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# Zooplankton advective losses may affect chlorophyll-*a* concentrations in fishless high-mountain lakes

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## ABSTRACT

**Zooplankton advective losses may affect chlorophyll-*a* concentrations in fishless high-mountain lakes**

Hydraulic washout of lakes and reservoirs is recognized as a major regulating factor of both, phyto- and zooplankton populations. We have analysed the changes in the chlorophyll-*a* concentration in 21 high-mountain lakes from Sierra Nevada. Eleven lakes proved to have superficial diffuse inlets and outlets (open lakes) while the other 10 have no outlets, or no permanent outlets (closed lakes), where the plankton losses by washout are not possible. The lakes were sampled on two occasions during the ice-free season: just after the spring thaw (July) and by late August, after plankton development. In July, chlorophyll-*a* was quite similar between the closed and open lakes while in August the open lakes had about three-fold more chlorophyll-*a* than did closed ones. Model selection analysis made with 12 variables than can affect chlorophyll-*a* indicated that, in July, chlorophyll-*a* was related mainly to the maximum depth of closed lakes although the type of lake (closed or open) had no effect. However, in August, the type of lake (more chlorophyll-*a* in open than in closed lakes) and the ratio between dissolved inorganic nitrogen and total phosphorus (DIN:TP ratio) (higher values, lower chlorophyll-*a*), was related to chlorophyll-*a* concentration. The results of DIN, soluble reactive phosphorus (SRP) and nutrient ratios did not indicate a different nutrient availability between open and closed lakes in either of the two periods considered. Nevertheless, the data available showed that the zooplankton was, on average, about two-fold more abundant in the closed lakes than in the open ones during the entire ice-free season. These results indicate that the advective losses caused by the water flow could be greater for the zooplankton than for the phytoplankton, interfering with the coupling of trophic chains. Thus, part of primary production in the open lakes could not be transferred to higher trophic levels because of the zooplankton losses.

**Key words**: High-mountain lakes, advective losses, zooplankton, phytoplankton, chlorophyll-*a*, grazing pressure, phytoplankton control

## *RESUMEN*

***Las pérdidas advectivas de zooplankton pueden afectar al contenido de clorofila-*a *en lagos de alta montaña sin peces***

*El desagüe de lagos y embalses es un factor importante que se reconoce como regulador de las poblaciones de fito y zooplancton. En este trabajo analizamos los cambios en las concentraciones de clorofila-*a *en 21 lagunas de alta montaña de Sierra Nevada, once de las cuales presentan salidas superficiales de agua permanentes (lagunas abiertos) mientras las otras diez no presentan salidas superficiales, o sólo en un corto periodo (lagunas cerradas), en las que la pérdide plancton por desagüe no es posible. Las lagunas se muestrearon en dos ocasiones durante la estación libre de hielo: en julio, justo tras el deshielo primaveral y al final de agosto, cuando el plancton se ha desarrollado. En julio, la concentración de clorofila-*a *fue similar en lagunas abiertas y cerradas, mientras que en agosto las lagunas abiertas presentaron aproximadamente tres veces más clorofila-*a *que las cerradas. Un análisis de selección de modelos efectuado sobre 12 variables que pueden afectar a la clorofila-*a *reveló que en julio la clorofila-*a *se relacionó con la profundidad de las lagunas cerradas, pero el tipo de laguna (abierta o cerrada) no tuvo ningún efecto sobre aquella. Sin embargo, en agosto, el tipo de laguna (más clorofila-*a *en las lagunas*

*abiertas que en las cerradas) y la razón nitrógeno inorgánico disuelto:fósforo total (DIN/TP) (a valores más altos, menos clorofila-*a*), mostraron efecto sobre los niveles de clorofila-*a. Los valores del DIN, del fósforo reactivo soluble (SRP) y razones de nutrientes no indican diferencia en la disponibilidad de nutrientes entre lagunas abiertas o cerradas en ninguno de los períodos considerados. No obstante, los datos disponibles muestran que la abundancia de zooplancton es, en promedio, el doble en las lagunas cerradas que en las abiertas durante un periodo de deshielo completo. Estos resultados señalan que las pérdidas advectivas producidas por el flujo de agua pueden ser más importantes para el zooplancton que para el fitoplancton, interfiriendo en el acoplamiento de las cadenas tróficas. Así, parte de la producción primaria de las lagunas no puede transferirse a niveles tróficos superiores debido a las pérdidas de zooplancton.*

## INTRODUCTION

Phytoplankton abundance is presumed to represent a balance between *in situ* growth (affected mainly by temperature, light, and nutrient availability) and loss processes, which may include sinking, grazing, parasitism, or washout. Zooplankton abundance is also a balance between growth (affected primarily by temperature and algal availability) and loss processes such as predation, parasitism, and washout. Thus, hydraulic washout is recognized as an important regulating factor of both, phyto- and zooplankton populations (Reynolds, 1984; Wetzel, 2001; Kalff, 2002) and the models for estimating plankton population growth include this parameter modulating the plankton growth rate (Reynolds, 1984; Lucas *et al.*, 2009). Hence, in the absence of others factors, plankton population abundance increases when population growth rate is faster than hydraulic loss and decreases when washout is faster than growth rate. Therefore, in a specific system, the effect of advective losses through lake out-flow is presumably greater on zooplankton, regardless of its size and swimming capacity, than phytoplankton, as the generation time of the former is significantly longer (Wetzel, 2001).

In this regard, some studies suggest that slower reproducing zooplankton may be more susceptible than phytoplankton to advective losses. For example, advective losses appear to be important in explaining differences between planktonic communities in lentic and lotic environments in the studies of Pace *et al.* (1992) and Thorp *et al.* (1994). Moreover, there is evidence that zooplankton abundance is positively correlated to water residence time but not with the phytoplankton abundance in rivers (Basu & Pick, 1996), reservoirs (Campbell *et al.*, 1998; Beaver *et al.*, 2013) or rapidly flushing lakes (Walz & Welker, 1998). Also, according to Havel *et al.* (2009) the observed decline in crustacean zooplankton downstream from dams reflects that reproduction is insufficient to balance high mortality by advective losses in the channel, where high algal biomass and rapid population growth rates allowed rotifers to dominate there.

For lakes with relatively similar morphometry, water residence time should be contrastingly lower in lakes with outlets (open lakes) than lakes without outflows or with no permanent ones (closed lakes). Consequently, advective losses should be greater in open lakes for zooplankton than for phytoplankton. For that reason, with similar resource availability for phytoplankton and without zooplankton predators, phytoplankton biomass should be less in closed lakes than in open lakes. Hence, we hypothesised that grazing pressure on phytoplankton should be higher in closed lakes with respect to open ones if no mechanism other than advection is removing zooplankton from the lakes. In this work, we compare the phytoplankton development in a group of 21 small, fishless, high-mountain lakes in Sierra Nevada (Spain) to test the aforementioned hypothesis.

## METHODS

### Site description

In the Sierra Nevada mountains of SE Spain ((36º 55' - 37º 15' N, 2º 31' - 3º 40' W; maximum altitude 3482 m a.s.l), ~50 small lakes of glacial

**Table 1.** Maximum depth, chlorophyll-*a* values and DIN:TP ratios in the Sierra Nevada lakes studied in July and August. Zooplankton, Rotifera, and Crustacea abundances averaged for an entire ice-free season are from Morales-Baquero & Conde-Porcuna (2000) (n: times sampled each lake). *Profundidad máxima, concentración de clorofila-a y cociente DIN:TP en los lagos estudiados de Sierra Nevada en julio y agosto. Los valores de abundancia media de zooplancton para el periodo libre de hielo están tomados de Morales-Baquero & Conde-Porcuna (2000) (n: número de veces que se muestreó cada lago).*

| Lake | Location UTM (30S) | Outflow[1] | Maximun depth (m) | Lake surface area (ha) | Chl-*a* (µg/L) July | Chl-*a* (µg/L) August | DIN:TP (weight) July | DIN:TP (weight) August | Zooplankton[2] (ind/L) | Rotifera[2] (ind/L) | Crustacea[2] (ind/L) | n |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Virgen 2 | VG664009 | 1 | 1.3 | 0.01 | 0.34 | 4.10 | 6.6 | 1.2 | 12.24 (7.13) | 0.55 (0.16) | 11.69 (6.57) | 5 |
| Yeguas | VG662013 | 1 | 2.5 | 0.33 | 1.22 | 3.21 | 49.5 | 2.6 | - | - | - | |
| Lanjarón 2 | VF641991 | 1 | 1 | 0.05 | 0.30 | 1.93 | 3.1 | 1.0 | 93.40 (53.84) | 28.94 (26.76) | 64.47 (48.91) | 3 |
| Mosca | VG723017 | 1 | 2.8 | 0.44 | 0.60 | 3.68 | 31.0 | 6.3 | 0.83 (0.32) | 0.13 (0.08) | 0.70 (0.28) | 3 |
| Gemela | VG714004 | 1 | 0.3 | 0.07 | 0.43 | 2.31 | 19.1 | 0.9 | 20.02 (9.06) | 1.58 (0.72) | 18.44 (10.23) | 6 |
| Aguas Verdes | VG674006 | 1 | 2.8 | 0.19 | 0.34 | 0.83 | 4.6 | 2.7 | 10.59 (5.64) | 0.60 (0.34) | 9.99 (5.97) | 4 |
| Siete lag. 2 | VG735014 | 1 | 3.5 | 0.34 | 0.51 | 0.51 | 13.8 | 2.9 | 58.30 (35.69) | 2.44 (1.34) | 55.86 (35.83) | 6 |
| Siete lag. 4 | VG737012 | 1 | 0.5 | 0.19 | 0.86 | 0.58 | 21.8 | 6.1 | 2.14 (0.42) | 0.44 (0.19) | 1.70 (0.44) | 6 |
| Borreguil | VG734009 | 1 | 2 | 0.18 | 1.11 | 2.31 | 14.1 | 2.7 | 0.98 (0.16) | 0.74 (0.20) | 0.24 (0.11) | 4 |
| Siete lag. 7 | VG739004 | 1 | 0.8 | 0.53 | 0.38 | 1.15 | 14.8 | 4.4 | 5.10 (1.85) | 0.32 (0.07) | 4.78 (3.34) | 6 |
| Peñón Negro | VF738983 | 1 | 2 | 0.67 | 2.82 | 5.39 | 6.20 | 0.6 | 47.22 (17.71) | 21.70 (18.75) | 25.52 (11.36) | 4 |
| Lanjarón 1 | VF646993 | 1-0 | 2.5 | 0.29 | - | 1.09 | - | 0.8 | 111.98 (73.48) | 109.08 (108.73) | 2.90 (1.31) | 3 |
| Majano | VG712003 | 1-0 | 0.8 | 0.27 | 0.68 | 3.21 | 24.1 | 1.1 | 54.26 (31.49) | 36.24 (32.55) | 18.02 (8.01) | 5 |
| Río Seco | VG694009 | 1-0 | 2 | 0.42 | 0.47 | 0.46 | 6.0 | 1.9 | 89.06 (52.30) | 1.48 (0.92) | 87.59 (51.73) | 4 |
| Río Seco Inf. | VG697008 | 1-0 | 0.5 | 0.12 | 3.21 | 2.30 | 3.3 | 0.5 | 101.17 (43.25) | 94.35 (42.44) | 6.82 (4.61) | 3 |
| Larga | VG704017 | 0 | 7 | 1.77 | 0.21 | 0.43 | 26.6 | - | 22.88 (14.23) | 0.98 (0.78) | 21.90 (13.55) | 2 |
| Río Seco Sup. | VG692008 | 0 | 1.5 | 0.07 | 0.97 | 0.83 | 11.2 | 1.9 | 18.97 (6.85) | 7.24 (6.84) | 11.73 (5.25) | 4 |
| Lanjarón 3 | VF636985 | 0 | 1.5 | 0.11 | 1.07 | 2.70 | 1.0 | 0.6 | 78.65 (25.40) | 73.65 (29.35) | 5.00 (4.15) | 2 |
| Caldera | VG708012 | 0 | 7 | 2.10 | 0.21 | 0.25 | 54.4 | 5.7 | 15.87 (5.05) | 0.88 (0.41) | 14.99 (5.48) | 5 |
| Caballo | VF612968 | 0 | 4 | 0.48 | 0.17 | 0.38 | 1.3 | 1.5 | 54.08 (30.675) | 9.30 (8.90) | 44.78 (39.35) | 2 |
| Cuadrada | VF618975 | 0 | 5 | 0.24 | 0.21 | 0.77 | 3.3 | 2.2 | 54.875 (42.6) | 28.30 (2.80) | 26.58 (14.58) | 2 |

1): 1: Open lakes; 0: Closed lakes; 1-0: lakes open in July and closed in August
2): Averages (standard error); n= number of samples

origin lie at an elevation of ~2800–3100 m a.s.l. These mountain lakes are typically small (surface area < 1 ha) and shallow (maximum depth < 10 m) and may undergo variable reductions in their water level depending on annual meteorological conditions. In this study, the lake surface area and maximum depth ranged from 0.01 to 1.0 ha and 0.3 to 8 m, respectively (Table 1). Further details can be found in Morales-Baquero *et al.* (1999). The lakes are fishless and Secchi disk visibility exceeds the water depth. Normally these water bodies remain ice-covered from November to June and do not thermally stratify during the summer. Lake waters are relatively soft: conductivity ranged from 5 to 77 µS/cm; total alkalinity from 50 to 400 µeq/L and pH from 6.5 to 9.5 (mean values at the time of this study). The phytoplankton is dominated by nannoplanktonic species (Sánchez-Castillo *et al.*, 1989) and the dominant zooplankton species are *Mixodiaptomus laciniatus* and *Diaptomus cyaneus* among copepods, *Daphnia pulicaria* among cladocerans and *Hexarthra bulgarica* and *Euchlanis dilatata* among rotifers (Cruz-Pizarro, 1983, Morales-Baquero *et al.*, 1989).

**Sampling and analyses**

A total of 21 lakes were studied (Table 1). The lakes were sampled just after the ice thaw and later, in the middle of the growing season, when the plankton communities were well developed. On each occasion, we collected the samples from all the lakes in the shortest time period possible. The first sampling took place over a 12-day period between 15-27 July 1991, and the second over a 13-day period between 21 August and 3 September of the same year. Hereafter, these periods will be referred to as "July" and "August", respectively. Samples were taken at the deepest point of each lake. An equal volume of water was extracted (using a centrifugal electric pump) from four evenly spaced levels of the water column, pre-filtered through a 40-µm NYTAL® net to remove the zooplankton, and then mixed together. Aliquots for analysis were taken from this mixed sample, and those for chemical analysis were stored in the dark at 4 ºC and analysed within 24 h of sampling.

Chlorophyll-*a* was determined by filtering 1 L of water through a GF/C filter, immediately after collection. The filter was placed in a glass vial, 10 ml of 95 % methanol was added, and the vial was stored in the dark at 4 ºC during transport. The vial was then frozen at -10 ºC for 24 h and the extract measured and corrected for pheopigments using a Hewlett Packard scanning spectrophotometer. Total nitrogen (TN) and total phosphorus (TP) were determined in unfiltered aliquots of the water samples, digested using a mixture of potassium persulphate and boric acid at 120 ºC for 30 min, and subsequently analysed by the ultraviolet method (APHA, 1989) and the ascorbic-acid method of Murphy & Riley (1962), respectively. SRP (soluble reactive phosphorus) and DIN ($NH_4^+$-N + $NO_2^-$-N + $NO_3^-$-N) were analysed from 0.45-µm (GF/C filters) filtered aliquots and determined using the Murphy & Riley (1962), the blue indophenol method (Rodier, 1990), the Strickland & Parsons (1968) and the sodium salicylate method (Rodier, 1990), respectively. According to Morris & Lewis (1988), we used several indexes (DIN:TP, DIN: SRP and TN:TP) to examine the nutrient limiting for phytoplankton growth. Concentrations and elemental ratios are reported by weight.

The zooplankton of the lakes included in the present study was previously quantified in a more detailed sampling program (Morales-Baquero, 1985). The lakes were sampled in 1981 and 1982, during the entire ice-free period but more intensively in 1982 (four times in each lake in most cases; see Table 1). A total of 20 L of water from the littoral zone were filtered through 40-µm mesh. Zooplankton average abundance of rotifers and crustacean [cladocerans + copepods (nauplii, copepodites and adults)] are shown in Table 1, and were previously published in Morales-Baquero & Conde-Porcuna (2000).

**Statistical analyses**

Statistical analyses were performed using program R 3.4.0 (R Foundation for Statistical Computing). We analysed the relationship of chlorophyll-*a* with the environmental variables that might affect it. The 12 environmental variables include lake morphometric data (type of

basin [open or closed], maximum depth, lake surface, catchment area, and the relation of lake surface to the catchment area), nutrient availability [dissolved inorganic nitrogen (DIN), soluble reactive phosphate (SRP), total nitrogen (TN) and total phosphorus (TP)], nutrient ratios (DIN:SRP, DIN:TP and TN:TP) and other environmental variables such as temperature and presence of littoral vegetation. The categorical variable "type of basin" indicated the presence of outlets in both sampling periods (open lakes) or no permanent or absence of outlets (closed lakes). In the last block, we included four lakes that had weak outlets in July and were closed in August. These lakes showed no significantly different pattern from those of the rest of closed lakes. In both open and closed lakes the inflows were diffuse. Continuous environmental variables were log-transformed (base 10) prior to statistical analyses in order to achieve normality and homoscedasticity as well as to linearize their relationships.

The environmental variables related to the chlorophyll-*a* concentration were assigned by model selection. We also included in the models the interactions of the type of basin with the other environmental variables. The R package "MuMIn" (Bartoń, 2018) was used to rank models, and two model-selection tests were performed, one with July data and the other with August data. Previously, high collinear variables (Variance Inflation Factor (VIF) > 10) were excluded from the set of variables of each model using the R package "usdm" (Naimi *et al.*, 2014). Model selection was based on the second-order Akaike information criterion ($AIC_c$) because of the relatively small sample sizes (Burnham & Anderson, 2002). If differences in $AIC_c$ between each model and the model with the minimum $AIC_c$ ($\Delta AIC_c$) is lower than 2, the models have relatively equal support (Burnham & Anderson, 2002). Additionally, the Akaike weights were summed (cumulative $AIC_c$ weights) over all possible models containing a given variable to measure the relative importance of each independent variable (Burnham & Anderson, 2002; Burnham & Anderson, 2004). Variables with a cumulative weight ≥ 0.5 show strong evidence of inducing a response in the dependent variable according to Barbieri and Berger (2004). Assump-
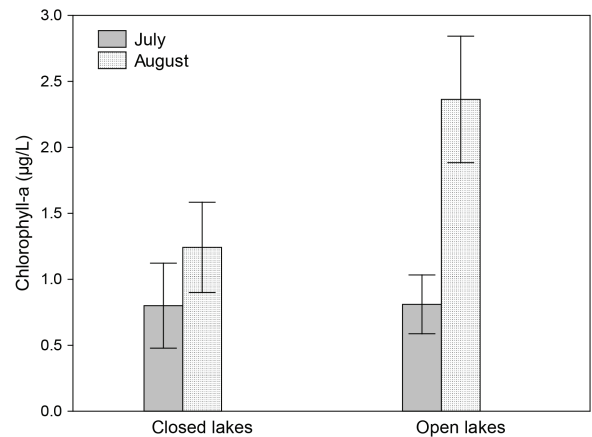


**Figure 1.** Mean value of chlorophyll-*a* concentrations in the open and closed lakes in July and August of 1991. Whiskers show the standard error. *Valor medio de la concentración de clorofila-*a *en las lagunas abiertas y cerradas en julio y agosto de 1991. Las líneas de dispersión muestran el error estándar.*

tions of linear models (normality of residuals, homoscedasticity, outliers and no multicollinearity) were checked for the best models.

We also used an unpaired t-test to analyse differences in the zooplankton abundance averaged for the ice-free period between open and closed lakes. Because zooplankton abundance data showed a mean-variance relationship according to Taylor power law, these data were powered to ¼ (Downing, 1979).

## RESULTS

In July the chlorophyll-*a* concentrations were quite similar between open and closed lakes while in August open lakes showed some three-fold more chlorophyll-*a* than did closed ones (Fig. 1). Model selection with July data showed that variables with the highest cumulative $AIC_c$ weights were the type of basin (0.84), depth (0.72), and the interaction between the type of basin with depth (0.66). Consequently, these variables had the highest relative importance. Other variables with $AIC_c$ weights higher than 0.5 were the ratio TN:TP (0.62) and the interaction between type of basin and the ratio TN:TP (0.53). In fact, the best selected model includes those five variables ($\Delta AIC_c > 4$ with

**Table 2.** Best model for the effect of environmental variables on the chlorophyll-*a* concentration of lakes in July and August. Statistically significant values ($p < 0.05$) are indicated in bold. SE = Standard error. *Mejor modelo obtenido para el efecto de las variables ambientales sobre la concentración de clorofila-*a *en las lagunas en julio y en agosto. Los valores estadísticamente significativos (*p < 0.05*) se indican en negrita. SE = error estándar.*

| July | AIC$_c$ = 15.24 | | | |
| --- | --- | --- | --- | --- |
| | Estimate | SE | t value | *p* |
| Intercept | 0.042 | 0.126 | 0.338 | 0.740 |
| Type of basin (open) | -0.279 | 0.154 | -1.806 | 0.092 |
| Depth[+] | -1.009 | 0.289 | -3.495 | **0.004** |
| Type of basin x Depth | 1.255 | 0.381 | 3.294 | **0.005** |
| August | AIC$_c$ = 14.56 | | | |
| | Estimate | SE | t value | *p* |
| Intercept | 0.078 | 0.101 | 0.778 | 0.447 |
| Type of basin (open) | 0.438 | 0.137 | 3.199 | **0.005** |
| DIN:TP ratio[+] | -0.726 | 0.205 | -3.544 | **0.002** |

[+]log transformed

respect to the rest of the models). However, that model has too many variables for the number of cases we had, and some VIF values in the model were higher than 10. The second-best model includes only the three variables with the highest AIC$_c$ weights and, in this case, VIF values were low (< 3) (Table 2). According to that model, the maximum depth of closed lakes could exert a negative effect on chlorophyll-*a* (Table 2).

Model selection with August data showed that the only variables with cumulative AIC$_c$ weights higher than 0.5 were the ratio DIN:TP (0.96) and the type of basin (0.82), which suggest that these variables were the most relevant variables to explain the chlorophyll-*a* concentrations. Although 9 models were selected with a ΔAIC$_c$ < 2, the best model with significant slopes and assumptions was the model including only the DIN:TP and the type of basin (Table 3, Fig. 2). The slopes of the regression lines linking the DIN:TP ratio and chlorophyll-*a* in both types of lakes did not significantly differ ($p > 0.05$), but the highly significant differences

in the interception points of the two lines suggest a clear effect of the outflow in the chlorophyll-*a* content. According to this model, open lakes had a higher chlorophyll-*a* concentration than did closed lakes for similar DIN:TP ratios (Table 2, Fig. 2).

For contrasting purposes, when we used those variables (type of basin and the ratio DIN:TP) to model chlorophyll-*a* concentration with July data, we found no significant relationships. Consequently, in August, when the algal and zooplankton communities have had enough time to develop, the phytoplankton shows a higher biomass in open lakes than in closed ones regardless of the nutrient supply. The latter results agree with the idea of lower grazing pressure in the open lakes due to washout losses of zooplankton. This idea is also supported by the available data on the zooplankton of Sierra Nevada lakes (Table 1). Figure 3 shows the abundance of zooplankton in open and closed lakes averaged for the ice-free period. Rotifers has an average of 36.2 ind/L in closed lakes
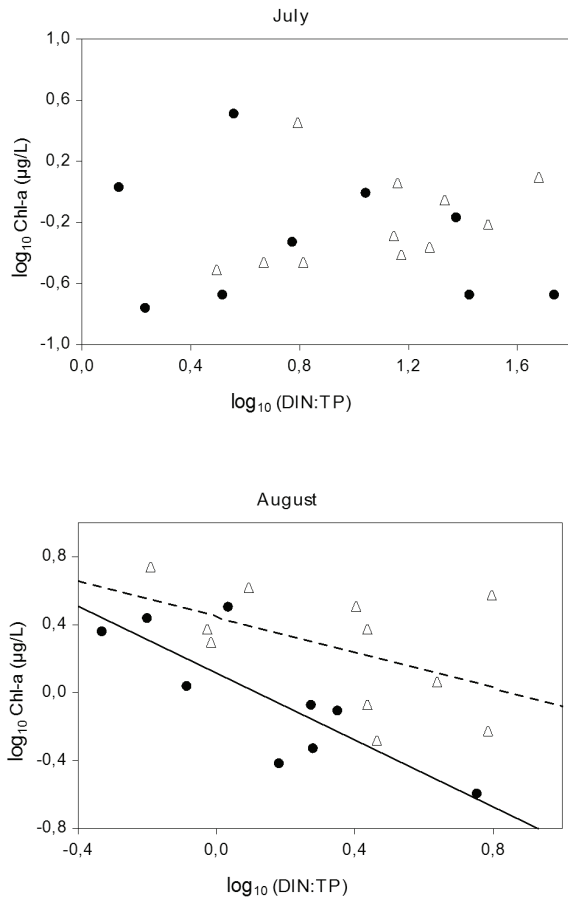
July



August

**Figure 2.** Relationships between the DIN:TP ratio and the chlorophyll-*a* concentrations in open and closed lakes in a) July and b) August. Significant regression lines are shown for open (triangles) and closed (solid circles) lakes in August. *Relación entre el cociente DIN:TP y la concentración de clorofila-*a *en las lagunas abiertas y cerradas en a) julio y b) agosto. Se muestran las líneas de regresión significativas para lagunas abiertas (triángulos) y cerradas (círculos) en agosto.*
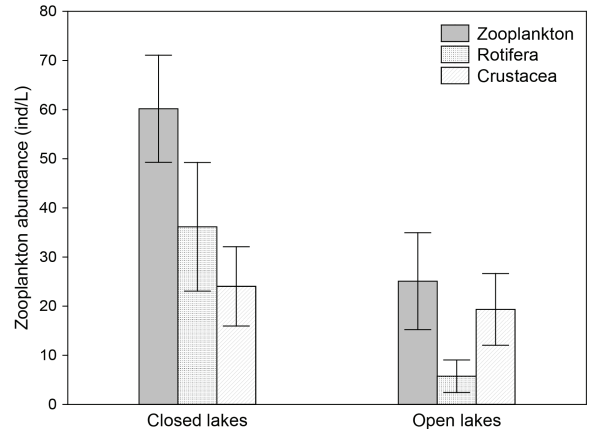


**Figure 3.** Averaged zooplankton, Rotifera and Crustacea abundance for the entire ice-free period (see Table 1) in open and closed lakes. *Abundancia media de zooplancton, rotíferos y crustáceos para el período libre de hielo (ver Tabla 1) en lagunas abiertas y cerradas.*

## DISCUSSION

The results of model selection from the 12 variables considered that can affect phytoplankton provided evidence that both the nutrient availability and the type of basin (open or closed) simultaneously controlled the phytoplankton biomass in the lakes of Sierra Nevada in August. The effect of the factor "type of basin" can be related to the grazing pressure of the zooplankton, which can be altered by the advective transport.

In July, when the planktonic community had not yet developed, there was a negative relationship of depth with chlorophyll-*a* in closed lakes, i.e. greater phytoplankton densities in shallow closed lakes in comparison to deeper closed ones. It is possible that in the shallow lakes, having less water volume, less time is needed to recolonize the pelagic zone from the sediment by the overwintering resting forms. The lack of a relationship between the chlorophyll-*a* and DIN:TP ratio in both types of lakes in July, when chlorophyll-*a* levels were low, was likely the result of a low nutrient limitation at this time. Seasonal changes in the strength of nutrient limitation in lakes have been reported elsewhere (Elser *et al.*, 1995). Particularly, nutrient limitation is often more relevant during the summer in non-stratified lakes

which is about 6-fold more than in open lakes (5.7 ind/L) and this difference is statistically significant (unpaired t-test: t = 2.62; *p* = 0.019). Also, the average crustacean abundance tended to be higher in closed lakes (24.0 ind/L) than in open ones (19.3 ind/L), although not statistically significant. Altogether, the closed lakes (55.5 ind/L) had more than twice the average zooplankton abundance of the open lakes (24.1 ind/L), this difference being statistically significant (unpaired t-test: t = -2.85; *p* = 0.011; n = 10 lakes in each group).

(Hansen *et al.*, 1997). Seasonal studies in the Sierra Nevada lakes have also shown evidence of changes in nutrient limitation from the beginning of the ice-free period to the middle of the growing season when the plankton has developed (Morales-Baquero *et al.*, 1999). In fact, bioassays have demonstrated that nutrient limitation is greater during the middle than at the beginning of the growing season (Villar-Argaiz, 1999).

In August, once the plankton has been developed, nutrient limitation could be stronger in all lakes and the relationship between the relative N and P availability and the phytoplankton abundance could be apparent in these lakes. N limitation appears when DIN values are very low (< 6 µg-N/L; Morris & Lewis, 1988) and DIN:TP values are below 3.4 (by weight) (Bergström, 2010). In August the DIN:TP ratios decreased in all lakes (Table 1) and the continuous negative relationship between chlorophyll-*a* and DIN:TP ratios in this month but not in July (Fig. 2) could reflect an increment of the degree of nutrient limitations as summer progresses and phytoplankton populations grow. Despite that this limitation appeared to occur similarly in both open and closed lakes, according the model performed, open lakes had a greater chlorophyll-*a* concentration than did closed lakes for similar DIN:TP ratios. Thus, the observed higher chlorophyll-*a* contents in the open lakes in comparison to the closed ones does not appear to depend on differences in nutrient supply.

Another factor that can cause differences in phytoplankton abundances is sedimentation below the euphotic zone, as light tends to be limited with depth. Since the systems with high water residence time can increase the rates of sedimentation of algae and light limitation (Søballe & Bachmann, 1984), phytoplankton losses due to sedimentation should be higher in the closed lakes than in the open ones. Nevertheless, this does not plausibly explain the lower chlorophyll-*a* contents observed in the closed lakes with respect to open ones, because in Sierra Nevada the light generally reaches the lake bottom.

Higher grazing pressure of zooplankton on phytoplankton in closed lakes is a more plausible explanation for the observed trends. Evidence indicates that changes in the outflow losses of zooplankton can change the standing stock of phytoplankton without alterations in the trophic status of the systems. For instance, Campbell *et al.* (1998) showed that after the conversion of a rapidly flushing lake in a reservoir, with much longer water residence time, the zooplankton biomass increased by one order of magnitude while the phytoplankton biomass fell below former values. These changes occurred independently of primary production or nutrient availability. Moreover, Morales-Baquero *et al.* (1994) has shown a negative relationship between water residence time and the chlorophyll-*a* per zooplankton biomass in reservoirs of contrasting trophic conditions. This is further evidence of high zooplankton grazing pressure over phytoplankton in waters with longer residence time.

The positive relationship between the water residence time and zooplankton abundance is well reported in rivers (Basu & Pick, 1996), lakes (Walz & Welker, 1998; Rellstab *et al.*, 2007), floodplains (Bozelli, 1994; Dias *et al.*, 2017), and reservoirs (Campbell *et al.*, 1998; Beaver *et al.*, 2013), pointing out the importance of the advective losses of zooplankton. In fact, the export of zooplankton through the outflow of reservoirs can be a major source of food for the fish community of the waters (Akopian *et al.*, 1999). Also, the increase in lake water residence time has been indicated as a possible explanation of the observed long-term trends of *Daphnia* increases associated with warming in Sierra Nevada lakes (Jiménez *et al.*, 2015, 2018)

In Sierra Nevada, zooplankton advective losses can occur in open lakes but not in closed ones, and the available data supports this contention. The zooplankton data in Table 1 are from a robust sampling survey, and the composition and relative abundance of the zooplankton communities in the lakes is quite similar throughout several study years (Carrillo *et al.*, 1996, Morales-Baquero *et al.*, 2006, Pérez-Martínez *et al.*, 2007). Hence, although zooplankton and phytoplankton were not sampled in the same year, we can attribute higher zooplankton predation pressure on phytoplankton in closed lakes than in open ones. Furthermore, the zooplankton samples were taken from the littoral zone. In this zone, the effect of advection over the zooplankton is proba-

bly less influential than in the open waters. In fact, contrary to high flushing rate habitats, inshore habitats act as storage zones for zooplankton (Reckendorfer *et al.*, 1999; Walks, 2007); nevertheless, the open lakes in Sierra Nevada contain less zooplankton than do closed ones. On the other hand, we found more zooplankton in closed lakes, despite that littoral sampling probably underestimates some populations, such as *Daphnia* sp., which tend to avoid the shore (Ringelberg, 1969) and have a strong grazing impact on phytoplankton. Thus, as expected from our initial hypothesis, the lower phytoplankton biomasses in the closed lakes in comparison to the open ones, for similar nutrient availability, can be attributed to a high grazing pressure due to lack of advective losses of zooplankton in the closed lakes.

In summary, this study indicates that in the small lakes of Sierra Nevada, outflow can interfere in the phytoplankton-zooplankton coupling. Our results suggest that, in the open lakes, part of the primary production cannot be transferred to higher trophic levels because of zooplankton losses. Advective losses are great in small lakes, but also in reservoirs with low water residence times because of high rates of water extraction. This abiotic mechanism of rarefaction can interfere with the articulation of the trophic chains and, together with other factors, can help to explain failures of the trophic-cascade hypothesis (Carpenter *et al.*, 1985) when it is applied to reservoirs in comparison to natural lakes.

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