



21 **Abstract**

22 Organisms adjust their size according to temperature and supposedly also respond to its negative  
23 covariate, oxygen. To what extent is size a response to temperature or oxygen? We analyzed the  
24 thermo-oxygenic niche for the community of 188 rotifer species. Evolution toward ranges of  
25 thermal tolerance occurred separately from evolution toward their optima. Body size was  
26 adjusted to both temperature and oxygen, but the cues for body size response differed; size was  
27 either driven by optimal temperatures or by the oxygen tolerance range. Animals are clearly  
28 separated into generalists or specialists, and their evolutionary body size adjustment is realized  
29 through differential responses to environmental factors. Oxygen is as important as temperature in  
30 the evolution of body size and ecological niche preference. An important conclusion from this  
31 study is that oxygen deprivation following global warming seems to be as problematic for the  
32 studied organisms as the temperature increase itself.

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## 42 **Introduction**

43 To understand the link between the performance of organisms and their environment is one of the  
44 *Grand Challenges* in organismal biology (Schwenk *et al.* 2009), especially in the context of  
45 abrupt climatic change (Allen *et al.* 2018). The general prediction is that body size decrease is a  
46 third universal response to global warming, especially in aquatic systems, following the  
47 geographic and phenological shifts in species distribution (Daufresne *et al.* 2009). Understanding  
48 the body size response to environmental factors is essential because this trait is unique  
49 (Kozłowski 2006). It can be perceived mutually as a morphological, physiological and life history  
50 trait, interconnecting the fields of ecology, evolution, and physiology. Despite the fundamental  
51 role of body size response, the key question of how animals become the size they are still awaits  
52 a satisfactory answer (Callier & Nijhout 2014). What kind of negative changes should we expect  
53 in the communities exposed to warming? Can we prevent at least some of these negative  
54 changes? Which actions should we undertake? What organisms do we save first? These urgent  
55 questions are not trivial for many reasons; however, the fundamental question of how organisms  
56 interact with their thermal environment remains unanswered. The crucial role of body size is that  
57 this trait is the major target of selective response on the organismal level (Kozłowski 2000), and  
58 such individual response affects the whole community through the plethora of possible ways  
59 including trophic interactions, dispersal abilities, habitat exploitation, nutrient cycling and others  
60 (Hildrew *et al.* 2007).

61 Ambient temperature is the most influential variable that shapes organismal strategies, from  
62 physical effects, through evolutionary influence, to ecological interactions (Schmidt-Nielsen  
63 1990; Willmer *et al.* 2000; Begon *et al.* 2006). All life forms are equipped with mechanisms to  
64 detect and react to changing temperatures. Body size adjustment is an example of such a reaction.

65 It is a phenomenon observed both genetically and phenotypically and is an assumed driver for  
66 interspecific Bergmann's rule (Bergmann 1847) and the intraspecific temperature-size rule  
67 (Atkinson 1994). Body sizes changed with seasonal variations in temperature according to the  
68 rule "when it's hot, shrink" in both small-scale (Kiełbasa *et al.* 2014) and large-scale studies  
69 (Horne *et al.* 2016; Horne *et al.* 2017). The empirical evidence for the direction of thermally  
70 induced body size changes on both the genetic and phenotypic levels in the same species is  
71 scarce, and it provides an ambiguous view of either positive covariance, as in *Brachionus*  
72 *plicatilis* rotifer (Walczyńska *et al.* 2017) or the negative one, as for the case of *Drosophila*  
73 *pseudoobscura* (Taylor *et al.* 2015).

74 Most other environmental variables are at least partly positively correlated with temperature.  
75 Oxygen is an exception because it is negatively correlated with temperature (Wetzel 2001 for  
76 aquatic systems). Oxygen stress often occurs at higher temperatures, because the energetic  
77 demands of an organism grow faster than the oxygen supply (Verberk *et al.* 2011). This  
78 phenomenon is especially important for organisms that inhabit aquatic environments (Forster *et*  
79 *al.* 2012; Horne *et al.* 2015; Horne *et al.* 2017). It is caused by the fact that breathing under water  
80 is much more challenging than in air because of the much slower oxygen diffusion in the former  
81 system (Verberk *et al.* 2011). Oxygen stress at higher temperatures has been suggested to cause  
82 the cell size to decrease to enhance the efficiency of oxygen transport to the mitochondria  
83 (Woods 1999), and as a result, the whole body shrinks (Atkinson *et al.* 2006). Animals anticipate  
84 oxygen deficiency when experiencing a temperature increase (Walczyńska *et al.* 2015). Thus,  
85 they face the ecophysiological dilemma of either accepting this challenge and adjusting their cell  
86 (and body) size to overcome the reduced aerobic metabolism efficiency or behaviorally adapting  
87 to more favorable conditions. Those able to decrease their size will respond until the conditions

88 are too stressful, while those lacking the ability to respond are immediately at risk when escape is  
89 not possible. To understand whether and how organisms respond to change, we first need to  
90 identify the exact environmental response cues and initiation mechanisms. Including oxygen  
91 concentrations as a covarying parameter in the studies on size-to-temperature response facilitates  
92 the interpretation of the results (Kiełbasa *et al.* 2014; Walczyńska & Sobczyk 2017). A main  
93 question has emerged: what exactly drives body size responses?

94 Here, we used an exceptional field model to study evolutionary outputs that could not be  
95 experimentally tested in the laboratory. We scrutinized data published 30 years ago in the new  
96 context of thermo-oxygenic niche construction within an aquatic community. The original data  
97 were provided by Bērzinš and Pejler, who reported the results of planktic, periphytic and benthic  
98 rotifer sampling from approximately 600 different water bodies (lakes, ponds, rivers and mires)  
99 in Sweden for approximately 40 years. The authors related the occurrence of each species to  
100 temperature (Bērzinš & Pejler 1989b) and oxygen concentration (Bērzinš & Pejler 1989a), among  
101 other factors. The data, supplemented with information on species-specific body sizes, gave us a  
102 unique opportunity to study the interspecific size response to subtle aspects of the environment at  
103 the macroevolutionary scale, in the context of ecological niche.

104 The evolution of the thermal niche is still an ecological riddle; natural selection shapes the  
105 thermal optimum, breadth of tolerance range and performance limit, but the correlations between  
106 these traits remain unknown, preventing an understanding of the ecological diversity of life  
107 (Mongold *et al.* 2008). An extension of this issue is whether species with a specific thermal range  
108 specialize at the same level along other niche axes (Sheth & Angert 2014). To answer these  
109 questions, we analyzed how similar the preferences for different thermal and oxygenic conditions  
110 were within a community of rotifers. We also refer to an important demarcation line in the

111 strategy of dealing with the environment, through a distinction between generalists, organisms  
112 that display the relatively high and flat performance across environments, and specialists, those  
113 that perform better in one type of environment than in all the others (Levins 1968). We examined  
114 how species-specific standard body size was affected by the joint thermal and oxygenic  
115 conditions of living. To find the subtle environmental cues for the possible size differences we  
116 preceded with the multivariate analyses to reveal how the species preferences described by the  
117 temperature and oxygen optima, ranges and tolerance limits were interrelated at the community  
118 level. We predicted the species body size to be affected by both the temperature and oxygen.  
119 We provide the first evidence of sharing the two-dimensional ecological niche, and of  
120 interspecific evolutionary body size response to this niche, for the large community of aquatic  
121 organisms.

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## 123 **Material and methods**

124 **Preparation of the dataset.** Each rotifer species was characterized by eight parameters  
125 describing its environmental preferences, namely, minimum/maximum/optimum/range of  
126 temperature/oxygen concentration in the living habitat. We obtained data on these species-  
127 specific parameters from two publications: Bērzinš and Pejler (1989a)(minimum, maximum,  
128 optimum and range of tolerance to oxygen concentration) and Bērzinš and Pejler (1989b) (the  
129 same parameters describing tolerance to temperature). We interpreted the values from the figures  
130 using millimeter paper, with an accuracy of 0.5 mg/L for O<sub>2</sub> and 1 °C for temperature. In both  
131 cases, the tolerance range was calculated as *max - min*, while the optimal value was assumed to  
132 be the value with the maximal abundance, as presented in the respective original figures. To  
133 compare the variability within the environmental variables examined, we estimated the

134 coefficient of quartile variation (CQV), calculating  $(Q_3 - Q_1) / (Q_3 + Q_1)$ , where  $Q_1$  is a first quartile  
135 and  $Q_3$  is a third quartile, for each variable separately.

136 The authors of the original articles did not provide data on the sizes of the species, but we were  
137 interested in the relative differences in rotifer species-specific body size rather than their local  
138 adaptations. Thus, we analyzed the association between the species standard body length ( $\mu\text{m}$ ) of  
139 rotifers, collected from the available databases, including Bielańska-Grajner *et al.* (2013),  
140 Bielańska-Grajner *et al.* (2015), Kreutz and Foissner (2006), Segers (1995), Segers and Shiel  
141 (2005) and the database from the website of the National Institute for Environmental Studies  
142 (<http://www.nies.go.jp/>), by searching for the species Latin name in a website browser. We  
143 standardized the length of the body by using the values provided for fixed, nonstretched  
144 individuals and excluding the length of toes and other appendages. In the cases when a size range  
145 was provided, we calculated the mean value. When more than one dataset was provided,  
146 especially for cited websites, we calculated the mean for all the subsources. The dataset for the  
147 188 rotifer species, with the species-specific body lengths and sources of information, are  
148 provided in the supplementary materials (Table S1).

149 **PCA analysis.** To determine how the parameters describing the thermal and oxygenic  
150 preferences grouped at the interspecific level, we conducted the principal component analysis  
151 (PCA), with rows representing the species and columns represented by eight environmental  
152 parameters. We log-transformed and standardized the data to provide the correlation matrix and  
153 we conducted the analysis in CANOCO 5.0 (Ter Braak & Šmilauer 2012).

154 **Phylogenetic analysis.** We performed all phylogenetic analyses in the R computational  
155 environment (v3.4.0) (R Core Team 2017). We obtained the phylogeny of 188 rotifer species  
156 from the open tree of life (Hinchliff *et al.* 2015) and ‘rotl’ package v3.0.3 (Michonneau *et al.*  
157 2016). Because the branch lengths were not available, we automatically estimated them using a

158 method proposed by Grafen (1989) and the ‘ape’ package v4.1 (Paradis *et al.* 2004). All the path  
159 lengths from the root to the tips were equal. The tree contained polytomies, where more than two  
160 branches descended from a single node. Simulation studies have generally found that independent  
161 contrasts and phylogenetically generalized least squares (PGLS) are fairly robust to errors in both  
162 phylogenetic topology and branch length (Symonds & Blomberg 2014). We tested the  
163 relationship between species body size (length, after natural logarithm transformation) and  
164 species-specific environmental characteristics in a phylogenetic comparative model. We applied  
165 PGLS using the `gls()` function in ‘nlme’ in the caper package ([https://CRAN.R-](https://CRAN.R-project.org/package=caper)  
166 [project.org/package=caper](https://CRAN.R-project.org/package=caper)) considering body size as a response variable and four environmental  
167 parameters (ranges and optima) as explanatory variables. In the model, we assumed Brownian  
168 motion (BM) evolution, which is the most commonly assumed model of phenotypic evolution by  
169 comparative phylogenetic methods (Revell 2010; Lajeunesse & Fox 2015). BM evolution treated  
170 random genetic drift as a primary process resulting in the loss of similarity from ancestral  
171 characteristics (Martins & Garland 1991). Traits, e.g., body size and morphology, exhibit strong  
172 phylogenetic signals and most likely evolve by gradual changes over time according to the BM  
173 model of evolution (Symonds & Blomberg 2014). We assumed the correlation structure based on  
174 Pagel’s  $\lambda$  (Pagel 1999) fixed at 1.

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## 176 **Results**

### 177 **Thermo-oxygenic niche of rotifer assemblage**

178 PCA analysis showed three informative principal components (PCs) for data interpretation  
179 (eigenvalue > 1), explaining 37.47 % (PC1), 29.17 % (PC2) and 15.08 % (PC3) of the variance  
180 (Fig. 1A, B). The evolutionary adaptation may be studied as a process or a product (Mongold *et*  
181 *al.* 2008). Treating the community of rotifers as a gene pool, we actually observed a product of



182 evolution (Kimura 1974) at the assemblage level; the evolution of generalists was affected by  
183 both temperature and oxygen, as explained by PC1 (horizontally oriented arrows for temperature  
184 and oxygen tolerance ranges in Fig. 1A and their high loadings in Fig. 1B), and was clearly  
185 separated from the evolution of specialists, as explained by PC2 (vertically oriented arrows for  
186 temperature and oxygen optima in Fig. 1A and their high loadings in Fig. 1B). The generally  
187 opposite position of the parameters representing temperature vs. oxygen in PC2 (Fig. 1A, C)  
188 reflects the importance of their natural negative correlation in evolutionary processes. PC3 shows  
189 the ecological force of minor importance that caused a non-uniformity in the pattern of breath of  
190 thermal tolerance and hypoxia tolerance: according to PC3 the relationship between  $T_{\text{range}}$  and  
191  $O_{2\text{min}}$  is positive, as compared to their negative link according to PC1 in Fig. 1 B).

192 According to the CQV analysis, the lowest variability was observed for  $T_{\text{max}}$ , oxygen optimum  
193 ( $O_{2\text{opt}}$ ) and oxygen maximum ( $O_{2\text{max}}$ ), followed by optimum temperature ( $T_{\text{opt}}$ ), temperature  
194 range ( $T_{\text{range}}$ ), and oxygen tolerance range ( $O_{2\text{range}}$ ) (Fig. 3C).

### 195 **Body size of species assembling the niche**

196 We tested the body size relationship to the parameters representing the PC1 (ranges) and PC2  
197 (optima). Phylogenetically corrected regression analyses based on data obtained from the open  
198 tree of life (Hinchliff *et al.* 2015) for 188 species (Fig. 2) revealed that rotifer body size increased  
199 with increasing tolerance to oxygen range ( $O_{2\text{range}}$ ,  $p < 0.01$ ; adjusted  $R^2 = 0.054$ ), decreased with  
200 increasing optimal temperature ( $T_{\text{opt}}$ ,  $p < 0.01$ ; adjusted  $R^2 = 0.048$ ), and had no relationship with  
201 the remaining two parameters ( $p = 0.20$  and adjusted  $R^2 = 0.003$  for  $T_{\text{range}}$  and  $p = 0.57$  and  
202 adjusted  $R^2 = -0.004$  for  $O_{2\text{opt}}$ ; Fig. 3). Body size evolved in response to both variables,  
203 temperature and oxygen, but the evolutionary cues for response were different; species were  
204 smaller when specializing to a high optimal temperature or to a narrow oxygen tolerance range  
205 (Fig. 3).

206

## 207 **Discussion**

208 We found that within the rotifer community that involved 188 species of different ecology  
209 (planktic, periphytic and benthic), representing various aquatic habitats (i) species-specific  
210 thermal tolerance range and oxygen tolerance range evolved in the same direction, (ii) optima for  
211 temperature and oxygen evolved in opposite directions. These results raise an intriguing question  
212 regarding the possible different physiological mechanisms behind the selective forces of  
213 adaptation to tolerance ranges *vs.* specialization to specific optima. They also mean that, in  
214 general, a species characterized by the wide range of thermal tolerance should be expected to  
215 have the wide range of tolerance to oxygen availability as well.

216 The analysis of body size relationship with eight environmental variables which describe the  
217 thermo-oxygenic niche showed that, at the interspecific level, body size decreased in response to  
218 both temperature and oxygen, but in different ways; the target of size response was optimum in  
219 the case of temperature and the tolerance range in the case of oxygen.

220 The interspecific variability in each of the eight parameters shows that oxygen changes will affect  
221 the organisms similarly to temperature changes as a consequence of the current climate changes.

222 Araújo *et al.* (2013) estimated the variance in cold tolerance ( $CT_{min}$ ) *vs.* heat tolerance ( $CT_{max}$ ) in  
223 different groups of ectotherms. They found that the cold tolerance variance was almost twice as  
224 high as the heat tolerance variance. Their interpretation of the results was that ectotherms are  
225 more vulnerable to an increase in maximum temperatures than in minimum temperatures;  
226 tolerance to cold is labile and subject to natural selection, whereas tolerance to heat is  
227 physiologically conserved. In this respect, our results are in agreement; the tolerance to cold was  
228 more variable than the tolerance to heat (7.82 *vs.* 5.98 of variance for  $T_{min}$  and  $T_{max}$ , respectively).

229 A similar comparison between oxygen and temperature requires the use of a scale-independent

230 parameter, such as CQV. Provided that the reasoning of Araújo *et al.* is correct, the rotifer  
231 community is most vulnerable to changes in these variables, while the least conserved are  $T_{\min}$   
232 and low oxygen (hypoxia!) tolerance ( $O_{2\min}$ ). The result obtained for species-specific oxygen  
233 optimum and maximum seems to be especially important considering the global ocean is  
234 undoubtedly warming (Cheng *et al.* 2019), and its oxygen concentration is decreasing through  
235 different synergistic mechanisms (Breitburg *et al.* 2018). Our CQV analysis showed that rotifer  
236 community appeared sensitive not only to potential changes in upper thermal limits ( $T_{\max}$ ) but  
237 similarly much to oxygen deprivation below the optimal concentrations at which the species  
238 perform best ( $O_{2\text{opt}}$ ; Fig. 1C). This result acts as a specific warning: aquatic ectotherms are  
239 potentially very vulnerable to climate warming because the successive absolute reduction in  
240 oxygen concentration in water may be too challenging for them to quickly adapt.

241 The exceptional feature of the data published thirty years earlier is that by tracking the members  
242 of the community in their natural habitat, we can actually observe the ecological, realized niche,  
243 which is a component of the fundamental niche remaining for usage in consequence of  
244 interactions with other organisms (Hutchinson 1957). Hence, it seems justified to claim that the  
245 here-studied rotifer community construct their ecological niche by detecting and responding to  
246 subtle environmental cues. Within their thermo-oxygenic niche, the parameters limiting the  
247 interspecific body size adjustment are maximal temperature, optimal oxygen and maximal  
248 oxygen concentration.

#### 249 **Generalist-specialist continuum implications**

250 The PCA showed that evolution toward ranges of temperature and oxygen tolerance occurred  
251 separately from evolution toward specialization for high performance at pick values of both  
252 parameters. This clear trade-off between evolution toward generalist and specialist strategies  
253 agree with current theoretical predictions (Levins 1968; Huey & Hertz 1984). As a consequence

254 of distinguished patterns of adaptation to ranges of tolerance or specialization for certain optimal  
255 values, the simple observation of decreasing body size with increasing temperature within the  
256 thermo-oxygenic niche may be an outcome of two different physiological mechanisms leading to  
257 the same result: a large size at low temperatures and high relative oxygen availability and a small  
258 size at high temperatures and low relative oxygen availability (Fig. 4). This retorts to the matter  
259 whether decreasing in heat is indeed equivalent to increasing in cold (Walczynska *et al.* 2018).  
260 The former is driven by oxygen-limited aerobic metabolism (Woods 1999; Verberk *et al.* 2011)  
261 and may evolve along with a narrow tolerance to oxygen conditions (this study), while the latter  
262 may be driven by the limitation of proper genome maintenance in cold conditions (Xia 1995;  
263 Woods *et al.* 2003; Hessen *et al.* 2013) and/or the energetic costs of cell membrane maintenance  
264 (Szarski 1983) and may reflect specialization toward a low optimal temperature. The existence of  
265 a common explanation for body size-to-temperature observations was questionable (Angilletta &  
266 Dunham 2003; Angilletta Jr 2009). The dual causative mechanism we report here constitutes  
267 such a possible common, though complex, clarification.

### 268 **Global change implications**

269 It is imperative to elucidate whether the cues species experience are clear enough to respond. The  
270 warming effect is not symmetrical, as the minimum temperature is rising faster than its maximum  
271 (Easterling *et al.* 1997), and species with preferences for low optimal temperatures or high  
272 optimal oxygen concentrations are challenged differently than species with preferences in the  
273 upper thermal range. High optimal oxygen concentration is distinctive in this regard because  
274 species are apparently specialized for specific  $O_{2opt}$  by some mechanisms alternative to the body  
275 size adjustment at the interspecific level (Fig. 3); neither they would be able to escape when  
276 facing a large-scale process of oxygen deprivation, such as global warming. With regard to the  
277 questions posed at the beginning of this text, our result on the high conservativity regarding

278 preferred oxygen levels has important implications for conservation strategies. We should  
279 immediately start by focusing on saving the ecosystems with the highest risk of a sharp decline in  
280 oxygen availability.

281 To conclude, in this study we show that the community of aquatic animals displays clear  
282 preferences within a thermo-oxygenic niche, which reflects in the species-specific body size  
283 response to subtle cues of both environmental variables studied. This is the first evidence of such  
284 a clear pattern of within-community response to two-dimensional ecological niche, that may act  
285 as a base for any ecological large-scale studies and models, especially regarding the  
286 consequences of global warming. Our strong message is that oxygen should be taken into account  
287 as a variable similarly important to temperature, if we aim to understand, or to counteract, the  
288 effects of climatic changes on communities.

289  
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445 **Figure legends**

446 **Fig. 1.** The evolution of the thermo-oxygenic niche in 188 rotifer species. The results of the PCA  
447 analysis: **A** – factor plane (PC1-PC2) projection (each point represents a species), **B** – parameter  
448 loadings for PC1-PC3 in a multivariate space, **C** – coefficient of quartile variation (CQV) of each  
449 parameter represented by bubble size on the PC1/PC2 plane. The parameters are in the same  
450 order as in (**A**); temperature parameters are marked in red and oxygen parameters are in blue. The  
451 CQV value of each parameter is provided next to the respective bubble. Exemplary rotifer species  
452 are presented in **D**.

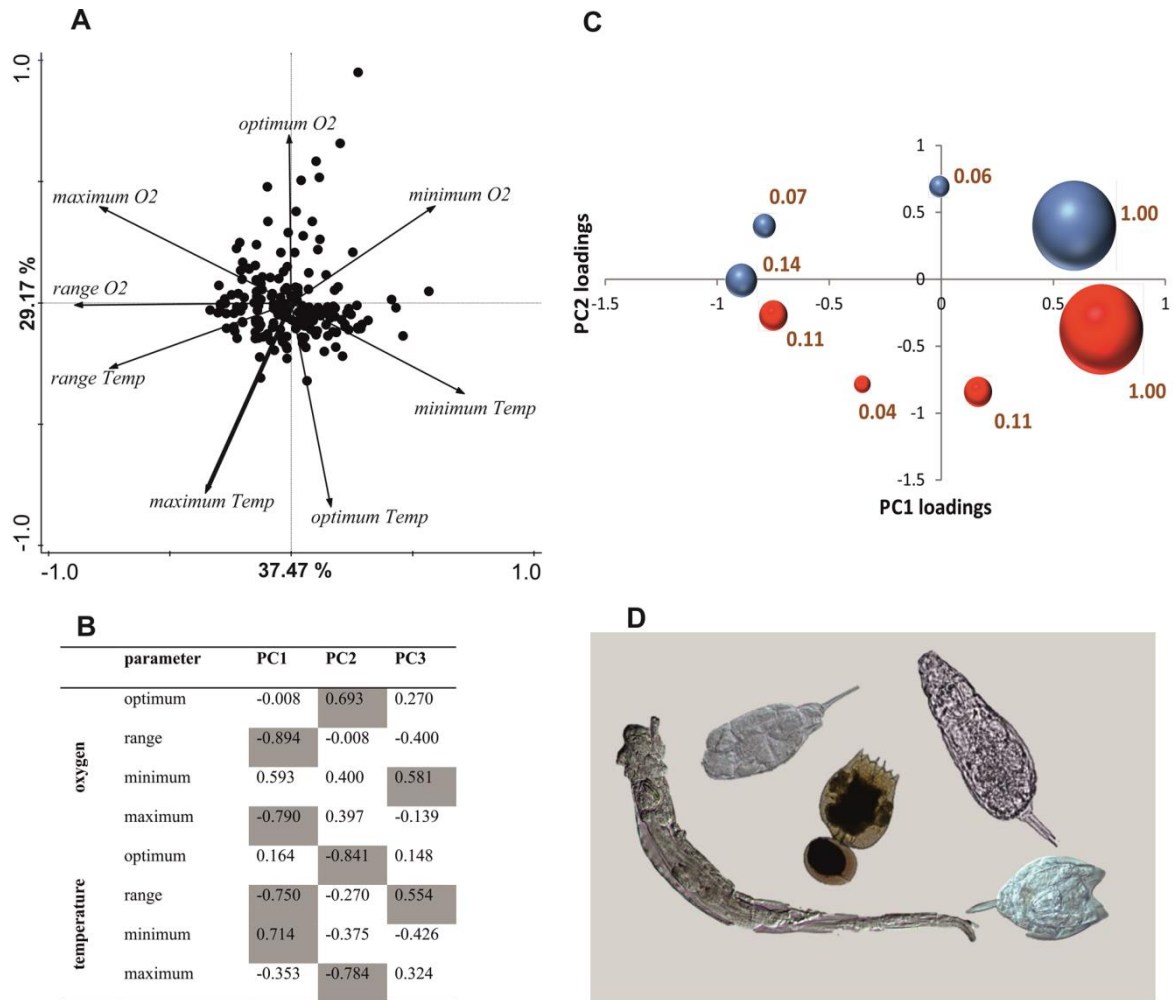
453 **Fig. 2.** Phylogenetic tree for all rotifer species representing a dataset for those species with  
454 available phylogenetic data (N = 188). Data were collected from the tree of life web project. The  
455 branch lengths were arbitrarily estimated using the method proposed by Grafen (1989) and the  
456 ‘ape’ package v4.1 (Paradis *et al.* 2004).

457 **Fig. 3.** Body size evolution toward a thermo-oxygenic niche in 188 rotifer species. The simple  
458 regression estimates of the relationship of body length with four environmental parameters that  
459 drove the PCA: ranges (A, B) and optima (C, D) of oxygen concentration and temperature. Each  
460 point represents an original value for a given species, while the estimation was phylogenetically  
461 corrected. Significant relationships are shown with their linear estimations.

462 **Fig. 4.** The thermo-oxygenic niche and its relationship to body size evolution in the assemblage  
463 of 188 rotifer species. The communities consist of large species at low temperature/high O<sub>2</sub>  
464 conditions and small species at high temperature/low O<sub>2</sub> conditions. This common observation  
465 may result from two compatible processes: low temperature may limit the small size, while low  
466 O<sub>2</sub> availability may constrain the large size. We associated this pattern with the evolution toward  
467 the generalist or specialist strategy, which was clearly divided according to our PCA. The pattern  
468 of O<sub>2</sub> decrease with increasing temperature (in water) is based on data from Wetzel (2001).

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**Fig. 1.**



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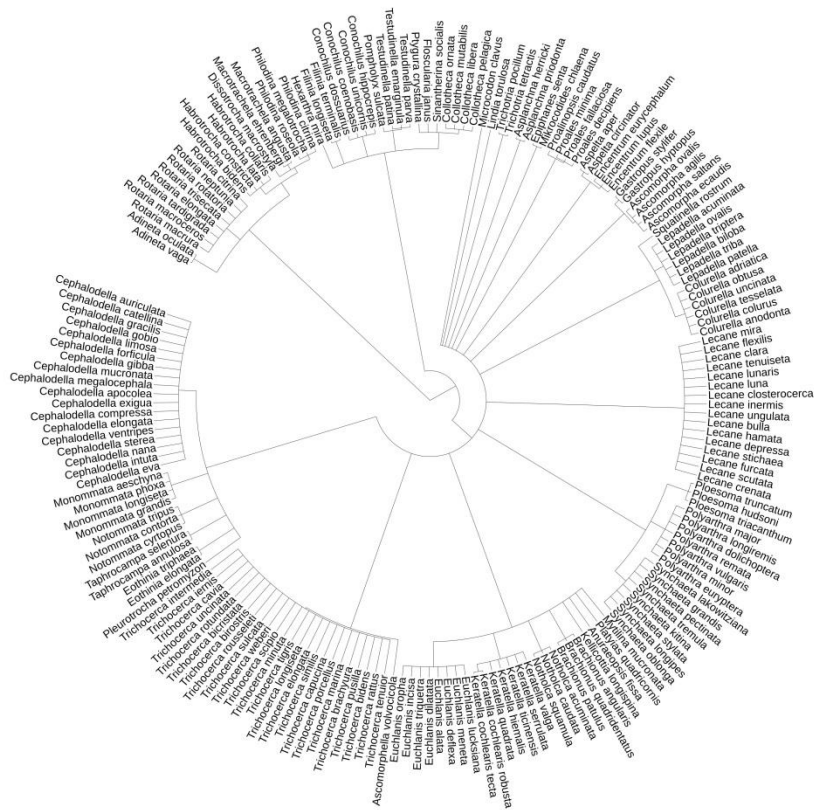
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Fig. 2.



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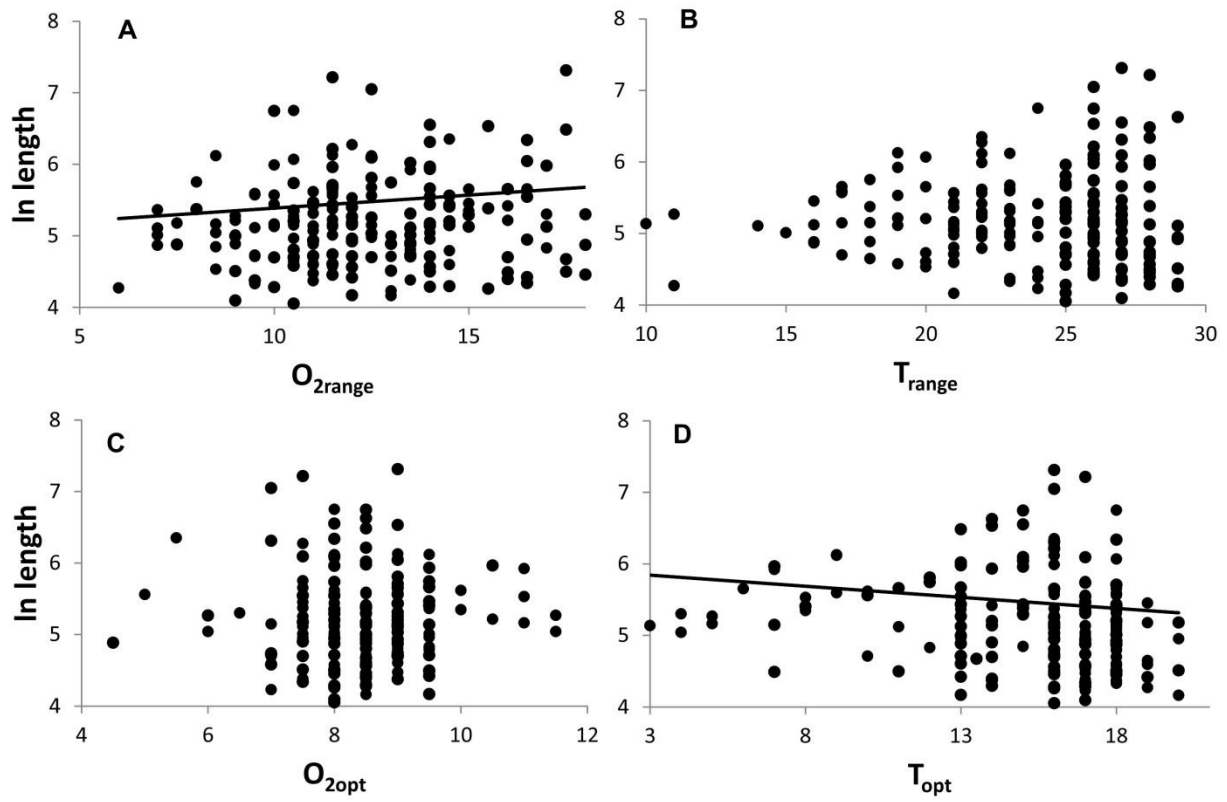
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Fig. 3.



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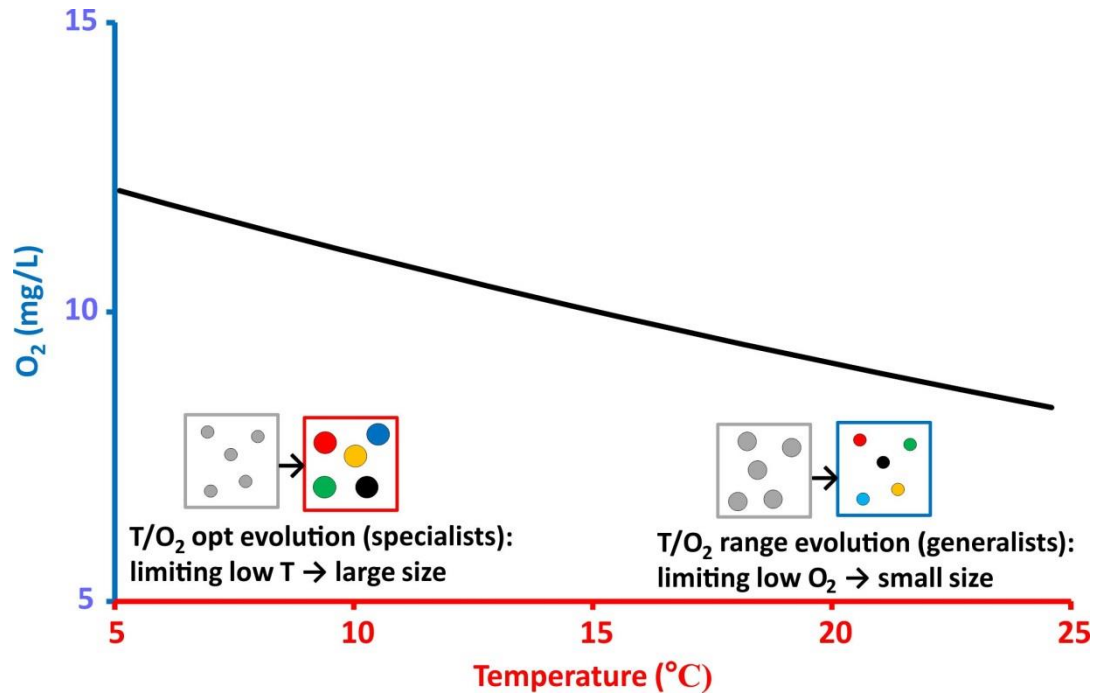
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**Fig. 4.**



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