



# **Biotic and abiotic drivers of aquatic plant communities in shallow pools and wallows on the sub-Antarctic Iles Kerguelen**

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**Biotic and abiotic drivers of aquatic plant communities in shallow pools and wallows on the sub-Antarctic Iles Kerguelen**

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**Author contributions statement**

25 AKB, FMB, LS, DR, and CS conceived and designed research. AKB and PD collected the  
26 data. FV performed the chemical analyses of the samples. AKB, PED, and SD analysed data.  
27 All authors contributed to the writing and approved the manuscript.

## **Abstract**

In the sub-Antarctic region, climate change is particularly rapid, while their freshwater ecosystems, such as ponds, host plant species with limited spatial distributions. These particular systems and their plant communities remain however poorly known and the context of their changing habitat calls for deeper insights into these systems. We performed an extensive survey of 45 ponds over three locations of the Iles Kerguelen during the winter and summer seasons of two years, which included the measurement of 12 abiotic parameters and the assessment of the plant community composition. Overall, our results showed that Iles Kerguelen ponds are shallow freshwater ecosystems harboring species-poor plant communities with high evenness, of which structure did not vary among the sampled locations. Three different habitats were identified among ponds in relation with nutrient supply by marine animals, which ultimately influenced plant community structure and cover. We highlight that these habitats are mostly determined by water depth, nutrients, and temperature (mean and variance). Present plant community composition was more strongly correlated with plant community composition in the previous year than with abiotic conditions. Overall, this study provides new knowledge on the aquatic plant communities and the functioning of Iles Kerguelen freshwater ecosystems, which can serve as a basis for future studies dealing with the impact of climate change in the sub-Antarctic region.

## **Keywords**

Aquatic plants; Ponds; Seasonality; Plant community structure; Habitat; Spatio-temporal variability.

## Introduction

There is now ample evidence that climate change affects a broad range of organisms (see e.g. Walther et al. 2002), with severe effects for plant species in freshwater habitats (Lacoul and Freedman 2006). In polar regions, including the sub-Antarctic region, climate change is particularly rapid (Lebouvier et al. 2011; Leihy et al. 2018), calling for deeper insights into sub-Antarctic ecosystems, characterized by species with restricted spatial distributions (Frenot et al. 2001; van der Putten et al. 2010; Chau et al. 2021). This seems especially true for freshwater ecosystems harboring aquatic plant species. Indeed, to our knowledge, their ecology remains poorly known, probably because of technical difficulties associated with sampling in harsh climatic conditions and remote locations.

Freshwater ecosystems of the sub-Antarctic region include numerous rivers and lakes, but aquatic plants preferentially colonize shallow and lentic systems of small sizes, *i.e.* ponds (Hennion et al. 1994) including elephant seal wallows. So far, most works dealing with sub-Antarctic aquatic plants have focused on their morphological (Hennion et al. 1994; Hennion and Walton 1997) and genetic variations (Siljak-Yakovlev et al. 2020; Chau et al. 2021), while no study has considered community assembly mechanisms. Nevertheless, two main processes, dispersal and environmental filtering, often play key influences on plant community assembly through space and/or time (Vellend 2010). Yet, the actual influence of these processes in sub-Antarctic ponds has never been tested. Dispersal and environmental filtering in sub-Antarctic ecosystems should be strongly modulated by the specific traits of aquatic plants, and by the harsh environmental conditions encountered by the species. Dispersal in the sub-Antarctic aquatic plant species appears limited, as they do not display specific adaptations to zoochory, and seem alternatively prone to hydrochory (Hennion and Walton 1997). Dispersal limitation usually results in spatial differentiation in plant communities. Specificities of climate and ecosystem functioning in the sub-Antarctic region

suggest that temporal (*i.e.* seasonal) variations in abiotic parameters may differ from what is usually observed in regions and habitats from lower latitudes (Smith 1985). In particular, Hennion et al. (1994) reported that alternating periods of evaporation and heavy precipitation (seasonality) resulted in frequent and sometimes drastic fluctuations in the water depth of ponds of the sub-Antarctic Iles Kerguelen. It is thus likely that these seasonal variations of the pond habitat characteristics ultimately result in temporal modifications of the plant community composition.

Seasonal variations of water depth should therefore shape pond habitat *per se* or by modulating other physical and chemical parameters (Lacoul and Freedman 2006). Ponds are small water bodies, and as such, their physical and chemical characteristics would highly differ from larger sub-Antarctic water bodies, such as lakes (see e.g. Camacho 2006; Saunders et al. 2008). Changes in water temperature, which are linked to pond size (Bornette and Puijalon 2011), should be especially critical in the rapidly warming sub-Antarctic region (Lebouvier et al. 2011). Water temperature influences not only plant metabolism (Bornette and Puijalon 2011) but also microbial processes involved in nutrient cycling (MacKay et al. 2009; Biddanda 2017; Velthuis et al. 2017), as well as the concentrations of dissolved gasses (Bornette and Puijalon 2011). Additionally, ponds are enriched by nutrients conveyed by marine animals, with these enrichments being temporarily variable depending on animal migrations (Smith and Froneman 2008). Characterizing water depth, water temperature, and nutrient availability within these freshwater ecosystems is thus crucial to improve our understanding of the drivers of the assembly of aquatic plant community through time.

The current lack of knowledge on the sub-Antarctic aquatic biodiversity combined with recent works demonstrating that sub-Antarctic plants are already strongly affected by climate change (Le Roux et al. 2005; Frenot et al. 2006; Le Roux and McGeoch 2008; Bergstrom et al. 2015) call for scrutinizing how variations in abiotic and biotic characteristics

of ponds drive plant community assembly. Indeed, there is now ample evidence of climate change across the sub-Antarctic region. In the Iles Kerguelen and Marion Island, temperatures have increased while annual precipitation is strongly reduced (Lebouvier et al. 2011; McClelland et al. 2018). In Macquarie Island, however, summers and winters become respectively drier and wetter (Bergstrom et al. 2015). Given that under harsh environmental conditions, the abiotic filter is known to be a strong driver of plant community composition, in particular within sub-Antarctic terrestrial ecosystems (Bazzichetto et al. 2021; Momberg et al. 2021), it is critically important to characterize and monitor the main abiotic parameters that are known to affect the physiological status, and thus survival, of aquatic plants like *Ranunculus* species (Hennion et al. 2012; Labarrere et al. 2019).

A preliminary large field prospection performed during the Austral summer 2017, together with the existing literature (Hennion et al. 1994), indicated that the ponds located along the shore are the main habitat for aquatic plant species at the Iles Kerguelen. Thus, in this study, we surveyed the aquatic plant communities of 45 ponds over three locations across the shore of the main island of the Iles Kerguelen over two years. We expected that (i) pond plant communities should vary both through time (season, year) and space (location) in the Iles Kerguelen because of the stochastic impacts of marine animals on nutrient dynamics in ponds, and the low dispersal ability of aquatic plant species, reducing potential community homogenization among ponds; (ii) water depth, water temperature, and nutrient availability should be major parameters characterizing ponds habitat; and (iii) under the harsh climatic conditions of the archipelago, abiotic characteristics of ponds should prevail over biotic ones (past plant community composition), in determining the present plant community composition.

## Materials and methods

## Study area

This study was conducted on the main island of the Kerguelen archipelago (hereafter referred to as ‘Kerguelen’) located in the sub-Antarctic region (South Indian Ocean) (48°30’00” to 50°00’00” S, 68°27’00” to 70°35’00” E). The climate is characterized by cool temperatures (annual mean of 4.6 °C with little variation over the year, from 2.1 °C to 7.7 °C) (Frenot et al. 2001, 2006; Lebouvier and Frenot 2007), and low to abundant precipitations *i.e.* ranging from 500 mm to 3200 mm per year along an East-West gradient (Frenot et al. 1998). However, Kerguelen currently experiences rapid climate change with increasing mean and variability of temperatures, and strongly reduced precipitation (Lebouvier et al. 2011). Overall, this leads to harsh environmental conditions for species adapted to cold and stable temperatures.

We focused on the shallow freshwater ponds located near the eastern shoreline, including elephant seal wallows. These ponds are supplied with water largely by rainfall, and with nutrient inputs from marine animal (mostly elephant seals and birds) (Smith and Froneman 2008). These specific habitat conditions are comparable to those found in Spit Bay seal wallows of Heard Island (Bergstrom and Selkirk 2000).

Overall, the Kerguelen are characterized by a low species richness, hosting 22 native Angiosperms only (van der Putten et al. 2010). Based on the literature (Schotsman 1961; Lourteig 1952, 1964; Hennion and Couderc 1992; Hennion et al. 1994), six perennial plant species were expected to occur in the ponds, although aquatic plant communities of the Kerguelen have never been thoroughly described so far. These six aquatic plant species were: *Limosella australis* R.Br. (Scrophulariaceae), *Callitriche antarctica* Engelm (Plantaginaceae), *Juncus scheuchzerioides* Gaudich. (Juncaceae), and three species of Ranunculaceae: *Ranunculus biternatus* Smith, *R. pseudotrullifolius* Skottsb., and *R. moseleyi* Hook.f. Individuals of these six species are able to horizontally propagate through clonal growth (van Groenendael et al. 1996). Due to water depth fluctuations, we also anticipated that additional



non-aquatic plant species (*i.e.* species that usually colonize terrestrial habitat) would colonize ponds from their edges.

### *Field survey*

Sampling was conducted from the Austral summer 2018 to the Austral summer 2020, at three locations on the main island of the Kerguelen archipelago. The locations were distributed along the sea shore at low altitudes, and described a West-East gradient: Molloy, Isthme Bas, and Cap Ratmanoff (Table 1, Fig. S1). Location flatness and proximity to the sea shore allows the presence of sea animals. At each location, fifteen ponds were randomly selected to describe a gradient of pond size (Table 1, Fig. S2). Size was variable among ponds but also within ponds, due to important fluctuations of water depth (Hennion et al. 1994). GPS coordinates (precision of 1 m) of pond positions were recorded at each location. These positions were then used in GIS (QGIS 3.20.3) to calculate the distances between ponds and the sea shore as a proxy for the frequency of visitation by marine animals.

Within each pond and at each sampling date, we randomly positioned three to five 1 m<sup>2</sup>-quadrats to measure a range of biotic and abiotic parameters. The number of quadrats depended on the pond size: five quadrats were realized for ponds whose surface was >5m<sup>2</sup>, while three to four quadrats were used for ponds <5m<sup>2</sup>. At each sampling date, quadrat positions and size allowed to sample both ponds' edges and centers. To characterize the composition of pond plant communities, the abundance (*i.e.* percentage of quadrat surface covered) of the different species, and of bare soil, were estimated for each quadrat. All these abundances were recorded twice a year, once in November, and another time in May, which correspond to the beginning and to the end of the vegetation growth season, respectively. This growing season (November-April) will hereafter be referred as to summer, while the rest of the year (May-October) will corresponds to winter (Bergstrom et al. 2015). In addition,

physical and chemical parameters were measured every three months, in November, February, May, and August over the two consecutive years of the study. Within each quadrat, we measured the mean water depth (based on three measurements), pH, specific conductance, and mean dissolved oxygen concentration (calculated from three measurements performed near the surface, in the middle of the water column, and at the bottom of the pond; HQ20, HACH<sub>TM</sub>, Dusseldorf, Germany). At each pond, one 30 mL-water sample, and one 50 mL-sediment sample were also collected for further nutrient analyses in the lab. For these two samples, water and sediments were collected from several points within the pond to account for possible heterogeneity in nutrient distribution.

#### *Nutrient analyses*

After collection, water and sediment samples were stored at 6 °C and brought within 24h to the laboratory. Water samples were further filtered through Whatman GF/F filters (pore size: 0.7µm, Cytiva, Amersham, United Kingdom). All samples (water and sediments) were then stored at -20 °C for several weeks before chemical analyses. N-NH<sub>4</sub><sup>+</sup>, N-NO<sub>3</sub><sup>-</sup> and P-PO<sub>4</sub><sup>3-</sup> concentrations were determined based on standard colorimetric methods ([Grasshoff et al. 1983](#)) using a sequential analyzer (SmartChem200, AMS Alliance, Frépillon, France).

Nitrogen and organic carbon concentrations in sediments were measured following the “capsule” method ([Brodie et al. 2011](#)). Specifically, an amount of five mg (± 10%) of homogeneous sediment was acidified with 2M HCl in silver capsules to eliminate carbonates. Liquids were then evaporated on a 65 °C hot plate for 12 hours, before the entire drying out of capsules at 80 °C for a few days. Carbon and nitrogen analyses were then performed using an elementary analyzer (FlashEA 1112 NC Analyzers®, Thermo Fisher Scientific, Waltham, Massachusetts, USA). Following [Ni et al. \(2016\)](#), available organic phosphorus was extracted from 50 mg of dry sediment samples with 5 ml of NaOH (1 M). After extraction during 16

hours on a rotatory shaker at room temperature, the supernatant was collected, and its pH was stabilized at 7 with addition of HCl (4 M). The extracted P that has been converted into orthophosphate was quantified using the molybdate/ascorbic acid blue method (Murphy and Riley 1962), and results were reported in mg of available P (phosphorus available to plant growth) per g of dry sediment.

#### *Water temperature simulation*

Water temperature was recorded every half hour (HOBO Pendant MX2202, ONSET™, Cape Cod, Massachusetts, USA) from September 2020 to October 2021, in the middle of the water column of each pond. We performed linear regression analyses to determine the relationship between water temperature within each pond and air temperature recorded at the meteorological station of the Kerguelen (Météo France 2020-2021 records, Port-aux-Français), after confirming normality of the distribution of model residuals. All relationships between air and water temperatures were significant ( $R^2 \geq 0.55$ ;  $P < 0.05$ ), with the exception of two ponds. These relationships were then used to infer water temperature occurring during the whole study period (summer 2018-2020) based on Météo France 2018-2020 records.

From these estimated data, we calculated the mean and the variance (square of standard deviation) of daily water temperatures over a period of three months *i.e.* between two dates separating other abiotic parameters measurements.

#### *Data analyses*

Before statistical analyses, abiotic parameters and plant species abundances recorded from different quadrats per pond were averaged for each sampling date. We used plant species abundances to calculate their relative frequencies *i.e.* proportion of vegetation cover occupied by the species. Species richness, Shannon diversity, and evenness (Pielou index) of plant

community were calculated using the *vegan* R package (Oksanen et al. 2016). Pairwise correlations between these indexes were tested using non-parametric Spearman's rank correlation tests. Species richness and evenness were not correlated while Shannon diversity was strongly correlated with the two other indexes ( $r > 0.75$ , and  $P < 0.05$ ). We therefore removed Shannon diversity from subsequent analyses.

We tested the effects of year, season, and location (Molloy, Isthme Bas, and Cap Ratmanoff) on the two community indexes using general mixed-effect models with pond as random factor (Bolker et al. 2009). Richness index was square-rooted to satisfy normal distribution of residuals. The statistical significance of the fixed factors and their interactions was determined through type II ANOVA. Calculation of marginal and conditional  $R^2$  associated to the model was performed with the *performance* R package (Nakagawa et al. 2017).

We further used Between-Classes Principal Component Analysis (BCA-PCA; Dolédec and Chessel 1987) as implemented in the *ade4* R package (Dray et al. 2007; Thioulouse et al. 2018) to quantify the amount of variance delivered by time (season, year) and space (location, pond) and explaining plant community composition (*i.e.* relative frequencies of plant species in each pond at each sampling date). BCA-PCA belongs to the constrained analysis techniques (ter Braak and Verdonschot 1995) and is a particular case of PCA in which the sample grouping (e.g. in time or space) is accounted for in the analysis. The index characterizing the effect of time (or space) is quantified by between-class variance, which represents the ratio of the amount of variance explained by the sample grouping and the total variance among samples. Between-class variance was tested against simulated values obtained after 9,999 permutations of the rows of the plant species composition tables and allowed to assess the significance of the effects of year, season, location, and pond on plant community composition.

To characterize the habitat of plant species, we performed Principal Component Analysis (PCA) on the pond abiotic parameters using the *FactoMineR* R package (Lê et al. 2008), followed by Hierarchical Clustering on PCA scores (Ward method based on Euclidean distances) to discriminate pond clusters based on abiotic parameters. The mean of each abiotic parameter was then compared between clusters through ANOVA tests based on general linear models, and significant differences between clusters were assessed through Tukey HSD *post-hoc* tests ( $P < 0.05$ ). A similar procedure was applied to determine the relationships between clusters and (i) the plant community indexes, and (ii) the total vegetation cover within the pond. The parameter values were log or arctan transformed when necessary, to satisfy the assumption of the normal distribution of residuals.

To investigate the relationships between plant community composition and abiotic parameters across seasons, we used the STATICO method (Simier et al. 1999; Thioulouse et al. 2004). The method has been shown to be useful to investigate the temporal dynamics of the relationship between environment and diatoms (Mendes et al. 2009), Copepods (Marques et al. 2011), and macroinvertebrate assemblages (Shieh and Chi, 2010). This method allows the simultaneous analysis of several pairs of tables in one run. In our case, we had four tables *i.e.* two seasons (for each season, we averaged the measurements of two dates) across two years, from summer 2018 to winter 2020 (see Fig. S3). This multivariate method combines the potential of (i) co-inertia analysis that allows matching two tables providing a new table (cross-covariance table), which describes the covariance between each environmental variable and each plant species (Dolédéc and Chessel 1994; Dray et al. 2003), and (ii) partial triadic analysis allowing the simultaneous analysis of several tables in one run with the aim of depicting their similarity (Thioulouse and Chessel 1987), *i.e.* in our case, across seasons and years. In practice, the first step (co-inertia analysis) crossed the two tables (*i.e.* the physical and chemical parameters and the plant community composition) associated to each season of

each year. This step ended up with the production of four cross-covariance tables depicting the covariance between each environmental variable and each plant species, at each season of each year. In a second step, the series of cross-covariance tables (one per season and per year) were analyzed with partial triadic analysis, looking for temporal variations in the species-environment relationships. The strengths of these relationships between plant communities and abiotic parameters across seasons were then assessed using the  $R_v$ -coefficient, which is a multidimensional equivalent of the ordinary correlation coefficient between two variables (Robert and Escoufier 1976). We further evaluated the statistical significance of each of the four computed  $R_v$ -coefficients by a random permutation test using 999 replicated matches of two tables (after random permutations of array rows), and we compared the observed  $R_v$ -coefficient and the distribution of values obtained from permutations.

Finally, to assess the relative contributions of biotic (plant community composition of preceding seasons) vs. abiotic (physical and chemical parameters of preceding seasons) characteristics of ponds to the variability of the plant community composition observed at a given date, we used the analysis proposed by Townsend et al. (2003) and used by de Castro et al. (2017). This analysis was performed between the plant community composition recorded in summer 2020 (at the end of the survey), and the biotic and abiotic characteristics of ponds from summer 2018 to summer 2020. Let  $PCP_i$  and  $PCC_i$  denote respectively the physico-chemical parameter table and the plant community composition table at the  $i$ th sampling date, and consider  $PCC_{S20}$  as the plant community composition table in summer 2020. Assuming that the physico-chemical parameters at the  $i$ th sampling date would be a more efficient driver of the plant community composition in summer 2020 than the plant community composition, we tested the null hypothesis  $R_v(PCP_i, PCC_{S20}) = R_v(PCC_i, PCC_{S20})$  against the alternative  $R_v(PCP_i, PCC_{S20}) > R_v(PCC_i, PCC_{S20})$  and we performed bootstraps on the statistics  $t = R_v(PCP_i, PCC_{S20}) - R_v(PCC_i, PCC_{S20})$  using 999 replicates. We assessed the statistical

significance of the observed difference between pairs of  $R_v$ -coefficient by comparing it to the distribution of the simulated values. We used this approach for the 36 pairs of potential differences between the four physical and chemical parameters and the four plant community composition tables.

All data simulations and statistical analyses were performed using R 4.1.2. (R Core Team 2021).

## Results

### *Description of plant communities within ponds at Kerguelen*

Pond plant communities of the Kerguelen were characterized by a low species richness (S), and a high evenness (E). On average, ponds provide a habitat for five to six plant species, while evenness was on average  $> 0.7$  suggesting that plant species displayed similar relative frequencies. Marginal  $R^2$  indicated that year and season explained a very low percentage of species richness and evenness variance respectively, while these indices did not vary with location (Table 2). However, pond explained a large percentage of variance of both indices (conditional  $R^2$ , Table 2). Species richness was higher in the second sampling year ( $S_{\text{summer2019}}=5.77 \pm 1.27$ ;  $S_{\text{winter2020}}=5.64 \pm 1.26$ ) as compared with the first one ( $S_{\text{summer2018}}=5.45 \pm 1.33$ ;  $S_{\text{winter2019}}=5.22 \pm 1.57$ ), while evenness slightly increased in winter ( $E_{\text{winter2019}}=0.81 \pm 0.14$ ;  $E_{\text{winter2020}}=0.79 \pm 0.12$ ) compared to summer ( $E_{\text{summer2018}}=0.76 \pm 0.15$ ;  $E_{\text{summer2019}}=0.72 \pm 0.16$ ).

Analyzing the composition of plant communities showed that relative frequencies of aquatic species varied from 0.00 to 0.35 across all locations and dates of sampling (Figs. 1, S4). Overall, the most abundant aquatic species was *Juncus scheuchzerioides* while the rarest ones were *Limosella australis* and *Ranunculus moseleyi*. *R. moseleyi* was almost absent from the three sampled locations during the two winter periods, suggesting that the species was

sensitive to seasonal variations in environmental factors. Non-aquatic species altogether [*i.e.* *Poa annua*, *Acaena magellanica*, *Agrostis magellanica*, and *Montia fontana*] generally dominated pond communities with mean relative frequencies ranging from 0.20 to 0.55 (Figs. 1, S4).

The first three axes of the BCA-PCA performed on the mean relative frequencies of plant species, explained 49.3% of total inertia. Permutation tests revealed that the season had no significant effect on plant community composition in ponds (between-class variance = 0.011; *simulated-P* > 0.05), while the year and the location respectively explained only 1.0% (between-class variance = 0.010; *simulated-P*=0.046) and 7.1% (between-class variance = 0.070; *simulated-P*=0.001) of their variation. In contrast, a high proportion of variation in plant community composition (*i.e.* between-class variance= 0.595; *simulated-P*=0.001) was explained by differences among ponds, suggesting that mechanisms driving plant community structure operated at the local scale of the pond.

#### *Physical and chemical characteristics of ponds*

Kerguelen ponds appeared as shallow freshwater ecosystems (mean water depth < 16cm, although they can easily exceed 30 cm in depth depending on rain supply) with highly variable availability in nutrients (Tables 3, S1). pH was very close to neutral on average but its range indicated strong variation between ponds of the same location (Table 3). Water temperature was cool and overall stable among days (mean temperature and temperature variance, Tables 3, S1) but variations in pond size and water depth could cause strong within-day variation (up to  $\pm 10$  °C, A.-K. Bittebiere - field observation). Locations and time (year, season) affected all these physical and chemical parameters with varying strengths (Tables 3, S1, S2), either alone or in interactions, with no general pattern in the observed responses. For instance, all abiotic parameters varied with the sampling year except  $\text{NH}_4^+$ , and this response



350 additionally depended on the season (mean and variance of water temperature, specific  
351 conductance, dissolved oxygen, C/N,  $N_{\text{tot}}$ ) and/or the location (temperature variance,  
352 dissolved oxygen,  $\text{PO}_4^{3-}$ ). Yet, most of the variance in physical and chemical parameters was  
353 explained by ponds (Table S2).

354 We performed a PCA on all abiotic parameters measured in the ponds. The first-  
355 three axes explained 46.7% of total variance (Fig. 2). The first axis described the trophic  
356 status of ponds based on water and sediment nutrient concentrations, and dissolved oxygen  
357 concentration (Fig. 2a), while the second and third axes were related to physical parameters,  
358 (temperature variance and water depth on the one hand, mean temperature and pH on the  
359 other hand; Fig. 2b). We detected three different pond clusters regardless of time (year,  
360 season), and locations (Table S3). Cluster 1 corresponded to ponds with a high variance in  
361 water temperature together with high concentrations of dissolved oxygen, low concentrations  
362 of phosphorus (bioavailable P in sediments and  $\text{PO}_4^{3-}$ ) and nitrogen ( $N_{\text{tot}}$  in sediments,  $\text{NH}_4^+$ ,  
363  $\text{NO}_3^-$ ) (Fig. 2c). Cluster 2 was characterized by the deepest water, low temperature variance,  
364 and intermediate concentrations of phosphorus (bioavailable P and  $\text{PO}_4^{3-}$ ) (Fig. 2c). Cluster 3  
365 was related to occasionally extreme values in nutrient concentrations in water and sediments  
366 ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ,  $\text{PO}_4^{3-}$ , and bioavailable P), high water temperature and low dissolved oxygen.

367 We detected a significant difference in pond distance to sea shore among pond  
368 clusters (ANOVA,  $F_{2/332}=25.4$ ,  $P < 0.001$ ; Fig. 3a). Ponds of clusters 2 and 3 were  
369 significantly closer to sea shore than ponds of cluster 1. In addition, the total vegetation cover  
370 significantly changed with the pond cluster (ANOVA,  $F_{2/164}=13.8$ ,  $P < 0.001$ ; Fig. 3b).  
371 Indeed, ponds of cluster 1 had significantly lower vegetation cover than those of cluster 2,  
372 while cluster 3 showed intermediate vegetation cover. Regarding plant community structure,  
373 species richness did not depend on the pond cluster (ANOVA,  $X_{1/165}=1.7$ ,  $P > 0.05$ ; Fig. 3c),

while evenness was respectively the highest and the lowest in ponds of cluster 2 and 1, those of cluster 3 displaying an intermediate evenness (ANOVA,  $F_{2/164}=8.0$ ,  $P < 0.001$ ); Fig. 3d).

#### *Abiotic and biotic drivers of plant community composition*

The composition of the plant community was significantly related to the physical and chemical parameters of ponds with a slightly stronger response in summer compared to winter (Table 4). No strong covariation of relative frequencies was detected among aquatic plant species (Figs. S5a, c, e, g). The plant community composition was similar in summers 2018 and 2019 (Figs. S5a, e), and more seasonally stable during the second sampling year in comparison with the first year (Figs. S5a, c, e, g). In addition, plant community composition was very similar between locations of sampling (Figs. S6a, c, e, g). Importantly, regardless of the sampling season and year, bioavailable P ( $P_{\text{bio}}$ ) and total N ( $N_{\text{tot}}$ ) in the sediments, together with the variance of daily water temperature were the strongest drivers of the plant community composition unlike mean water temperature (Figs. S5b, d, f, h). Ponds with higher water temperature variance among days were preferentially inhabited by *Juncus scheuchzerioides* (summer 2018-2019 and winter 2020; Figs. S5a, e, g). During winter, the importance of abiotic parameters for plant community composition was more variable across sampling years, but nitrogen availability in water and sediments seemed determinant ( $N_{\text{tot}}$ , and  $\text{NO}_3^-$  or  $\text{NH}_4^+$ ) (Figs. S5d, h). Sampling locations were mostly differentiated by the abiotic parameters especially along the second STATICO axis (Fig. S6) *i.e.* by the specific conductance of water (winter 2019 and summer 2019, Figs. S5d, f), and by the availability in  $\text{NH}_4^+$  (winter 2020, Fig. S5h).

Most tested relationships between the present (summer 2020) and past (summer-winter 2018-2020) physical and chemical parameters and plant community composition, and the present plant community composition (summer 2020; Table 5) were statistically

significant, indicating the influence of past physical and chemical parameters (in winter and summer 2019 only), and of all past plant community compositions on present plant community composition. The differences in  $R_v$ -coefficients between plant community composition and physical and chemical parameters were all negative when significant (Table S4), indicating that the relationships between present and past plant community compositions were overall stronger than the relationships between present plant community composition and present and past physical and chemical parameters. In addition, plant community composition of the two preceding seasons (winter 2020  $PCC_{M20}$  and summer 2019  $PCC_{N19}$ ) similarly influenced present plant community composition (summer 2020). Indeed, differences in the strengths of the relationships between present plant community composition and  $PCC_{M20}$  on the one hand, and  $PCC_{N19}$  on the other hand, were not significant (Table S4).

## Discussion

In this study, indices of plant community structure mostly varied between ponds with an additional low effect of time (either season or year depending on the index). Water depth, water temperature, and nutrient availability were the major parameters differentiating sub-Antarctic ponds habitat. Surprisingly, and contrary to our hypothesis, abiotic characteristics of ponds did not prevail over biotic characteristics in determining the plant community composition.

### *Pond habitats and their plant communities through space and time*

The studied ponds were shallow freshwater ecosystems characterized by intermediate level of specific conductance and dissolved oxygen concentration, which were consistent with rainfall supply. Indeed, [Voisin et al. \(2018\)](#) showed that specific conductance of urban wetlands receiving stormwater runoff ranged from 50 to 200  $\mu S.cm^{-1}$ . Nutrient availability in the water

was highly variable, and pH was on average close to neutral (*i.e.* softwater habitat; see [Lacoul and Freedman 2006](#)) although the maximal and minimal values indicate heterogeneity among the ponds, likely reflecting different levels of photosynthetic activity (e.g. [Buapet et al. 2013](#)). At similar latitudes, equivalent mean values of pH were recorded within lakes of the Macquarie and Kerguelen islands ([Tyler 1972](#)), and of South Georgia ([Hansson et al. 1996](#)). However, these ecosystems display higher specific conductance and lower phosphate concentration ([Hansson et al. 1996](#)) than that observed in our studied ponds. Phosphorus appears to be the limiting nutrient as usually observed in freshwater systems ([Toivonen and Huttunen 1995](#); [Sarneel et al. 2011](#)). Indeed, the mean N:P atomic ratio in the water column (using  $\text{N-NH}_4^+$ ,  $\text{N-NO}_3^-$  and  $\text{P-PO}_4^{3-}$  concentrations reported on Table 3) was strongly higher (55:1) than the Redfield N:P ratio of 16:1, suggesting that P was a more limiting nutrient for primary producers than N ([Geider and La Roche 2002](#)). Mean daily temperature of water was about 4.7 °C, and was similar on average among consecutive days, albeit strong within days' variations were recorded.

Location and time (year, season) affected all of the considered physical and chemical parameters measured from the ponds with varying strengths, either alone or in interaction. Although we could not detect any general pattern in the observed changes through location and time, we evidenced that pond explain a large part of the variability in most physical and chemical parameters. These complex patterns may result from factors such as variations in pond morphometry (pond size, depth) ([Hanson et al. 2007](#); [Nõges 2009](#)) and climatic conditions (air temperatures, rainfall) ([Dodds et al. 2019](#)) interacting through space and time, although this hypothesis remains to be thoroughly tested.

The studied ponds were poorly colonized by plants, with only five to six species being present, which is consistent with former studies performed at high latitudes on aquatic ecosystems ([Lacoul and Freedman 2006](#); [Heino and Toivonen 2008](#)). Field observations also

reveal the presence of algae and cyanobacteria in these systems (AK. Bittebiere, field observations). Plant species like *Poa annua* and *Acaena magellanica* colonized ponds from their edges, likely benefiting from more frequent decrease in water depth related to rainfall reduction (Casanova and Brock 2000). These species even became the most frequent, although not dominant (high evenness), probably because they display faster expansion rate than the aquatic species like *Ranunculus bitermatus* and *R. pseudotrullifolius* (Chapuis et al. 2004). Different mechanisms were suggested to explain high evenness of aquatic plant communities in the literature, such as low pressure of vegetative propagules, high water level (Li et al. 2015), or disturbance (e.g. through grazing, reducing the dominance of the most abundant species) (Wood et al. 2012). In the shallow ponds of Kerguelen, as all species are clonal, we can hypothesize that disturbance, probably by sea animals, is the mechanism preventing species from becoming dominant.

Contrary to our expectations, this composition of pond communities did not vary much at the spatial scale, suggesting that aquatic species dispersal was not limited within Kerguelen (Capers et al. 2010). Our observations reveal that most variation of the plant community composition was actually detected among ponds, and not between sites. Mechanisms driving pond community assembly thus operated at local scale, likely involving abiotic and biotic filters (Violle et al. 2012) such as water trophic level (Alahuhta 2015) and species interactions (Sarneel et al. 2011). Based on our results, we argue that future studies of pond systems of the Iles Kerguelen, have to be conducted at this spatial scale.

#### *Different pond habitats*

Three pond clusters, *i.e.* habitats, related expectedly to water nutrients, water depth, and temperature, could be discriminated across ponds. Importantly, not only the mean temperature, but also its variance, determined pond habitats. These differences could be, at

least partially, explained by the distance to sea shore, *i.e.* by the frequency of visitation by sea animals such as elephant seals and penguins. Indeed, inland ponds that were the most distant from the sea shore (cluster 1) displayed the lowest nutrient concentrations. By contrast, ponds close to the sea shore, *i.e.* more easily reachable by sea animals, showed intermediate (cluster 2 – coastal ponds) to high concentrations of phosphorus (P bioavailable,  $\text{PO}_4^{3-}$ ) and nitrogen ( $\text{N}_{\text{tot}}$ ,  $\text{NH}_4^+$ ,  $\text{NO}_3^-$ ) leading to eutrophication episodes associated with high water temperature, and involving low dissolved oxygen (cluster 3 - wallows). Differences in nutrient availability between coastal ponds and wallows may have resulted from stochastic visitation of different sea animals. Indeed, penguins and elephant seals may supply pond water into varying source of nitrogen ( $\text{NH}_4^+$ ,  $\text{NO}_3^-$ , urea, and uric acid) that can be differentially assimilated by plant species (Erskine et al. 1998). In addition, seasonal variation in feeding habits and use of ponds of sea animals (Cherel et al. 2007) may explain the observed nitrogen dynamics in water and sediments. This interpretation suggests that these ecosystems rely heavily on trophic exchanges with surrounding oceanic waters, as suggested by Smith (2008) and Lebouvier et al. (2011). Similar influence of sea animals that deposit substantial amount of feces, urine, and molted skin or feathers together with carcasses, have been demonstrated in other Antarctic and sub-Antarctic ecosystems such as terrestrial grasslands at Marion Island (Panagis 1985) or larger freshwater ecosystems (lakes) in the Antarctic (Smith 1985). Interestingly, the habitat differentiation that we have highlighted here, occurred within restricted area (at the location scale) along a few hundred meters.

Ultimately, changes in pond habitat caused variations in plant community structure and aquatic ecosystem functioning. Coastal ponds (with intermediate level of nutrient availability) had higher vegetation cover and evenness compared to wallows and inland ponds. In plant communities with low evenness, fertilization usually enhances the dominance of already successful competitors, and accelerates local extinction rates (Wilsey and Polley

2004). However, in our studied ponds, dominance within plant communities was low and the different species were equally abundant. It has been shown in the literature that sea animal fertilization could enhance species persistence, by increasing population sizes (Hillebrand et al. 2007) and stimulating greater plant growth. Nevertheless, higher fertilization did not seem to benefit to plant cover in the present survey, probably because of eutrophication phenomenon, involving water turbidity that would have limited light availability.

Finally, our results reemphasize the tight trophic interaction that exists between marine and terrestrial ecosystems at the sub-Antarctic islands (Smith 1985; Convey and Lebouvier 2009). Elephant seals may additionally structure aquatic plant communities through trampling and soil compaction (Tejedo et al. 2009) although this effect was not considered in our study. To this end, this suggests that decrease in sea animal populations, as observed for example in Macquarie Islands (Hindell et al. 2017), could have serious impacts on the plant community dynamics in ponds (Jenkin 1997; Erskine et al. 1998).

#### *Physical and chemical parameters of ponds determine plant community composition*

Our result showed how the physical and chemical parameters of ponds could drive plant community composition. This abiotic control appeared slightly stronger in summer, *i.e.* the growing season, than in winter, and was mostly attributed to the availability of nutrients in sediments ( $P_{\text{bio}}$ ,  $N_{\text{tot}}$ ) and to the variance in daily water temperature within ponds, regardless of the sampling season and the year. In Kerguelen, water temperature appears independent from pond area and water depth (Douce et al., 2023). Ponds with higher variation in water temperature among days were preferentially inhabited by *J. scheuchzerioides*, suggesting that this aquatic species could be more tolerant than the five others to the instability of temperatures resulting from climate changes of Kerguelen, although this assertion remains to be tested. This finding additionally raises the question of the ability of the other species to

cope with these changes in the upcoming years (Lebouvier et al. 2011). Aquatic plant species found in the sub-Antarctic region have restricted distribution areas (*R. moseleyi* may even be endemic to Kerguelen) (Hennion et al. 1994; Chau et al. 2021), and conservation issues are also of concern in the face of climate change. More widely, since aquatic plants play a major role in the functioning of freshwater ecosystems (Jeppesen et al. 1997; Iacarella et al. 2018; Reitseman et al. 2018; Lürig et al. 2020), the resistance of pond ecosystem to the on-going rising of water temperature is highly questionable.

A new finding emanated from our study, which demonstrated that the past abiotic conditions in ponds acted more significantly on the present plant community composition than present abiotic conditions. However, and contrary to our expectations, these abiotic conditions did not prevail on the past plant community composition in determining the present plant community composition. Indeed, it may be expected that under the harsh abiotic conditions of Kerguelen combined to the high variability in pond abiotic parameters observed through years and seasons, abiotic conditions would be the strongest determinant of plant community composition. The effect of past plant community composition in aquatic plants can be explained by two non-mutually exclusive hypotheses related to their clonal properties (as studied communities were dominated by perennials). First, aquatic plants display both ramets and connections of which lifespans exceed the year, ensuring the stability of their population sizes (Winkler and Fischer 2002; Stöcklin and Winkler 2004) within ponds. Connections are in particular involved in resources storing (carbohydrates) through time, to support further individual growth (Stuefer and Huber 1998, 1999). Second, clonal plants are known to integrate their past interactions and adjust their present growth accordingly (Turkington et al. 1991; Bittebiere and Mony 2015), ultimately determining their persistence within the present community (Bittebiere and Mony 2015). This process is based on the availability of resources: ramets share information on resource depletion by neighbors within



the clone, depending on the integration distance that mimic the duration of clone memory of the habitat conditions (Louâpre et al. 2012). Therefore, longer term studies are needed to precisely determine the actual duration of the influence exerted by abiotic and biotic parameters on pond plant communities.

## *Conclusions*

To our knowledge, this study is the first focusing on pond ecosystems at Kerguelen. It provides new insights on the relationships between the dynamics and functioning of pond plant communities and their habitat, which can serve as a basis for future studies dealing with the impact of climate change within the whole sub-Antarctic region. Indeed, other archipelagos like Prince Edward Islands (Chau et al., 2021), and Heard and MacDonald Islands (Bergstrom and Selkirk 2000) harbor the same aquatic species and pond habitats.

In particular, we have demonstrated the importance of past abiotic and biotic parameters on pond plant communities, highlighting the need for long-term studies dedicated to these threatened ecosystems, especially in a context of climatic warming. Our study additionally showed that water temperature variability influences plant community composition and subsequently the whole pond ecosystem. This result motivates for conducting new studies on the mechanisms underlying the responses of aquatic plants to temperature by investigating how thermal stress may affect plant growth (Riis et al. 2012) and physiological strategies (Arbona et al. 2013).

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855

856

857 **Table 1.** Description of the surveyed ponds at the three locations. Means and ranges of pond  
858 size were calculated based on measurements replicated on three consecutive years.

	Molloy	Isthme Bas	Cap Ratmanoff
Latitude, longitude	49.36°S - 70.06°E	49.36°S - 70.24°E	49.32°S - 70.53°E
Mean altitude of ponds above sea ( $\pm$ SD, m, $N = 15$ )	11.2 (8.4)	3.4 (2.6)	8.8 (3.6)
Mean pond size ( $\pm$ SD, m <sup>2</sup> , $N = 45$ )	58.9 ( $\pm$ 56.9)	140.3 ( $\pm$ 344.7)	54.0 ( $\pm$ 62.9)
Pond size range (min-max, m <sup>2</sup> , $N = 45$ )	2.4 – 166.5	4.8 – 1,252.2	2.9 – 164.6

859

860 **Table 2.** Variations of plant community indexes in space (location) and time (year, season) assessed with mixed-models and pond as random  
861 factor. The significance of the explanatory variables was determined by ANOVA tests. Marginal R<sup>2</sup> indicates the percentage of index variance  
862 explained by the fixed effects; Conditional R<sup>2</sup> indicates the percentage of index variance explained by the whole model (including fixed and  
863 random effects).

	Marginal R <sup>2</sup>	Conditional R <sup>2</sup>	Year (df=1)		Season (df=1)		Location (df=2)		Year×Season (df=1)		Year×Location (df=2)		Season×Location (df=2)		Year×Season×Location (df=2)	
			X <sup>2</sup>	P	X <sup>2</sup>	P	X <sup>2</sup>	P	X <sup>2</sup>	P	X <sup>2</sup>	P	X <sup>2</sup>	P	X <sup>2</sup>	P
Richness	0.07	0.48	6.1	*	1.7	ns	2.8	ns	0.2	ns	2.3	ns	0.5	ns	2.3	ns
□																
Evenness	0.07	0.50	3.3	t	12.8	**	1.2	ns	0.7	ns	2.3	ns	1.0	ns	0.3	ns

864 Notes:  $t 0.1 < P < 0.05$ , \*  $P < 0.05$ ; \*\*\*  $P < 0.001$ ; ns = not significant. □ square-root-transformed.

865

866 **Table 3.** Mean ( $\pm$ SD) abiotic parameters measured in the ponds of Iles Kerguelen depending on the year, season, and location of sampling.

867 Values in italic indicate the maximum and the minimum (*i.e.* the range) recorded for each season per year on each location.

Date	Location	Mean Temp. (°C)	Temp. variance (°C <sup>2</sup> )	Water depth (cm)	Conduct. ( $\mu$ S.cm <sup>-1</sup> )	pH	DO (mg.L <sup>-1</sup> )	N-NH <sub>4</sub> <sup>+</sup> ( $\mu$ g.L <sup>-1</sup> )	N-NO <sub>3</sub> <sup>-</sup> ( $\mu$ g.L <sup>-1</sup> )	P-PO <sub>4</sub> <sup>3-</sup> ( $\mu$ g.L <sup>-1</sup> )	P <sub>bio</sub> (mg.g <sup>-1</sup> )	C/N	N <sub>tot</sub> (g.g <sup>-1</sup> )
Summer 2018	Isthme	4.55	2.72	16.07	240.23	7.00	7.25	233.27	201.14	24.98	0.88	13.84	0.017
	Bas	( $\pm 1.34$ )	( $\pm 2.10$ )	( $\pm 11.75$ )	( $\pm 85.82$ )	( $\pm 0.81$ )	( $\pm 4.73$ )	( $\pm 298.24$ )	( $\pm 268.35$ )	( $\pm 38.97$ )	( $\pm 0.59$ )	( $\pm 2.35$ )	( $\pm 0.007$ )
		<i>6.34-</i>	<i>7.66-0.57</i>	<i>50.53-</i>	<i>452.00-</i>	<i>8.98-</i>	<i>14.65-</i>	<i>1597.00-</i>	<i>1379.50-</i>	<i>197.85-</i>	<i>2.26-</i>	<i>19.99-</i>	<i>0.032-</i>
		<i>1.82</i>		<i>3.60</i>	<i>142.35</i>	<i>5.88</i>	<i>0.08</i>	<i>76.60</i>	<i>45.50</i>	<i>0.00</i>	<i>0.17</i>	<i>8.95</i>	<i>0.003</i>
	Molloy	4.64	6.03	15.04	155.66	7.04	9.84	566.13	254.30	25.65	0.79	12.63	0.013
		( $\pm 1.95$ )	( $\pm 1.48$ )	( $\pm 7.55$ )	( $\pm 51.85$ )	( $\pm 0.56$ )	( $\pm 4.86$ )	( $\pm 1415.07$ )	( $\pm 329.60$ )	( $\pm 54.60$ )	( $\pm 0.56$ )	( $\pm 1.43$ )	( $\pm 0.007$ )
		<i>6.92-</i>	<i>9.34-3.59</i>	<i>35.11-</i>	<i>336.80-</i>	<i>8.07-</i>	<i>15.90-</i>	<i>7545.00-</i>	<i>1275.50-</i>	<i>221.97-</i>	<i>2.34-</i>	<i>17.32-</i>	<i>0.029-</i>
		<i>1.89</i>		<i>4.22</i>	<i>102.54</i>	<i>6.10</i>	<i>0.15</i>	<i>59.55</i>	<i>28.00</i>	<i>0.00</i>	<i>0.00</i>	<i>10.06</i>	<i>0.002</i>
	Cap	4.41	4.05	10.66	255.34	6.73	5.77	924.19	236.54	33.95	0.83	11.75	0.021
	Ratmanoff	( $\pm 1.63$ )	( $\pm 1.08$ )	( $\pm 4.86$ )	( $\pm 77.13$ )	( $\pm 0.74$ )	( $\pm 4.16$ )	( $\pm 1677.30$ )	( $\pm 435.32$ )	( $\pm 50.44$ )	( $\pm 0.58$ )	( $\pm 1.25$ )	( $\pm 0.009$ )
		<i>6.55-</i>	<i>6.02-2.15</i>	<i>27.50-</i>	<i>482.00-</i>	<i>9.09-</i>	<i>17.95-</i>	<i>7840.00-</i>	<i>2341.00-</i>	<i>199.55-</i>	<i>1.95-</i>	<i>13.87-</i>	<i>0.043-</i>
		<i>2.15</i>		<i>1.47</i>	<i>164.46</i>	<i>5.58</i>	<i>0.14</i>	<i>68.10</i>	<i>29.50</i>	<i>0.95</i>	<i>0.01</i>	<i>8.02</i>	<i>0.006</i>
Winter 2019	Isthme	3.31	3.00	14.10	238.10	6.78	8.57	95.48	103.31	31.38	0.70	16.38	0.015
	Bas	( $\pm 1.46$ )	( $\pm 2.43$ )	( $\pm 8.83$ )	( $\pm 117.77$ )	( $\pm 1.23$ )	( $\pm 4.39$ )	( $\pm 123.55$ )	( $\pm 39.55$ )	( $\pm 35.80$ )	( $\pm 0.32$ )	( $\pm 2.26$ )	( $\pm 0.006$ )
		<i>5.17-</i>	<i>9.87-0.52</i>	<i>37.33-</i>	<i>605.20-</i>	<i>10.25-</i>	<i>13.54-</i>	<i>681.50-</i>	<i>198.50-</i>	<i>147.62-</i>	<i>1.24-</i>	<i>21.69-</i>	<i>0.029-</i>
		<i>0.31</i>		<i>2.33</i>	<i>125.86</i>	<i>3.62</i>	<i>0.15</i>	<i>22.10</i>	<i>35.50</i>	<i>1.25</i>	<i>0.15</i>	<i>12.03</i>	<i>0.003</i>
	Molloy	2.66	6.64	14.76	121.07	6.70	10.11	117.56	583.78	13.74	0.72	14.40	0.010
		( $\pm 1.91$ )	( $\pm 2.10$ )	( $\pm 8.00$ )	( $\pm 48.22$ )	( $\pm 0.60$ )	( $\pm 4.33$ )	( $\pm 247.10$ )	( $\pm 1085.69$ )	( $\pm 35.39$ )	( $\pm 0.37$ )	( $\pm 1.36$ )	( $\pm 0.005$ )
		<i>5.04-</i>	<i>12.03-3.25</i>	<i>29.20-</i>	<i>230.34-</i>	<i>7.70-</i>	<i>16.14-</i>	<i>1401.70-</i>	<i>4793.00-</i>	<i>181.17-</i>	<i>1.45-</i>	<i>17.45-</i>	<i>0.022-</i>
		<i>0.30</i>		<i>0.83</i>	<i>66.92</i>	<i>5.28</i>	<i>0.15</i>	<i>8.15</i>	<i>50.50</i>	<i>0.00</i>	<i>0.04</i>	<i>11.49</i>	<i>0.003</i>
	Cap	2.79	4.46	12.48	184.62	6.63	8.44	112.08	267.74	20.90	0.71	14.05	0.014
	Ratmanoff	( $\pm 1.63$ )	( $\pm 1.49$ )	( $\pm 4.23$ )	( $\pm 53.31$ )	( $\pm 0.38$ )	( $\pm 4.17$ )	( $\pm 111.13$ )	( $\pm 711.41$ )	( $\pm 35.12$ )	( $\pm 0.38$ )	( $\pm 1.76$ )	( $\pm 0.006$ )
		<i>5.20-</i>	<i>7.76-1.95</i>	<i>22.93-</i>	<i>319.00-</i>	<i>7.55-</i>	<i>15.84-</i>	<i>651.05-</i>	<i>3813.00-</i>	<i>152.66-</i>	<i>1.51-</i>	<i>18.66-</i>	<i>0.025-</i>
		<i>0.33</i>		<i>5.00</i>	<i>116.84</i>	<i>5.70</i>	<i>0.14</i>	<i>29.55</i>	<i>25.00</i>	<i>2.08</i>	<i>0.17</i>	<i>10.89</i>	<i>0.004</i>



Summer 2019	Isthme	5.68	4.51	17.00	197.00	6.85	10.87	71.61	75.38	28.24	0.70	16.11	0.015
	Bas	(±2.37)	(±4.02)	(±9.25)	(±29.18)	(±0.70)	(±2.74)	(±61.49)	(±24.59)	(±21.27)	(±0.45)	(±2.13)	(±0.005)
		9.77-	16.82-0.62	44.93-	275.33-	8.47-	16.83-	324.50-	136.50-	79.62-	1.70-	20.92-	0.026-
		2.12		2.80	147.86	5.41	4.40	31.00	13.50	4.30	0.23	12.25	0.004
	Molloy	6.44	9.99	18.63	141.47	7.21	12.99	629.84	72.15	6.97	0.96	15.23	0.010
		(±3.50)	(±4.40)	(±6.57)	(±115.35)	(±0.86)	(±2.76)	(±1195.31)	(±54.43)	(±6.72)	(±0.60)	(±2.42)	(±0.005)
		10.99-	20.51-3.93	32.73-	688.00-	9.53-	19.03-	5630.50-	259.50-	32.53-	2.25-	19.92-	0.019-
	Cap Ratmanoff	2.20		8.25	73.52	6.00	6.39	51.00	21.50	0.00	0.09	10.63	0.002
		5.88	6.71	13.84	198.35	6.89	11.91	770.34	380.83	18.62	0.65	14.86	0.014
		(±2.87)	(±3.05)	(±5.92)	(±81.01)	(±0.48)	(±1.89)	(±1566.66)	(±1121.00)	(±33.20)	(±0.40)	(±1.41)	(±0.006)
		9.27-	13.22-2.35	28.33-	411.40-	8.23-	17.09-	7330.00-	4590.00-	145.38-	1.69-	17.76-	0.022-
		2.40		4.00	5.70	5.61	9.03	31.50	23.00	0.00	0.11	11.19	0.003
Winter 2020	Isthme	5.04	3.72	22.65	212.45	7.11	10.40	126.34	116.59	49.57	0.59	14.29	0.018
	Bas	(±1.60)	(±3.33)	(±13.93)	(±43.76)	(±0.41)	(±2.19)	(±46.37)	(±102.82)	(±50.71)	(±0.39)	(±1.83)	(±0.007)
		7.37-	13.94-0.51	67.07-	333.60-	8.09-	14.88-	223.00-	425.00-	237.60-	1.52-	18.65-	0.041-
		2.22		6.43	151.18	6.39	4.62	65.50	3.00	5.01	0.01	9.72	0.004
	Molloy	5.41	8.24	17.41	112.13	6.83	10.97	395.76	180.07	7.04	0.59	13.61	0.011
		(±2.36)	(±3.68)	(±7.84)	(±54.82)	(±0.46)	(±2.90)	(±804.68)	(±324.14)	(±6.86)	(±0.35)	(±1.48)	(±0.005)
		8.34-	16.99-3.21	34.67-	332.67-	8.10-	14.15-	3334.50-	1575.00-	33.61-	1.65-	17.47-	0.027-
	Cap Ratmanoff	2.31		4.00	54.94	6.08	1.70	37.50	3.00	0.00	0.07	11.30	0.004
		5.04	5.54	13.79	192.86	6.80	10.93	299.50	77.70	5.29	0.59	12.48	0.018
		(±1.95)	(±2.54)	(±6.86)	(±53.34)	(±0.64)	(±3.17)	(±908.52)	(±182.17)	(±4.21)	(±0.37)	(±2.39)	(±0.008)
		7.37-	10.96-1.92	31.33-	326.00-	7.68-	14.38-	4836.00-	1030.50-	21.50-	1.48-	16.86-	0.034-
		2.48		4.00	120.98	4.63	3.86	21.00	5.00	0.00	0.14	8.05	0.005

868 Notes: Temp. temperature; Conduct. specific conductance; Pbio: phosphore bioavailable. N = 30.

869 **Table 4.** Relationships between the plant community composition and the physical and  
870 chemical parameters recorded in ponds at each sampling date. Parameters from the  
871 permutation test in STATICO analysis are given with  $R_v$  quantifying the strength of the  
872 relationship (on a 0-1 scale), and *simulated-P* as the statistical significance of the observed  
873  $R_v$ .

	$R_v$	<i>Simulated-P</i>
summer 2018	0.285	***
winter 2019	0.229	**
summer 2019	0.276	***
winter 2020	0.238	**

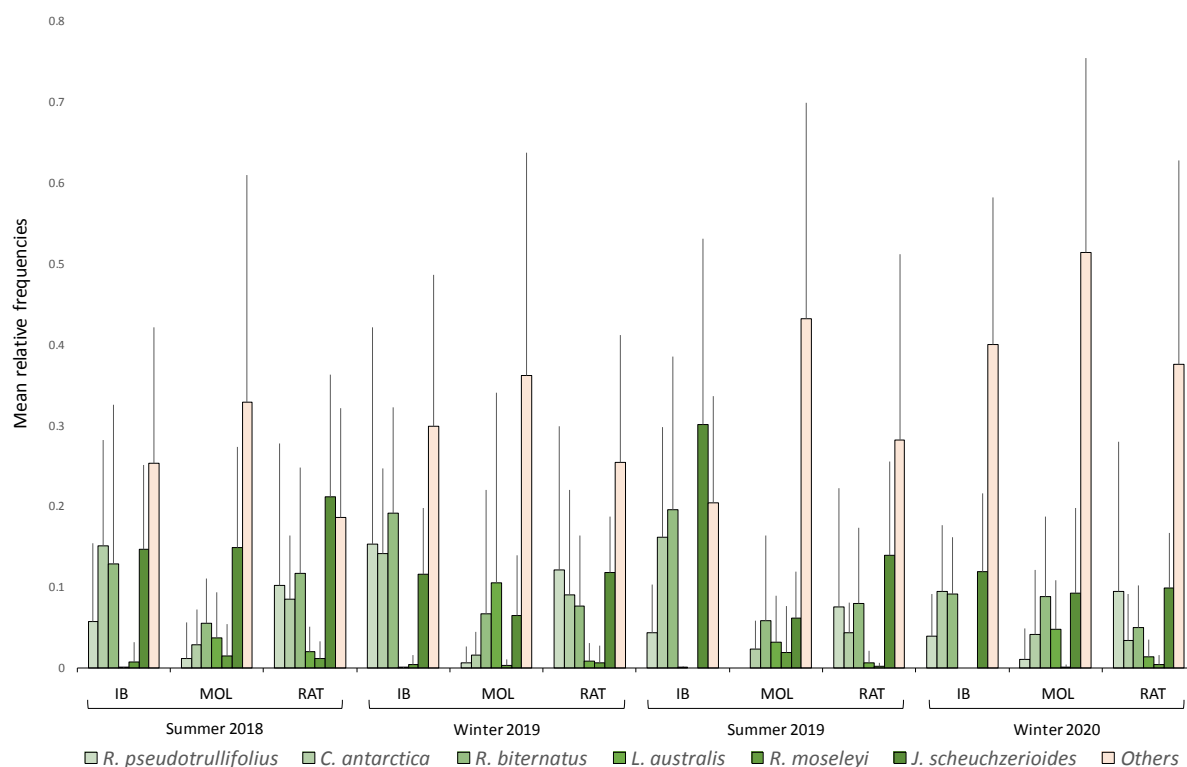
874 *Notes: \*\*  $P < 0.01$ ; \*\*\*  $P < 0.001$ .*

875

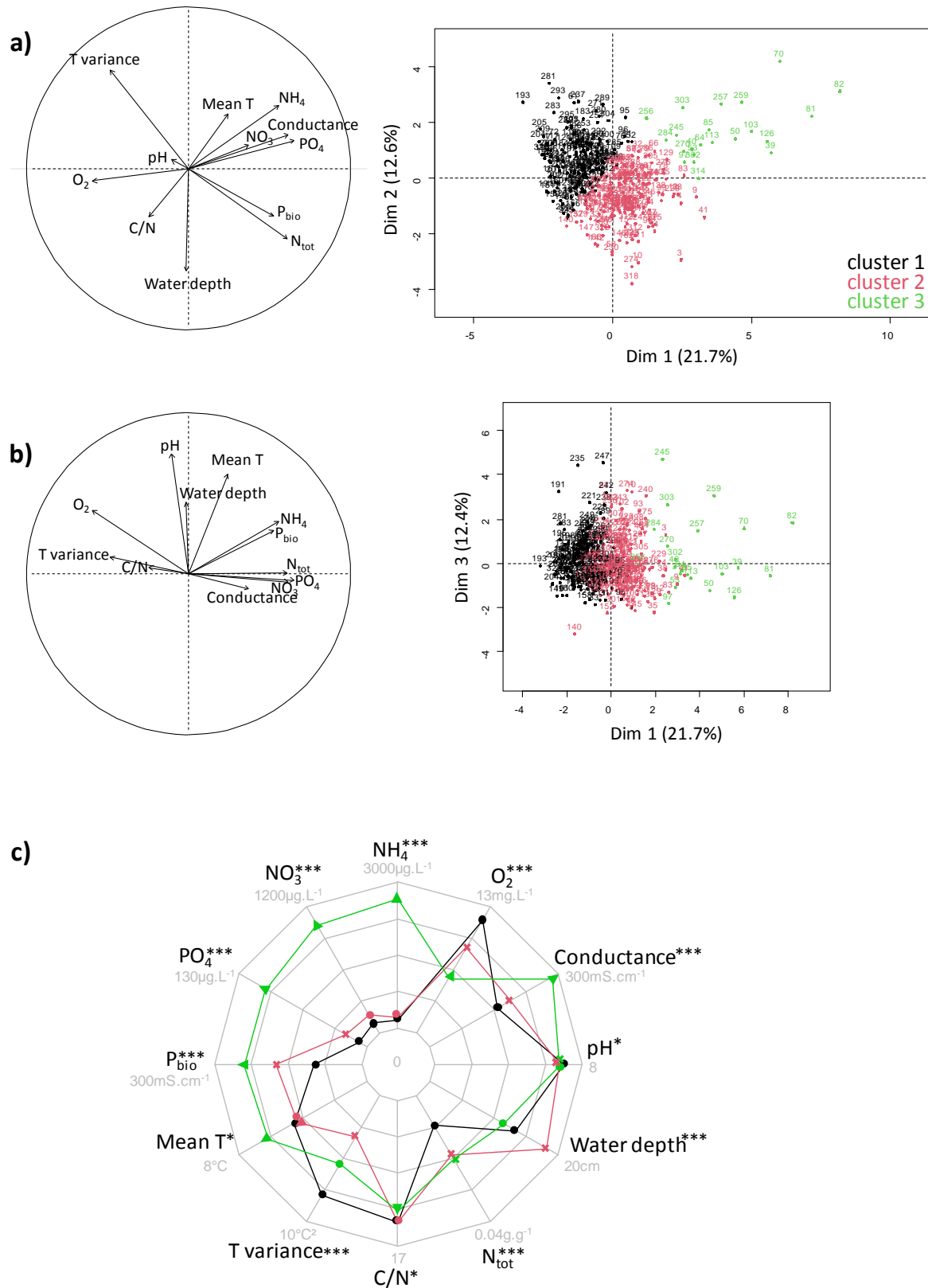
876 **Table 5.** Relationships between present plant community composition *i.e.* recorded during the  
877 last campaign of survey (summer 2020), and past plant community compositions (PCC), and  
878 past and present physical and chemical parameters (PCP, *i.e.* recorded during summer 2020  
879 and the preceding seasons). *R<sub>v</sub>*: strength of the relationship; *simulated-P*: statistical  
880 significance of the observed *R<sub>v</sub>*.

	<i>R<sub>v</sub></i>	<i>Simulated-P</i>
PCC, winter 2020	0.575	<0.001
PCC, summer 2018	0.568	<0.001
PCC, summer 2019	0.553	<0.001
PCC, winter 2019	0.458	<0.001
PCP, winter 2019	0.227	<0.030
PCP, summer 2019	0.216	<0.040
PCP, winter 2020	0.196	ns
PCP, summer 2020	0.191	ns
PCP, summer 2018	0.179	ns

881



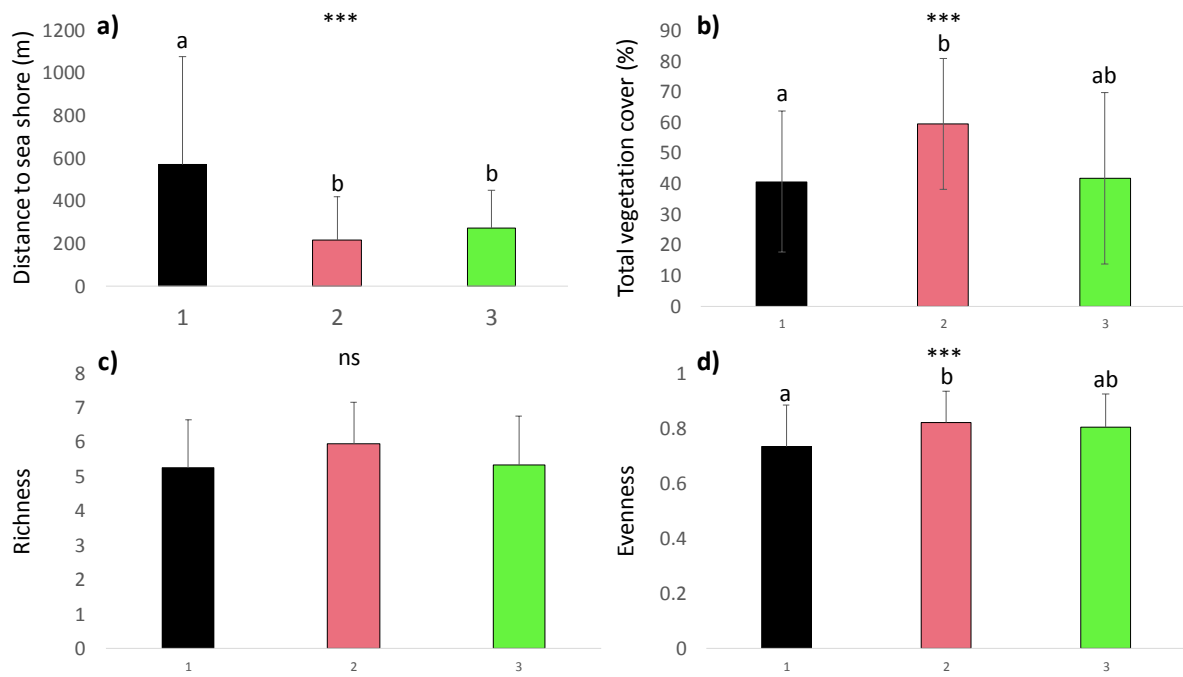
**Figure 1.** Mean ( $\pm$ SD) composition of pond plant communities depending on the season and year. Varying shades of green represent different aquatic plant species (see accompanying legend), while light orange was used to represent relative frequencies of all non-aquatic species. IB: Isthme Bas, MOL: Molloy, RAT: Cap Ratmanoff.



888

889 **Figure 2.** Principal component analysis and hierarchical clustering performed on the abiotic  
 890 parameters of all ponds. Abiotic parameters and pond scores projected along a) axes 1 and 2,  
 891 and b) axes 1 and 3. c) Mean abiotic parameters characterizing the clusters shown with

892 different colors). Asterisks indicate significant difference of mean abiotic parameters between  
893 clusters (ANOVA tests) \* $P < 0.05$ ; \*\*\*  $P < 0.001$ , different symbols indicate different means  
894 (Tukey post-hoc tests). Tvariance – temperature variance, mean T – water temperature,  $O_2$  –  
895 dissolved oxygen,  $P_{bio}$  – bioavailable P. In a) and b) different numbers refers to different  
896 ponds at varying dates of sampling, and within different locations ( $N = 360$  in total).  
897



**Figure 3.** Mean ( $\pm$ SD) cluster values (labelled from 1 to 3 along the first axis of the PCA performed on abiotic parameters) for a) distance from sea shore, b) percentage of vegetation cover within the pond, c) plant community richness, d) plant community evenness. Asterisks above the figures indicate the significance of the clustering on the response variable (ANOVA tests), ns  $P > 0.05$ ; \*\*\*  $P < 0.001$ , different letters indicate significant differences in mean response variable among clusters (Tukey post-hoc tests).