Climate-driven changes on phytoplankton–zooplankton coupling and nutrient availability in high mountain lakes of Southern Europe

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SUMMARY
1. The effect of climate variability on phytoplankton and zooplankton dynamics and nutrient availability was studied in two high mountain fishless lakes (La Caldera and Río Seco) of contrasting morphology, hydrology and dissolved inorganic nitrogen : soluble reactive phosphate (DIN : SRP) ratios during 1986 and after a 10-year-long drought in 1996 and 1997.
2. Thaw was delayed and water temperatures were lower in both lakes in 1996 than in 1986 and 1997. However, the lake-specific DIN : SRP ratio was maintained in the 3 years studied, reflecting its local control.
3. On other hand, the presumptive limiting nutrient in each lake, P in La Caldera and N in Río Seco, showed higher concentrations in 1996 versus 1986 and 1997. Significant positive correlations between temperature and chlorophyll $a$ were found in both lakes in 1996 but these relationships were negative or not significant in 1986 and 1997. Zooplankton biomass showed lower values in 1996 than in 1986 or 1997.
4. These findings can be explained by a decoupling of the phytoplankton–zooplankton interaction because of a constraint on zooplankton growth by low temperatures in the coldest year studied. This observation furnishes evidence that regional climatic control on the phytoplankton–zooplankton link can modulate the overall demand for nutrients.

Keywords: climate-driven changes, mountain lakes, nutrient availability, phytoplankton–zooplankton coupling, resource limitation

Introduction
A key question in ecology is how shifts in climate affect aquatic ecosystems. While some ecologists have emphasised the importance of lake-specific factors (Webster et al., 1996), others have shown that ecosystems are subject to regional climatic effects (Baines et al., 2000). Interactions between local and regional factors and between abiotic and biotic factors create a complex lake response, making it difficult to draw general conclusions about the impact of climate change on food web structure and functioning.

Key climate factors in high mountain lakes include temperature, which influences lake chemistry via abiotic and biotic activity (Sommaruga-Wögrath et al., 1997) and rainfall and length of ice-free period, which affect trophic dynamics (Park et al., 2004). Beside the effects of regional climate, mountain lakes may be particularly susceptible to local controls. Thus, Baron & Caine (2000) found that individual lake characteristics (i.e. position in the landscape) overwhelmed the regional response to climate in two geologically and morphologically similar nearby mountain basins, especially for non-conservative solutes such as nitrate.
Among abiotic factors, variations in nitrogen and phosphorous concentrations are of special interest because both can be limiting factors for plankton growth. The concentrations of these non-conservative solutes in lakes are partially controlled by food web dynamics. Nitrate and phosphate are taken up by autotrophic plankton and recycled by the heterotrophic components of food web. Transference of either nutrient from inorganic to zooplankton organic form depends on the coupling efficiency of nutrient–phytoplankton–zooplankton interactions. Functionally, temperature affects these couplings by controlling the growth rates of phytoplankton (Reynolds, 1984) and the filtering, assimilation, excretion and growth rates of zooplankton, as well as the development time of their eggs (Downing & Rigler, 1984). Despite recognition of the importance of temperature in both aquatic and terrestrial ecosystems, very few studies have examined how temperature changes can influence the dynamics of interacting populations. Evidence to date indicates that temperature can affect phytodin plankton–zooplankton coupling. For instance, long-term studies of Lake Windermere (George & Harris, 1985; George & Taylor, 1995) and Lake Washington (Winder & Schindler, 2004a,b) reported uncoupled phytoplankton–zooplankton growth because of warm temperatures. Field observations are supported by mesocosm experiments that showed disruption of the coupling of zooplankton–phytoplankton growth at increased temperatures (Beisner, McCauley & Wrona, 1997).

Sierra Nevada is the southernmost mountain range in Europe with elevations above 3000 m a.s.l. In this study, the nitrogen and phosphorus availability and phytoplankton and zooplankton dynamics were examined in two neighbouring lakes of Sierra Nevada (Río Seco and La Caldera) in 1986 and, after a decade of severe drought, in 1996 and 1997. Although these lakes differ in their morphometry, hydrology and availability of nitrogen and phosphorus (Morales-Baquero et al., 1999), they both support very simple communities, with zooplankton as the highest trophic level. The aim of this study was to analyse the effects of local lake-specific features and regional climate, mainly rainfall and temperature, on N and P availability and phytoplankton–zooplankton dynamics in two nearby high mountain lakes.

Study sites
La Caldera (around 2 ha surface area and 23.5 ha catchment area) and Río Seco (around 0.4 ha surface area and 9.9 ha catchment area) are remote high mountain lakes above the tree-line. La Caldera is an oligotrophic system and Río Seco is oligomesotrophic system. These ecosystems are located in the Sierra Nevada mountain range (S Spain, 36°55′–37°15′N, 2°31′–3°40′W) on siliceous bedrock, mainly mica-schist with graphite and mica-schist with feldspar, at an altitude of around 3050 m a.s.l in two neighbouring basins. The soils are very poorly developed and do not support agriculture or forestry use. These lakes are ice-covered from around October–November until June–July.

La Caldera lake (mean depth of 4.3 m) has no visible inlets or outlets. Hydrological loading and evaporation are responsible for the water level fluctuation between years, with a decline in the maximum depth from 12 to 1.4 m between 1987 and 1995 due to a severe decade-long drought, followed by a return to the maximum depth of 14 m in 1996 (Villar-Argaiz, Medina-Sánchez & Carrillo, 2002). The maximum depth of Río Seco lake (almost 3 m) is determined by a surface outflow. As the summer period progresses, the outflow ceases and the water level lowers, although never below 2 m.

The plankton community of both lakes is extremely simple (Carrillo et al., 1995; Medina-Sánchez et al., 1999; Barea-Arco, Pérez-Martínez & Morales-Baquero, 2001; Villar-Argaiz et al., 2002). Phytoplankton is dominated by nanoplancktonic species (mainly Chrysophyceae, Bacillariophyceae and Chlorophyceae). The main zooplankton species are the copepod Mixodiaptomus laciniatus, the cladoceran Daphnia pulicaria and the rotifer Hexarthra bulgarica. Both lakes are fishless, show water mixing and present total Secchi disc visibility during the ice-free period. La Caldera lacks littoral vegetation while Río Seco’s shoreline is dominated by bryophytes.

Methods
Río Seco and La Caldera were sampled according to a similar schedule in 1986, 1996 and 1997. Both lakes were sampled on the same day at 3 to 4-day intervals during the ice-free period of 1986. They were also sampled at weekly intervals during the ice-free
periods of 1996 and 1997. Samples were taken at the
deepest point of the lake using an 8 L Van Dorn
 sampler at evenly distributed depths, except for Río
Seco in 1996 and 1997, when an electric pump was
used. Water for temperature measurements and chem-
ical analysis was obtained from an integrated water
column sample at the deepest point of the lake. Water
temperature was measured using a YSI probe (Yellow
Springs Instruments, Yellow Springs, OH, U.S.A.).
Water for chemical analyses was filtered through
0.45-µm Sartorius disposable filter before determin-
ation of dissolved substances. Soluble reactive phos-
phate (SRP) was analysed by the ascorbic acid method
of Murphy & Riley (1962). NH$_4^+$-N was determined
using the blue indophenol method (Rodier, 1981) and
NO$_2^-$-N following the method of Shinn (1941). NO$_3^-$-N
was determined by reduction to nitrite with cadmium
amalgam in 1986 and by ionic chromatography
(DW 300, Dionex Corporation, Sunnyvale, CA,
(DIN) is the sum of the three nitrogen compounds.
DIN : SRP ratios are given by mass.

Zooplankton samples were taken at the deepest
point of the lake using a 8 L Van Dorn sampler at
evenly distributed depths in La Caldera and, in 1986,
in Río Seco. In 1996 and 1997, Río Seco was divided
into five evenly distributed sectors, and one integra-
ted sample representative of the water column was
taken from each sector with an electric pump;
zoooplankton abundance for each sample date was
considered the average of the five samples. Zooplank-
ton were concentrated by filtering through a nytal
mesh (40 µm), preserved in 4% formaldehyde (final
concentration) and counted with an inverted micros-
cope (1000×). The biomass of crustaceans and rotifers
were determined after filtration of 2–5 L of 40-µm
screened lake water and a 24-h cold pigment extrac-
tion (Whatman GF/C) in methanol (Jeffrey &
Humphrey, 1975). Chlorophyll $a$ was not measured
in Río Seco during 1996 and 1997, therefore carbon
estimates from phytoplankton cell counts were trans-
fomed into chlorophyll $a$ values by dividing by 50
(Reynolds, 1984) to enable interannual comparisons.
Río Seco phytoplankton was sampled using the same
methods as for zooplankton (see above). Phytoplank-
ton samples (100 mL) were preserved in Lugol’s
iodine and counted using an inverted microscope
(1000×). Phytoplankton cell volume was calculated by
approximating each cell to its nearest geometrical
shape using image analysis (Quantimet 500, Leica,
Wetzlar, Germany); the cell volume was converted
to carbon units according to cell volume–weight rela-
tionships for phytoplankton (Rocha & Duncan, 1985).

Rainfall and air temperature were obtained from
the nearest meteorological station to the lakes (Alber-
gue Universitario 2550 m a.s.l.; 742 mm, mean preci-
pitation from 1960–61 to 1997–98). Because June, July,
August and September are the driest months in Sierra
Nevada, the volume of the lakes at the beginning of
the ice-free period represents most of the water
received during the hydrological year (from October
to September).

Results

The dynamics of winter air temperatures in Sierra
Nevada in 1995–96 were different from those in 1985–
86 and 1996–97 (Fig. 1). The winter of 1985–86 was
cold but precipitations were extremely low. High
accumulation of snow and ice in Sierra Nevada in the
spring of 1996 was favoured by a combination of low
preceding winter–spring air temperatures and an
even distribution of rainfall among winter and spring
months. The 1996–97 winter also registered high
precipitations but mainly in December, with very
low precipitations and high temperatures during the
spring of 1997. Thus, ice cover of Sierra Nevada lakes
persisted for a month longer in 1996 than in 1986 or
1997. As a consequence, water temperatures remained
low for a longer period of time in both lakes during
1996 (Fig. 2). On the other hand, the 1987–95 period
was extremely dry in Sierra Nevada. This had an
extreme effect on the water level of La Caldera, which
reached its minimum depth during this period (less
than 2 m in 1995). The water level in Río Seco
remained within a narrow range (less than 1 m of its
maximum depth) during the same period.

The DIN : SRP ratio was very different in both lakes
(Table 1): in La Caldera, this ratio was almost always
above 100, whereas in Río Seco it was usually well
below 100 and on many days below 10. These ratios
suggest a shortage of phosphorus in La Caldera and of nitrogen in Río Seco. Nevertheless, the annual average values of DIN and SRP in each lake showed interannual differences for both lakes (Fig. 3). U-test (Wilcoxon–Mann–Whitney) comparisons among annual mean values demonstrated significantly higher DIN in 1996 versus 1986 and 1997 in Río Seco lake and significantly higher SRP in 1996 versus 1986 and 1997 in La Caldera lake. Therefore, the potentially limiting nutrient in each lake according to the DIN : SRP ratio, either N or P, was more available during the coldest studied year.

In 1986 and 1997, phytoplankton biomass was low when temperatures were high in both lakes, but an inverse trend was observed in 1996 (Fig. 2). There were significant positive correlations between temperature and chlorophyll a in Río Seco (r = 0.65; P < 0.05) and in La Caldera (r = 0.66; P < 0.05) in 1996, but these relationships were negative or not significant in 1986 and 1997.

A Wilcoxon matched pairs test to compare biomass taking account of seasonal development showed that zooplankton biomasses were lowest in 1996 (Table 2). Although zooplankton biomass was about an order of magnitude higher in Río Seco than in La Caldera during the study period (Fig. 4), the interannual dynamics of both lakes showed similar patterns. Thus, zooplankton biomasses started to grow early in 1997 and 1986, reaching high biomasses in August in both lakes (Fig. 4). However, during the cold year of 1996, the growth was delayed and maximum values were not reached until mid- to late-September.

**Discussion**

Local lake-specific controls and meteorological forces affected the studied lakes differently throughout the survey period. With respect to the chemical signature, each lake maintained a distinct DIN : SRP ratio regardless of interannual climatic variability. On the other hand, despite major differences in lake morphology and hydrological regime between Río Seco and La Caldera, the general temperature, chlorophyll a and zooplankton biomass dynamics showed clear similarities between these lakes in the coldest year (1996).

**Nutrient resource control**

The DIN : SRP ratio remained relatively constant in each lake during the 3 years studied. The higher DIN : SRP ratio in La Caldera than in Río Seco can be partially explained by the differences in their catchment areas. La Caldera has a larger catchment area than Río Seco, and unfertilised lands usually export more nitrogen than phosphorus (Downing & MacCauley, 1992). In a previous study of Sierra Nevada lakes, Morales-Baquero et al. (1999) showed that the size of the catchment basins could explain between 24% and 38% of the variability in DIN : SRP ratios. Nevertheless, the high proportion of unexplained variability and the large differences in DIN : SRP ratios between La Caldera and Río Seco mean that other lake-specific factors must contribute to the N and P availability in these lakes. For instance, the influence of catchment characteristics, as vegetation cover class or catchment slope, on nutrient limitation of upland lakes have been shown (Maberly et al., 2003). Interestingly, the long drought had no
effect on the DIN : SRP ratios, despite the fact that La Caldera almost dried as a consequence of the drought and was refilled within a few months in 1996.

At any rate, differences in DIN : SRP ratios between the studied lakes suggest distinct nutrient limitation patterns. Thus, high DIN : SRP ratios indicated that P is most likely a limiting factor in La Caldera. Bioassays performed in this lake confirm the strong response of phytoplankton to phosphorus enrichment (Villar-Argaiz et al., 2002). In addition, episodic atmospheric inputs of Saharan dust, which can be rich in phosphorus, are reflected by sudden increases in chlorophyll a (Carrillo, Cruz-Pizarro & Morales-Baquero, 1990; Morales-Baquero, Pulido-Villena & Reche, 2006). Conversely, Río Seco showed symptoms of nitrogen shortage. In fact, the concentration of DIN became as low as 2.4 µg L⁻¹, whereas SRP reached measurable values (>1 µg L⁻¹). DIN concentrations below 6 µg L⁻¹ indicate N limitation (Morris & Lewis, 1988).

It is worth noting that the probable limiting nutrient in each lake was found at its highest concentration in 1996. The mitigation of the nutrient limitation conditions in that year may be the result of increased nutrient inputs from the rainfall and/or greater postdrought weathering in the catchments. In fact, high nitrate and orthophosphate enrichment has been reported after the postdrought refilling of a reservoir (Stent, 1981). Nevertheless, weathering of the catchments in Sierra Nevada would have increased N and/or P in similar ways in both lakes, because the soil characteristics of the two neighbouring watersheds are similar, instead of exclusively increasing the limiting nutrient in each lake (Fig. 3). In addition, the non-limiting nutrient in each lake in 1995, N in La Caldera and P in Río Seco, did not

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**Table 1** Mean DIN : SRP ratios (by mass) and standard errors in the lakes during the ice-free periods of the 3 years studied

<table>
<thead>
<tr>
<th>Years</th>
<th>La Caldera (µg L⁻¹)</th>
<th>Río Seco (µg L⁻¹)</th>
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<tbody>
<tr>
<td>1986</td>
<td>732.0 ± 106.5</td>
<td>20.7 ± 5.9</td>
</tr>
<tr>
<td>1996</td>
<td>165.6 ± 28.4</td>
<td>33.7 ± 6.2</td>
</tr>
<tr>
<td>1996</td>
<td>451.5 ± 63.0</td>
<td>11.4 ± 2.1</td>
</tr>
</tbody>
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DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphate.
increase with respect to wet year of 1986 (Fig. 3). Furthermore, rainfall amounts and solute dynamics are more easily established for conservative elements such as calcium and chloride than for non-conservative solutes, which can be controlled by lake food webs and show less concordance with rainfall (Baines et al., 2000; Baron & Caine, 2000; Webster, Soranno & Baines, 2000). For these reasons, food web controls may provide a more plausible explanation for the availability of N and P in the lakes studied. However, the question arises as to how food web dynamics could account for the significant increase in the potentially limiting nutrient during the wet and cold year of 1996.

![Graphs showing dissolved inorganic nitrogen (DIN) and soluble reactive phosphate (SRP) in La Caldera and Río Seco lakes.](image)

**Fig. 3** Annual averages and standard errors of dissolved inorganic nitrogen (DIN) and soluble reactive phosphate (SRP) in La Caldera and Río Seco lakes. Probability values (Wilcoxon–Mann–Whitney U-test) refer to differences in mean values between marked years.

**Table 2** Interannual paired comparisons of zooplankton biomass in La Caldera and Río Seco lakes

<table>
<thead>
<tr>
<th>Years compared</th>
<th>La Caldera</th>
<th>Río Seco</th>
</tr>
</thead>
<tbody>
<tr>
<td>1986–96</td>
<td>2.412 (P &lt; 0.05)</td>
<td>2.432 (P &lt; 0.05)</td>
</tr>
<tr>
<td>1996–97</td>
<td>2.667 (P &lt; 0.01)</td>
<td>2.665 (P &lt; 0.01)</td>
</tr>
<tr>
<td>1986–97</td>
<td>−0.289 (NS)</td>
<td>0.651 (NS)</td>
</tr>
</tbody>
</table>

Z values, Wilcoxon matched pairs test.

Climate effect on phytoplankton–zooplankton dynamics

Temperature is a major determining factor in plankton development and in the control of zooplankton hatching, growth and filtering rates. Because zooplankton has longer generation times, their growth would be more delayed by low temperatures compared to that of phytoplankton. This seems to have been the case in 1996, when a delay in zooplankton growth was observed in La Caldera and Río Seco. These observations are consistent with the long-term dynamics of *Daphnia* described by George et al. (1990), and with the overriding control of zooplankton growth by temperature (Straile, 2000). These results also agree with the significant positive relationships found between temperature and abundance of the dominant rotifer in Río Seco and La Caldera (Morales-Baquero, Carrillo & Cruz-Pizarro, 1995). Therefore, low biomasses of zooplankton can be expected when temperatures are low, regardless of the food supply. This was in fact the case for La Caldera and Río Seco in 1996 (Table 2). Moreover, the zooplankton biomass in La Caldera was especially low in 1996 because zooplankton had to
recolonise the ‘empty’ water volume after the massive postdrought water input.

Thus, top-down control on phytoplankton was weaker in 1996 than in 1986 and 1997. We suggest that phytoplankton in 1996 would be mainly controlled by abiotic factors such as temperature or by bottom-up resource control. Two pieces of evidence point to temperature as the main control factor: first, there was a positive correlation between chlorophyll $a$ and temperature in La Caldera and Rio Seco in 1996; second, the more limiting nutrient in each lake, P in La Caldera and N in Rio Seco, showed a higher concentration in 1996 than in 1986 or 1997.

Fig. 5 schematises our vision of plankton-nutrient dynamics. It is likely that phytoplankton growth was higher in 1986 and 1997 than in 1996 because of higher temperatures, but an effective top-down control by zooplankton prevented phytoplankton biomass accumulation. Conversely, a weak top-down control in 1996 may have allowed the accumulation of a high phytoplankton biomass despite a lowering of phytoplankton growth rates by the cold temperatures. On the other hand, the reduced growth rates of phytoplankton in 1996 would have weakened the demand for inorganic nutrients, which would therefore accumulate as solutes. In fact, this may explain why the most deficient nutrients for phytoplankton growth, P in La Caldera and N in Rio Seco, significantly increased in 1996 versus 1986 and 1997.

From a trophic web perspective, unusually low temperatures would have the effect of partially uncoupling the phytoplankton and the zooplankton, decreasing the global nutrient transfer up the trophic chain (Fig. 5). Uncoupling or segregation of the response window between phytoplankton and zooplankton has been previously reported, but as the result of an increase in temperature. Thus, occasional warm summers promoted early thermoclines that favoured the development of edible phytoplankton, hence dissolved nutrients were consumed before zooplankton had time to develop (George & Harris, 1985). Consequently, warm years show lower zooplankton biomasses that suffer strong food limitations, in what could be designated a ‘hot temperature decoupling’. Likewise, Winder & Schindler (2004a,b) documented a desynchrony between diatom bloom timing and Daphnia peak density in recent decades in Lake Washington as a result of the long-term water temperature increase.

Our results add to evidence of the critical role of temperature in decoupling food web interactions. However, in contrast to previous studies, this response was caused by abnormally low temperatures, in what could be designated a ‘cold temperature decoupling’. Most studies on variations in resource–consumer interaction have overlooked the effect of temperature changes. The present study suggests that the strength of the top-down control for a given system is temperature sensitive.

Water temperature and plankton dynamics in Sierra Nevada are not solely a by-product of air temperatures in the ice-free season but also of climate during the preceding winter, consistent with observations in central-European lakes (Straile, 2000; Livingstone & Dokulil, 2001) and North-American lakes (Park et al., 2004). In Sierra Nevada, early summer water temperature is especially dependent on total winter

Fig. 4 Time course of zooplankton biomass in La Caldera and Rio Seco during the 3 years studied.
precipitation, which is extremely variable in South-West of Spain (Ministerio de Medio Ambiente, 2000). Thus, low winter air temperatures alone do not necessarily produce low water temperatures in the following summer (see 1986, Figs 1 & 2). The more frequent droughts and higher temperatures at lower latitudes predicted by European climate models (Watson, Zinyowera & Moss, 1998; Watson, 2002) would increase the demand for nutrients, further enhancing nutrient limitation in the high mountain lakes of Sierra Nevada.

In conclusion, this study shows that local lake-specific factors outweighed the effects of meteorological forcing in determining lake-specific N : P ratios. This agrees with the observation by Baron & Caine (2000) that mountain lakes are particularly prone to local controls. However, we also demonstrate that, despite the differences between the lakes, meteorological forcing can have similar effects on the consumer–resource coupling, determining its strength and the overall availability of the most limiting nutrient.

Acknowledgments

Financial support was obtained from CICYT Project AMB99-541; MCYT Projects REN2001-2840; REN03-0383 and EC Environment MOLAR Project (ENV4-CT95-0007). Sierra Nevada S.A. kindly provided the climatic data. We are grateful to two anonymous referees for improving an early version of this work.

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Climate, food web and nutrient availability 997


(Manuscript accepted 20 February 2006)