

## First reports of short-term temporal variations in crustacean species richness in north Patagonian coastal temporary pools

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### ABSTRACT

Temporary pools are freshwater bodies of significant interest due to their high species diversity, and the colonization and extinction processes observed at temporal scales. The aim of the present study was to perform a short-term ecological comparison in a group of shallow temporary pools in northern Chilean Patagonia in two periods, August and September 2018. The results revealed marked differences in terms of species richness in the study pools at different conductivities and chlorophyll a concentrations. The dominant species was the calanoid copepod *Boeckella gracilis* Daday, 1902, while the presence of the rare Anostracan *Branchinecta rocaensis* Cohen, 1982 was reported in conditions of low chlorophyll a and low conductivity. The community was studied using co-occurrence null models that revealed the absence of structured patterns in species associations, and the presence of niche overlap, with many repeated species that have a similar ecological niche. The present results are identical with observations from temporary pools in southern Chilean Patagonia.

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## KEYWORDS

*Boeckella gracilis*, *Branchinecta rocaensis*, Chile, Cladocera, Copepoda, crustacean, zooplankton

## INTRODUCTION

Temporary pools are interesting ecosystems due to their high species richness (Spencer *et al.*, 1999; Schwartz and Jenkins, 2000), and to the population mechanisms involved in these ecosystems, specifically local colonization and extinction processes (Eitam *et al.*, 2004a; 2004b). This is because the reproduction of the species that inhabit the pools is based on dormant eggs that lie latent during the dry period (Takhur and Pardhi, 2018; Serra *et al.*, 2019). Associated with these reproductive behaviors is the added complexity of metapopulation and metacommunity dynamics, since each pool is an independent unit with individual exchange due to migration, extinction and colonization processes (Rojas-Castro and Araya-Crisóstomo, 2019), and simultaneous genetic selection of individuals (Norambuena *et al.*, 2019).

Temporary pools are associated mainly with regular seasonal events, for example, some originate from rainy periods in flood zones close to rivers, or flood zones in lowland sites after rain, or even due to snow melt. When pools are present, they have high alpha diversity because many of the species that inhabit them produce diapause eggs, with high dispersion and colonization abilities (Schwartz and Jenkins, 2000; Alekseev, 2007a; 2007b; Alekseev *et al.*, 2007; Meland *et al.*, 2019; Raza and Sharip, 2019; Sun *et al.*, 2019). These ecosystems are important for the study of conservation procedures (Eitam *et al.*, 2004a; 2004b) because interactions occur with associated fauna, for example aquatic birds that use the pools for nesting and feeding, and the presence of these birds enhances the dispersion capacity of dormant eggs (Green *et al.*, 2005).

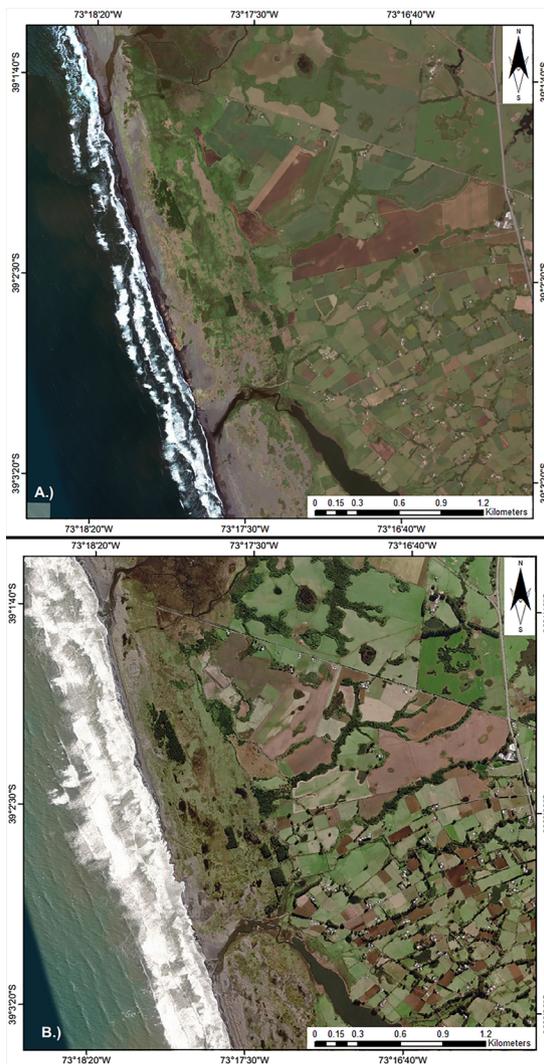
In Chile, the presence of temporary pools in Patagonia (38–53°S) is reported mainly during the southern late winter or early spring, specifically August to September. Pools studied on the southern

Patagonian plains (46–53°S) originate from snow melt (De los Ríos-Escalante *et al.*, 2018); however, in the mountainous zones of northern Patagonia (38°S; De los Ríos and Roa, 2010), or in coastal zones at the same latitude, they are due to winter rains (De los Ríos-Escalante and Carreño, 2018). The aim of the present study was to carry out the first short-term comparisons of richness, abundance and species composition among crustaceans, using null models (species co-occurrence and niche overlap), in northern Patagonian coastal temporary pools during two different periods.

## MATERIAL AND METHODS

### Study site

The study site is an area called Puaucho dunes, located on the sandy coast of the Araucania Region, northern Chilean Patagonia (38°S). The site has numerous temporary shallow pools present between early June and late September, approximately during the rainy period; they are absent (due to the absence of rain) during the rest of the year (De los Ríos-Escalante, 2018; Fig. 1). The site was visited in the middle of August and middle of September. Water temperature, conductivity and total dissolved solids were measured *in situ* using a Hanna sensor HI98130; chlorophyll a was measured in the laboratory using acetone pigment extraction (Strickland and Parsons, 1972), and zooplankton was collected by filtering a volume of 10 L with a screen of 100 µm mesh size. The samples were fully screened to avoid underestimating species richness and abundance (Soto and De los Ríos, 2006; De los Ríos-Escalante, 2018). The specimens collected were fixed in absolute ethanol (70 %), identified according to specialized literature (Araya and Zúñiga, 1985; Reid, 1985; Bayly, 1992; González, 2003) and quantified in the laboratory.



**Figure 1.** Aerial photograph of study site. **A:** Winter (August, 2018); **B:** Summer (January, 2018).

### Data analysis

As a first step, a niche overlap analysis was performed using an individual matrix in which rows and columns represented species and pools, respectively. This matrix was used to test if the niche overlap differed significantly from the corresponding value under the null hypothesis (random assemblage). These analyses were applied to the data and were based on the Pianka index (Gotelli and Ellison, 2013). This model shows the probability of niche sharing compared to the niche overlap of a theoretically simulated community (Gotelli and Ellison, 2013). The niche amplitude can either be retained or reshuffled; when it is retained it preserves the specialization of each species, whereas when it is reshuffled, it

covers a wide utilization gradient of specialization. Furthermore, zero occurrence in the observed matrix can be maintained or omitted. In the present study, we used the RA3 algorithm (Gotelli and Ellison, 2013; Carvajal-Quintero *et al.*, 2015), which retains the amplitude and reshuffles the zero conditions (Gotelli and Ellison, 2013). This null model analysis was carried out using the R software (R Development Core Team, 2009) and the EcosimR package (Gotelli and Ellison, 2013; Carvajal-Quintero *et al.*, 2015).

As a second step, a species presence/absence matrix was constructed, with the species in rows and the pools in columns. From this matrix we calculated a Checkerboard score (“C-score”), which is a quantitative index of occurrence that measures the extent to which species co-occur less frequently than expected by chance (Gotelli, 2000). A community is structured by competition when the C-score is significantly larger than expected by chance (Gotelli, 2000; Tondoh, 2006; Tiho and Josens, 2007). Lastly, we compared the co-occurrence patterns with null expectations via simulation. Gotelli and Ellison (2013) suggest using the statistical null model Fixed-Fixed, as in this model, the row and column sums of the matrix are preserved. Thus, each random community contains the same number of species as the original community (fixed column), and each species occurs with the same frequency as in the original community (fixed row). The null model analyses were likewise performed using the R software (R Development Core Team, 2009) and the EcosimR package (Gotelli and Ellison, 2013; Carvajal-Quintero *et al.*, 2015).

As a third step, a redundancy analysis was applied to the study variables – conductivity, total dissolved solids, chlorophyll a concentration, water temperature, and species abundance of zooplankton – in order to determine the importance of these variables for classifying the study pools. This analysis was performed using the R software (R Development Core Team, 2009). A matrix correlation analysis was carried out to determine the associations between the study variables, using parametric Pearson correlation coefficient, after verification of normality and homoscedasticity conditions. The software packages used were Hmisc R (Harrell, 2016), ade4 (Dray *et al.*, 2020) and Vegan (Oksanen *et al.*, 2019).

## RESULTS

The study site included five pools during the August sampling and ten pools in September; this variation was due to the late occurrence of the rainy season. The samples presented low conductivity, total dissolved solids (TDS) and chlorophyll a concentrations, and a relatively low number of species (2–5) with marked dominance of the calanoid copepod *Boeckella gracilis* (Daday, 1902), and ostracods of the genus *Cypris* O.F. Müller, 1776 (Tab. 1); perhaps the most remarkable feature was the presence of the anostracan *Branchinecta rocaensis* Cohen, 1982, in one pool (Tab. 1).

The results of the null model analysis revealed that there was no niche sharing between the species, and consequently no interspecific competition (Observed index = 0.190; Mean index = 0.136; Variance of simulated index = 0.001;  $P = 0.101$ ); meanwhile the null model species association revealed that the species associations were random (Observed index = 3.607; Mean index = 3.311; Variance of simulated index =

0.032;  $P = 0.057$ ), due to the presence of many repeated species in many pools.

The correlation matrix shows direct significant association of TDS with conductivity, TDS with *Bo. gracilis*, TDS with *Ceriodaphnia dubia* Richard, 1894, conductivity with *Bo. gracilis*, conductivity with *C. dubia*, pH with *Daphnia* O.F. Müller, 1785 and pH with *Br. rocaensis* and significant inverse associations were found for chlorophyll a with pH, and *Simocephalus* Schoedler, 1858 with *Daphnia* sp. (Tab. 2). The redundancy analysis results revealed that the main contributor variables for axis 1 were total dissolved solids, conductivity, and *Bo. gracilis*, whereas the main contributor variables for axis 2 were temperature and chlorophyll a, *Bo. gracilis* and *Cypris* sp. (Tab. 3).

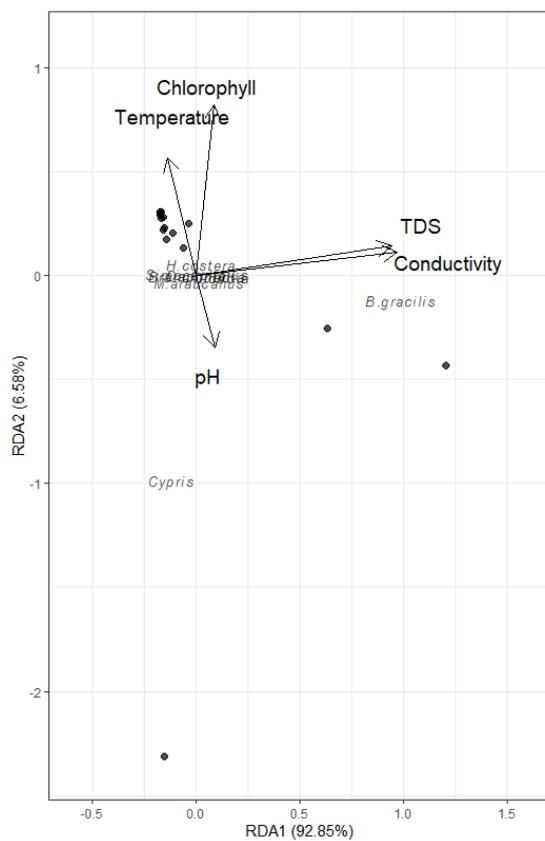
The redundancy analysis results revealed that pools 1; 2; 3; 4; 8; 10; 13; 14; 15 contained low abiotic and biotic levels, whereas pool 5 had high pH and abundance of *Cypris* sp., and pools 7 and 12 had high abundance of *Bo. gracilis* and high conductivity and TDS values (Fig. 2).

**Table 1.** Date of sampling, temperature, total dissolved solids (TDS), conductivity, crustacean species abundances (ind./L) for study pools.

Site	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12	P13	P14	P15
Sampling date	Aug-18	Aug-18	Aug-18	Aug-18	Aug-18	Oct-18									
Temperature (°C)	17.0	13.5	13.0	15.4	13.4	14.6	15.3	13.5	13.7	13.1	13.8	13.1	20.0	18.1	17.3
TDS (mg/L)	0.05	0.11	0.11	0.09	0.08	0.18	0.25	0.18	0.14	0.10	0.11	0.15	0.14	0.08	0.20
Conductivity (mS/cm)	0.13	0.23	0.23	0.17	0.17	0.35	0.50	0.36	0.28	0.19	0.23	0.30	0.26	0.17	0.40
pH	9.00	6.47	7.11	7.25	7.80	8.07	7.97	7.58	7.79	7.57	7.25	6.73	6.96	6.64	7.88
Chlorophyll a (µg/L)	4.3	14.9	5.5	8.8	4.6	38.1	38.5	37.3	70.4	70.7	72.0	41.4	41.2	70.7	41.0
<i>Branchinecta rocaensis</i> Cohen, 1982	0.01	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Daphnia</i> O.F. Müller, 1785	0.25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
<i>Ceriodaphnia dubia</i> Richard, 1894	0.00	0.00	0.00	0.00	0.01	29.56	38.67	0.01	8.89	0.00	0.22	0.01	2.22	0.44	0.67
<i>Simocephalus</i> Schoedler, 1858	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.11	0.00	0.11	0.00	0.11
<i>Boeckella gracilis</i> (Daday 1902)	0.01	0.25	0.00	7.50	25.00	29.56	328.67	4.44	26.67	0.00	3.56	195.56	3.89	0.78	15.33
<i>Mesocyclops araucanus</i> Löffler, 1962	0.00	0.01	0.01	0.01	5.00	0.00	6.44	0.00	0.01	0.00	0.00	0.00	0.11	0.11	0.00
<i>Cypris</i> O.F. Müller, 1776	0.00	0.01	1.00	7.50	165.00	0.00	6.44	4.44	8.89	0.33	1.33	12.22	5.33	1.78	4.56
<i>Hyalella costera</i> González and Watling, 2001	0.00	0.00	0.01	0.00	0.01	0.00	6.44	0.11	17.78	0.01	0.22	12.22	2.56	0.33	0.01
Taxa number	3	3	2	4	5	2	5	4	5	2	4	4	6	5	5

**Table 2.** Correlation matrix for variables considered in the present study, *P* values in parentheses (*P* values lower than 0.05 denotes significant correlation).

	Sp. number	<i>H. costera</i>	<i>Cypris</i>	<i>M. araucanus</i>	<i>Bo. gracilis</i>	<i>Simocephalus</i>	<i>C. dubia</i>	<i>Daphnia</i>	<i>Br. rocaensis</i>	Chlorophyll a	pH	Conductivity	TDS
Temperature	-0.13 (0.64)	0.13 (0.64)	-0.20 (0.46)	-0.08 (0.77)	-0.33 (0.23)	-0.12 (0.65)	-0.23 (0.40)	-0.05 (0.86)	-0.05 (0.86)	0.51 (0.86)	0.00 (0.06)	-0.05 (0.98)	-0.06 (0.84)
TDS	0.23 (0.40)	0.29 (0.30)	0.38 (0.15)	0.14 (0.61)	0.63 (0.01)	0.18 (0.51)	0.68 (< 0.01)	-0.42 (0.11)	-0.42 (0.11)	0.21 (0.11)	0.10 (0.44)	0.99 (0.01)	
Conductivity	0.22 (0.42)	0.28 (0.30)	0.39 (0.14)	0.16 (0.56)	0.65 (< 0.01)	0.16 (0.56)	0.69 (< 0.01)	-0.37 (0.18)	-0.37 (0.18)	0.19 (0.18)	0.13 (0.50)		
pH	-0.13 (0.65)	-0.04 (0.89)	0.15 (0.58)	-0.23 (0.39)	0.06 (0.82)	-0.08 (0.76)	0.35 (0.20)	0.64 (0.01)	0.64 (< 0.01)	-0.19 (< 0.01)			
Chlorophyll a	0.21 (0.44)	0.35 (0.19)	0.04 (0.89)	-0.17 (0.54)	0.04 (0.89)	0.29 (0.29)	0.09 (0.74)	-0.36 (0.18)	-0.36 (0.18)				
<i>Br. rocaensis</i>	-0.20 (0.47)	-0.14 (0.63)	-0.10 (0.73)	-0.11 (0.70)	-0.13 (0.65)	-0.13 (0.63)	-0.12 (0.65)	1.00 (< 0.01)					
<i>Daphnia</i>	-0.20 (0.47)	-0.14 (0.63)	-0.10 (0.73)	-0.11 (0.73)	-0.13 (0.70)	-0.13 (0.65)	-0.12 (0.63)						
<i>C. dubia</i>	0.00 (0.99)	0.22 (0.43)	-0.08 (0.78)	-0.17 (0.78)	0.66 (0.53)	-0.19 (< 0.01)							
<i>Simocephalus</i>	0.43 (0.10)	-0.16 (0.55)	0.50 (0.06)	0.23 (0.06)	-0.20 (0.41)								
<i>Bo. gracilis</i>	0.23 (0.40)	0.47 (0.07)	-0.05 (0.86)	-0.16 (0.86)									
<i>M. araucanus</i>	-0.01 (0.95)	-0.19 (0.50)	0.59 (0.02)										
<i>Cypris</i>	0.24 (0.39)	-0.15 (0.60)											
<i>H. costera</i>	0.33 (0.22)												



**Figure 2.** Redundancy analysis results for data obtained in the present study.

**Table 3.** Redundancy analysis for variables considered in the present study.

Environmental variables		
	RDA1	RDA2
Temperature	-0.1375	0.5674
TDS	0.9406	0.1433
Conductivity	0.9675	0.1136
pH	0.0896	-0.3431
Chlorophyll a	0.0862	0.8203
Biotic variables		
	RDA1	RDA2
Branchinectidae		
<i>Branchinecta</i> sp.	-0.0001	-0.0001
Daphniidae		
<i>Daphnia</i> sp.	-0.0039	-0.0031
<i>Ceriodaphnia dubia</i>	1.1449	-0.0128
<i>Simocephalus</i> sp.	0.0006	0.0043
Centropagidae		
<i>Boeckella gracilis</i>	11.9842	-0.3998
Cyclopidae		
<i>Mesocyclops araucanus</i>	0.1498	-0.0095
Cyprididae		
<i>Cypris</i> sp.	-1.4882	-3.2085
Hyalellidae		
<i>Hyalella costera</i>	0.2767	0.1780

## DISCUSSION

The results denoted the low number of species in the study pools (two to six species). This is similar to observations of shallow temporary pools in central Patagonia caused by snow melting during late winter and early spring, *i.e.*, between August and October (De los Ríos *et al.*, 2018). Although the classic literature on temporary pools denotes high numbers of crustacean species, up to seven or eight species, this richness is based on northern hemisphere references (Spencer *et al.*, 1999; Schwartz and Jenkins, 2000; Meland *et al.*, 2019; Razak and Sharip, 2019; Sun *et al.*, 2019). In spite of these differences, the literature about southern Patagonian inland waters revealed that the number of crustacean zooplankton species is higher in shallow temporary and permanent pools than in lakes (Soto *et al.*, 1994; Menu-Marque *et al.*, 2000).

These species numbers observed in shallow temporary pools in southern Patagonian are similar to the results of observations on sub-Antarctic islands (Diaz *et al.*, 2019).

The presence of the calanoid *Bo. gracilis* agrees with the classic literature on South American inland waters which report the dominance of the genus *Boeckella* Guerne and Richard, 1889, because this species is more tolerant of oligotrophy and high conductivity in the water than are cladocerans (Soto and De los Ríos, 2006; De los Ríos-Escalante, 2010). Although the conductivity values were low in comparison to the high conductivity in southern Patagonian pools, which can reach 4,000–30,000 mS/cm (Soto *et al.*, 1994; Soto and De los Ríos, 2006), in the present site — just as in southern Patagonian pools — a direct association was found between conductivity and calanoid abundance (Soto and De los Ríos, 2006).

In northern Chile, although the mineral content of inland waters is markedly high, a direct association can be found between calanoid copepods and salinities lower than 90 g/L; indeed, this taxon is the exclusive component at salinities between 5–90 g/L (De los Ríos-Escalante, 2010).

The presence of a representative of the genus *Branchinecta* is an interesting finding, as records of this group in Chile are scarce, mainly in central and southern Patagonian temporary pools (De los Ríos-Escalante, 2010; De los Ríos *et al.*, 2018; Rogers *et al.*, 2020). The record of this species at the study site was an important finding for its zoogeography in Chile. It was reported under conditions of low chlorophyll *a* and conductivity, similar to the records for central and southern Patagonia (De los Ríos *et al.*, 2018). The presence of this genus in temporary pools would be an important key component, as it is not only detritivorous but also a potential predator on small-bodied zooplankton, mainly small copepods (Horváth *et al.*, 2013; Lukic *et al.*, 2018). In southern Patagonia and the sub-Antarctic islands, members of the genus *Branchinecta* Verrill, 1869 would be mainly detritivorous (Paggi, 1996; Hawes, 2008; Pocięcha, and Dumont 2008).

On the basis of evidence from the northern hemisphere, temporary pools have metapopulation and metacommunity structure and functioning due to continuous local extinction and colonization processes, and migration brought about by agents such as migratory water birds that carry dormant eggs (Green *et al.*, 2005; Ripley and Simovich, 2008; Horvarth *et al.*, 2013). Calanoid copepods and anostracans also play a key role in these environments (Horvarth *et al.*, 2013). This information would probably be similar to observations for southern Patagonian temporary pools that are feeding and nesting areas for aquatic birds, with an impact on the zooplankton community (De los Ríos-Escalante, 2010).

In conclusion, the pools evaluated in the present study would indicate that — in spite of the low species numbers — these temporary pools present similarities to their northern hemisphere counterparts, specifically in the key role of anostracans and copepods, and probably in their metapopulation and metacommunity functioning. More detailed ecological studies would be

necessary to understand the structure and functioning of these ecosystems.

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