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Species-specific phenological trends in shallow Pampean lakes (Argentina) zooplankton driven by contemporary climate change in the Southern Hemisphere

Zooplankton phenology in Southern Hemisphere

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Primary Research

Abstract

The relationship between the timing of recurrent biological events and seasonal climatic patterns (i.e., phenology) is a crucial ecological process. Changes in phenology are increasingly linked to global climate change. However, current evidence of phenological responses to recent climate change is subjected to substantial regional and seasonal biases. Most available evidence on climate driven phenological changes comes from Northern Hemisphere (NH) ecosystems, and typically involve increases in spring and summer temperatures, which translate into earlier onsets of spring population developments. In the Argentine Pampa region, warming has occurred at a much slower pace than in the NH, and trends are mostly restricted to increases in the minimum temperatures. We used zooplankton abundance data from lake Chascomús (recorded every two weeks from 2005 to 2015) to evaluate potential changes in phenology. We adopted a sequential screening approach to identify taxa displaying phenological trends, and evaluated if such trends could be associated to observed long-term changes in water temperature. Two zooplankton species displayed significant later shifts in phenology metrics (end date of *Brachionus havanaensis* seasonal distribution: 31-day/decade, onset and end dates of *Keratella americana* seasonal distribution: 59-day/decade and 82-day/decade, respectively). The timing of the observed shift in *B. havanaensis* phenology was coincident with a warming trend in the May lake water temperature (4.7°C per decade). Analysis of abundance vs. temperature patterns from six additional shallow Pampean lakes, and evaluation of previous experimental results, provided further evidence that the lake water warming trend in May was responsible for the delayed decline of *B. havanaensis* populations in autumn. This study is the first report of freshwater zooplankton phenology changes in the Southern Hemisphere (SH).

Introduction

The relationship between the timing of recurrent biological events and seasonal climatic patterns (i.e., phenology) is a crucial ecological process. As a major driver of population dynamics, dispersal and migration, phenology has strong implications for population demography. In addition, by differentially affecting co-occurring species, phenological changes may have profound effects on species interactions, such as increased (or reduced) competition for resources, trophic mismatch, disruption of plant-pollinator interaction, etc. (Hampton, 2005; Thomson, 2010; Thackeray *et al.*, 2013). Changes in phenology are increasingly linked to global climate change (Ovaskainen *et al.*, 2013). However, current evidence of phenological responses to recent climate change is subjected to substantial regional, habitat-specific, taxon-specific and seasonal biases (Brown *et al.*, 2016). By far, most of the available evidence on climate driven phenological changes comes from Northern Hemisphere (NH) ecosystems (Cohen *et al.*, 2018). Moreover, the larger proportion (>80%) of Southern Hemisphere (SH) datasets originated in Australasia, while Africa and South America are remarkably under-represented. In these regions, most studies have focused on terrestrial and marine environments (Chambers *et al.*, 2013). Within freshwater systems, an overwhelming proportion of phenological studies have been conducted in deep stratifying lakes (many of which freeze) from temperate European countries (Vadadi-Fülöp & Hufnagel, 2014). Most such studies focused on the spring period, during the ice free period, with much less attention on autumn and winter periods (Chen & Folt, 1996; Manca & DeMott, 2009; Gallinat *et al.*, 2015).

Documented responses in freshwater systems typically involve advancements (=earlier shifts) in the date of initiation of population growth or the date on which the population maximum is reached. In general, the advancement of spring phenological events has been attributed to earlier ice break-up dates and/or higher spring water temperature (Table 1 in

Vadadi-Fülöp & Hufnagel, 2014). Within planktonic animals, most examples of phenological shifts over time involve fast growing, parthenogenetic species, e.g., cladocerans and rotifers (Winder & Schindler, 2004; Hampton, 2005; Thackeray *et al.*, 2012), as opposed to organisms with longer or more complex life cycles, such as copepods (Gerten & Adrian, 2002; Winder *et al.*, 2009).

The paucity of phenological studies form the Southern Hemisphere limits our ability to draw general conclusions on phenological trends at a global scale (Cohen *et al.*, 2018), not only because of a large underrepresented region, but also and most importantly, because of the disparities in ongoing and projected climate trends between both hemispheres, which result from the uneven distribution of land masses (for a review see Chambers *et al.*, 2013). Studies from the SH (mostly on terrestrial plants and birds) display large disparities in the timing and direction of phenological changes, with a moderately higher proportion of trends towards earlier events in autumn (Chambers *et al.*, 2013).

Quite pertinent for the present study is the fact that climate trends in temperate Argentina differ those in other areas of the world. In most parts of the country, warming has taken place at a much slower pace than in the NH (Nuñez *et al.*, 2008; Barros *et al.*, 2015). In contrast to most other land areas of the world, there has been net cooling over most of Argentina (about -0.04°C/decade), and a remarkable decrease in the diurnal temperature range north of 40°S. This is associated with a noticeable reduction in the maximum temperature (-0.12°C/decade) along with a weak warming trend in the minimum temperature (0.05°C/decade). A long-term modelling study of lake Chascomús water temperature concluded that the lake had warmed at a rate of 0.3°C per decade over the study period (1966 to 2012), with most significant increases during the warmer season (Elisio *et al.*, 2015).

Contrary to most known examples of lakes displaying long-term phenological shifts, shallow Pampean lakes remain mixed during the whole year and do not freeze in winter.

Thus, the physical environment can be expected to provide fewer (and from a researcher's perspective, less predictable) cues to trigger phenological responses of plankton. On the positive side, the noticeable seasonal succession of Pampean lakes zooplankton (Diovisalvi *et al.*, 2015b) entails a progressive replacement of species, which are likely adapted to different ranges of temperature and other environmental conditions. Under such circumstances, studies with whole year coverage maximize the chances of detecting phenology trends, and for analogous reasons the use of metrics that capture the whole seasonal distribution of activity for the focal organisms should be preferred (Miller-Rushing *et al.*, 2008). Fortunately, time schemes of many plankton monitoring programs allow such an approach (Thackeray *et al.*, 2012).

Based on seasonal temperature trends, both at hemispherical and local scales, we anticipated that the chances of detecting phenology shifts should be maximum in autumn. Nevertheless, phenology shifts may not only arise from direct species-specific temperature responses, but also from indirect effects related to bottom-up and top-down processes (Velthuis *et al.*, 2017). Such trophic interactions restrict our ability to predict the magnitude and direction of changes (Cohen *et al.*, 2018). In order to investigate the phenological changes of a zooplankton community in the Pampean region, we analyze three phenological metrics (onset, central and end point of the growing season) of lake Chascomús zooplankton, over the period 2005-2015. In addition, we used shorter (2-year) time-series datasets from 6 additional shallow Pampean lakes to validate the patterns observed in lake Chascomús.

Materials and methods

Study area

The Pampa region of Argentina is one of the largest wetland areas of South America (Diovisalvi *et al.*, 2015a). The region is a predominantly flat area, crossed by rivers and scattered with shallow lakes. The climate is warm temperate (mean annual temperature 13 to 16°C) and characterized by pronounced thermal seasonality. The mean annual precipitation decreases along a NE-SO gradient from 1,000 to 400mm, and displays large inter annual variability, resulting in dry and wet periods (see Diovisalvi *et al.*, 2015a). Our main study site, lake Chascomús (35º36'S, 58º02'W), is a large shallow lake (surface area 30.1 km^2, mean depth of 1.9 m), which is a part of a chained system lakes within to the Salado River watershed. Lake Chascomús was sampled every other week from June 2005 to January 2015. In addition, six other Pampean shallow lakes were included: El Triunfo (35º51'S, 57º52'W), La Limpia (35º37'S, 57º48'W), La Salada de Monasterio (35º47'S, 57º52'W), Grande de Otamendi (34º13'S, 58º52'W), La Barrancosa (37°20'S, 60°7'W) and El Chifle (37°24'S, 59°47'W) (Figure 1). These lakes were sampled monthly from October 2012 to January 2015.

Sampling

Routine sampling included zooplankton collections along with measurements of a standard set of limnological variables. Water temperature, pH (Orion pH meter), conductivity (Hach conductimeter), dissolved oxygen concentration (YSI 5000 oximeter) and Secchi disk readings were measured *in situ*. Chlorophyll *a* concentration (Chl *a*), suspended particulate matter (seston) and nutrients (N and P) were measured following Izaguirre *et al.,* (2015). Zooplankton samples were collected from a central sampling site (Diovisalvi *et al.*, 2015c) by pouring 45L of lake surface water (upper ~30cm) through a 45-µm mesh net and preserved in 4% formalin. Rotifers and copepod nauplii were counted under a compound microscope on 1-mL Sedgwick-Rafter counting cell; cladocerans and copepods (copepodids and adults) were counted under a dissecting microscope in a 5-mL Bogorov counting chamber. Zooplankton were identified to species (more rarely to genus) level. Rotifers were identified following Ruttner-Kolisko (1974) and Koste (1978) and crustaceans according to Reid (1985) and Paggi (1995).

Air and water temperature

We used the hourly air temperature time series from the nearest official meteorological station (Dolores city, 85 km from the lake). We used the series from Dolores city because data were certified by the Argentine National Meteorological Service and the time coverage included our study period (2005 to 2015). Using over 3 years of simultaneous measurements, we have confirmed that the mean daily air temperature from Dolores was strongly correlated with measurements from our own meteorological station located near the lake ($r^2 = 0.981$, $p<0.0001$, $n=1305$). Missing data (9% of total data series) were replaced by linear interpolation estimates. In order to assess long-term temperature changes, within each month, daily temperature values were regressed vs. the year.

Measurements of lake water temperature were available for every sampling date. Given that water temperature had a strong seasonal component (Elisio *et al.*, 2015) and considering that the lake was sampled every other week (i.e., not at fixed dates), monthly water temperature estimates could be affected by among-years differences in the actual date of sampling. To circumvent this problem, we developed a simple model to remove both the seasonal component and the dependence of the measured values on the date of sampling. The rationale for this was to develop a model relating water temperature to sampling date (expressed as day of year, DOY). The model residuals represent deviations from the expected temperature for any given DOY. Therefore, instead of analyzing trends of actual temperature data, we could focus our attention on the deviations from the expected values, and ask if such deviations showed significant trends over the years. For each DOY we computed $f = sin(2\pi(80.8 - DOY)/365.25)$, which is proportional to the expected (i.e., astronomical) incident radiation; and its derivative $f' = cos(2\pi(80.8 - DOY)/365.25)$, which represents the rate of change of incident radiation at any given DOY. The number 80.8 was chosen so that the maximum value of f occurs on the longest day of the year (December 21 in the SH).

Then, we used multiple regression analysis to fit temperature measurements to a regression model including f and f' as independent variables. The underlying rationale for these models is that changes in water temperature result from the additive effects of thermal conduction at the water–atmosphere interface and the incoming and emitted radiation (Elisio *et al.*, 2015). In general, irradiance values are useful to distinguish between summer and winter. On the other hand, the rate of change of irradiance (as estimated by $f^{'}$) is more effective for distinguishing between autumn and spring. We anticipated that temperature would be related to irradiance (as estimated by f) and that the addition of f' would improve the model fit. The long-term changes in temperature residuals were investigated using linear regression vs. the year. The procedure was repeated for each month.

Zooplankton analysis

The analysis of zooplankton abundance data from lake Chascomús was intended to identify species (or groups) that displayed phenological trends, and subsequently assess if such trends could reasonably be associated with long-term changes in water temperature. The procedure involved four sequential steps or criteria:

1. First, we defined the group of "common taxa" (species or groups of species) as those taxa that exceeded 5% of the total abundance in, at least, five of 214 samples analyzed. Within the latter group,
2. We searched for taxa whose time series of population abundance displayed a substantial seasonal component. For this purpose, the log-transformed series of abundance values (i.e., $\log(x+1)$), was fitted using Seasonal Decomposition of Time Series by Loess (stats package of R) and only taxa showing > 20% of the variation explained by the seasonal component were retained for further analysis.
3.

4. Phenological metrics for each "growth cycle" were estimated for "common taxa" displaying a substantial seasonal component. The beginning of each growth cycle was set to the first of August, as most species showed minimal abundance values around that date. We fitted two phenological models to the abundance data to quantitatively describe the population abundance distribution for each focal organism (Miller-Rushing *et al.*, 2008). The central point of the growing season was estimated as the center of gravity; while the onset, middle, and end points of the growing season were estimated as the 10%, 50%, and 90% quantiles of the area under the adjusted Weibull-type curve, respectively (Rolinski *et al.*, 2007; Feuchtmayr *et al.*, 2012). Long-term trends in phenology were assessed by regressing the estimated phenological metrics vs. year (Feuchtmayr *et al.*, 2012).
5. For those taxa displaying long-term trends in a given phenological metrics, we regressed such metrics vs. the mean value of environmental variables (temperature, Chl *a*, seston and phosphorus content of seston) (Feuchtmayr *et al.*, 2012) computed for the window of time on which the phenological shift occurred. For example, if a given phenological metric was observed to occur between "date a" and "date b" over the 10-year study; then, we used the average of all samples collected between "date a" and "date b" for each year, to estimate the mean value of the corresponding environmental variable.

For those taxa that satisfied the four previous criteria, we compared their patterns of population abundance vs. temperature observed in lake Chascomús, with those observed in the other six Pampean shallow-lakes. Due to large differences in absolute abundance, both between years and among lakes, abundance values were re-scaled by standardizing each observation for each lake and "growth cycle" as $z = (x - \mu) / \sigma$; where z is the standard score, x is the log-transformed abundance value (i.e., $\log(x + 1)$), and μ and σ are the mean and standard deviation within each lake and "growth cycle", respectively. The standardized abundance values were plotted against lake water temperature, and the upper boundary, conditional on temperature was estimated using quantile regression for the 95% quantile (i.e., s = 0.95) (Koenker, 2000).

Results

Environmental variables

Air and water temperature displayed strong seasonal patterns (Figure 2 a and b, and Table 1). Significant increasing trends in monthly air temperature were observed for May (0.2°C per year), August (0.16°C per year), October (0.12°C per year), and December (0.25°C per year); while a significant decreasing trend was observed only for July (-0.18°C per year) (Table 2). On the other hand, the regression model relating lake water temperature to the day of the year (DOY) explained 86% of total variance ($R^2= 0.86$, $p< 0.0001$, $n= 230$). Thus, the model effectively removed the seasonal component from the water temperature time-series (coincidently, also 86% of total variance, Table 1). Model residuals represent the observed temperature deviations from the expected seasonal values of lake water temperature. Overall, we found a weak, but significant positive trend in the annual model's residuals vs. year (0.13°C per year; $R^2= 0.027$, $p= 0.0127$, $n= 230$). However, by performing stratified analyses by month, we found that significant trends occurred only during May, revealing an average warming of lake water temperature of about 0.47°C per year for that month ($R^2= 0.27$, $p= 0.0268$, $n= 18$). The seasonal behavior of other potentially relevant environmental variables was much less marked (Figure 2 c, d, and e). The seasonal contribution to total variance was 2%, 14% and 23% for Chl *a*, seston, and phosphorus content of seston, respectively (Table 1).

Zooplankton

Forty-eight taxa (5 copepods, 9 cladocerans, and 34 rotifers) were identified during the study period. Common taxa were cyclopoid *Acanthocyclops robustus*, calanoid *Notodiaptomus incompositus* the cladoceran *Bosmina* and 10 rotifer species (or genera) (Table 3). Some very common species, such as *Keratella tropica*, were frequent and highly abundant (representing >5% in 160 out of 214 samples), but did not show marked seasonality. On the other hand, several species occurred only sporadically. Overall, only nine "common taxa" (criterion 1) displayed a recurrent seasonal pattern (criterion 2): *N. incompositus* nauplii, *N. incompositus* adults + copepodids, and the rotifers: *Brachionus caudatus*, *B. havanaensis*, *B. plicatilis*, *K. americana, Polyarthra*, *Trichocerca* and a group made up of all rotifers excluding *B. havanaensis* and *K. americana* (see explanation below) (Figure 3 and Table 3). Most "common taxa" showed a clear and repetitive pattern displaying maxima during the warm season and minima (sometimes absence) in winter. The amplitude of abundance variation was, in some cases, up to 3 orders of magnitude (i.e. *B. havanaensis*, *B. caudatus*). *Brachionus plicatilis*, *K. americana* and *Trichocerca* were absent from the water column on some cycles. For each one of these nine taxa, the four phenological metrics (central point of the growing season, i.e., center of gravity; onset, middle and end points of Weibull distribution) were regressed against year (Table 4). Twenty three out of 36 phenology metrics (64%) showed increased trends over time (i.e., delays), while the remaining 13 (36%) displayed decreased trends (i.e., advancements). However, the statistical strength of most trends was weak. The strongest relationships (i.e., $p < 0.05$) were observed for three cases: *B. havanaensis* end of the growing season (i.e., Weibull percentile 90%, delayed from April 22 to May 26), *K. americana* onset of the growing season (i.e., Weibull percentile 10%, delayed from September 24 to November 25) and *K. americana* end of the growing season (i.e., Weibull percentile 90%, delayed from May 30 to August 20) (Table 4 and Figure 4 a-c). The remaining taxa, i.e., copepods, cladocerans and rotifers (excluding *B. havanaensis* and *K. americana*) did not show significant trends in phenological metrics. Each of the 3 phenological metrics that showed changes over time, were regressed vs. the mean value of environmental variables (temperature, Chl *a*, seston and phosphorus content of seston). We found a significant relationship between the end of the growing season of *B. havanaensis* and the mean water temperature (R^2= 0.507, p= 0.0314, n= 9), computed for the window of time on which the phenological shift occurred (Figure 4d).

The normalized abundance of *B. havanaensis* recorded in lake Chascomús and in 6 additional Pampean shallow-lakes (El Triunfo, La Limpia, La Salada de Monasterio, Grande de Otamendi, La Barrancosa, and El Chifle) is plotted in Figure 5. The figure shows that *B. havanaensis* abundance was strongly constrained by temperature, resulting in an upper limit that increases with temperature. The upper edges of the distributions for both data sets were estimated using quantile regression. No statistical differences between the two data sets were observed (i.e., the 95% confidence intervals of the slopes overlapped: Chascomús 0.077 to 0.167°C^{-1}; other Pampean lakes 0.004 to 0.138°C^{-1}).

Discussion

Lake Chascomús showed a weak (albeit significant) increase in mean annual water temperature during the study period. This is consistent with previous studies that reported that, for most part of Argentina, warming has occurred at a much slower pace than in the NH (Nuñez *et al.*, 2008; Barros *et al.*, 2015). However, when water temperature trends were analyzed on a monthly basis, it became apparent that the warming of lake water had occurred solely during the month of May (mid-autumn), with an average increase of 0.47°C per year. This May increasing trend in lake Chascomús water temperature is coincident with the concurrent increase in air temperature observed in the nearby area (0.2°C per year, data from

Dolores Observatory). However, other observed trends in air temperature (increases in July, August and December and a decrease in June) did not translate into significant trends in lake Chascomús water temperature. Lake water temperature typically correlates with regional-scale air temperatures (Adrian *et al.*, 2009), but among-year differences in thermal inertia due to changes in water depth add a stochastic component to the heat balance between air and water (Winslow *et al.*, 2017). Elisio and co-workers (Elisio *et al.*, 2015) modeled lake Chascomús water temperature for the period 1966-2012 and concluded that the lake had warmed at a rate of 0.04°C per year, over the whole period of 47 years, with most significant warming occurring during the warmer seasons. For the period of our study (2005-2015), we have also estimated an annual warming trend of 0.13°C per year; but the stratified analysis showed that the only significant warming trend corresponded to the month of May. Summarizing, studies around Chascomús area are consistent in showing significant warming trends during the warmer season, but show subtle differences in warming magnitude and timing (i.e., the actual months on which the changes occurred).

Despite the lack of distinct seasonal landmarks in lake Chascomús (e.g., ice melting, water column stratification), several zooplankton species display striking recurrent seasonal patterns or phenologies (Diovisalvi et al., 2015b, 2015c and Figure 3). In this paper however, we focused exclusively on species that showed significant phenological changes in the long run. Two taxa displayed long-term phenology trends (i.e., they met criteria 1 to 3). In both cases, the trends involved delays of some phenological metrics: *B. havanaensis* showed a 31-day/decade delay on the end date of its seasonal distribution (i.e., the 90% quantile of the Weibull function) and *K. americana* showed 59-day/decade and 82-day/decade delays on the onset and end dates of its seasonal distribution (i.e., 10% and 90% quantiles of the Weibull function). However, *B. havanaensis* was the only species that displayed a shift in phenology, which could confidently be associated with a concurrent trend in lake water temperature (i.e., it also met criterion 4). This is consistent with previous reports of phenological trends in freshwater zooplankton. In general, only one or a handful zooplankton species has been reported to undergo phenological shifts within individual lakes (discussed below), even when the number of occurring species may be large (up to several tens). This may be partly due to statistical constrains (i.e., trend detection may be restricted to the most abundant and/or frequently occurring species), and also to inter-specific differences in tolerance to environmental change (i.e., some species may not respond, or respond weakly, to changes in temperature, as long as it remains within their physiological ranges).

Brachionus havanaensis is a warm stenotherm rotifer (Ruttner-Kolisko, 1974) that develops dense populations in shallow Pampean lakes. In lake Chascomús, it becomes the dominant species during late summer and autumn, but it is absent from the water column during the coldest months of the year (Diovisalvi *et al.*, 2015b). The delay in the end date of its seasonal distribution (i.e., the 90% quantile of the Weibull function) implies the widening of its temporal niche (Hampton, 2005) towards the end of the autumn. This shift has occurred during the month of May and was concurrent with the increase in the mean monthly water temperature. Additional evidence (discussed below) provides further support to our claims that the *B. havanaensis* shift in phenology was a direct response to the increase in water temperature during the declining phase of its seasonal cycle. Phenological responses could also result from indirect effects via altered bottom up or top-down forcing (Manca *et al.*, 2007; Visconti *et al.*, 2008; Stoks *et al.*, 2014; Vadadi-Fülöp & Hufnagel, 2014; Velthuis *et al.*, 2017). For example, the delays in the onset and end dates of *K. americana* population could not be associated with concurrent trends in temperature; nor could it be related to trends in the other environmental variables considered in this study. Given that shifts in *K. americana* phenological metrics occurred during periods of fish recruitment (Iglesias *et al.*, 2011), there is a potential for indirect trophic interactions, which were not addressed in the present study.

The continued persistence of *B. havanaensis* into late autumn was coincident with the increasing water temperature that was observed in May, but it was seemingly unrelated to changes in resource availability (as estimated by Chl *a* and seston concentrations). Moreover, two independent pieces of information provide further support to our interpretation that the shift in *B. havanaensis* phenology was a direct response to increases in May water temperature. The first piece of evidence comes from the study by Diovisalvi and co-workers (Diovisalvi *et al.*, 2015b), who performed life table experiments at two different temperatures (9°C and 20°C). They reported that the egg production of *B. havanaensis* was high at 20°C, but halted at 9°C. Moreover, these authors also showed that, under favorable temperature conditions (i.e., at 20°C), *B. havanaensis* was insensitive to changes in the quality of food naturally occurring in lake Chascomús. These findings contrasted with the results obtained for *K. tropica* and *B. caudatus* (assayed simultaneously), which reproduced at both temperatures and did better when fed on natural seston produced in winter (higher food quality) as compared to seston produced in summer (lower food quality). The second piece of evidence comes from the cross-system comparative analysis of *B. havanaensis* abundance vs. temperature distribution reported here. This analysis showed that *B. havanaensis* abundance (normalized by lake and growth cycle) was strongly constrained by temperature (Scharf *et al.*, 1998) and that the upper limit (conditional to temperature) to data distributions for lake Chascomús was virtually identical to that of other six Pampean shallow-lakes. Within a given region, the patterns of temperature change among lakes tend to be highly coherent (Kent *et al.*, 2007). In contrast, concentrations of nutrients and zooplankton populations vary much less coherently over time (see Feuchtmayr *et al.*, 2012). Thus, the observed similarity in *B. havanaensis* responses to temperature among lakes provide strong support to our interpretation that the shift in B. *havanaensis* phenology was a direct consequence of the warming trend in lake water temperature in May, which we presume, resulted in the lengthening of the amictic (asexual) phase of its reproductive cycle (i.e., delayed production of resting eggs). Warmer autumn temperatures have been reported to delay insect diapause (Gallinat *et al.*, 2015), but experimental work suggesting that temperature may control the induction of mixis in rotifers is lacking (John Gilbert, pers. comm.).

A number of zooplankton phenology studies in NH lakes have reported earlier spring events in response to climate warming (Vadadi-Fülöp *et al.*, 2012 and references therein). Most studies reported shifts in cladoceran populations (particularly, *Daphnia*), while only a handful studies reported changes in rotifer phenologies. For example, the strong advancement of the spring peak in the rotifer *Keratella* (Winder & Schindler, 2004) and the greater window of time for the growth of *Conochilus* populations (Hampton, 2005) have been linked to the long-term warming trend of Lake Washington. Similarly, *Keratella* populations have been reported to develop earlier in Lake Muggelsee (Gerten & Adrian, 2000), although subsequent studies have not reported any further shits in rotifer phenologies (Adrian *et al.*, 2006; Scharfenberger *et al.*, 2013). Finally, Molinero *et al.* (2006) analyzed a 29-year (1969–98) time-series of physical and biological data for Lake Geneva. These authors did not report changes in rotifer phenology, but they did observe an abrupt shift (around 1987) in the size structure of the rotifer community, which was linked to water temperature increases. Similarly, mesocosm studies (Zhang *et al.*, 2015; Velthuis *et al.*, 2017) have demonstrated advancements in rotifer phenological metrics due to experimental increases in temperature. It must be noted however, that the pervasiveness of spring phenology trends observed in the NH have strongly influenced the design of experimental studies, reducing the chances of capturing delayed phenology trends, as those experiments were not intended to mimic the persistence of warmer temperature in autumn.

Reported phenological rates of change in NH environments are lower for rotifers (*Keratella*; advancement shift: 6 to 7 days per decade) than for cladocerans (*Daphnia*; advancement shift: 1 week to 5 months per decade). Rotifer populations may be negatively impacted through exploitative or interference competition with large-sized cladocerans (Gilbert, 1988), potentially obscuring phenological responses to in-lake long-term temperature trends (Hampton, 2005). In lake Chascomús, large cladocerans are virtually absent, and although rotifers develop the highest densities during the warmer months, they are present (as a group) during the whole annual cycle. This fact, together with a continuous monitoring program (Gallinat *et al.*, 2015; Zipf *et al.*, 2017), allowed us to capture the change in the declining phase of the annual *B. havanaensis* population, which according to our estimates experienced a delay of roughly a month per decade.

To the best of our knowledge, this is the first study documenting phenological shifts in SH freshwater zooplankton. The observed delayed trend in the ending phase of *B. havanaensis* population in autumn differ from most published phenological changes in NH zooplankton (i.e., most frequently earlier spring trends), but is consistent with air temperature trends reported for central Argentina (Nuñez *et al.*, 2008; Barros *et al.*, 2015). There are fewer long-term ecological datasets for the SH than for the NH. In particular, there is a scarcity of data for Africa, Pacific nations and South America (Chambers *et al.*, 2013). Moreover, the length of the data sets available for SH locations (ten years in our case) is often shorter than that of similar datasets for NH. Chambers and co-workers (2013) investigated the potential biases introduced when analyzing shorter series of data. They concluded that there was no impact of the data series length on the likelihood of detecting earlier or later trends over time. However, the length of the data series influenced the magnitude of the observed trend. In general, the magnitude of the phenological trend was greater for shorter data series (Chambers *et al.*, 2013, appendix S4). In order to address this potential shortcoming, we have examined additional sources of evidence that provide further support to the *B. havanaensis* phenological shift inferred from the 10-year time-series of lake Chascomús. On the one hand, the long-term trends in air and lake water temperature reported here (2005-2015) are supported by the evidence of lake Chascomús warming over a longer period of time (1966-2012) (Elisio *et al.*, 2015). Moreover, the temperature dependence of *B. havanaensis* population density was also supported by the comparative analysis of its populations density distribution in other shallow Pampean lakes. This cross-system comparison allowed us to analyze the temperature dependence of *B. havanaensis* populations in twelve additional annual cycles (i.e., 6 lakes x 2 years) reinforcing our confidence in the conclusions drawn from the 10-year time series of lake Chascomús.

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Figure legend

Fig. 1. Geographic location of studied shallow lakes (Buenos Aires province, Argentina). Inset: the position of Buenos Aires Province within South America.

Fig. 2. Time series of main environmental variables: Air temperature from Dolores city (a), and water temperature (b), Chl *a* (c), seston (d) and phosphorus content of seston (e) from lake Chascomús.

Fig. 3. Time series for the set of zooplankton taxa that displayed recurrent seasonal patterns in lake Chascomús. Note that # refers to the abundance of all rotifers excluding *B. havanaensis* and *K. americana* (see text for explanation).

Fig. 4. Temporal change of phenological metrics for *B. havanaensis* (a- end date of Weibull function), and *K. americana* (b- onset, and c- end date of Weibull function). Relationship between the end date of Weibull function of *B. havanaensis* and water temperature (d) computed for the window of time on which the phenological shift occurred (see text for explanation).

Fig. 5. Relationship between *B. havanaensis* standardized abundance and water temperature. The lines correspond to the 95th quantile regression for 6 shallow lakes (black) and for lake Chascomús (grey).

Table 1. Relative contribution to the total variance of decomposition based on Loess (STL) of main physical and chemical variables.

	Seasonal	**Trend**	**Residual**
Tair	0.74	0.00	0.26
Tw	0.86	0.01	0.12
Chl *a*	0.02	0.49	0.33
Seston	0.14	0.28	0.49
Phosphorus content of seston	0.23	0.19	0.53

Table 2. Daily air temperature (°C) mean, minimum and maximum values for each month and linear trends for mean temperature by month. Statistical parameters: R Square, slope parameter and *p* value of F statistics, * $p < 0.05$. Samples (n).

	n	**Mean**	**Min**	**Max**	**R Square**	**Slope**	***P*-value**
Jan	341	22.0	14.1	30.5	0.004	0.057	0.253
Feb	282	21.3	14.3	27.7	0.007	-0.076	0.177
Mar	310	18.6	9.8	24.8	0.010	-0.107	0.073
Apr	300	15.0	5.4	23.9	0.004	0.070	0.298
May	310	11.6	3.2	21.8	0.029	0.203	0.003*
Jun	300	8.6	0.5	20.5	0.007	-0.095	0.135
Jul	310	8.1	0.9	19.2	0.020	-0.184	0.013*
Aug	310	9.6	1.5	22.8	0.017	0.162	0.022*
Sep	300	11.9	3.7	23.3	0.006	0.086	0.188
Oct	310	15.0	8.5	24.3	0.013	0.122	0.041*
Nov	300	18.1	9.3	26.1	0.002	0.053	0.426
Dec	310	20.6	10.5	28.8	0.047	0.248	0.000*

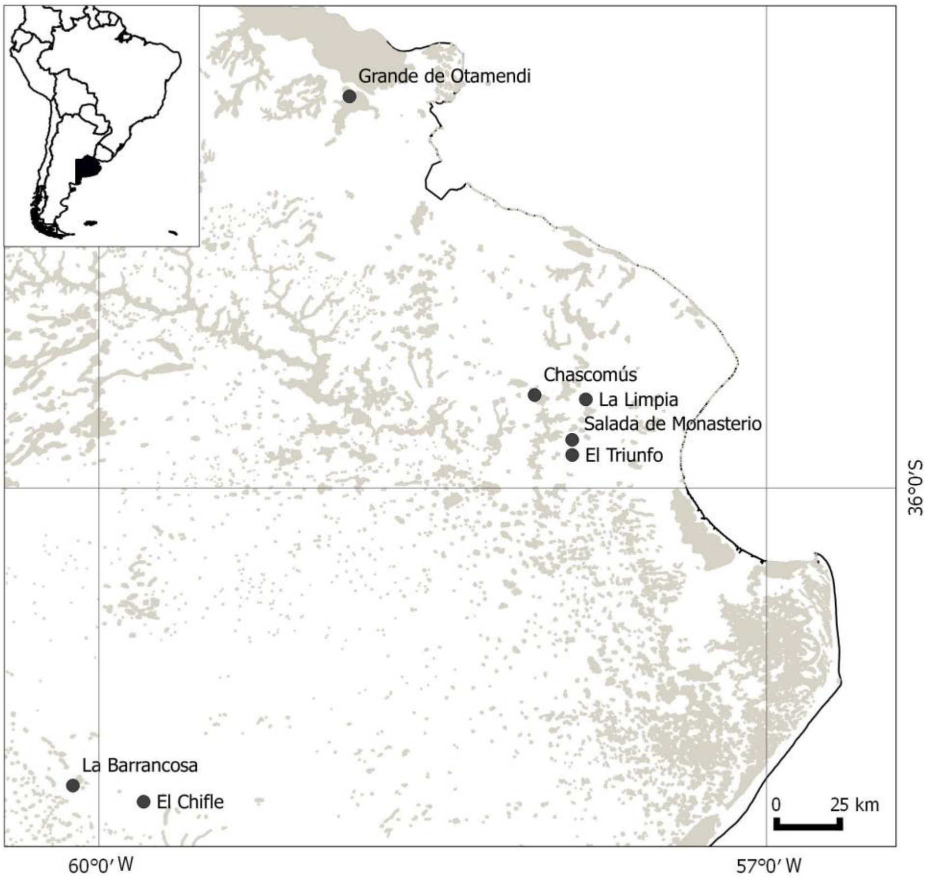
Table 3. Common taxa of zooplankton. Species or group of species exceeded 5% of the total abundance in, at least in 5 of 214 samples analyzed. Relative contribution to the total variance of decomposition based on Loess (STL). # Rotifers total abundance with the exception of *B. havanaensis* and *K. americana.*

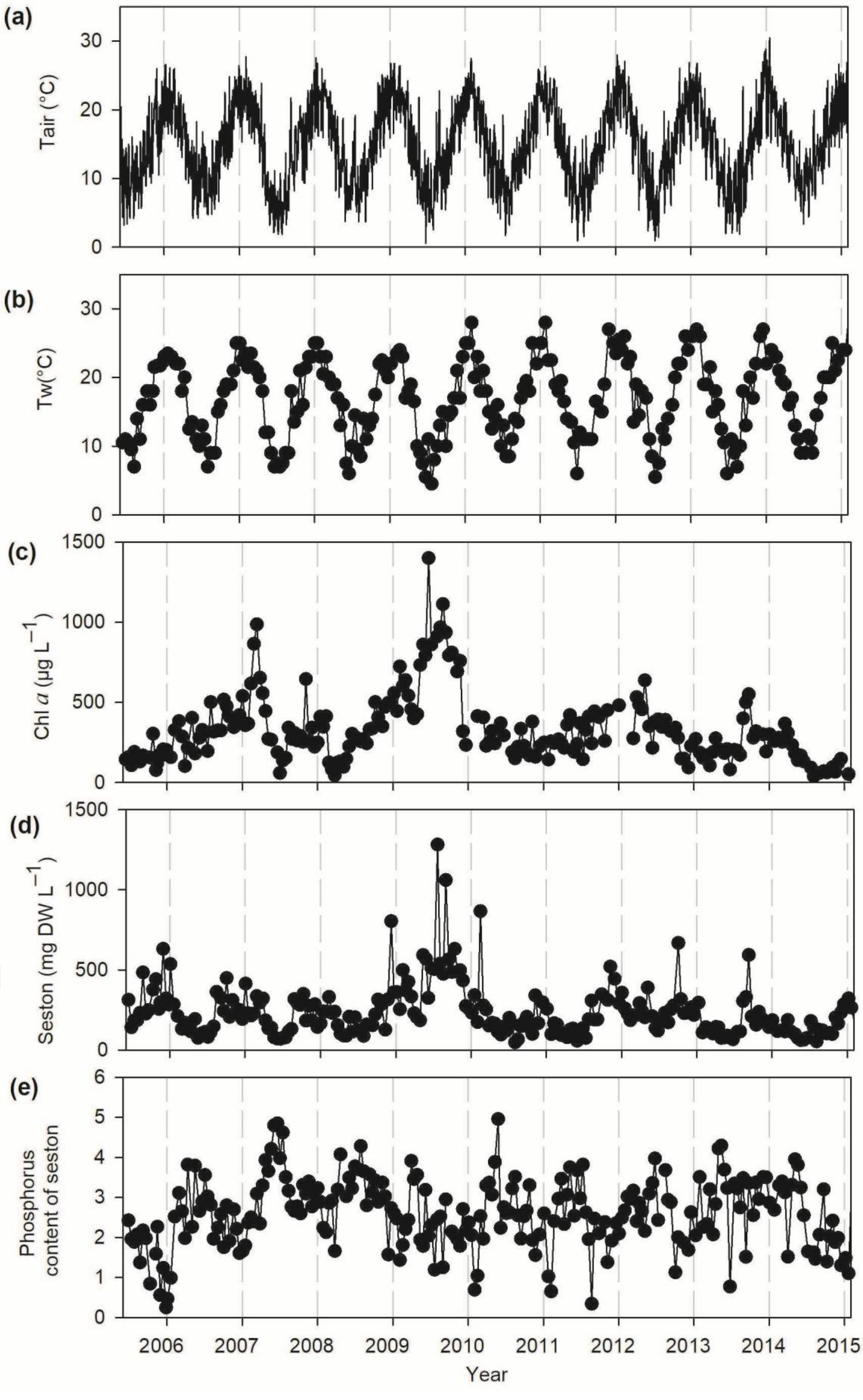
Zooplankton groups	**Samples with >5% abundance**	**Relative variance STL**		
		Seasonal	**Trend**	**Residual**
Cyclopoid nauplii	191	0.14	0.19	0.58
Cyclopoid copepods	122	0.13	0.20	0.63
Calanoid nauplii	65	0.34	0.09	0.52
Calanoid copepods	15	0.21	0.21	0.47
Cladocerans	71	0.10	0.40	0.41
Bosmina	57	0.10	0.54	0.25
Rotifers$^{\#}$	209	0.41	0.25	0.34
B. havanaensis	74	0.81	0.05	0.11
B. caudatus	138	0.62	0.12	0.26
B. plicatilis	10	0.26	0.28	0.37
B. calyciflorus	5	0.06	0.18	0.74
K americana	60	0.28	0.40	0.28
K. tropica	160	0.05	0.21	0.59
Filinia	15	0.11	0.22	0.56
Polyarthra	8	0.30	0.17	0.44
Pompholyx	9	0.12	0.38	0.48
Trichocerca	7	0.42	0.11	0.41

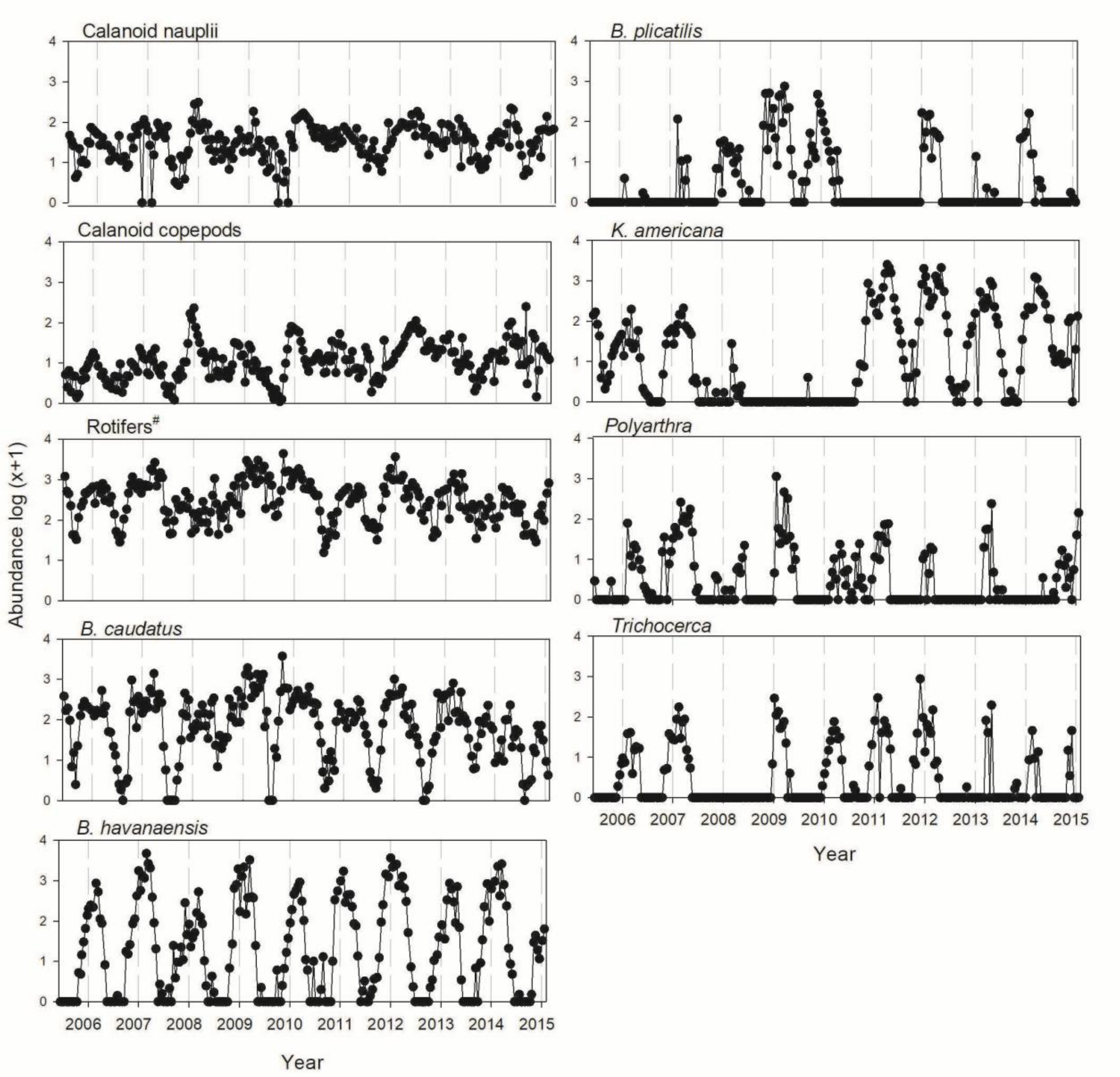
Metrics sp/ group	**Calanoid nauplii**	**Calanoid copepods**	**Rotifers$^{\#}$**	***B. havanaensis***	***B. caudatus***	***B. plicatilis***	***K. americana***	***Polyarthra***	***Trichocerca***
Weibull									
N		7	5	9	9	6	6	9	8
Onset									
Mean	20-Sep	21-Oct	16-sep	02-Oct	17-Oct	29-Nov	29-Oct	07-Dec	07-Dec
RSquare	0.49	0.14	0.14	0.10	0.03	0.04	0.74	0.29	0.28
Decadal Rate of Change (days)	-67	82	33	-38	22	-36	59	131	88
P-value	0.08	0.404	0.528	0.415	0.636	0.720	0.014*	0.137	0.173
Middle									
Mean	25-Jan	27-Jan	19-ene	12-Feb	19-Jan	09-Jan	28-Feb	03-Mar	08-Feb
RSquare	0.51	0.19	0.09	0.00	0.11	0.22	0.22	0.21	0.04
Decadal Rate of Change (days)	143	66	-83	-5	84	-51	-62	97	-32
P-value	0.07	0.322	0.634	0.932	0.377	0.349	0.292	0.219	0.641
End									
Mean	16-Jun	07-May	16-jul	10-May	21-Jul	21-May	19-Jul	09-May	08-May
RSquare	0.43	0.29	0.06	0.51	0.19	0.07	0.85	0.01	0.41

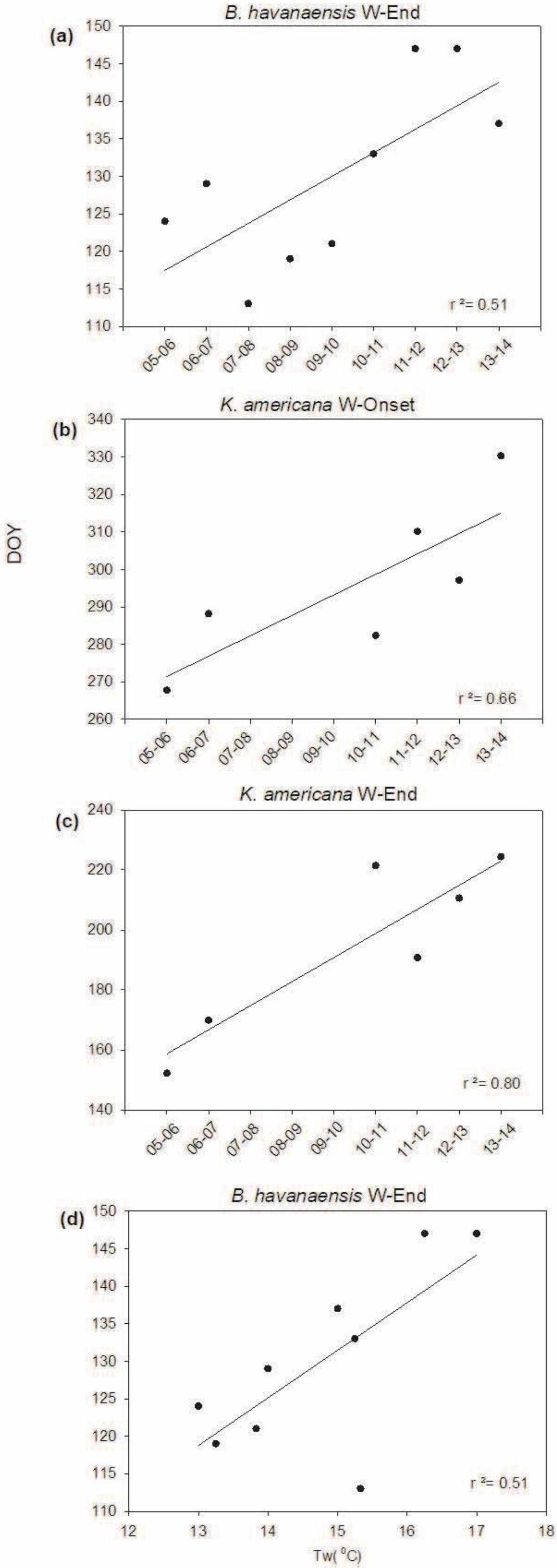
Decadal Rate of Change (days)	89	86	-6	31	39	-29	82	-20	-18
P-value	0.11	0.212	0.685	0.032*	0.240	0.622	0.003*	0.836	0.085
D									
N	9	9	9	9	9	9	9	9	9
Mean	02-Feb	03-Feb	4-feb	05-Feb	06-Feb	27-Feb	10-Feb	20-Mar	19-Feb
RSquare	0.07	0.21	0.32	0.22	0.09	0.19	0.10	0.05	0.01
Decadal Rate of Change (days)	10	32	15	20	14	-43	68	34	7
P-value	0.508	0.212	0.111	0.199	0.422	0.278	0.453	0.566	0.846

Table 4. Phenological metrics of zooplankton groups with seasonal periodicity. Weibull distribution (onset, middle and end points) and Center of gravity (D): Mean and parameters of lineal tendency through the years (R Square, decadal rate of change and p value of F statistics, * $p < 0.05$). # Rotifers total abundance with the exception of *B. havanaensis* and *K. americana*. Number of growth cycle analyzed (N).


Grande de Otamendi
Chascomús
La Limpia
Salada de Monasterio
El Triunfo
La Barrancosa
El Chifle
0
25 km
36°0′S
60°0′ W
57°0′ W


(a)
Tair (°C)
0
10
20
30
(b)
Tw(°C)
0
10
20
30
(c)
Chl *a* (µg L^{-1})
0
500
1000
1500
(d)
Seston (mg DW L^{-1})
0
500
1000
1500
(e)
Phosphorus content of seston
0
1
2
3
4
5
6
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year


Calanoid nauplii
Calanoid copepods
Rotifers#
B. caudatus
B. havanaensis
B. plicatilis
K. americana
Polyarthra
Trichocerca
Abundance log (x+1)
0
1
2
3
4
2006
2007
2008
2009
2010
2011
2012
2013
2014
2015
Year


B. havanaensis W-End
(a)
r ²= 0.51
K. americana W-Onset
(b)
r ²= 0.66
DOY
K. americana W-End
(c)
r ²= 0.80
B. havanaensis W-End
(d)
r ²= 0.51
Tw(°C)
05-06
06-07
07-08
08-09
09-10
10-11
11-12
12-13
13-14
110
115
120
125
130
135
140
145
150
260
270
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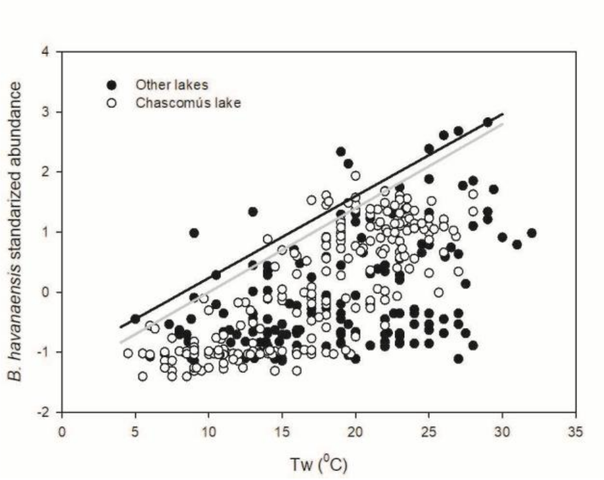
Other lakes
Chascomús lake
B. havanaensis standarized abundance
Tw (⁰C)
4
3
2
1
0
-1
-2
0
5
10
15
20
25
30
35