Transitional dynamics in macrobenthic diversity and submerged plant growth: disentangling environmental and habitat causes in a subtropical coastal lagoon

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Title

Transitional dynamics in macrobenthic diversity and submerged plant growth: disentangling environmental and habitat causes in a subtropical coastal lagoon

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Abstract

Coastal lagoons are highly dynamic transitional ecosystems where mechanisms driving temporal changes in structure and functioning are highly complex. We examined changes in macrozoobenthic assemblages, climatic trends, and environmental conditions, including salinity, water depth, temperature, nutrients, and submerged macrophyte biomass, over a seven-year environmental monitoring dataset (2018–2024) in Garzón, a coastal lagoon in Uruguay. This period was characterized by regional climatic anomalies providing a broader context for the observed ecological dynamics. Two distinct phases in assemblage structure were identified: 2018–2022, characterized by high environmental variability and dominance of the estuarine gastropod *Heleobia* aff. *australis*, and 2023–2024, marked by stable low salinity, increased water depth, excessive growth of the submerged macrophyte *Myriophyllum quitense*, and the rise of freshwater-tolerant opportunistic taxa such as *H. parchappii* and chironomids. Breakpoint analysis identified a shift in benthic structure between 2021 and 2023. IS-lasso models revealed concomitant effects of multiple physico-chemical drivers on benthic species abundance and disentangled the influence of environmental variables from habitat effects driven by macrophyte presence. Between 2018 and 2022, species responses were influenced by salinity and temperature, whereas in 2023–2024, responses were dominated by a set of factors including macrophyte biomass. Overall, our results demonstrate how monitoring data can be used to detect structural reorganization, assess ecosystem stability, and identify environmental conditions associated with increased ecological risk.

Keywords: benthic community; temporal changes; monitoring; aquatic vegetation; transitional ecosystems

Introduction

Coastal lagoons are transitional environments increasingly threatened by multiple disturbance factors that undermine their ecological integrity. Changes in land use and the expansion of coastal development worldwide have substantially elevated nutrients and sediment inputs into estuarine and coastal habitats triggering negatively impacts on biodiversity and ecosystem functioning, and consequent loss of ecosystem services (Galloway and Cowling 2002). The influx of anthropogenic nutrients has driven widespread eutrophication causing contamination and the subsequently enhancement of primary production, leading to a decline in ecosystem health (Cloern, 2001). Given their association with contiguous draining basin and limited exchange with adjacent ocean waters, coastal lagoons are particularly vulnerable to nutrient inputs from agricultural runoff (Cloern et al. 2016; Rodríguez-Gallego et al. 2017; Fernández-Alías et al. 2022), which can cause significant shifts in benthic communities and disrupt entire trophic webs (Pérez-Ruzafa et al. 2019).

Additionally, global climate change resulting in rising water temperatures, affecting precipitations and increased export of freshwater and nutrients (Jeppesen et al. 2009; Ockenden et al. 2017; Meerhoff et al. 2022) are expected to exacerbate coastal eutrophication in the future years (Rabalais et al. 2009; Anthony et al. 2009). Such conditions amplify seasonal and interannual variability in key physicochemical parameters including salinity and transparency, altering ecosystem structure including composition and distribution of submerged macrophyte assemblages (Rodríguez-Gallego et al. 2010; Christia et al. 2018) with relevant consequences for benthic communities structures and ecosystem functioning (Rodríguez-Gallego et al. 2010; Ouisse et al. 2011; Jones et al. 2022).

Macrobenthic fauna plays a fundamental role in coastal ecosystems by contributing to nutrient cycling, water filtration, organic matter decomposition, food web dynamics, and sediment bioturbation (Pearson and Rosenberg 1978; Zaldívar et al. 2008). Their high diversity, relatively long life cycles, low mobility, and differing sensitivities to environmental conditions make them effective indicators of ecological integrity (Borja et al. 2000; Mir et al. 2025; Saddiki et al. 2025). Changes in their abundance, biomass, and diversity often reflect ecological stress caused by both natural and anthropogenic drivers (Coelho et al. 2015; Lacoste et al. 2023). Furthermore, disturbance events such as hypoxia, terrestrial runoff, macroalgal blooms, and changes in water exchange with the adjacent ocean can strongly influence ecological processes affecting functional and taxonomic diversity of benthos, as well as nutrient fluxes in coastal lagoons (Zaldívar et al. 2008; Lacoste et al. 2023). Consequently, temporal studies are critical to understanding shifts in benthic communities and accurately detecting changes in ecological status (Pérez-Ruzafa et al. 2007; Ligorini et al. 2022; Giampaoletti et al. 2025). However, there is still a notable lack of studies using a multi-year approach addressing the ecological responses of transitional ecosystems, particularly regarding benthic communities (Pitacco et al. 2018; Giampaoletti et al. 2025).

Along Uruguay's Atlantic coast, there are located a series of coastal lagoons that experience periodic connections with the ocean, leading to fluctuating salinity regimes that strongly determines the composition and abundance of aquatic flora and fauna, including benthic communities (Bonilla et al. 2005; Meerhoff et al. 2013; Rodríguez-Gallego et al. 2015; Amaral et al. 2016). Since 1974, nutrient inputs into these lagoons have increased due to intensified land use in their catchment areas, where agricultural

activities have expanded significantly (Rodríguez-Gallego et al. 2017). Moreover, as a response to touristic urbanization, in 2015 a bridge was built over the sand bar of the lagoon. Unexpectedly, in the summer of 2023, the native submerged macrophyte *Myriophyllum quitense* rapidly and massively colonized the previously unvegetated lagoon, significantly altering ecosystem services and generating widespread public concern. This phenomenon was unique to Laguna Garzón, with no comparable changes observed in the nearby coastal lagoons.

In this lagoon, as in other coastal lagoons in Uruguay, macrozoobenthos is generally characterized by a low number of species (Giménez et al. 2005, 2006), with macroinfaunal diversity and abundance closely linked to sediment characteristics (Giménez et al. 2006). Despite considerable temporal variability in abundance, community structure has remained relatively stable over time, as documented in previous temporal studies (Giménez et al. 2005; Meerhoff et al. 2013; Bergamino et al. 2025). However, it remains unclear whether and how benthic fauna in coastal lagoons respond to shifts between contrasting ecosystem states, particularly in terms of changes in species abundance, replacement, and persistence within communities (e.g., Giampaoletti et al. 2025).

These dynamics highlight the need for holistic approaches that combine temporal ecological understanding including climatic variability and habitat heterogeneity, in order to anticipate and respond to ecosystem shifts driven by both natural variability and human pressures (Ligorini et al. 2022). In this context, we focus on patterns of changes in both environmental variables and in the abundance of dominant macrozoobenthic species, during the process of growth of submerged *M. quitense* plants, which covered the entire

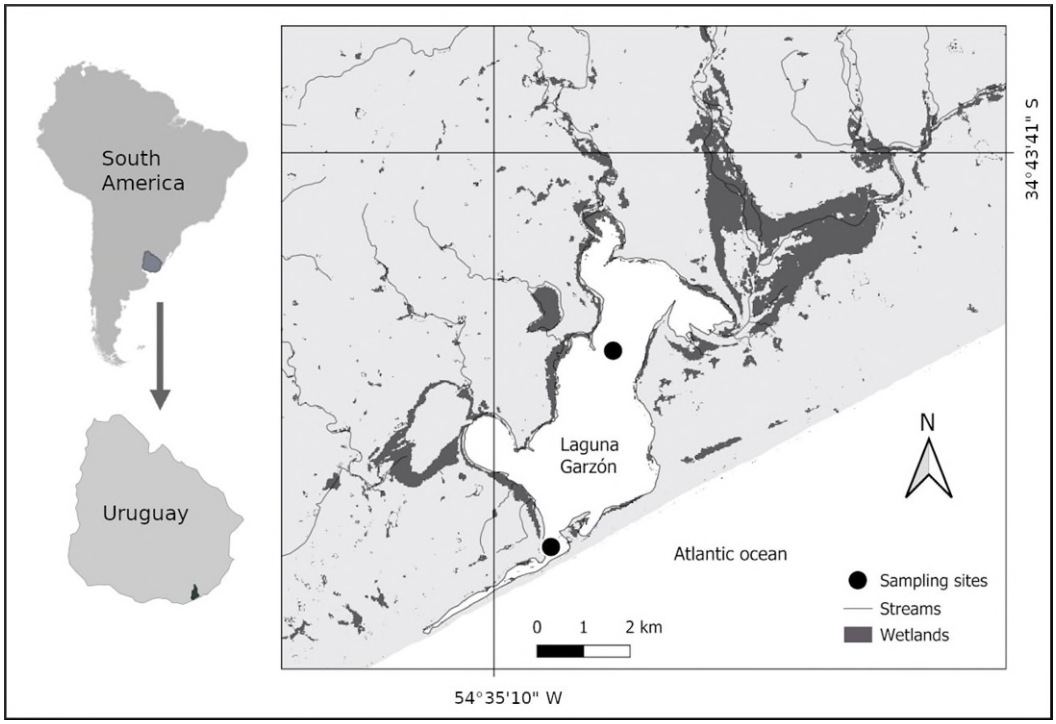
coastal Laguna Garzón in less than a year. Specifically, we examined how variations in macrozoobenthic abundance and biodiversity were associated with changes in environmental parameters, and identified the species associated with such changes. Based on seven seasonal monitoring, and by applying a multivariate approach, we evaluated the relative importance of physico-chemical drivers versus habitat changes derived from macrophyte proliferation in explaining the observed patterns of macrozoobenthic assemblages structure. A temporal (pre- vs. post-) framework remains relatively underused in benthic ecology, where studies more commonly rely on spatial control–impact comparisons (Lacoste et al., 2023), yet this approach is particularly valuable to capture ecosystem responses to abrupt habitat changes such as submerged macrophyte proliferation. This integrated assessment seeks to advance our understanding of ecological trajectories and their responses in transitional ecosystems facing increasing anthropogenic and climatic pressures.

Methods

Study area

Laguna Garzón (33°36′01″S, 26°54′01″E) is a coastal lagoon located along the southwestern shoreline of Uruguay (Fig. 1). Classified as a choked lagoon, it is intermittently connected to the Atlantic Ocean and primarily fed by three tributaries, with Arroyo Garzón, located in the northern sector, being the main freshwater contributor. The lagoon has a shallow average depth of approximately 1.6 m (Rodríguez-Gallego et al. 2017), covers a surface area of 18 km², and drains a watershed of 695 km² (Rodríguez-Gallego et al. 2017). It is a mixohaline brackish system, with salinity levels decreasing from seaward to landward (ranging between 0.2 and 26.2 PSU), influenced by proximity to the sandbar located along its southern margin (Rodríguez-Gallego et al. 2017). Like

153 other estuarine systems in the region, Laguna Garzón is subject to a highly variation in
154 its connection to the sea. Bar openings are generally more frequent during autumn to
155 spring months. The artificial opening of the sandbar of Laguna Garzón is periodically
156 carried out by the local municipality, mainly during winter, in order to manage water
157 levels.
158



159

160 **Fig. 1.** Location of study sites within Laguna Garzón, east coast of Uruguay.
161

162 **Field and laboratory activities**

163 Sampling was conducted in Laguna Garzón (Rocha, Uruguay), as part of the
164 Interinstitutional Monitoring Program of Coastal Lagoons of Uruguay, coordinated by
165 the Ministry of the Environment (Dirección Nacional de Control y Evaluación
166 Ambiental) in collaboration with Universidad de la República (Centro Universitario
167 Regional del Este), the National Public Waters Agency (OSE), the Municipality of Rocha
168 and the Ministery of Livestok, Agriculture and Fishery (Dirección Nacional de Recursos

169 Acuáticos). Field campaigns were seasonally distributed (winter, spring, summer, and
170 autumn), with sampling occurring from 2018 to 2024. The monitoring in Laguna Garzón
171 consists of two sampling stations located along a spatial gradient from seaward to
172 landward zones (Fig. 1), and encompassing a range of environmental conditions
173 previously described by Meerhoff et al. (2013) and Rodríguez-Gallego et al. (2017),
174 among other authors. The seasonal sampling design over multiple years at two contrasting
175 sites helps capturing the temporal dynamics of the system, while keeping a simple and
176 low-cost long-term monitoring programme. Sample size consisted of 28 data per variable
177 at each sampling site (n=56), without replication (except for plants biomass, see the
178 corresponding section). The nearest meteorological field station is located in Rocha
179 (Estación Meteorológica de Rocha del Instituto Uruguayo de Meteorología). Rainfall in
180 this study is presented as daily data. The cumulative values of the 30 days prior to
181 sampling were determined and used in further analysis. Annual precipitation anomalies
182 were calculated relative to the 35-year historical mean. Positive anomalies reflect years
183 with higher precipitation than the long-term average, while negative anomalies indicate
184 years that were drier than normal conditions.
185
186 **Water and sediment**
187 At each sampling site, *in situ* salinity, turbidity, pH, temperature, and dissolved oxygen
188 (expressed as % saturation) were measured with a multi-parameter sensor (Horiba U-52).
189 A 5-L sub-surface water sample was collected at each lagoon site and kept fresh in the
190 dark (for approximately three hours) until laboratory processing, where water was filtered
191 to determine chlorophyll *a* (Chl-*a*), dissolved nutrients, suspended solids, and organic
192 matter. Chl-*a* concentration (µg/L) was determined by spectrophotometry according to
193 Jespersen and Christoffersen (1987) using GF/F Whatman filters. Water filtered through

GF/C filters was used to determine ammonium (NH_4^+; detection limit of 10 µgN/L; Koroleff, 1970), nitrite (NO_2^-; detection limit of 1 µgN/L; Bendschneider and Robinson, 1952), nitrate (NO_3^-; detection limit of 10 µgN L-1; Mackereth et al. 1978), and phosphate (PO_4^{3-}; detection limit of 10 µgP/L; Murphy and Riley 1962). Total nutrients were determined in unfiltered water, previously digested by the Valderrama (1981) procedure, and analyzed according to Murphy and Riley (1962) for phosphorus (TP; detection limit of 10 µgP/L) and Mackereth et al. (1978) for nitrogen (TN; detection limit of 10 µgN/ L). Silicate (SiR; detection limit 2.8 µg/L) was analysed in unfiltered water samples according to the method of Müllin and Riley (1955). All nutrients forms were analysed spectrophotometrically.

Total suspended solids (TSS) content was determined by filtering of water onto GF/C Whatman filters and by weight difference after drying at 105 °C for 24 h, while the suspended solid organic matter (SSM) was obtained by weight difference after ignition of the previously dried filters at 498 °C for 30 min.), according to APHA (1985).

For sediment determination, subsamples from an undisturbed grab sample were collected using an Ekman grab (0.05 m^2) penetrating 10 mm deep into the sediment at each sampling station and sampling date for subsequent analyses. To determine chlorophyll *a* in sediments (Chl-a sed), the top 0.5 cm layer of sediments was collected and estimated following a modification of Lorenzen (1967). To determine organic content and total phosphorus and nitrogen, the top 1 cm layer of sediments was collected following the same procedure. The organic content (OMsed) was determined by weight difference after drying (98 °C for 48 h) and loss on ignition (498 °C for 1 h), respectively (APHA, 1985). Nutrient content in sediments was obtained following a Valderrama (1981) digestion

procedure, with total nitrogen (TNsed) determined using the methods of Muller and Widemann (1955) and total phosphorus (TPsed) by Murphy and Riley (1962).

Macrozoobenthos and submerged aquatic vegetation (SAV)

At each station, one sediment sample was collected seasonally using an Ekman grab sampler (0.05 m²). For a detailed description of the collection methods of macrozoobenthos, see Bergamino et al. (2025). Benthic organisms were sorted, counted, and identified to the lowest possible taxonomic level. Abundances were expressed as individuals per square meter (ind·m^{-2}). At each station, water depth was measured *in situ* using a graduated ruler at the point of sample collection.

Submerged aquatic vegetation (SAV) biomass was included as part of the environmental monitoring throughout the entire study period (2018–2024), using the Ekman grab as a sampler. From 2018 to 2022, no detectable SAV biomass was recorded across sampling sites, and values were consistently zero. Following the rapid colonization of the lagoon by submerged vegetation in 2023, SAV biomass was quantified using triplicate samples at each sampling point, collected with a floating wooden quadrat (0.09 m^2, during the surveys of May, August, and November 2023, and February 2024). All the plant biomass within the water column inside the quadrat was collected and rapidly sealed in a plastic bag, transported on cold, and processed during the sampling day. SAV was consistently dominated by the native *Myriophyllum quitense*, which represented the only macrophyte species recorded across sampling sites, except in the first SAV record were Charophytes were also present. Fresh biomass was weighted after removal of excess water. Subsamples were then oven-dried at 60 °C for 48 h to determine dry weight, and the relationship between fresh and dry biomass was used to estimate total dry plant biomass

per site. These values were extrapolated to 1 m² (following Rodríguez-Gallego et al. 2015).

Data analysis

By means of Principal Component Analysis (PCA) based on the correlation matrix, the variation in the main environmental gradients was identified from standardized variables. Variable contributions to each principal component were quantified, and biplots were generated to visualize relationships among variables and temporal patterns of the samples. Input data for the PCA were standardized (z-score normalization), and observations containing missing values were excluded prior to the analysis.

Beta diversity across years was assessed using the Sørensen dissimilarity index (βsor), calculated from presence/absence data to quantify interannual variation in benthic community composition. This index was decomposed into two components: species turnover (βsim), representing species replacement between years, and nestedness (βnes), indicating species loss patterns that may reflect environmental filtering (Baselga 2010). More precisely, turnover occurs when species present at one location are absent at another one but are replaced by different species absent from the first site. Nestedness corresponds to species that are present at one site are absent at another, but are not replaced by additional species (Dornelas et al. 2014). Pairwise dissimilarity matrices were computed using the betapart package (Baselga and Orme, 2012), based on Sørensen dissimilarities. These matrices were then visualized using hierarchical cluster analysis with the average linkage based on Sørensen distances. To evaluate whether interannual differences in species composition grouped into distinct temporal clusters, a PERMANOVA test was applied to the Sørensen distance matrix. To ensure robustness of these results,

homogeneity of multivariate dispersion among groups was assessed using PERMDISP, allowing verification that observed differences were driven by shifts in community composition rather than differences in within-group variability. Subsequently, a SIMPER (Similarity Percentage) analysis was conducted to identify which species contributed most to the compositional dissimilarity between defined year groups (e.g., 2018–2022 vs. 2023–2024, Clarke and Warwick, 1994), which were defined based on the ecological framework of the study aimed at evaluating the system transition associated with macrophyte expansion (before and after submerged plants growth). For these analyses, species abundance data were transformed using a fourth-root transformation to reduce the influence of highly abundant taxa. The contribution of each species to the observed dissimilarity was quantified and ranked to determine the taxa most responsible for the temporal turnover.

To identify the breakpoints at which species abundance changes taking into account for environmental factors, we conducted a segmented (broken-line) regression analysis using segmented R package (Muggeo, 2008; R Core Team 2023). In this model, the breakpoint was specifically estimated on the time variable (year). Segmented models describe relationships between a response variable and one or more explanatory variables as piecewise linear, consisting of two or more straight-line segments joined at unknown values known as breakpoints. Estimates of the regression parameters, including the coefficients (β) and breakpoints (ψ), were obtained by maximizing the log-likelihood function (Muggeo, 2003). A pscore test (Muggeo, 2016) was applied to evaluate whether the slopes before and after a proposed breakpoint differed significantly.

293 Although lasso regression offers an elegant and widely used approach for simultaneously
294 performing variable selection and parameter estimation, it is well known to have
295 limitations due to the nonsmooth nature of its objective function. This can create
296 challenges, for example, in computing standard errors. To address these drawbacks and
297 enable valid inference, the lasso penalty has been replaced with a smooth alternative,
298 known as IS-lasso. This method uses a smooth approximation of the L1 penalty, based
299 on the induced smoothing paradigm. As a direct result of this smoothing, point estimates
300 from IS-lasso are never exactly zero, ensuring that all available confounders remain
301 included in the model (Cilluffo et al. 2020). Two Poisson regression models for total
302 benthic macrofauna abundance within Laguna Garzón over the study period were
303 conducted. To explicitly evaluate the role of submerged plant biomass as a predictor of
304 benthic macrofauna abundance, the dataset was divided into two periods: before and after
305 the observed increase in plant biomass (2018–2022 and 2023–2024, respectively).
306 Poisson regression models were fitted separately for each period using IS-lasso, including
307 environmental variables and plant biomass, to identify key predictors of total benthic
308 macrofauna abundance. Model performance was evaluated using root mean square error
309 (RMSE). Statistical inference was based on coefficient estimates, standard errors, and
310 associated Wald-type tests within the IS-lasso framework.
311
312 **Results**
313 ***Environmental characteristics***
314 Annual rainfall anomalies relative to the 35-year climatological mean revealed
315 pronounced interannual variability. Years 2018 and 2021 showed values close to the long-
316 term average, while 2019 exhibited a strong positive anomaly (> 300 mm above average),
317 reflecting unusually high precipitation. In contrast, 2020 and the period from 2022 to

2024 presented negative anomalies, with annual deficits of approximately 250 mm (Fig. 2B). It is noteworthy that prior to 2022 one or several moths per year reported monthly accumulated rainfall higher to 150 mm, which determines a pulse of enormous amount of water in short periods of time. This kind of events were not recorded since 2022. In contrast, 2023 was a particularly dry year, with minimum 30 days accumulated rainfall of 4.3 mm and 14 mm recorded before the summer and winter samplings, respectively (Fig. 2A). Additionally, in summer 2023 a heat wave was recorded in February, being the unique during the monitoring period.

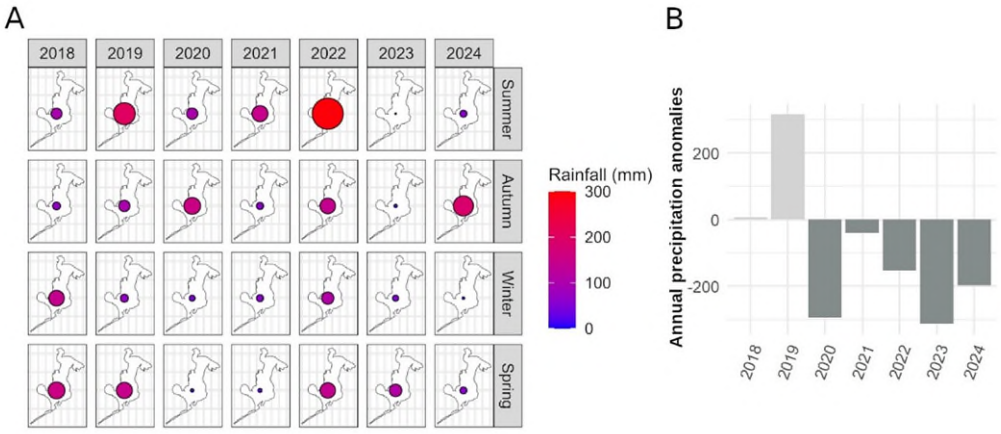


Fig. 2. (A) Temporal variability of rainfall accumulated during the 30 days preceding each sampling event in Laguna Garzón (2018–2024). (B) Annual precipitation anomaly for the 2018–2024 period relative to the 1990–2024 annual accumulated precipitation mean (dark grey bars indicate negative anomalies, light grey bars indicate positive anomalies).

The environmental parameters showed significant differences between years except for temperature. Lagoon depth showed comparatively stable and medium values between

2018 and 2020, but increased markedly in 2021, with the highest variability observed that year (mean ± standard deviation: 1.8 ± 0.7 m; Fig. 3). From 2022 onwards, depth stabilized at higher median values that ranged between 1.6 and 1.8 m (Fig. 3). The temporal variation of salinity showed marked interannual variability, ranging from 0.18 to 28.1 PSU. Salinity showed the highest mean values in spring and summer 2020 (22.06 ± 0.9 PSU) and 2021 (28 ± 0.1 PSU), but declined towards 2024 and kept low onwards, reaching the lowest values in autumn 2024 (0.6 ± 0.5 PSU; Fig. 3). Total nitrogen (TN) concentrations showed an overall increasing trend from 2019 to 2024, with higher mean values in 2022 and 2023 (854 ± 184 µgN/L). Total phosphorus (TP) exhibited a heterogeneous temporal pattern, with higher values in 2018 and secondary peaks in 2023. Dissolved inorganic nutrients (data not shown) showed marked interannual variability, with NO_3^- reaching maximum values in 2018 (up to ~223 µgN/L) and minimum values in 2023 (~5 µgN/L). Similarly, PO_4^{3-} reached maximum values in 2018 (up to 90 µgP/L) and minimum values (5 µgP/L) in multiple years including 2020, 2021 and 2022. NH_4^+ also exhibited strong variability, with maximum values recorded in 2024 (up to 188 µgN/L) and secondary peaks in 2023, while minimum values remained low (5 µgN/L) across 2020 and 2022. Sediment organic matter showed significant interannual differences, with higher values in 2024 (11.7 ± 6.3%) compared to 2018, 2022, and 2023. One-way ANOVA showed significant differences among years for salinity ($F=4.88$, $p = 0.0005$), depth ($F = 3.23$, $p = 0.008$, TN ($F =3.34$, $p = 0.007$), TP ($F =10.4.7$, $p = 1.49e-07$), and sediment organic matter ($F = 3.25$ $p =0.008$).

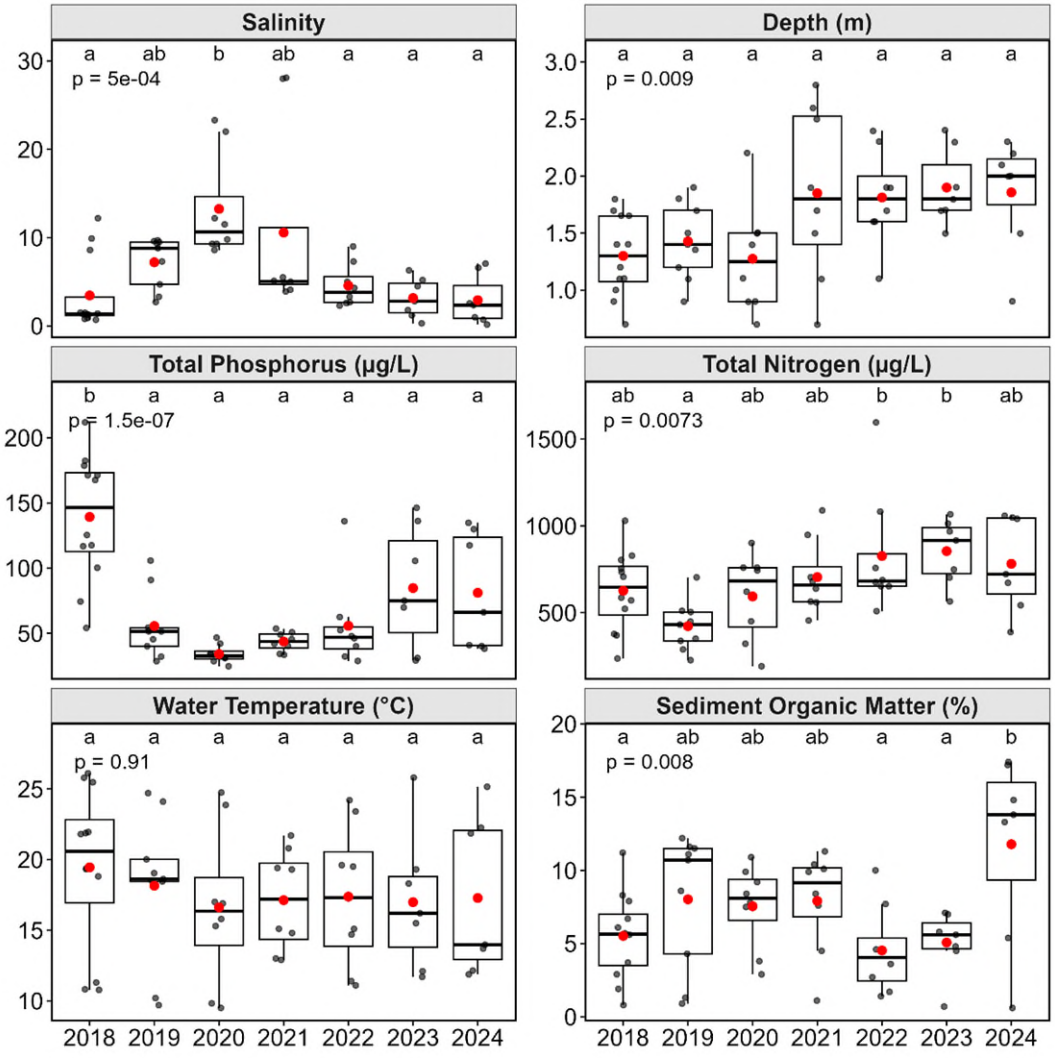


Fig. 3. Boxplots showing annual variations of environmental parameters in Laguna Garzón from 2018 to 2024. Overlaid ANOVA results (p-value) indicate the level of statistical significance in differences among years for each parameter. Letters (a, b, ab) indicate significant difference (Tukey $P < 0.05$). The black horizontal lines inside the boxes represent the median values, and the red dot indicates the mean. Extreme values are also shown.

The PCA (Fig. 4) showed a temporal separation among sampling years based on environmental conditions, although the first two axes explained a moderate proportion of the total variance (49.9%; PC1 = 31.5%, PC2 = 18.4%). PC1 mainly reflected an inverse

gradient between nutrient concentrations (TP, PO_4^{3-}, NO_3^-) and salinity, separating 2018 and 2023 from high salinity samplings. PC2 was primarily associated with suspended organic matter, and inversely to depth, and to a lesser extent to submerged plant biomass, contributing to the separation of 2023 relative to the other years (Table 1).

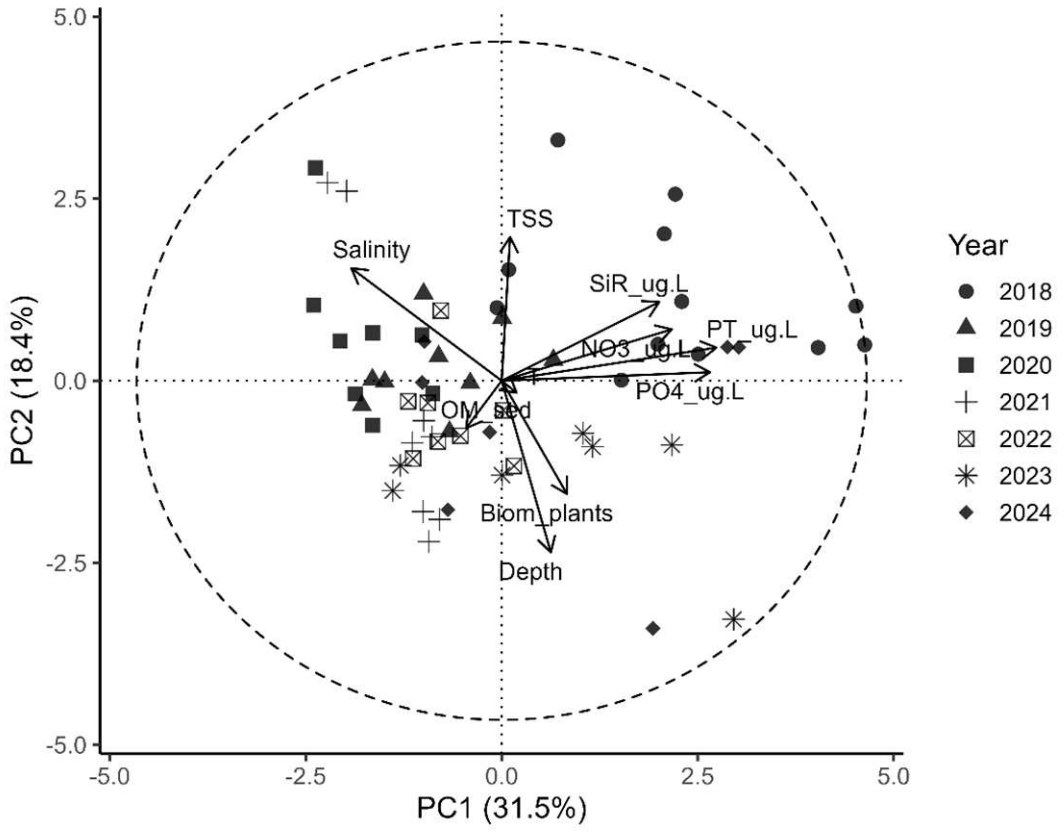


Fig. 4. Two-dimensional PCA plot of sampling years (2018–2024) in Laguna Garzón based on environmental variables. Arrows indicate the variables contributing most to the separation among periods.

Table 1. Correlations between the environmental variables and the first two principal component axes (PC1 and PC2) of the PCA performed for Laguna Garzón during the 2018–2024 period. Values indicate the strength and direction of the association between

each variable and the corresponding PCA axis. Total suspended solids (TSS, mg/L), Total phosphorus (TP, µg/L), Total nitrogen (TN, µg/L), Nitrate (NO_3-, µg/L), Orthophosphate (PO_4^{3-}, µg/L), Reactive silicate (SiR, µg/L), sediment organic matter (OM_sed, %), Macrophyte biomass (Biom-plants, gDW/m²).

Variable	PC1	PC2
Depth	0.210	-0.791
Salinity	-0.642	0.52
OM_sed	-0.152	-0.215
PO4	0.884	-0.190
PT	0.917	0.041
NO3	0.724	0.232
SiR	0.690	0.360
TSS	003	0.654
Biom_plants	0.24	-0.512

Macrophyte and macrozoobenthos features

The submerged plant *Myriophyllum quitense* suddenly appeared in the lagoon and dramatically increased its biomass in 2023 covering almost entirely the water body, followed by a partial decline in 2024 (Fig. 5), showing significant interannual variation ($F = 4.14$, $p = 0.0018$). Chlorophyll-a concentration exhibited not-significant interannual variability throughout the study period with values ranging from 0.5 µg/L in 2023 to 12.5 µg/L in 2022 (Fig. 5). In parallel, macrozoobenthic species richness peaked in 2023 and decreased in 2024 ($F = 4.06$, $p = 0.002$), whereas total abundance did not show significant differences among years (Fig. 5).

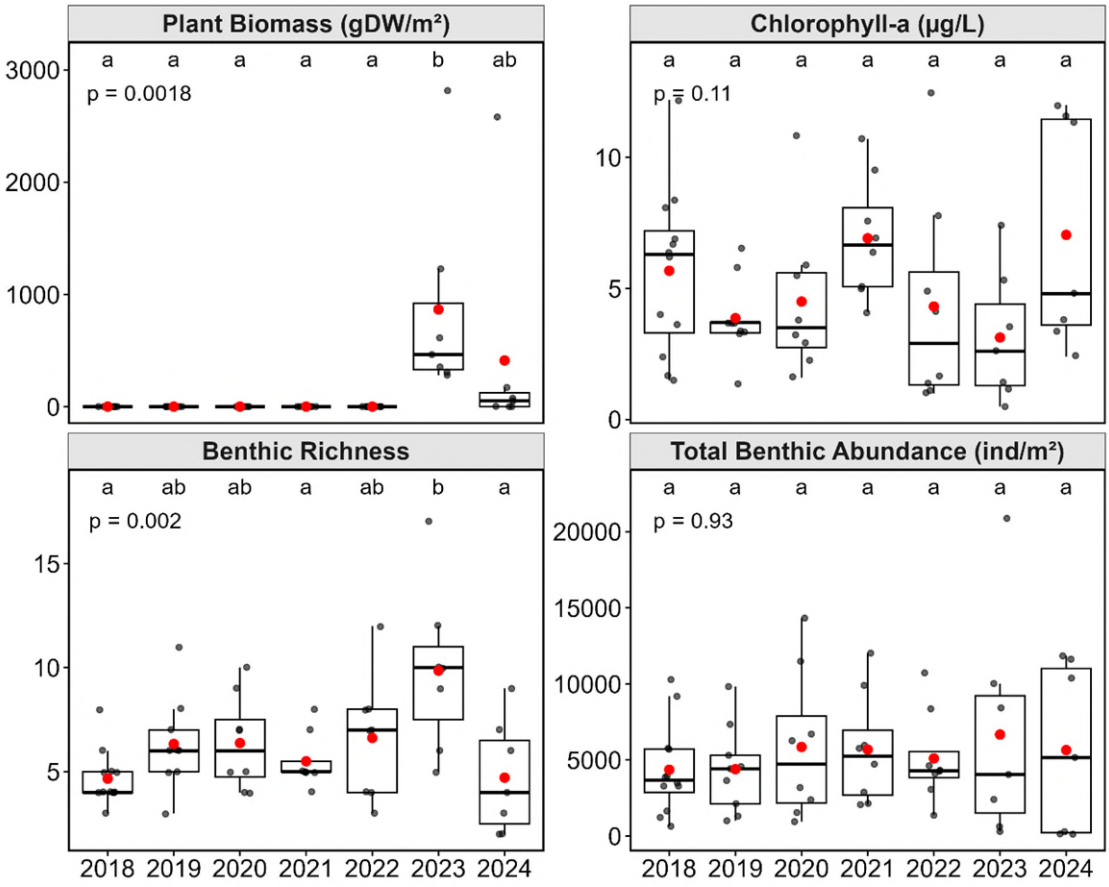


Fig. 5. Annual variation of biotic variables within Laguna Garzón including plant biomass of the macrophyte *Myriophyllum quitense* (gDW/m²), chlorophyll-*a* in the water column, macrozoobenthos species richness and total abundance values over the 2018-2024 study period. Overlaid ANOVA results (F-statistic and p-value) indicates the level of statistical significance in differences among years for each variable. Letters (a, b, ab) indicate significant difference (Tukey $P < 0.05$). The black horizontal lines inside the boxes represent the median values, and the red dot indicates the mean. Extreme values are also shown.

The gastropod *Heleobia* aff. *australis* exhibited a clear dominance pattern in terms of relative abundance from 2018 to 2022, frequently accounting for over 75% of the assemblages in multiple sampling events (Fig. 6). However, from 2023 onwards, a pronounced shift in species composition was observed, with *Heleobia parchappii*

increasing markedly in abundance, while *H.* aff. *australis* declined. This transition coincided with a broader increase in taxonomic diversity, marked by the more consistent presence of insect taxa such as Chironomidae indeterminate and isopods like *Cassidinidea fluminensis*, as well as crustaceans including Gammaridea sp. Bivalves such as *Erodona mactroides* also showing a high contribution in spring 2024.

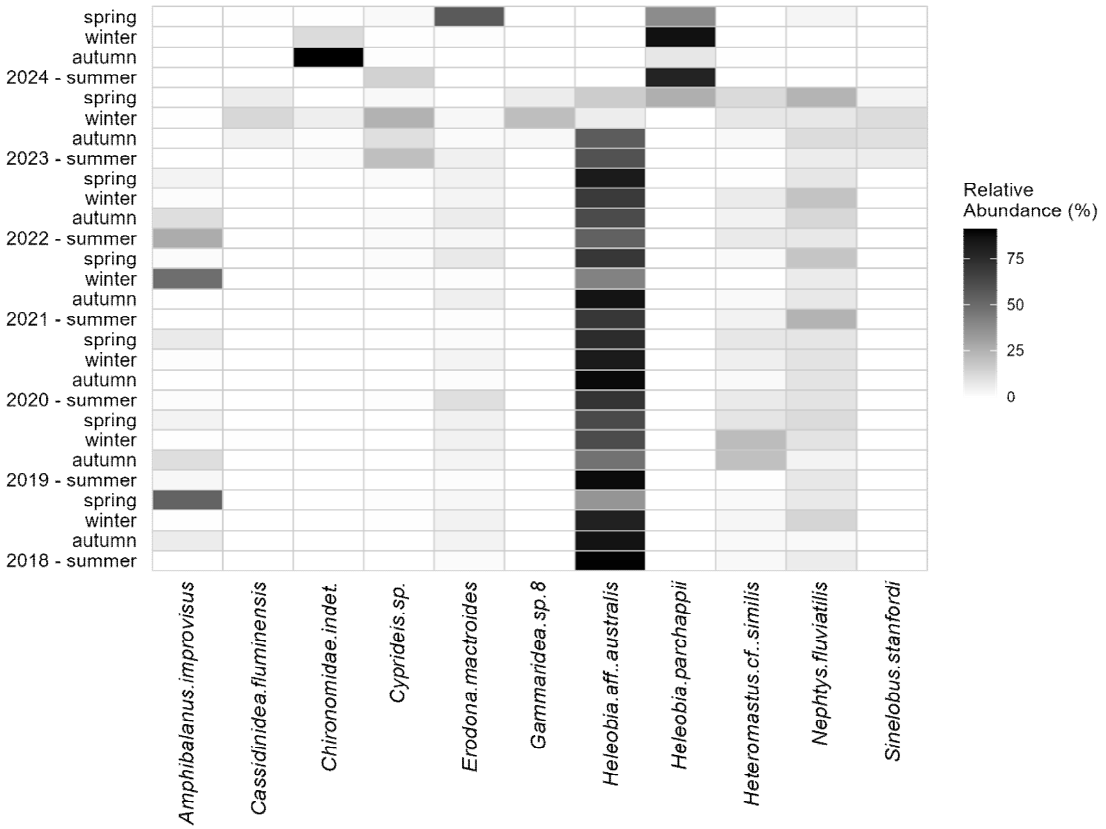


Fig. 6. Relative abundance heatmap of benthic macroinvertebrates across seasons and years collected within Laguna Garzón over the 2018–2024 period. Shading represents species' relative abundances in each year-season combination with lighter colours representing lower relative abundances while dark colours indicates higher relative abundances.

Beta diversity calculations assessing whether temporal changes in macrozoobenthic structure were driven by species replacement (βsim) or nestedness (βnes) indicated that species replacement was the dominant process (βsim = 0.41), although nestedness also contributed to overall dissimilarity (βnes = 0.18). The cluster analysis revealed a temporal shift in macrofaunal structure, separating the sampling years into two distinct groups (Fig. 7). One group encompassed the period from 2018 to 2022, showing relatively low dissimilarity among years ($\beta < 0.3$), while the other grouped 2023 and 2024, which were clearly separated from the earlier period. This grouping was supported by the PERMANOVA results (pseudo-F=5.94; $p = 0.04$). Homogeneity of multivariate dispersion among groups showed no significant differences in dispersion (PERMDISP: $F = 1.12$; $p = 0.34$), indicating that observed differences were driven by changes in community composition rather than variability within groups. Furthermore, the SIMPER analysis revealed the top discriminating species responsible for the similarity/dissimilarity among the study period 2018-2024 within the coastal Laguna Garzón (Table 2). The main discrimination occurred among both periods and mostly depended on the freshwater species *Heleobia parchappii*, as well as chironomids, gammarids, and Coenagrionidae (Odonata) which were more abundant in the years 2023-2024.

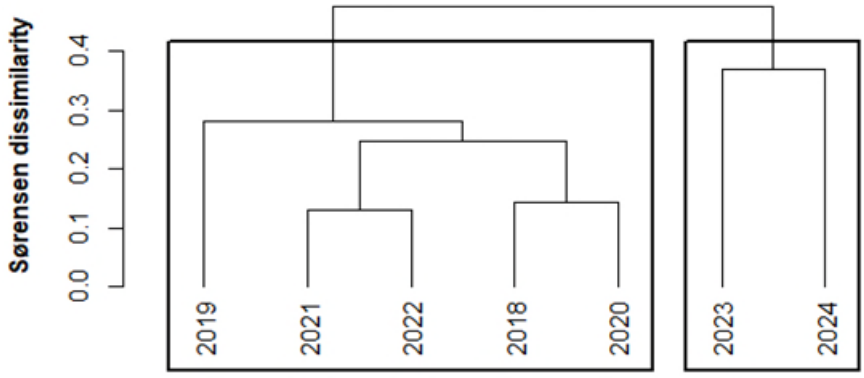


Fig. 7. Dendrogram illustrating the segregation in macrobenthos diversity over the study period 2018-2024 in the Laguna Garzón. The significance of segregation was assessed with the PERMANOVA.

Table 2. List of the species identified by the SIMPER analysis (individual contribution >3%) responsible for 100% of the distinction between the two periods (2018-2022 *vs* 2023-2024) in Laguna Garzón.

	Average dissimilarity	Contribution %	Cumulative %
Heleobia parchappii	0.03	7.10	7
Gammaridea sp.8	0.03	7.10	14
Chironomidae indet.	0.03	7.10	21
Coenagrionidae indet.	0.03	7.10	28
Sinelobus stanfordi	0.03	5.76	34
Cassidinidea fluminensis	0.02	4.39	39
Cyrtograpsus angulatus	0.02	4.29	43
Ficopomatus enigmaticus	0.02	4.19	47
Amphibalanus improvisus	0.02	4.02	51
Leptoceridae indet.	0.02	4.02	55
Berosus sp.	0.02	4.02	59
Laeonereis pandoensis	0.02	4.02	63
Nematoda indet.	0.02	4.02	67
Gammaridea sp.1	0.02	3.45	71
Gammaridea sp.5	0.02	3.42	74
Gammaridea sp.4	0.02	3.25	77

Gammaridea sp.7	0.02	3.25	80
Biomphalaria sp.	0.01	3.08	84
Copepoda indet.	0.01	3.08	87
Palaemonidae indet.	0.01	3.08	90
Polydora cornuta	0.01	3.08	93
Hirudinea sp. indet.	0.01	3.08	96

Breakpoint analyses revealed distinct temporal shifts in the abundance of dominant benthic taxa (Fig. 8). *Heleobia* aff. *australis* showed a continuous decline from 2018 until a significant breakpoint in 2023 ($p < 0.001$), after which abundances dropped sharply. *Erodona mactroides* remained at low abundances until 2022, followed by a pronounced increase and a significant breakpoint detected in 2023 ($p < 0.001$). In contrast, *Heteromastus* cf. *similis* declined steadily from 2018, with a breakpoint also identified in 2023 ($p < 0.001$), marking a further decrease in abundance. Finally, *Nephtys fluviatilis* exhibited an opposite pattern, with a significant increase up to 2022, when a significantly breakpoint was detected ($p < 0.001$), followed by a subsequent decline.

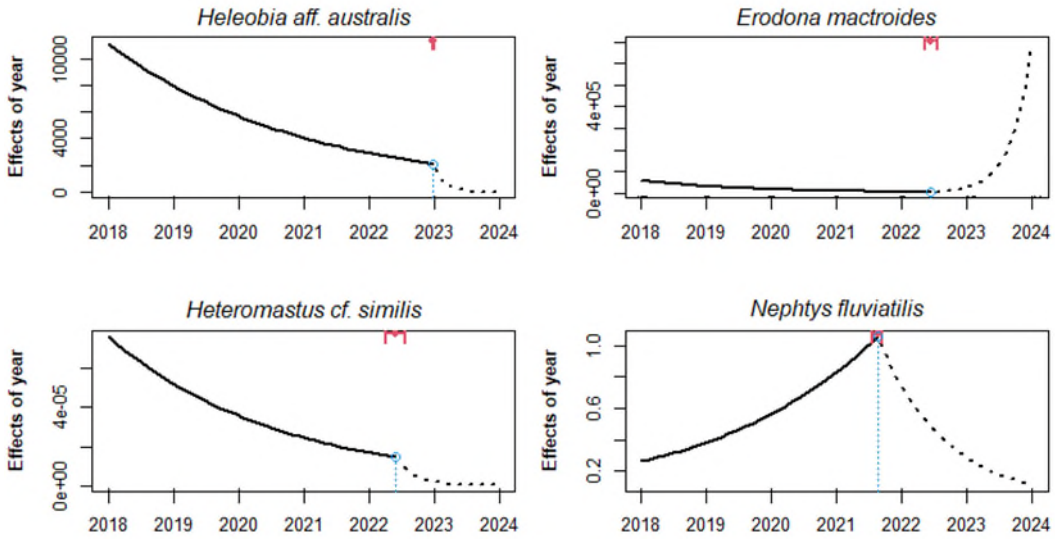


Fig. 8. Segmented temporal trends in the year effect (β) for the four dominant benthic species. Continuous lines represent the estimated regression effect of Year, with changes in slope indicating temporal breakpoints.

Temporal modelling

The IS-lasso models successfully identified the main environmental predictors shaping benthic species abundance across distinctive study periods (2018–2022 and 2023–2024; Fig. 9). For *Heleobia australis*, abundance was negatively associated with salinity and positively with temperature during 2018–2022, whereas in 2023–2024 the most relevant predictors shifted to sediment organic matter (negative effect) and NO_3^- and oxygen saturation (positive effect). *Erodona mactroides* also exhibited a marked change in predictor importance: temperature (positive effect) and salinity (negative effect) were the dominant drivers in 2018–2022, while in 2023–2024 most environmental coefficients became negative, with total suspended solids (TSS) emerging as the main predictor exerting a negative effect on abundance. For *Nephtys fluviatilis,* salinity and turbidity showed strong positive effects in 2018–2022, while total phosphorus and sediment organic matter had negative effects. Conversely, in 2023–2024 NO_3^-, oxygen saturation had a positive effect, whereas total suspended solids (TSS) and sediment chlorophyll-a negatively influenced abundance. Overall, these models capture the complexity and contextual effects underlying species abundance patterns, which result from the interplay of local environmental conditions such as water quality and broader climatic drivers, including temperature. For the polychaete *Heteromastus* cf. *similis,* abundance was positively related to NO_2 and, to a lesser extent, temperature and salinity in the first period. In contrast, during 2023–2024 its abundance was better explained by NO_3^-, (positive effect) and oxygen saturation with positive effects.

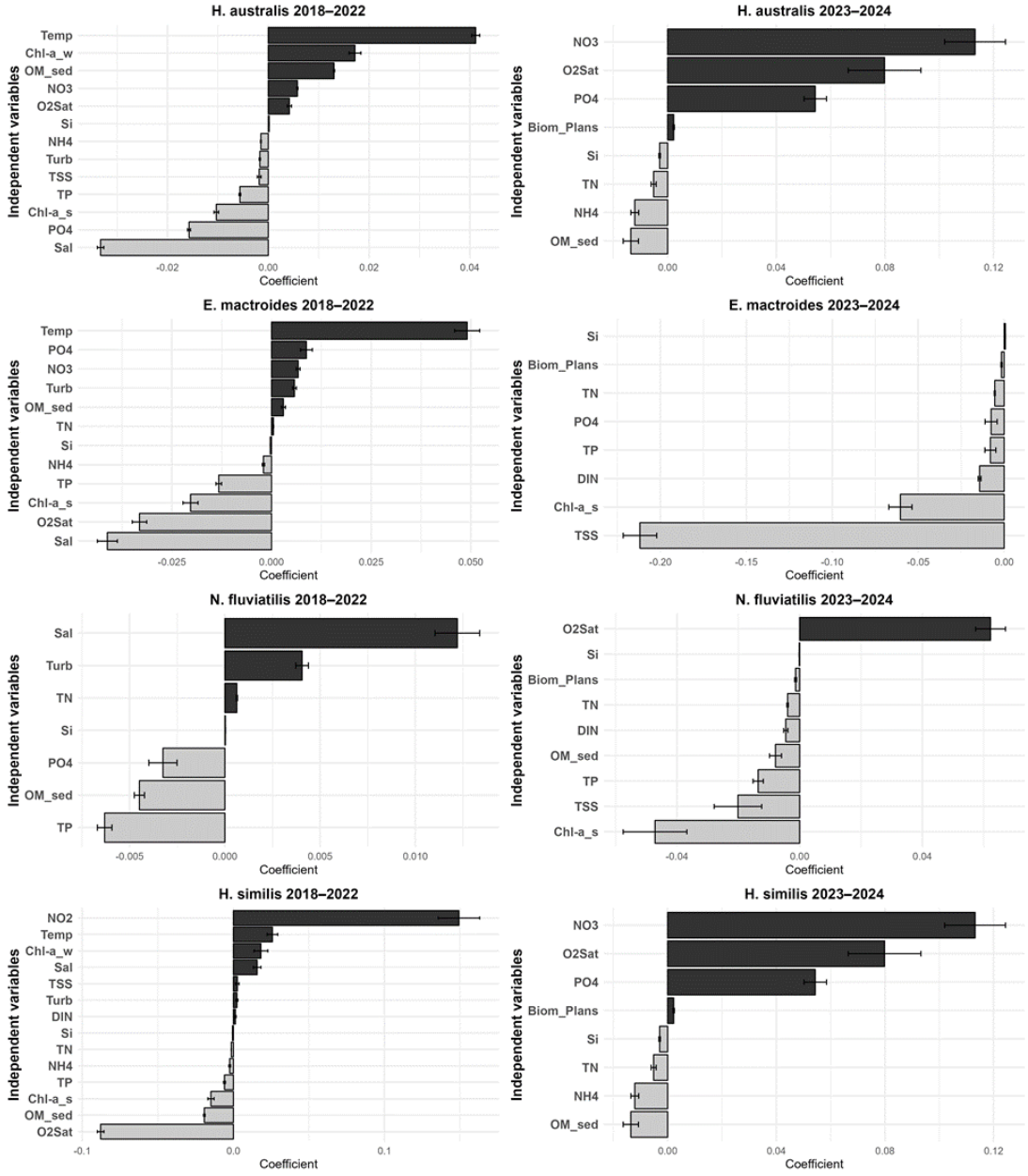


Fig. 9. Importance of environmental variables, expressed as standardized coefficients from the IS-lasso Poisson regression model, for total benthic macrofauna abundance in the coastal Laguna Garzón over the periods 2018–2022 and 2023–2024, the last one in the presence of submerged plants. Only variables with statistically significant effects ($p < 0.05$) were shown. Bar length represents the magnitude of the effect, and bar color

505 indicates the direction of the relationship: black for positive effects and grey for negative
506 effects. Variables are abbreviated as follows: Water temperature (Temp, °C), Depth (m),
507 Salinity (Sal, ‰), pH, dissolved oxygen saturation (O2Sat, %), Turbidity (Turb, NTU),
508 Chlorophyll-a in water (Chl-a_w, µg/L), Total suspended solids (TSS, mg/L),
509 Orthophosphate (PO_4^{3-}, µg/L), Total phosphorus (TP, µg/L), Total nitrogen (TN, µg/L),
510 Ammonium (NH_4^+, µg/L), Nitrite (NO_2^-, µg/L), Nitrate (NO_3^-, µg/L), Dissolved
511 inorganic nitrogen (DIN, µg/L), Reactive silicate (SiR, µg/L), Chlorophyll-a in sediments
512 (Chl-a_s, µg/gDW), Organic matter in sediments (OM-sed, %), Macrophyte biomass
513 (Biom-plants, gDW/m²). Data are shown separately for the most abundant macrobenthic
514 species previous to sumberged plants growth (according to Bergamino et al. 2025):
515 *Erodona mactroides*, *Heteromastus* cf. *similis*, *Heleobia* aff. *australis*, and *Nephtys*
516 *fluviatilis.*
517

518 **Discussion**

519 This study highlights the diverse responses of benthic communities to changes in
520 hydrological and habitat conditions, particularly those associated with salinity patterns,
521 nutrient availability, and the expansion of submerged aquatic vegetation in coastal
522 lagoons. In Laguna Garzón, the seven years monitoring period suggests a marked
523 ecological transition with changes characterized by shifts in environmental drivers
524 including the expansion of submerged aquatic vegetation, which was associated with a
525 substantial reorganization of macrozoobenthic assemblages.
526

527 During 2018-2024 monitoring, salinity exhibited a highly variable regime with a peak in
528 summer 2021, followed by a marked decline and stabilization with values below 4 PSU
529 in 2023 and 2024. This decline was likely linked to the prolonged closure of the lagoon-

530 sea connection starting in 2022, when the inlet remained closed for nearly a year and a
531 half, reducing seawater inflow and promoting nutrient accumulation and depth increase.
532 During this period, freshwater inputs occurred as small pulses that were insufficient to
533 trigger inlet opening, resulting in prolonged hydrological isolation. Consequently, gradual
534 freshwater accumulation increased water depth while maintaining low salinity due to the
535 absence of marine influence. This pattern is consistent with the inverse relationship
536 between salinity and nutrient concentrations described for coastal lagoons (Rodríguez-
537 Gallego et al. 2017), where nutrient-enriched freshwater inputs and the dilution effect of
538 marine water jointly regulate nutrient dynamics. Under high-salinity conditions, water
539 renewal tends to reduce nutrient concentrations, whereas prolonged isolation promotes
540 their accumulation. Therefore, a long period of time of low salinity, higher depth and
541 nutrients availability may have favoured the proliferation of the native macrophyte
542 *Myriophyllum quitense*. These findings support previous suggestions that decreasing
543 salinity patterns is associated with increased macrophyte biomass in coastal lagoons
544 (Rodríguez-Gallego et al. 2015; Hyman et al. 2021). Specifically, Hyman et al. (2021)
545 found that submerged aquatic vegetation (SAV) distribution decreases as salinity
546 increases, especially for species with low salinity tolerances. More broadly, connections
547 with the adjacent sea maintain a disturbance regimen, that regulates oligohaline-tolerant
548 macrophytes abundance, whereas prolonged isolation favours the spread of plants
549 abundance and its cascading effects throughout the entire ecosystem (Rodríguez-Gallego
550 et al. 2010; 2015).

551

552 Besides salinity, water depth plays a fundamental role as a more stable physical driver
553 influencing benthic and macrophyte communities. Variations in depth regulate light
554 penetration, water column stability, and the extent of suitable habitat for SAV, ultimately

555 affecting species colonization and persistence (Dixon et al. 2023). In the case of Laguna
556 Garzón, depth likely interact with salinity by modulating salinity fluctuations as was
557 proposed by Le Fur et al. (2018), and stabilizing habitat conditions favouring submerged
558 large-bodied species such as *M. quitense,* which may benefit from increased water depth
559 and reduced hydrological variability.

560

561 Previous data since 2005 documented for the macrozoobenthos in the Laguna Garzón the
562 dominance of the gastropod *Heleobia* aff. *australis* (Meerhoff et al. 2013; Giménez et al.
563 2005). However, during the 2023–2024 low-salinity period, benthic assemblages
564 underwent significant reorganization, with the emergence of opportunistic and
565 freshwater-tolerant species. Cluster analysis, supported by PERMANOVA results,
566 clearly separated period 2018–2022, characterized by relatively low interannual
567 dissimilarity ($\beta < 0.3$), from 2023–2024, which showed pronounced community shifts.
568 These shifts were mainly driven by the freshwater gastropod *Heleobia parchappii*, whose
569 abundance increase markedly in 2023–2024. In particular, in 2023-2024 the dominance
570 of *H. parchappii*, gammarids, and chironomids under salinity <4 PSU contrasts with
571 periods of higher salinity, which favoured *H. australis*, suggesting the existence of key
572 salinity thresholds structuring benthic community composition. *Heleobia parchappii* is
573 opportunistic, characterized by a high reproductive rate, rapid development, early
574 reproduction, and small body size (De Francesco and Isla 2004). The appearance of
575 chironomids is consistent with previous studies showing that reduced lagoon-sea
576 connectivity and environmental variability including salinity, favour their proliferation
577 (e.g., Jones et al. 2022), as well as SAV proliferation offers suitable habitat (Rodríguez-
578 Gallego et al. 2010).

Previous studies in Laguna Garzón indicated that benthic communities can remain stable over time despite variability in hydrological and salinity conditions (i.e., lagoon-sea connectivity) (Meerhoff et al. 2013; Bergamino et al. 2025). However, the stabilization of salinity at low values appears to have acted as a disturbance for stress tolerant species such as *H. australis*. At the same time, this stabilized regimen may have removed the controlling factors preventing low salinity tolerant SAV species to proliferate. The observed replacement of *H.* aff. *australis* by more freshwater-tolerant *H. parchappii*, as well as the appearance of chironomids, likely reflects the combined influence of altered hydrological conditions and macrophyte-driven habitat heterogeneity. Submerged vegetation increases niche diversity and provides refuges for benthic organisms (Magni and Gravina, 2023) and serves as a food source for grazers and detritus feeders (Vizzini and Mazzola, 2006), thereby amplifying the response of opportunistic freshwater species to environmental changes.

The lasso models revealed that during 2018-2022 benthic species abundances responded to a set of environmental drivers (i.e. salinity, temperature and nutrient concentrations), consistent with the fluctuating nature of shallow coastal lagoon (Como et al. 2024; Magni and Gravina, 2023). In contrast, during 2023–2024, when macrophytes became abundant, and salinity regimes stabilized at lower values while depth increased, species responses were mainly associated with nutrients levels, and TSS, and macrophyte biomass. This suggest a change from externally driven variability (such as marine inflow) towards increasing internal regulation which are associated with the structure of the assemblage. It should be noted that model inference is constrained by the set of measured

environmental predictors and does not explicitly account for temporal autocorrelation, which may influence parameter estimates. During the period of SAV proliferation and increased water transparency, changes in nutrient dynamics and enhanced primary production likely modified organic matter accumulation and habitat conditions for benthic fauna. This is consistent with recent studies highlighting the key role of both the quantity and nutritional quality of organic matter in structuring macrobenthic assemblages in coastal lagoons (Pusceddu et al., 2009; Giampaoletti et al., 2025). Periods of SAV decomposition combined with persistently low salinity could favour cyanobacteria blooms, potentially restructuring food web dynamics and ecosystem uses, as observed in other shallow coastal systems (Skarlato et al. 2018; Bouquet et al. 2022). The shift to a new stable state dominated by cyanobacteria should not be discharged, if freshwater to oligohaline conditions prevails and SAV continuous to retreat. This shows the necessity of taking management measures to reduce eutrophication and favour the re-establishment of the salinity regimen, though the sand bar opening among other measures in the catchment to reduce agriculture runoff.

Conclusions

By combining segmented regression and LASSO, this study disentangled and quantified the relative contributions of habitat modification by macrophytes and environmental drivers on benthic species abundances. Lagged responses among taxa highlight asynchronous reactions, with species-specific thresholds and sensitivities. The main drivers of benthic dynamics shifted from a mix of internal (lagoon) and external marine forcings during 2018–2022 to lagoon-internal processes in 2023–2024. Our results further suggest that this transition was initiated by the stabilization of the hydrological regime,

627 particularly reduced salinity variability and increased water depth following prolonged
628 lagoon isolation. Management strategies should focus on minimizing nutrient inputs form
629 agriculture fields and promoting water renewal to reduce hypoxia risks and sustain
630 ecological resilience (Zaldívar et al. 2008), and on re-establish the highly dynamic salinity
631 regimen. Overall, these results underscore that maintaining natural environmental
632 heterogeneity and disturbance regimes is crucial to preserve biodiversity, ecosystem
633 functioning, and resilience in coastal lagoons, while also demonstrating how habitat-
634 forming macrophytes can modulate benthic community trajectories under environmental
635 change. Such insights are crucial to strengthen lagoon resilience and to preserve their
636 ecosystem services under ongoing global change (Ligorini et al. 2022).

637

638 In this context, our findings contribute to a growing body of evidence highlighting the
639 sensitivity of coastal lagoons to changes in hydrological connectivity. Future efforts
640 should aim to integrate additional drivers, such as climatic variability and watershed
641 processes, and to extend this framework to other coastal lagoons, enhancing its
642 applicability for adaptive management and early detection of ecosystem shifts under
643 global change. Continued long-term monitoring will be critical to determine whether the
644 observed changes represent a persistent state. It will also help assess the potential for
645 alternative trajectories, including scenarios dominated by cyanobacteria in the absence of
646 submerged vegetation, or the possibility of reversal under changing environmental or
647 management conditions.

648

649

650 **Author Contribution Statement:**

All authors (L.B., G.C., S.S., C.L., S.C., F.S., S.P., E.R., A.M., S.V., and L.R.G.) contributed to the conceptualization of the study. S.S., S.C., C.L., S.P., E.R., A.M, and F.S. conducted fieldwork, with S.S. and F.S. also performing species identification. L.B. and G.C. carried out data analysis, and L.B. wrote the first draft of the manuscript. L.R.G. contributed to funding acquisition. All authors reviewed and edited the final manuscript and approved its submission.

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Declarations

All authors have read, understood, and have complied as applicable with the statement on "Ethical responsibilities of Authors" as found in the Instructions for Authors.

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