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1 **First report of vampyrellid predator-prey dynamics in a marine system**

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15

16 **Abstract**

17 We report for the first time the *in situ* dynamics of a vampyrellid in a marine system. A
18 high sampling frequency (twice-weekly) was applied in a tropical eutrophic lagoon (Rio de
19 Janeiro, Brazil) for five years (2012-2016). The vampyrellid *Hyalodiscus* sp. specifically fed on
20 the diatom *Chaetoceros minimus* during a short time-window (~3 months), although the prey
21 was intermittently detected as the dominant phytoplanktonic species over a longer period (~1
22 year). A classic Lotka-Volterra predator-prey dynamic was observed between the two partners,
23 with a significant modification of the short-term oscillations of the prey. Specific abiotic
24 preferences (i.e., relatively low temperature, intermediate salinity, and stratified conditions)
25 associated with prey availability seemed to define this narrow temporal window of occurrence.
26 Our results suggest that vampyrellids can be ecologically relevant in marine pelagic systems,
27 with their impact on planktonic dynamics strongly depending on complex interactions between
28 both biotic and abiotic factors.

29

30 **Main text**

31 Vampyrellids (Vampyrellida, Rhizaria) are a relatively easily recognizable group of
32 predatory amoebae feeding on protists and small metazoans in aquatic and terrestrial systems
33 (Berney et al. 2013). They display an impressive diversity of feeding strategies varying from
34 prey engulfment to protoplast-feeding, the later characterized by the perforation of the prey cell
35 wall and protoplast extraction (Hülsmann 1993). As all formally described vampyrellid species
36 so far have been recorded in freshwater or soil ecosystems, the group was initially thought to be
37 confined to non-marine habitats (Anderson and Patrick 1978, Hess et al 2012, Hülsmann 1993).
38 However, recent molecular surveys revealed a high diversity of these organisms in marine
39 microbial assemblages (Berney et al 2013). Although the understanding of vampyrellid
40 phylogeny and life-history has improved substantially in recent years (Gong et al 2015, Hess et
41 al 2012, Hess 2017a, Hess 2017b), little is known about their abundance and impact on prey
42 populations. Consumption of plankton prey has been observed mainly through feeding
43 experiments (Hess 2017b) or algal mass cultures (Gong et al 2015). The putative genus
44 *Asterocaelum* is the only known example of a vampyrellid preying on freshwater natural
45 plankton assemblages, where they are frequently reported as a significant source of mortality for

46 diatoms and cyanobacteria (Bailey-Watts and Lund 1973, Canter 1973, Cook 1976, Van
47 Wichelen et al 2006).

48 Here, we present the first record of the high-frequency predator-prey dynamics for a
49 vampyrellid in a marine system. We followed the phytoplankton dynamics in Rodrigo de Freitas
50 Lagoon (RFL), a eutrophic coastal system in Rio de Janeiro (Brazil), from January 2012 to
51 December 2016. Samplings were performed twice-weekly at five sampling stations (Fig. S1; see
52 Supplementary Materials for methodological details). This lagoon is dominated by small
53 eukaryotic phytoplankton (<20 µm), and high temporal variability (Alves de Souza et al. 2017).
54 The diatom *Chaetoceros minimus* was one of these dominant species highly fluctuating over the
55 time series (Fig. 1A). Predation on *C. minimus* by a vampyrellid was first detected in July 2013
56 in all sampling stations. Microscopic observations revealed that only *C. minimus* was consumed
57 by *Hyalodiscus* sp. in both field populations and feeding experiments using cultures from other
58 microalgal species occurring in RFL (listed in the supplementary materials). Although studies on
59 vampyrellids' prey-range are still insipient, both broad ranges of preys including fungi, algae and
60 nematodes (Old and Darbyshire 1978, Pakzad 2003) and trophic specialization (Hess 2017b)
61 were reported. Protoplast-feeding vampyrellids have been observed so far feeding on large-sized
62 algae (Cienkowski 1876, Hess et al 2012, Hess 2017a, Hess 2017b). To the best of our
63 knowledge, this is the first report of a vampyrellid with such feeding strategy preying on a
64 nanosized planktonic microalgae.

65 A culture of the vampyrellid (now lost) was established using a *C. minimus* strain
66 previously isolated from RFL as prey. Typical vampyrellid life-stages were recognized from
67 wild and cultured populations (Fig 1B-E). Both trophozoites (amoeboid free-living, feeding
68 stages) and digestive cysts having orange cytoplasmic coloration are typical for vampyrellids
69 (e.g., Hess et al 2012, Hess 2017a). The vampyrellid was identified as *Hyalodiscus* sp. based on
70 the sequencing (SSU rDNA) of the obtained culture. SSU rRNA gene sequences affiliated to
71 *Hyalodiscus* sp. accounted for 20-50% of the clone libraries (data not shown) when the highest
72 abundances of the vampyrellid occurred. Maximum likelihood analysis (Figs. 1F, S2) indicated
73 that SSU rRNA gene sequences of the cultured and environmental vampyrellids grouped
74 together within the *Hyalodiscus flabelus* clade (100% bootstrap) and fell into the lineage B3
75 (Berney et al 2013), which was recently suggested as encompassing the family Hyalodiscidae

76 (Hess 2017a). Sequences belonging to this clade have been reported worldwide in sediments of
77 coastal systems (Berney et al 2013).

78 We observed a strong interannual environmental heterogeneity in LRF, mainly related to
79 salinity and Brunt-Väisälä frequency (N_{BV} ; an estimate of stratification) (Fig. 2A). Dynamics of
80 the main phytoplankton groups also reflected such variability (Fig. 2B). Of special notice was the
81 long period of intermittent *C. minimus* predominance (June 2013 to August 2014) (Fig. 2C)
82 related to high $Si(OH)_4$ (Figs. S3, S4). *Hyalodiscus* sp. was observed only between July and
83 August 2013 (Fig. 2D). The high sampling frequency allowed the detection of classic Lotka-
84 Volterra dynamics between *C. minimus* and *Hyalodiscus* sp. (Fig 2E), with a 6-day time lag (t_{-6})
85 between the abundance peaks of the prey and its predator (cross-correlation $t_{-6} = 0.7$; $p > 0.01$)
86 (Fig. S5). No natural enemies (i.e., grazers or parasites) other than *Hyalodiscus* sp. showed such
87 a coupled dynamic with *C. minimus* during its period of predominance (Alves-de-Souza, data not
88 shown). Wavelet coherence analysis demonstrated a significant time-delayed negative interaction
89 between prey and predator (Fig. S6) whereas multiple polynomial regressions identified the
90 lagged (t_{-6}) *Hyalodiscus* sp. abundance as the main factor affecting the abundance of its diatom
91 prey during the period of their co-occurrence ($R^2 = 0.40$; $p < 0.001$) (Table S1).

92 *Hyalodiscus* sp. showed a narrow niche breadth when compared to its prey (Fig. 2F),
93 which was related to an apparent preference of the vampyrellid for temperatures lower than
94 23°C, intermediate salinities (12-16 PSU) and stratified conditions ($N_{BV} > 0.01 s^{-1}$) (2G). This
95 precise combination of abiotic parameters was observed only during *Hyalodiscus* sp. occurrence.
96 Polynomial regressions based on environmental variables and time lagged values (t_{-6}) of *C.*
97 *minimus* abundance indicated that *Hyalodiscus* sp. abundance was mostly affected by the
98 interaction between temperature, N_{BV} and lagged (t_{-6}) *C. minimus* abundance ($R^2 = 0.95$; p
99 > 0.01) (Table S2). A slightly different pattern was obtained for the period of *C. minimus*
100 predominance, with interaction between temperature and lagged (t_{-6}) *C. minimus* abundance,
101 explaining most variability in *Hyalodiscus* sp. abundance (partial $R^2 = 0.75$; $p > 0.01$). These
102 results indicated that N_{BV} was mostly important at the interannual scale and partly explained the
103 restricted occurrence of *Hyalodiscus* sp. in 2013. However, the importance of this variable
104 decreased when a shorter time period was considered because the water column was mostly
105 stratified during the period of predominance of *C. minimus*. During this restricted time-window,
106 the increase in temperature may explain why *Hyalodiscus* disappeared even when the water

107 column was still stratified and the prey present. Shifts in the relative importance of the
108 environmental variables according temporal scale have been also previously reported for other
109 microbial communities (Hatosy et al 2013, Reynolds 1990), including phytoplankton
110 assemblages of RFL (Alves-de-Souza et al 2017).

111 The restricted temporal occurrence of *Hyalodiscus* sp. in the RFL was in agreement with
112 an extensive study on vampyrellids' diversity in marine systems where only 13% of the
113 vampyrellid sequences were recorded from pelagic samples, with the major relative abundance
114 (87%) for this group detected in sediments (Berney et al 2013). While information on microbial
115 benthic assemblages is not yet available for RFL, further studies should assess the relevance of
116 resting cysts in the sediments for the *Hyalodiscus* sp. predator-prey dynamics in this system. The
117 conjunction of specific abiotic conditions (i.e., relatively low temperature, intermediate salinity,
118 and stratified waters) associated with specific prey availability seemed to determine the time-
119 window for *Hyalodiscus* sp. pelagic presence in RFL. Considering the worldwide distribution of
120 vampyrellids within the Hyalodiscidae clade (Berney et al 2013) and the cosmopolitan nature of
121 *C. minimus* (Tomas 1997), our results provide valuable insights into the population dynamics of
122 vampyrellids and the factors behind their elusive occurrence in plankton assemblages. They also
123 suggest that this group can indeed be ecologically relevant in marine plankton systems, with their
124 relative importance to the planktonic dynamics depending strongly on complex interactions
125 between both biotic and abiotic factors.

126

127 **Conflict of Interest**

128 The authors declare no conflict of interest.

129

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137

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181 reaches of the Schelde Estuary (Belgium). *Belgian Journal of Botany*: 153-166.

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186 **Caption of figures**

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188 Fig. 1 **a** Healthy diatom *Chaetoceros minimus*, the only microalgae observed to be preyed by
 189 *Hyalodiscus* sp. in mixed plankton assemblage from the Rodrigo de Freitas Lagoon (RFL) over
 190 the time series. **b-e** Different stages of *Hyalodiscus* sp.: trophozoite (amoeboid free-living,
 191 feeding stage) (b), feeding stage attached to *C. minimus* cells (c), digestive cysts (d), and resting
 192 cysts (e). **f** Maximal likelihood phylogeny (SSU rDNA) of vampyrellids showing the inclusion of
 193 *Hyalodiscus* sp. sequences (in blue) into Hyalodiscidae. Only bootstrap values higher than 70%
 194 are shown (expanded tree is given in Fig. S2; see Supplementary Material for details on the
 195 analysis). Small white arrows = feeding peduncle; Large black arrows = order of transition
 196 between the different *Hyalodiscus* sp. stages as observed in cultures from RFL (transition from
 197 resting cysts to the free-living stage is depicted with a question mark as it was not observed in
 198 cultures); Arrow heads indicate the four layers of resting cyst envelopes. Asterisks = individuals
 199 recorded from Lugols' samples during quantification (others originated from cultures).

200

201 Fig 2. **a-b** Interannual and seasonal variability of average values (for the five sampling stations)
 202 of water temperature ($^{\circ}\text{C}$), salinity (PSU), Brunt-Väisälä frequency (N_{BV}) (s^{-1}) as an estimate of
 203 water stratification, and phytoplankton biovolume ($\text{mm}^3 \text{L}^{-1}$). **c-d** Average abundance (cells ml^{-1})
 204 of *Chaetoceros minimus* (c) and *Hyalodiscus* sp. (d). **e** Detail showing the time-lagged
 205 interaction between *C. minimus* and *Hyalodiscus* sp. (grey area in a-d). **f** Outlying mean index
 206 (OMI) analysis showing the relative importance of environmental variables (blue vectors) to *C.*
 207 *minimus* (Cm) and *Hyalodiscus* sp. (H) realized niches (depicted by grey and orange polygons,
 208 respectively). Black points represent the average mean habitat conditions of the two species for
 209 the sampling domain (delimited by the dashed line). **g** Distribution of samples for the entire
 210 study period in a 3-D space determined by water temperature, salinity and N_{BV} . Samples where
 211 *Hyalodiscus* sp. was present were indicated in orange.

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1 Alves-de-Souza et al. **First report of vampyrellid predator-prey dynamics in a marine system.**
2 *The ISME Journal.*

3 **METHODS**

4 **Study area and sampling**

5 The Rodrigo de Freitas Lagoon (RFL) is a semi-confined eutrophic coastal lagoon in Rio de
6 Janeiro coast (22°57'02"S, 43°11'09"W; Fig. S1). It has a surface area of 2.5 km², volume of 6.5
7 million mm³, mean depth of about 2.8 m and maximum depth of 4 m. RFL receives part of the local
8 domestic and wastewater discharge coming from its drainage basin. The surroundings of RFL are
9 composed of hillside streams, rainforest, and residential areas. At the lowest basin areas, the land use
10 is predominantly urban with a high population density. Since the lagoon is surrounded by a highly
11 urbanized area, it receives influx of polluted waters from uncontrolled sewage systems and from
12 storm sewers. The lagoon is connected to the sea by the Jardim de Alah Channel, and receives
13 freshwater discharge from the Macacos and Cabeça rivers through the Piraquê Channel, situated on
14 the northeastern side of the lagoon.

15 Samples were collected twice a week from 2 January 2012 to 7 December 2016 in RFL at five
16 sampling stations (Fig. S1), with 1-4 day sampling intervals, coupled with the Phytoplankton
17 Monitoring Program developed by the Municipality of Rio de Janeiro. Subsurface (~10 cm depth)
18 water samples were collected for phytoplankton (250 mL) and nutrient analysis (500 mL). Additional
19 samples (2 L) were taken from station 3 located at the center of the lagoon for phytoplankton cultures
20 and molecular analyses. Water temperature and salinity were measured using a multiparameter probe
21 (YSI 63, Yellow Springs, Ohio, USA.) at 0.5 m intervals. Both variables were used to estimate the
22 density of the water (σ). The water stability (used as a measure of the degree of mixing) was given
23 by the Brunt-Väisälä frequency (N_{BV}), calculated based on the vertical distribution of water density
24 in the water column. Water transparency was estimated using a Secchi disk.

25 **Phytoplankton and vampyrellid quantification**

26 Sample processing was performed with special care to maximize resolution of the taxonomic
27 identification and confidence in assignments. Samples were immediately fixed after sampling with
28 Lugol's solution (1%) and the shape of phytoplankton cells and *Hyalodiscus* sp. was compared before
29 and after Lugol's fixation (the same was done on cultures).

30 Quantification was performed within 24 hours of sampling (to avoid excessive deformation
31 of cells and cell loss after fixation) by the Utermöhl method (1958) using an inverted microscope
32 (Zeiss AXIO Observer.A1, Göttingen, Germany) equipped with a digital camera (Zeiss AxioCam
33 ICc-3, Göttingen, Germany) after sedimentation in 10-ml columns. Cells smaller than 10 μ m were
34

35 quantified using an immersion objective (100× magnification) whereas cells larger than 10 µm were
36 enumerated under 40× magnification. In both cases, quantifications were performed in random fields
37 (Uehlinger 1964) until at least 100 units ($p < 0.05$) of the dominant species were enumerated (Lund
38 et al 1958). Quantification of grazers (ciliates and rotifers) between 2013 and 2015 was performed
39 simultaneously to the phytoplankton quantification (data not shown). We also checked for signals of
40 infection by eukaryote parasites (e.g., chytrids) on *C. minimus* during its period of predominance
41 (June 2013 to August 2014).

42 The usual discrepancy observed between small and large sized plankton normally results in
43 an over importance of small taxa when cell density values are included in statistical analysis. For
44 this reason, cell density values (cells ml⁻¹) were converted to biovolumes (mm³ L⁻¹) considering
45 equations for similar geometric shapes of the cells (Hillebrand et al., 1999) based on the cellular
46 dimensions of at least 50 individuals of each species.

48 **Vampyrellid cultivation and feeding experiments**

49 Living samples were used to establish a culture of the vampyrellid as soon as it was observed
50 in the quantification of the Lugol's samples. For that, digestive cysts were isolated by microcapillary
51 pipet (Andersen 2005), successively washed in at least six drops of culture media and added in separate
52 wells of 96-wells plates containing 200 µl of a *Chaetoceros minimus* strain isolated from RFL in 2012
53 (See Alves-de-Souza et al 2017 for details on *C. minutum* isolation). The obtained culture was further
54 transferred to a 24-well plate and thereafter transferred weekly into fresh *C. minutus* cultures in 50ml
55 culture flasks. Both vampyrellid and the diatom cultures were maintained in F/2 with the addition of
56 Na₂SiO₃ at 21-23°C and 120 µE m⁻²s⁻¹ in a 12/12 h light/dark cycle.

57 For the feeding experiments, we used cultures of the RFL predominant microalgae in 2012
58 and 2013 isolated as described by Alves-de-Souza et al. (2017). These cultures included diatoms
59 (*Cyclotella* sp., *Chaetoceros tenuissimus*), prymnesiophytes (*Chrysochromulina* sp., *Diacronema* sp.,
60 *Prymnesium* sp., *Isochrysis* sp.), chrysophytes (*Ochromonas* sp.), cryptophytes (*Hemiselmis* sp.),
61 chlorophytes (*Mantoniella* sp., *Pyramimonas* sp., *Nannochloris* sp.), eustigmatophytes
62 (*Nannochloropsis* sp), and cyanobacteria (*Synechocystis* sp). Cultures were acclimated to 14 PSU for
63 1 month before starting of the feeding tests. *Hyalodiscus* sp. (200 ml) was maintained using *C.*
64 *minimus* as prey. Feeding tests were started once most *C. minutus* cells were consumed and when
65 digestive cysts were the predominant vampyrellid stage (~5,000 digestive cysts ml⁻¹). Microalgal cell
66 densities were adjusted to ~5,000 cells ml⁻¹ in F/2 medium (supplemented with Na₂SiO₃ for diatoms).
67 *Hyalodiscus* sp. and microalgal cultures (1 ml each) were performed in triplicates in 24-well plates.
68 Inoculations using *C. minimus* were performed as control. Co-culture experiments were monitored
69 twice a week for one month to verify consumption of the microalgae by the vampyrellid.

70

71 **Genetic characterization of *Hyalodiscus* sp.**

72 *Hyalodiscus* sp. cultures (50 ml) were centrifuged and the resulting pellet was flash frozen in
73 liquid nitrogen. DNA extraction was performed using a modified guanidinium isothiocyanate
74 protocol (Chomczynski and Sacchi 2006) as described by Alves-de-Souza et al. (2011). The PCR
75 amplification of the SSU rDNA gene was performed using the primers 63F (forward: 5'- ACG CTT
76 GTC TCA AAG ATT A -3') and 1818R (reverse: 5'- ACG GAA ACC TTG TTA CGA -3'). The
77 PCR amplification mix (15 µL final volume per reaction) contained 1 µL of the DNA extract, 330
78 µM of each deoxynucleoside triphosphate (dNTP), 2.5 mM of MgCl₂, 1.25 U of GoTaq® DNA
79 polymerase (Promega Corporation, Madison, WI, USA), 0.17 µM of both primers, and 1× of buffer
80 (Promega Corporation). The PCR program included a denaturation step (95°C for 5 min), followed
81 by 35 cycles of denaturation (1 min at 95°C), annealing (1 min 30s at 55°C), and elongation (1 min
82 15 s at 72°C). The final elongation step lasted 7 min at 72°C. PCR products were cloned using the
83 TOPO TA Cloning® kit (Invitrogen) according to manufacturer's recommendations, and selected
84 clones were amplified by PCR following the protocol described above. PCR products were purified
85 using the ExoSAP-IT kit (USB) following the manufacturer's recommendations and directly
86 sequenced on an ABI Prism 3100 automatic sequencer (Applied Biosystems).

87 Environmental samples (1.5 L) from 3 July to 15 August 2013 (period where *Hyalodiscus* sp.
88 was observed in RLF) were first pre-filtered through 10 µm pore polycarbonate filters (47 mm,
89 Millipore) and collected on a 0.22 µm pore size polycarbonate filter (47 mm, Millipore) under gentle
90 vacuum (<5mm Hg). The filters containing the 0.2-10 µm fraction were transferred to 2 mL cryotubes
91 containing RNAlater™ (Ambion, Life Technologies Brazil), frozen in liquid nitrogen and stored at -
92 20 °C. For cDNA clone libraries, we selected the two sampling dates for which highest *Hyalodiscus*
93 sp. abundance were observed (8 and 29 July). Total RNA extraction followed by cDNA amplification
94 was performed as following the procedure described by Jeanthon et al. (2011). The hypervariable
95 region V4 of the 18S rDNA gene (about 600 bp) was amplified using the primers Euk528f (forward:
96 5'- CCG CGG TAA TTC CAG CTC -3'; Zhu et al. 2005) and S69 (reverse 5'- CCG TCA DTT CCT
97 TTR AGD TT -3'; Probert et al. 2014). These primers were selected as they offer a comparatively
98 good *in silico* phylogenetic coverage, allowing the recovery of a high diversity of sequences from
99 different taxonomic groups. PCR amplification and cloning were performed using the same
100 conditions as described above.

101 Phylogenetic assignment of sequences from both culture and cDNA clone libraries were
102 performed by *BLAST* analysis using the PR2 data base (<http://ssu-rrna.org/pr2>) (Guillou et al 2012).
103 Phylogenetic analysis included sequences obtained in this study and vampyrellid sequences available
104 in GenBank (<http://www.ncbi.nlm.nih.gov>) and used in previous studies (Berney et al 2013, Hess et

105 al 2012, Hess 2017a, Hess 2017b). Sequences were aligned using the online package MAFFT version
 106 7 (<https://mafft.cbrc.jp/alignment/software/>) based on the 18S rDNA secondary structure. Maximum
 107 likelihood (ML) analysis was performed using the online version of RAxML (Stamatakis 2006)
 108 available on the T-REX website (<http://www.trex.uqam.ca>) (Boc et al 2012), based on the model GTR
 109 + Γ + I. The robustness of the inferred topology was supported by bootstrap resampling (500
 110 replicates). The sequences obtained during this study were deposited in GenBank (accession nos.
 111 MH973259-MH973263).

112

113 **Statistical Analysis**

114 **Data transformation.** Based on low spatial heterogeneity observed in RFL for both phytoplankton
 115 and environmental conditions (Alves-de-Souza et al. 2017), all statistical analyses were based on
 116 average values for the five sampling stations. Average *Hyalodiscus* sp. and *C. minimus* abundances
 117 were previously transformed [$\log(x+1)$], whereas average environmental data were standardized to
 118 values between 0 and 1, based on the minimum and maximum values of each variable, using the
 119 formula

$$120 \quad x' = \frac{x - \min(x)}{\max(x) - \min(x)},$$

121 where x is an original value and x' is the standardized value.

122 All the statistical analyses described as follows were performed in R software (R Core
 123 Team, 2013) using packages freely available on the CRAN repository ([http://www.cran-r-](http://www.cran-r-project.org)
 124 [project.org](http://www.cran-r-project.org)).

125

126 **Wavelet analysis.** The effect of *Hyalodiscus* sp. on *C. minimus* short-term temporal dynamics during
 127 the period of predominance of the prey (June 2013 to August 2014) (N = 121) was evaluated by
 128 wavelet analysis (for a detailed description about the use of wavelet analysis in ecological time-series
 129 see Cazelles et al 2008). We also used this analysis to assess the importance of environmental
 130 variables on the short-time distribution of *C. minimus* during the entire sampling period (N = 507).
 131 To overcome the lack of periodicity between the sampling dates, we converted our irregularly
 132 distributed observations into a fixed interval dataset (following Carey et al 2016) by reassigning
 133 sampling days to the closest regularly spaced day on a 3-days interval throughout the time series, and
 134 linearly interpolating the missing data in the rare occasion in which sampling did not occur. Then, the
 135 correlation between the two time-series was assessed by wavelet coherence analysis in software R
 136 using the package ‘WaveletComp’ (Roesch et al 2014). Statistical significance level of the wavelet
 137 coherence was estimated using Monte Carlo permutation test.

138

139 **Cross-correlations (CCF).** We used the sample cross correlation function (*ccf*) of the package ‘*astsa*’
140 to identify time lags (*d*) in the interaction between *C. minimus* abundance (*x*-variable) and
141 *Hyalodiscus* sp. abundance (*y*-variable). For that, only the period of co-occurrence of the two species
142 was considered (June to August 2013; N = 36). Briefly, the cross-correlation analysis calculated
143 correlations between the *y*-variable at *t* and the *x*-variable at different time lags (e.g., $x_{t\pm 1}$, $x_{t\pm 2}$, $x_{t\pm 3}$,
144 and so on). Maximal CCF values indicated the optimal time lag (*d*), with a negative value of *d*
145 indicating a correlation between the *x*-variable at a time before *t* and the *y*-variable at time *t*.

146
147 **Niche analysis.** The niche breath of both *Hyalodiscus* sp. and *C. minimus* was estimated by the
148 outlying mean index (OMI) analysis (Dolédec et al 2000) considering the entire sampling period (N
149 = 507). This analysis allows to determine how the environmental variables affect the species
150 marginality, i.e., the Euclidean distance between the mean habitat condition used by the species and
151 the mean habitat condition observed in the entire sampling domain (Hausser et al. 1995), using the
152 function *niche* of the ‘*ade4*’ package (Dray and Dufour 2007). The reasoning behind the OMI analysis
153 was described in detail by Dolédec et al. (2000). Briefly, a PCA was first performed using the
154 environmental matrix to determine the position of the sampling units (SUs) in the multivariate space,
155 with the origin of the PCA axes corresponding to the center of gravity (*G*) of the SUs (i.e, represents
156 the average mean habitat of the sampling domain). Based on the distribution of the species in the
157 different SUs, a center of gravity was calculated for each species considering only the samples where
158 the species occurred. The OMIs for the different species were then estimated by the Euclidean
159 distance between the species center of gravity (representing the mean habitat condition used for the
160 species) and *G*. The OMI (i.e., species marginality) depends on the deviation from a theoretical
161 ubiquitous, uniformly distributed species that would occur under all available habitat conditions (i.e.,
162 observed in all SUs) (OMI = 0) and it is inversely relate to the tolerance index (an estimate of niche
163 breath). Thus, species with low OMI occur in typical (or common) habitat of the sampling region
164 (i.e., the mean habitat condition used by the species is similar to the mean habitat condition observed
165 in the entire sampling domain). They usually show high tolerance and are associated to a wide range
166 of environmental conditions (i.e., generalists). On the contrary, species with high OMI occur in
167 atypical habitats and are expected to have low tolerance associate to a distribution across a limited
168 range of environmental conditions (i.e., specialists) (Dolédec et al 2000).

169
170 **Polynomial regressions.** Polynomial regressions were used to fit no-linear relationships between the
171 response *y*-variable (*Hyalodiscus* sp. abundance) and explanatory *x*-variables (*C. minimus* abundance
172 and the environmental variables), using the *poly* function in the package ‘*stats*’. To evaluate whether
173 the effect of the different variables changed according to the temporal scale considered, the analyses

174 were performed considering two time-windows: 1) the entire sampling period (January 2012 to
 175 December 2016; i.e., interannual scale) (N = 507) and 2) only the period of *C. minimus* predominance
 176 (June 2013 to August 2014) (N = 121). In both cases, we first performed separated univariate
 177 polynomial regressions where the effect of the each environmental variable on *Hyalodiscus* sp. was
 178 evaluated individually. We also checked for interaction between the significant variables. When more
 179 than one variable (or interaction between variables) were found significant, their relative importance
 180 was assessed using multiple polynomial regression. As a 6-days time lag was detected between
 181 *Hyalodiscus* sp. and *C. minimus*, each *Hyalodiscus* sp. value was paired with the *C. minimus* value
 182 observed 6 days before (i.e., correlation between x_{t-6} and y_t). For the other variables, regressions
 183 considered simultaneous values simultaneously (i.e., correlation between x_t and y_t).

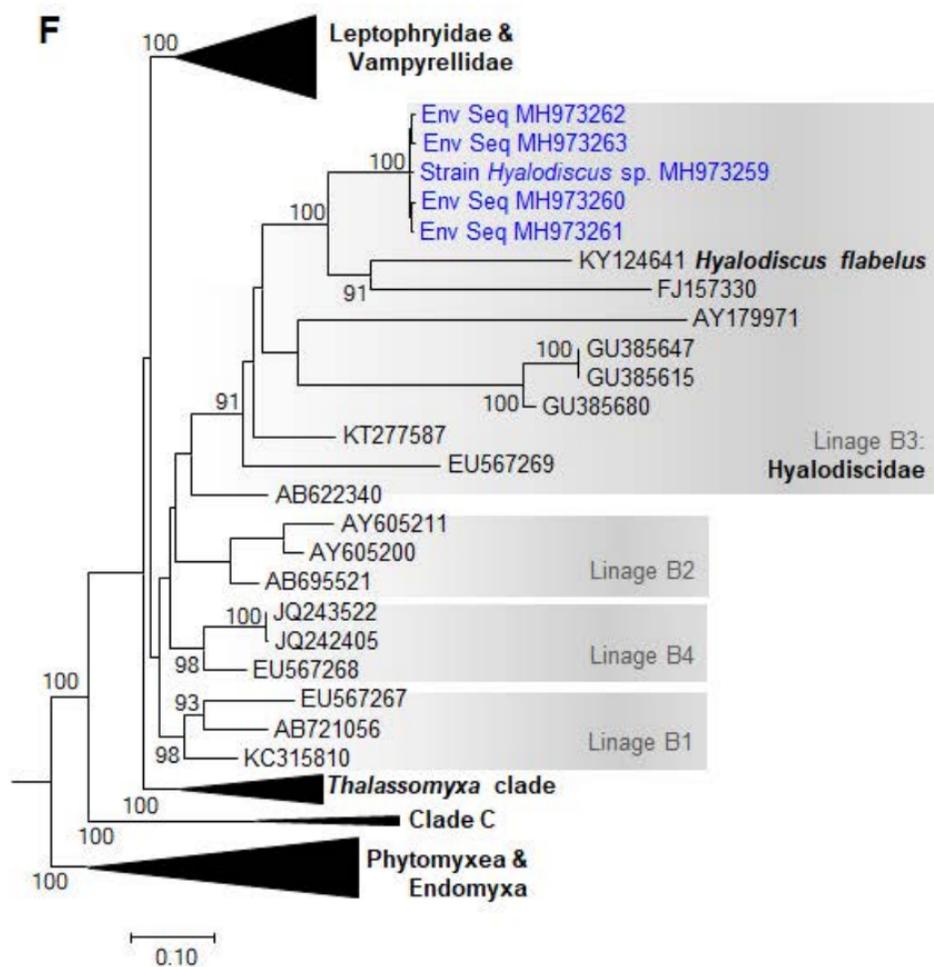
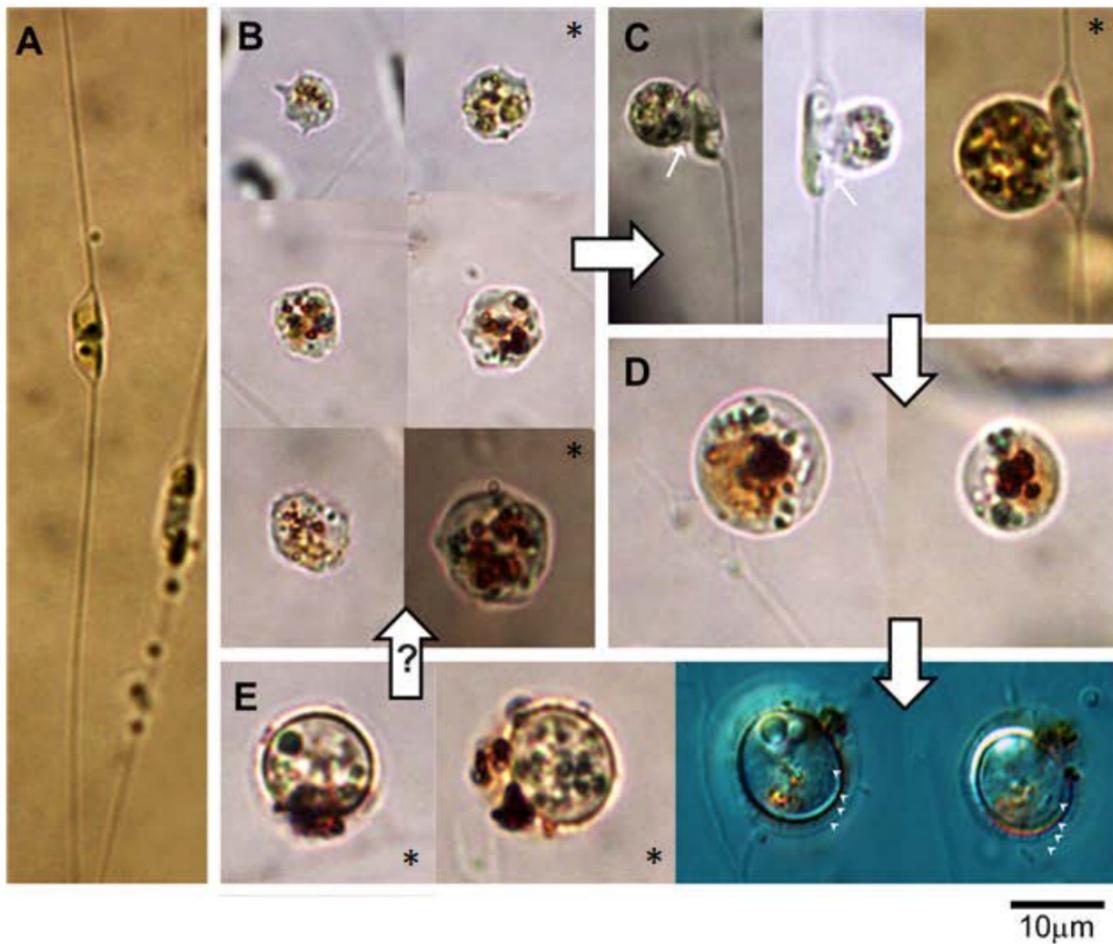
184 Polynomial regressions were also used to evaluate the relative importance of time-lagged (t -
 185 6) *Hyalodiscus* sp. and the environmental conditions for *C. minimus* distribution, following the
 186 procedure described previously. In this case, the two time-windows considered were: 1) the period of
 187 *C. minimus* predominance (June 2013 to August 2014; N = 121) and 2) the period of *C. minimus* and
 188 *Hyalodiscus* sp. co-occurrence (June to August 2013; N = 36).

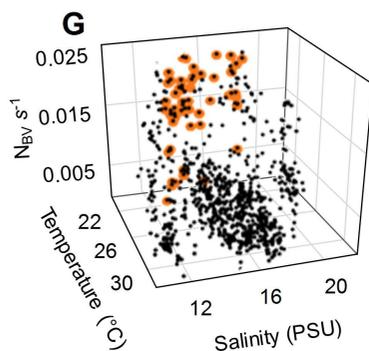
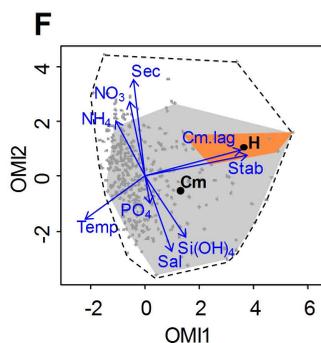
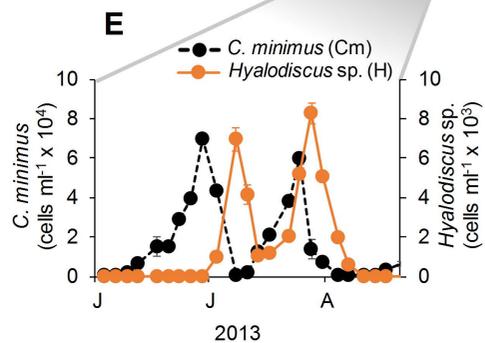
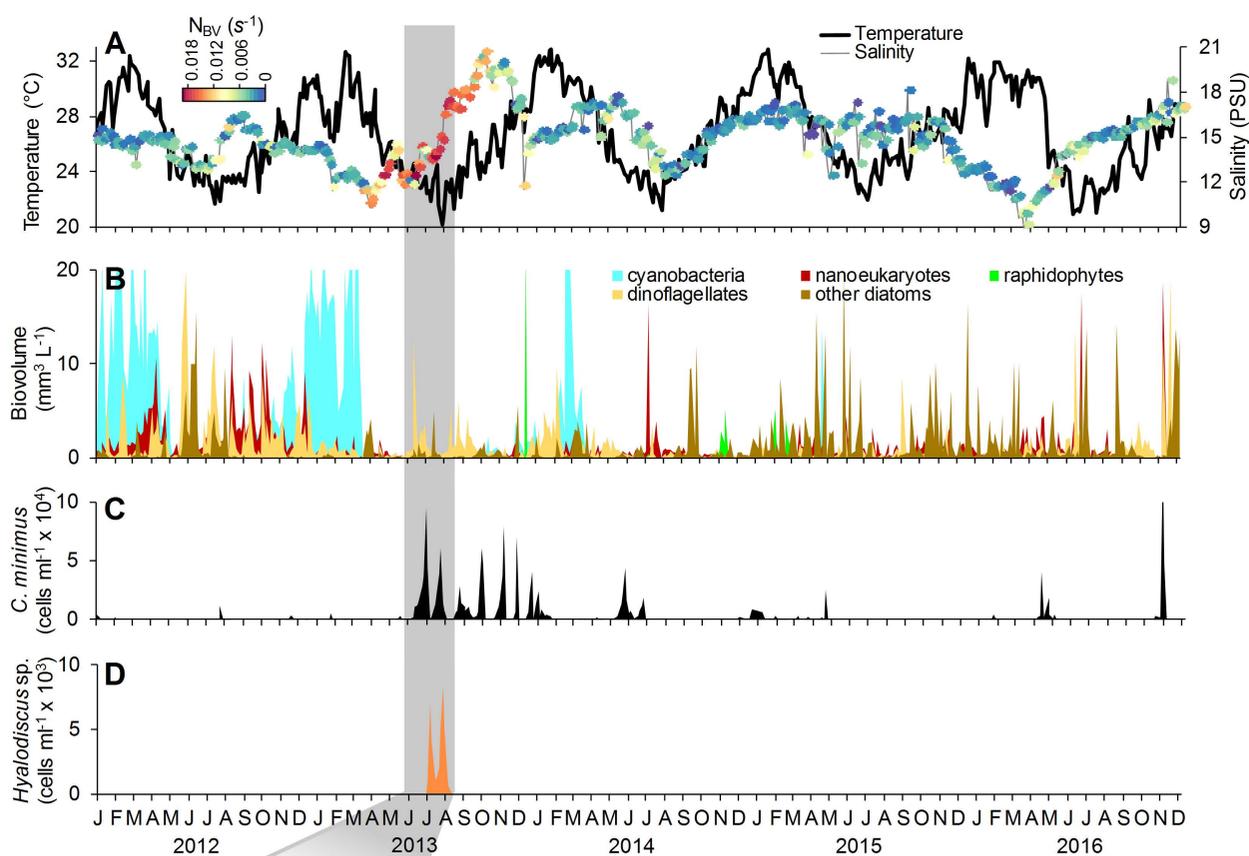
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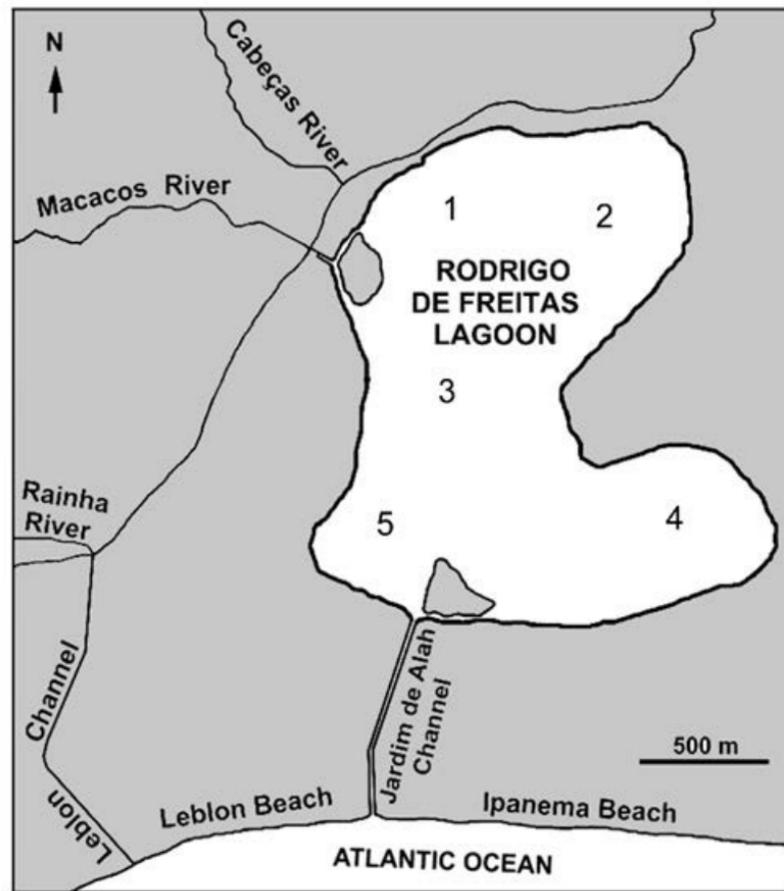
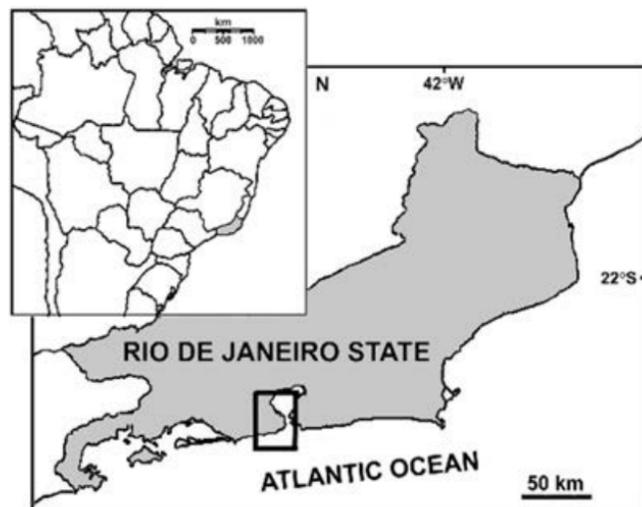


Fig. S1 Location of the five sampling stations in Rodrigo de Freitas Lagoon

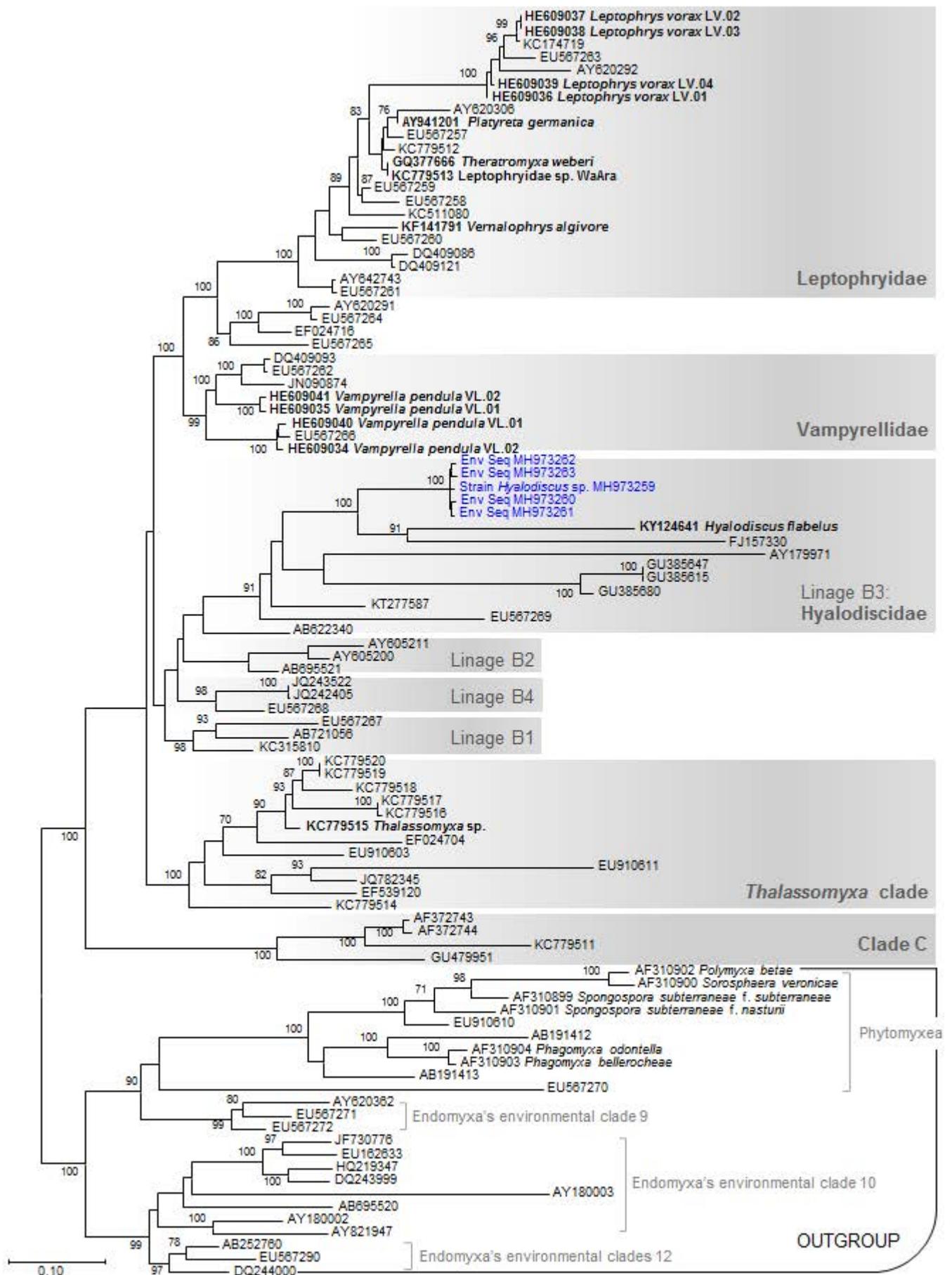


Fig. S2 Maximum-likelihood tree (SSU rDNA) of vampyrellids showing the phylogenetic position of *Hyalodiscus* sp. sequences (in blue) into the Hyalodiscidae clade. Only bootstrap values higher than 70% are shown.

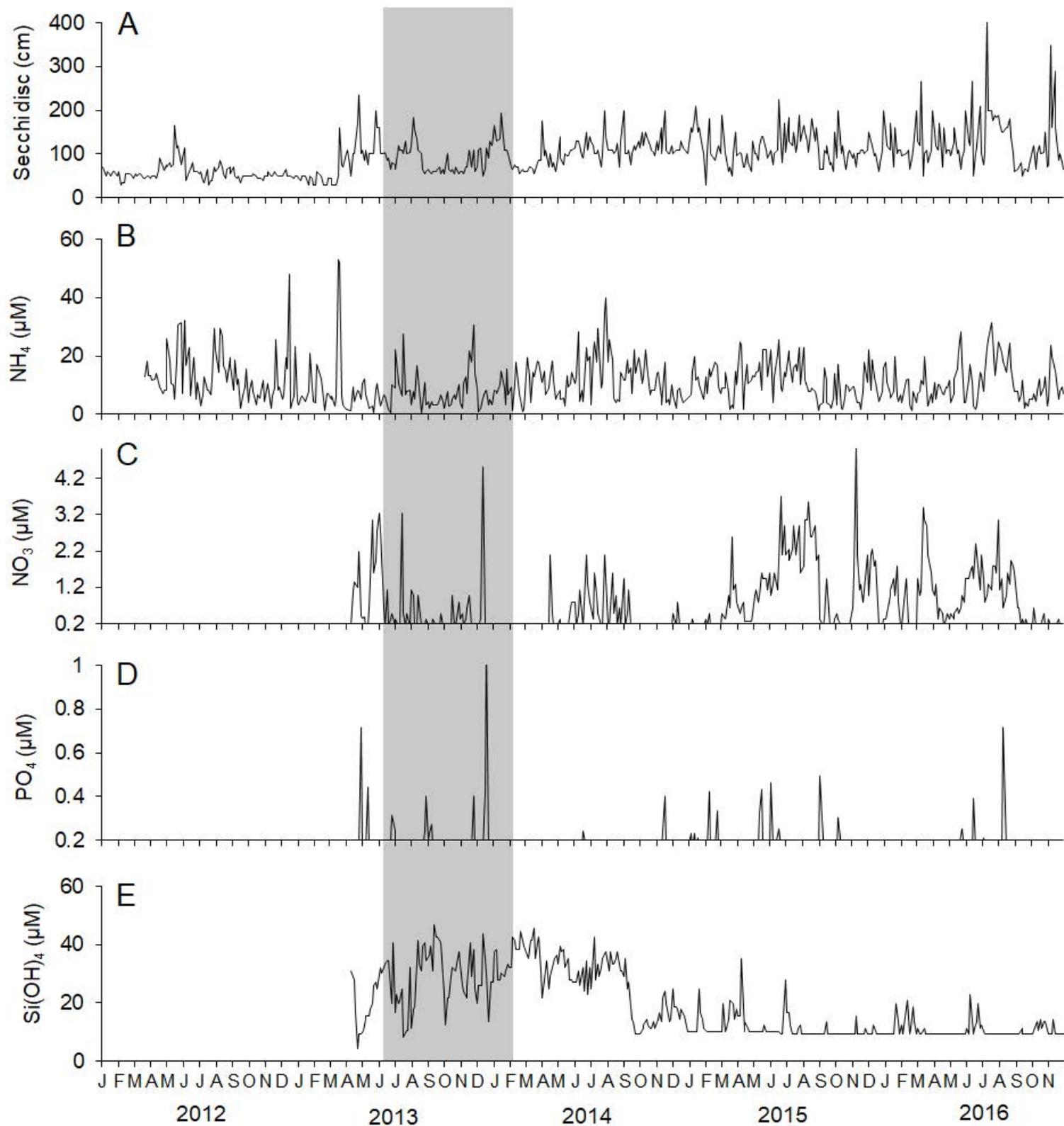


Fig. S3 a-e Interannual variability of water transparency (Secchi disc, cm) and nutrient concentration (μM). Period of intermittent predominance of *Chaetoceros minimus* is highlighted in grey.

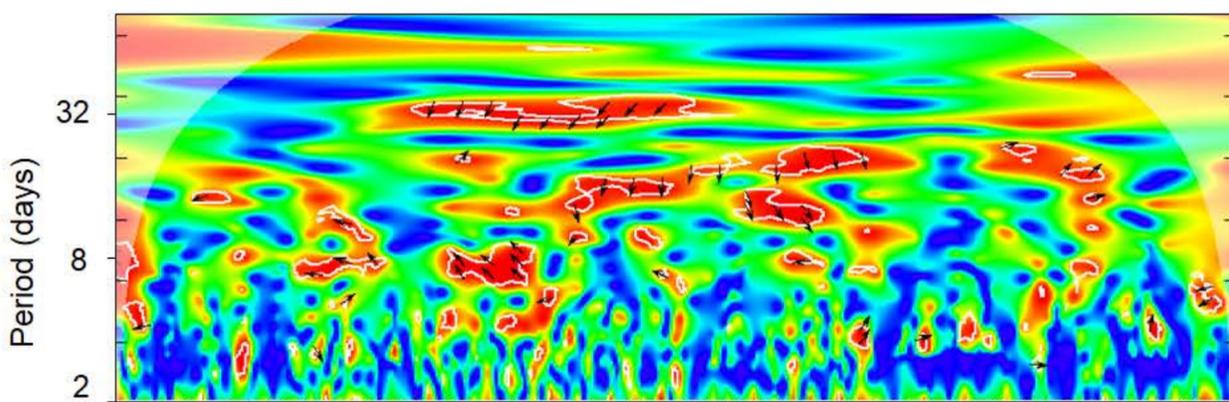
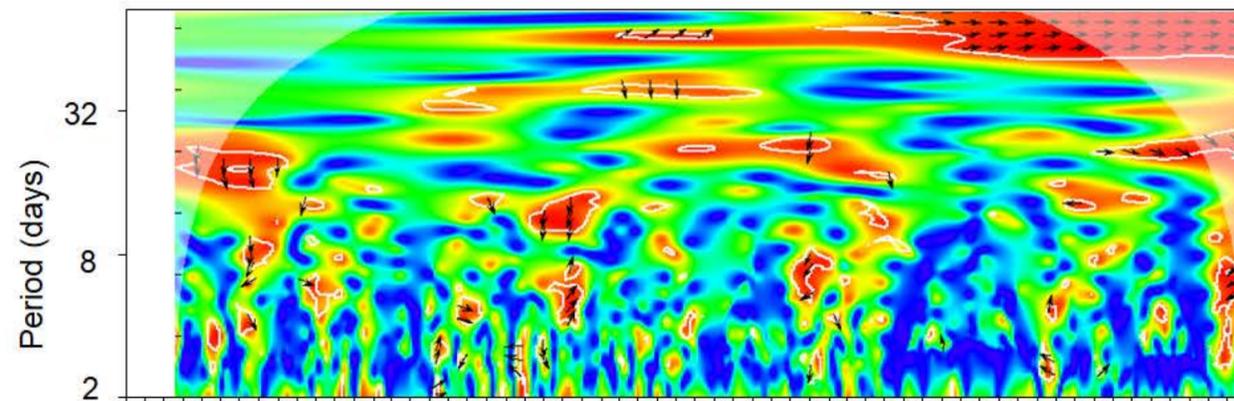
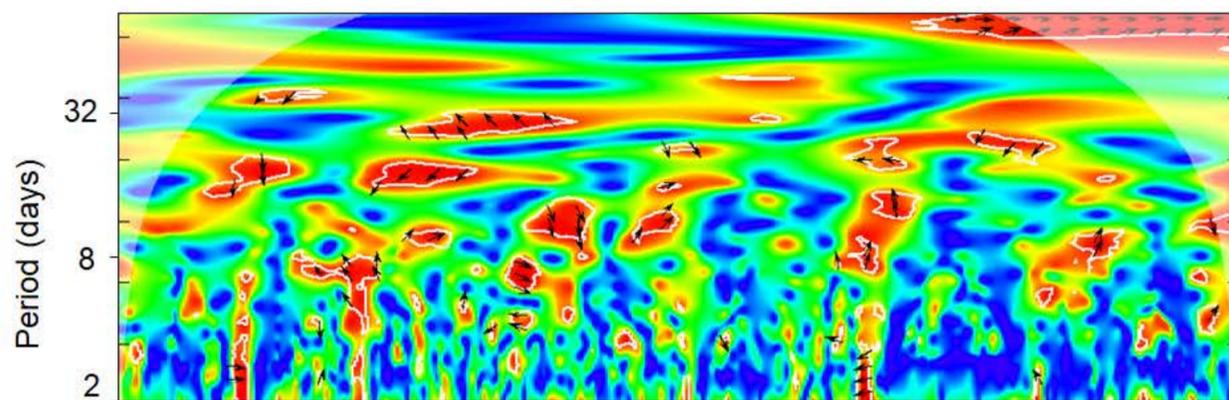
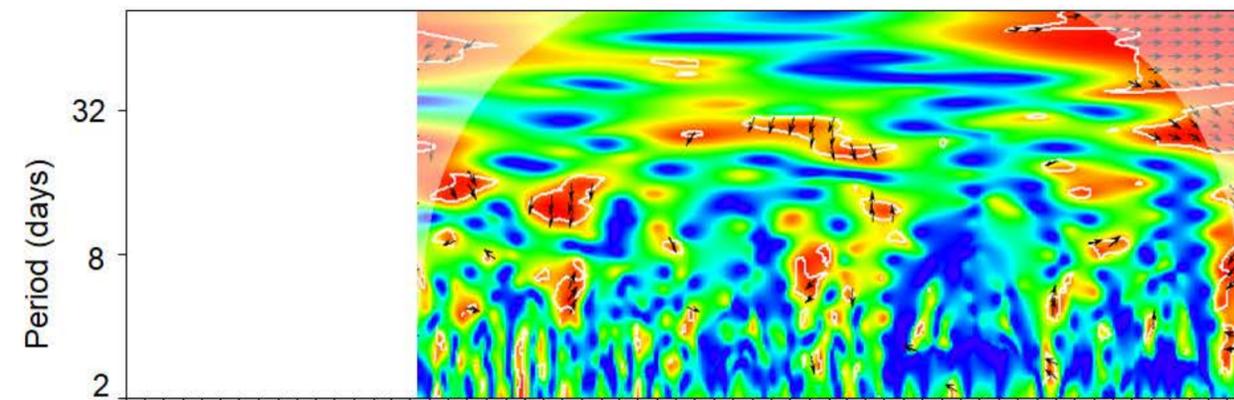
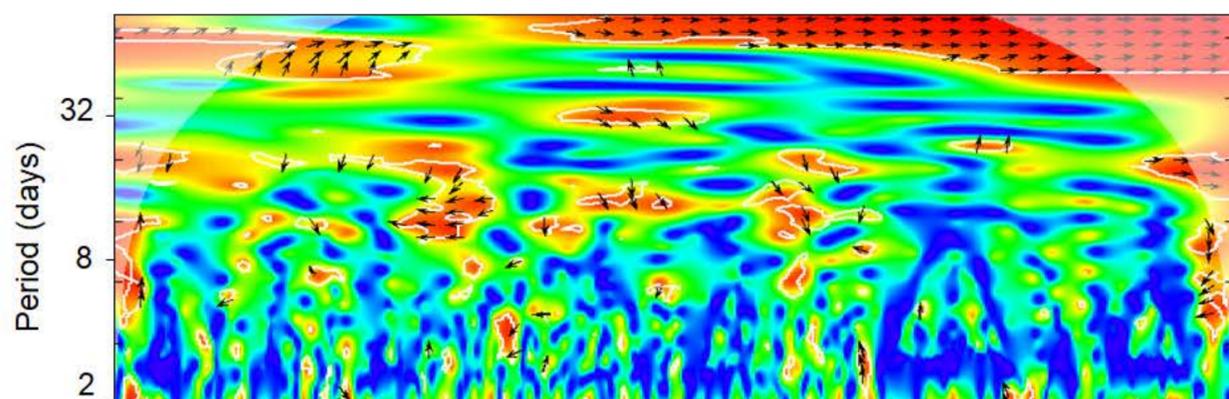
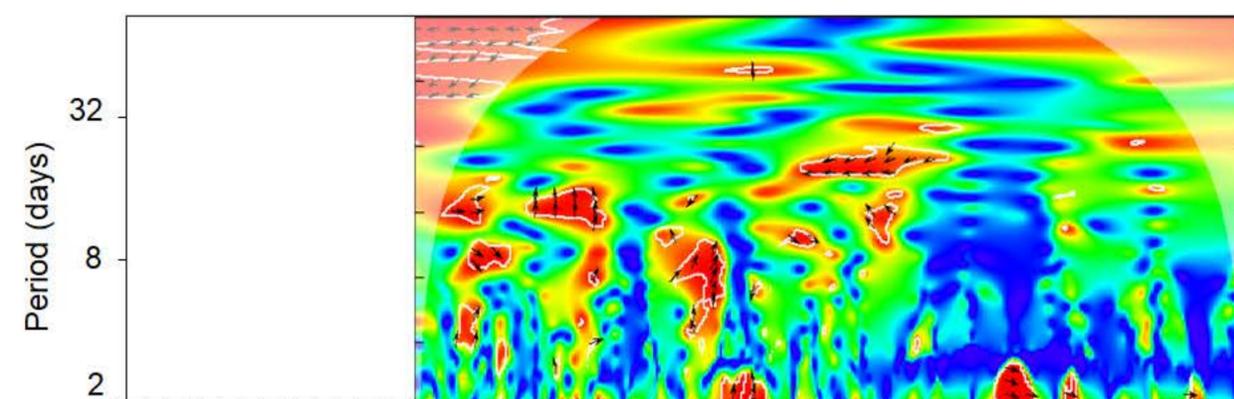
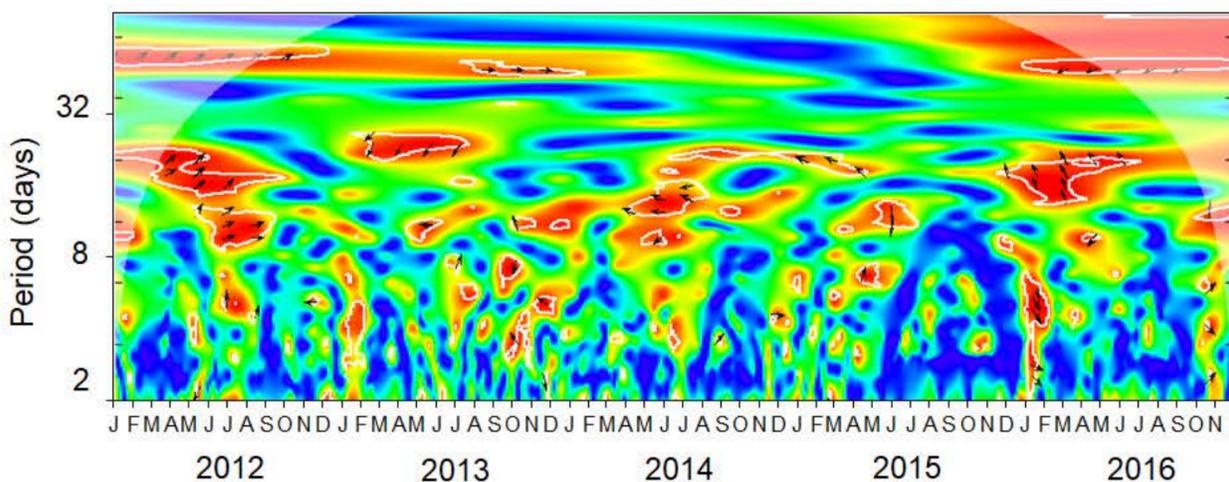
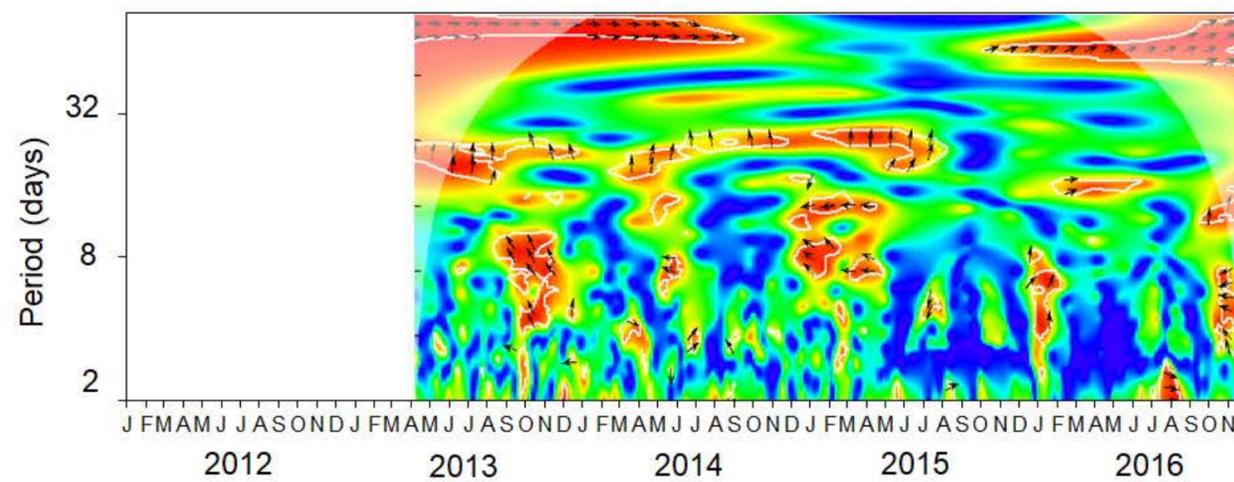
Temperature vs. *C. minutus*NH4 vs. *C. minutus*Salinity vs. *C. minutus*NO3 vs. *C. minutus*Secchi vs. *C. minutus*PO4 vs. *C. minutus*BV Frequency vs. *C. minutus*Si(OH)4 vs. *C. minutus*

Fig. S4 Wavelet coherence between environmental variables (considered as time-series x) and *Chaetoceros minutus* (considered as the time-series y). Periods with significant ($p > 0.05$) coherence are delimited by a thick white line whereas the transparent white area delimits the region where edge effects might distort the results. The phase angles (arrows) illustrate if the two time-series are in phase (positive correlation) or out of phase (negative correlation) and also if there is a time delay between them: in phase with x followed by y (\nearrow), out of phase with y followed by x (\searrow), out of phase with y followed by x (\nwarrow), out of phase with x followed by y (\swarrow). Series in phase and out of phase without a time delay are indicated by \rightarrow and \leftarrow , respectively. The period of intermittent *C. minutus* is indicated by the blue bars.

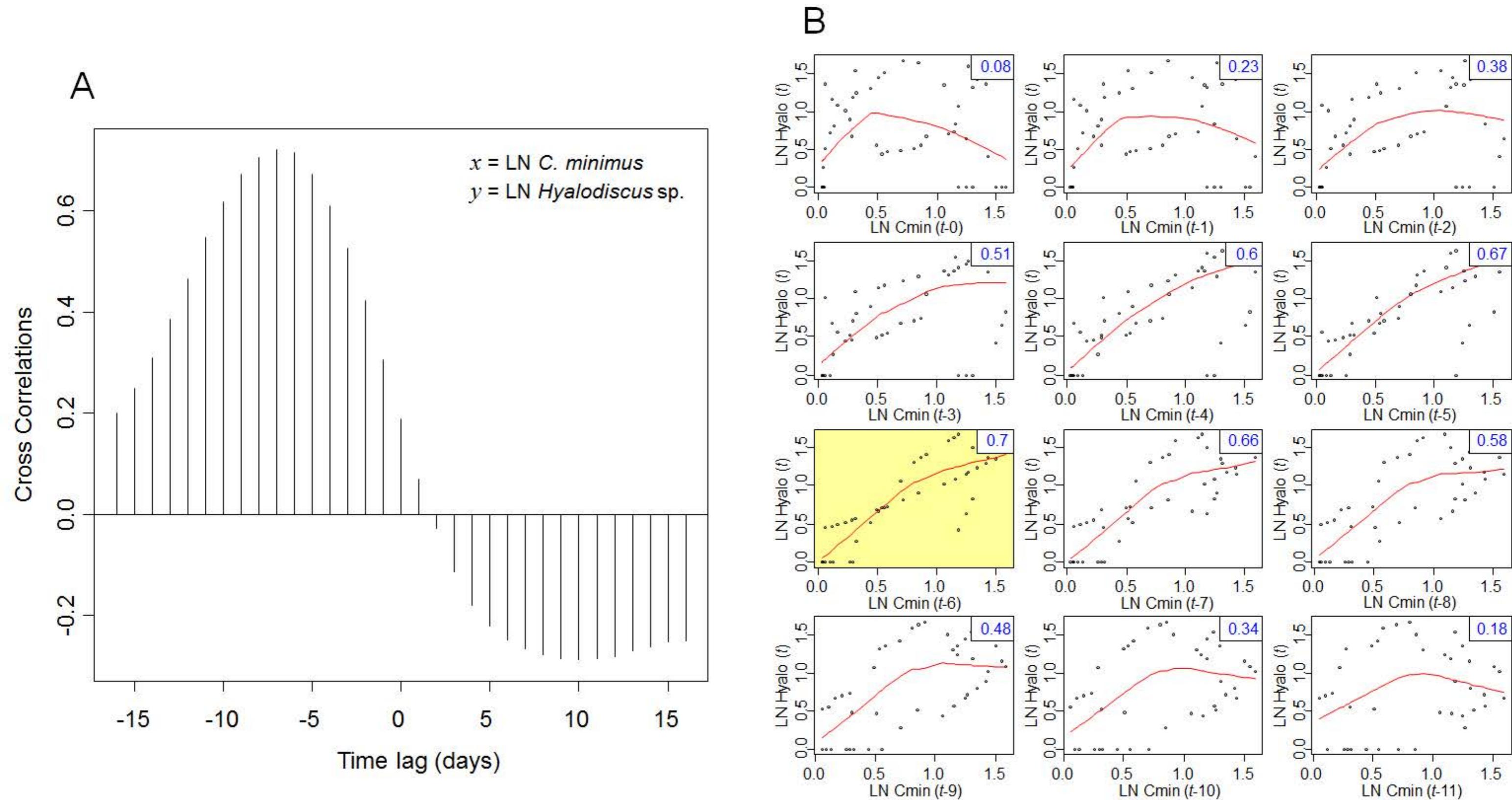


Fig S5 **a** Cross-correlation between *Chaetoceros minimus* (as the x -variable) and *Hyalodiscus* sp. (as the y -variable) at different time-lags (days). **b** Plots of cross-correlation at different time-lags (time-lag with highest cross-correlation is highlighted in yellow).

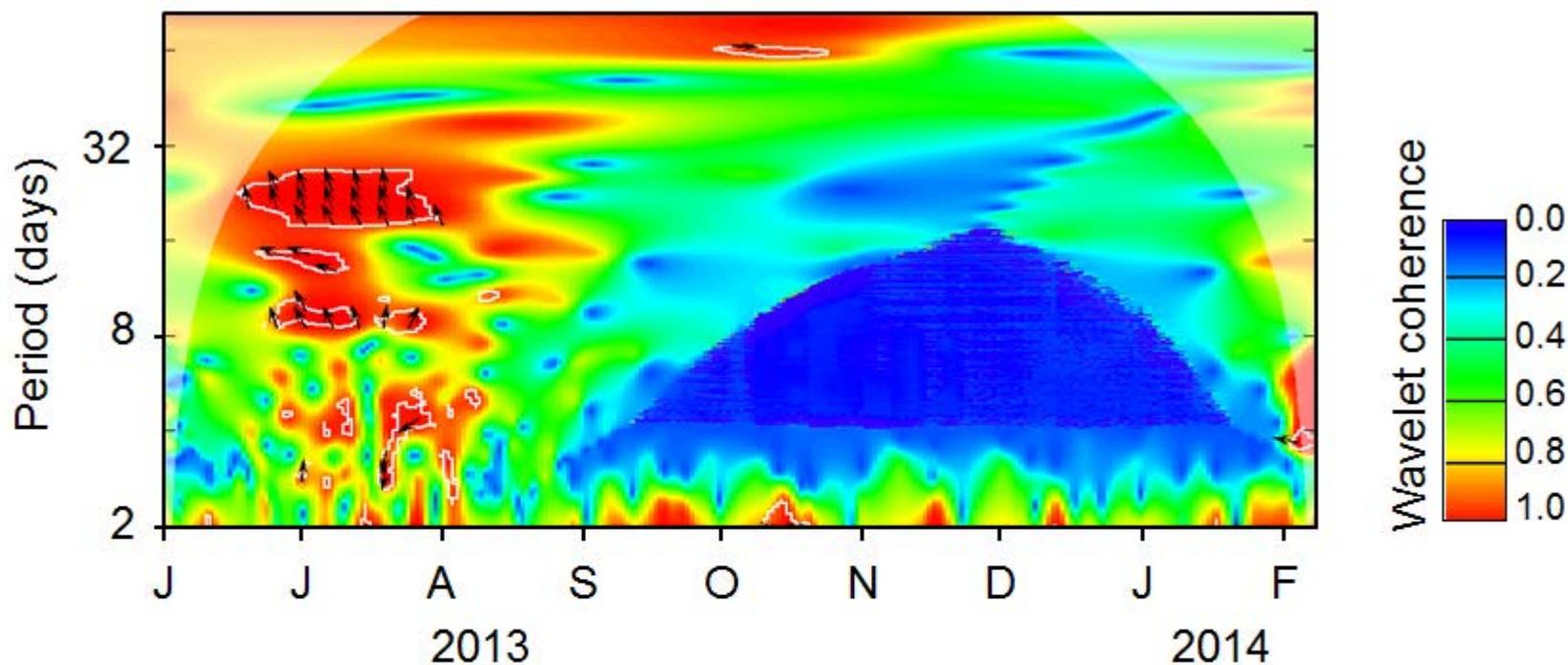


Fig. S6. Wavelet coherence analysis between *Hyalodiscus* sp. (considered as the time-series x) and *C. minimus* (considered as the time-series y). Periods with significant coherence ($p > 0.05$) are delimited by a white line whereas the transparent white area delimits the region where edge effects might distort the results. The predominant phase angles (ϕ) indicate that the two series were out of phase (negative correlation) with increase in x followed by a decrease in y .

Table S1. Result of polynomial regressions evaluating the effect of environmental variables and time-lagged (t_{+6}) *Hyalodiscus* sp. abundance (*Hyalo*) on *C. minimus* abundance. Temp = temperature, Sal = salinity, NBV = Brunt-Väisälä frequency (an estimate of water column stratification), Secchi = Secchi disk. *Interaction between variables; **Degrees for the different variables in the multiple polynomial regressions were the same used in the univariate polynomial regressions. Significant values are indicated in bold.

	Period of <i>C. minimus</i> predominance (January 2013 to August 2014)				Period of <i>Hyalodiscus</i> sp. occurrence (June to August 2013)			
	R ²	Partial R ²	<i>P</i>	degree	R ²	Partial R ²	<i>p</i>	degree
<i>Univariate polynomial regressions</i>								
Temp	0.05	-	0.81	2	0.20	-	0.019	
Sal	0.01	-	0.40	2	0.24	-	0.017	
N _{BV}	0.04	-	0.99	2	0.03	-	0.32	
Secchi	0.02	-	0.60	2	0.27	-	0.004	
<i>Hyalo</i> (t_{+6})	0.04	-	0.048	2	0.47	-	>0.001	
NO ₃	0.007	-	0.93	2	0.24	-	>0.001	
NH ₄	0.013	-	0.82	2	0.22	-	0.013	
PO ₄	0.14	-	>0.001	3	0.28	-	>0.001	
SiOH ₄	0.20	-	0.02	2	0.15	-	0.044	
<i>Significant interactions</i>								
SiOH ₄ * <i>Hyalo</i> (t_{+6})	0.27	-	0.036	*	-	-	-	
PO ₄ * <i>Hyalo</i> (t_{+6})	0.2	-	0.06	*	-	-	-	
SiOH ₄ * PO ₄ * <i>Hyalo</i> (t_{+6})	0.45	-	0.96	*	-	-	-	
SiOH ₄ * <i>Hyalo</i> (t_{+6}) + PO ₄	0.40	0.13	>0.001	*	-	-	-	
<i>Multiple polynomial regression</i>								
<i>Hyalo</i> (t_{+6})	-	-	-	-	0.47	0.47	>0.001	
<i>Hyalo</i> (t_{+6}) + Secchi	-	-	-	-	0.472	0.002	>0.001	
<i>Hyalo</i> (t_{+6}) + Secchi + PO ₄	-	-	-	-	0.56	0.088	>0.001	
<i>Hyalo</i> (t_{+6}) + Secchi + PO ₄ + NH ₄	-	-	-	-	0.58	0.02	>0.001	
<i>Hyalo</i> (t_{+6}) + Secchi + PO ₄ + NH ₄ + NO ₃	-	-	-	-	0.61	0.03	>0.001	
<i>Hyalo</i> (t_{+6}) + Secchi + PO ₄ + NH ₄ + NO ₃ + SiOH ₄	-	-	-	-	0.618	0.008	>0.001	

Table S2. Result of polynomial regressions evaluating the effect of environmental variables and time-lagged (t_{-6}) *C. minimus* abundance (*Cmin*) on *Hyalodiscus* sp. abundance. Temp = temperature, Sal = salinity, NBV = Brunt-Väisälä frequency (an estimate of water column stratification), Secchi = Secchi disk. *Interaction between variables; **Degrees for the different variables in the multiple polynomial regressions were the same used in the univariate polynomial regressions. Significant values are indicated in bold.

	Entire study period				Period of <i>Chaetoceros minimus</i> predominance (January 2013 to August 2014)			
	R ²	Partial R ²	P	degree	R ²	Partial R ²	p	degree
<i>Univariate polynomial regressions</i>								
Temp	0.24	-	<0.001	5	0.51	-	<0.05	3
Sal	0.002	-	0.237	2	0.16	-	<0.001	2
N _{BV}	0.23	-	<0.001	4	0.13	-	0.09	2
Secchi	0.008	-	0.13	2	0.15	-	0.1	2
<i>Cmin</i> (t_{-6})	0.19	-	<0.001	1	0.39	-	<0.001	1
<i>Significant interactions</i>								
Temp * <i>Cmin</i> (t_{-6})	0.74	-	<0.001	**	0.75	-	<0.001	**
Temp * N _{BV}	0.63	-	0.02	**	-	-	-	-
Sal * <i>Cmin</i> (t_{-6})	-	-	-	-	0.59	-	0.007	**
N _{BV} * <i>Cmin</i> (t_{-6})	0.78	-	<0.001	**	-	-	-	-
Temp * N _{BV} * <i>Cmin</i> (t_{-6})	0.95	-	<0.001	**	-	-	-	-
<i>Multiple polynomial regressions</i>								
Temp * <i>Cmin</i> (t_{-6})	-	-	-	-	0.75	0.75	<0.001	**
Temp * <i>Cmin</i> (t_{-6}) + Sal * <i>Cmin</i> (t_{-6})	-	-	-	-	0.77	0.02	<0.001	**