.,* 2018). As slow-growing and relatively stress tolerant zooplankton taxa, both *D. pulicaria* and *H. shoshone* appeared to be relatively tolerant of variation in ice timing, consistent with these groups legacy in alpine systems; Forbes (1893) first observed *H. shoshone* in Yellowstone Lake (2360 m ASL, Yellowstone National Park) in 1893, while Ward *et al.* (1904) reported *H. shoshone* and *Daphnia pulex* as the predominant zooplankton among alpine lakes above 2300 m ASL across the Colorado Rocky Mountains. Nonetheless, if earlier ice-out becomes more common in alpine and artic systems, it could create unfavorably warm conditions for alpine zooplankton or facilitate the upward invasion by more montane taxa (Holzapfel and Vinebrooke, 2005; Carter *et al.,* 2017; Preston *et al.,* 2020), leading to shifts in community composition and food web structure depending on dispersal opportunities. Extreme years with early ice clearance could pose threats to the calanoid copepods in GL4, as they are obligate sexual reproducers without a dormant egg bank for recolonization (Kramer *et al.,* 2011).

CONCLUSION

Our analysis of a decade of zooplankton population and individual-level data highlight the importance of ice clearance, water temperature and water clarity on zooplankton abundance, fecundity and trait expression. Results from statistical analysis indicate that despite the extremely variable environment, alpine zooplankton exhibit some degree of tolerance (Weidman *et al.,* 2014). Even though lower elevation cladocera are capable of tolerance strategies such as the synthesis of melanin (Weider *et al.,* 1987; Beaton and Hebert, 1988), these mitigation approaches are much more common in alpine zooplankton exposed to intense UVR. A large part of our current understanding of biologic patterns of community composition relies on sediment core analysis (Catalan *et al.,* 2002; Perga *et al.,* 2015; Jiménez *et al.,* 2018), rather than population surveys. By analyzing whole zooplankton communities, we characterized zooplankton population during the ice-free season and explored trait-based correlations to changes in ice cover. It is possible that as alpine lakes continue to experience greater reductions in ice cover (Brown and Duguay, 2010), the zooplankton communities within those lakes will also shift, likely to include greater abundances of large bodied cladocerans as these organisms may be released from some present thermal stress. With a growing scientific consensus around imminent change to alpine, arctic and other cold region lakes (Moser *et al.,* 2019; Sharma *et al.,* 2019), an increased understanding of their respective ecological baselines though long-term observation and experimentation is needed to penetrate the "invisible present" and shed light on future change (Magnuson, 1990).

DATA ARCHIVING

Data from this study are available through the Niwot Ridge Long Term Ecological Research Website (https://nwt.lternet.edu/), and exact citations for data versions can be found in the references of this document.

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