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1 **Origin and characteristics of the zooplankton phosphatase activity**  
2 **in a coastal ecosystem of the Mediterranean Sea (Toulon bay)**

3  
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11

12 **Abstract:**

13  
14 In Toulon Bay (France), very high phosphatase activities have been found in the zooplankton  
15 fraction  $>90\mu\text{m}$ . This work was intended to specify their origin. For that purpose, larvae, juvenile  
16 and adult Crustacea (Copepods: Calanoids, Cyclopoids, Branchiopods: Cladocera, and Cirripeds)  
17 were isolated. Their activities were measured using paranitrophenyl phosphate dissolved in sea  
18 water in order to calculate  $K_m$  (the enzyme half saturation concentration) and  $V_{\text{max}}$  (the reaction  
19 rate when the enzyme is saturated with substrate).  $V_{\text{max}}$  were referred to protein contents of the  
20 isolated organisms to calculate specific activities. For all zooplankton groups high and low  
21 affinity phosphatase activities were found. The low affinity enzyme was responsible for at least  
22 70 % of the total phosphatase activity. Its specific activity was higher for larvae than for  
23 copepodites and adults. In Cirriped nauplii this activity was particularly high with values which  
24 were several hundred times higher than that in other Crustacea. These enzymes had optimum pH  
25 close to 8.4, magnesium requirement and were competitively inhibited by orthophosphate.  
26 Experiments with intact and lysed Cirriped nauplii confirmed that living organisms had only a  
27 weak external activity and showed that most of the activity of these larvae was primarily  
28 intracellular.

29  
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32

33

34 **Introduction:**

35

36 Much of the hydrolysis of phosphate esters in the aquatic environment results from the presence  
37 of enzymes on the cell surface or in the periplasmic space of bacteria and phytoplankton  
38 (Ammerman, 1991, Ammerman and Glover, 2000). However, enzymatic activities are also  
39 secreted by bacteria and phytoplankton, or released into the medium following the death of  
40 planktonic organisms (Jansson et al, 1988; Hoppe, 2003; Nausch and Nausch, 2004). In  
41 phytoplankton, as in bacteria, phosphatases, particularly alkaline phosphatase, can be induced  
42 under conditions of phosphorus limitation; nevertheless, some forms of phosphatase activity are  
43 constitutive and their synthesis is independent of external phosphorylated compounds (Cembella  
44 et al., 1984).

45 Zooplankton are generally not regarded as an important contributor to phosphate ester hydrolysis.  
46 However, as early as 1951, Margalef speculated that soluble phosphatases could be produced by  
47 zooplankton (Boavida, 2005). Phosphatase secretion from zooplankton has been characterised  
48 notably in Daphnia, Bosmina, Holopedium, and Cyclops (Jansson et al.,1988) and may contribute  
49 to the overall phosphatase activity in fresh water. In sea water, we observed very high specific  
50 activities in homogenates of the >90 µm size class of the particulate material in Toulon Bay when  
51 the abundances of cirriped larvae were high (Gambin et al., 1999, Bogé et al., 2002; Jean et al.,  
52 2003; Bogé et al., 2006). This work was undertaken to give deeper insight into the origin of the  
53 zooplankton phosphatase activities originated from Toulon Bay. Phosphatases activities were  
54 investigated by measuring the biochemical constants, Km and Vmax. We first identified the  
55 taxonomic groups responsible for such activities. For that purpose, developmental stages of  
56 zooplankton groups (Copepodes : Calanoids, Cyclopoids, Branchiopodes : Cladocera, and  
57 Cirripeds) were isolated separately. So the influence of phytoplankton and detritus was excluded.  
58 A particular study was then carried out with Cirriped larvae to specify the localization of  
59 phosphatases. By using living larvae, it was possible to measure the internal, external and  
60 secreted activities. A study of its cation requirement, of its pH dependence and of the phosphorus  
61 inhibition has been also carried out.

62 **Material and Methods :**

63  
64 **Origin and isolation of zooplankton:** Zooplankton were collected using a 90  $\mu\text{m}$  net in May 2003  
65 and June 2005, from Toulon Bay on the French Mediterranean Coast. Zooplankton were  
66 immediately brought to the laboratory, and were frozen at  $-20^{\circ}\text{C}$  in natural seawater to  
67 immobilize the organisms and to facilitate their isolation. Zooplankton were then thawed at  
68 ambient temperature. We used a binocular lens and a dissecting needle with a wire loop ending to  
69 isolate and collect larval, juvenile and adult specimens of Copepoda (Calanoids and Cyclopoids),  
70 Branchiopods (Cladocera), Cirripeds and Malacostracea. About thirty individuals of each group  
71 were placed in Eppendorf tubes containing 250  $\mu\text{l}$  distilled water. We observed that when the  
72 congelation lasted between 2 and 20 hours, most zooplankton were immobile, except for some  
73 Cirriped larvae that were still alive. Enzyme activities were measured on dead organisms isolated  
74 from zooplankton frozen during at least 24 hours, except for the last experiment for which living  
75 Cirriped larvae were needed. In that case, the larvae were patiently isolated without previous  
76 congelation.

77  
78 **Phosphatase activity:** Batches of zooplankton were lysed by sonication at  $2^{\circ}\text{C}$  and the  
79 homogenates were used as enzyme sources. The substrate was paranitrophenyl phosphate  
80 (pNPP), dissolved in sea water which had been prefiltered through 0.45  $\mu\text{m}$  pore-size filters.  
81 Paranitrophenol (PNP) produced during the reaction was detected at 410 nm during one hour  
82 (Reichardt et al., 1967). The experiments were carried out at  $25^{\circ}\text{C}$ . The biochemical constants  
83  $K_m$  (the half saturation concentration) and  $V_{\text{max}}$  (the reaction rate when the enzyme is saturated  
84 with substrate) were calculated by means of an iteration program based on an analysis of Eadie  
85 Hofstee kinetics. Previous work showed that these kinetics are composed of two Michaelian  
86 components: a low affinity/high capacity mechanism and a high affinity/low capacity mechanism  
87 (Jean *et al.*, 2003, Bogé *et al.*, 2002, 2006). For each extract at least four substrate concentrations  
88 (0.01 to 10 mM, in geometrical progression) were tested and the  $V_{\text{max}}$  of each component was  
89 calculated.

90 Phosphatase activity is typically pH dependent (Hope, 2003). The pH dependence of Cirriped  
91 phosphatase activity was studied for a 15 mM substrate concentration prepared in prefiltered sea  
92 water. Glycine (50 mM) was used to buffer this medium at alkaline pH values(7.22 to 10.24).

93 Phosphatase activity is also influenced by specific cations (Mc Comb et al.,1979). The cation  
94 dependence of the Cirriped activity has been studied. Experiments were carried out with synthetic  
95 water made of distilled water adjusted to pH 8.4 with glycine (final concentration: 50 mM), and  
96 containing  $Mg^{2+}$ (10-100 mM),  $Ca^{2+}$ (5-20 mM),  $Na^+$ (50-500 mM) and  $K^+$ (5-20 mM) (all added as  
97 chloride salts) and pNPP (15 mM).

98 Orthophosphate competitively inhibits phosphatase activity at high phosphorus concentrations  
99 (Chrost, 1991). To investigate the influence of phosphorus on the zooplankton phosphatase  
100 activity, Cirriped larvae homogenates were used. The effect of 5 mM  $K_2HPO_4$  on the hydrolysis  
101 of pNPP (0.083 mM to 6.7 mM) was studied. At this concentration, phosphorus precipitates in  
102 sea water. To avoid this precipitation and to demonstrate the relevance of phosphate inhibition for  
103 seawater, natural seawater was replaced by synthetic sea water, containing 500 mM NaCl, 50  
104 mM MgCl, and 20 mM KCl, buffered at pH 8.4 with glycine.

105 An experiment was devoted to the study of the localization of the phosphatase activity in Cirriped  
106 larvae. Phosphatases are either internal or external enzymes (Jansson et al., 1988). The hydrolysis  
107 of substrates in the water is due to external enzyme activities whereas that of intracellular  
108 compounds results from internal enzymes. To characterize external enzyme activity, living  
109 Cirriped nauplii were placed in sea water with 15 mM pNPP. The PNP concentrations were then  
110 followed over one hour. The same experiment was carried out using larvae homogenates to  
111 characterise total phosphatase activities, including internal and external enzymes. We also looked  
112 for secreted phosphatase activity in water conditioned by larvae for 24 hours.

113  
114 Proteins: To evaluate specific phosphatase activities, protein concentrations of the plankton  
115 extracts were determined according to Lowry's method (1951). Specific activity was defined as  
116 the ratio between enzyme activity and protein concentration.

117  
118 Statistics: At least three batches of zooplankton were used for each taxonomic group (10  
119 experiments for Calanoids, 3 experiments for cirriped larvae, and 5 for the other taxonomic  
120 groups). Standard errors are presented. Non parametric tests were used for  $V_{max}$  intergroup  
121 comparisons (Mann Whitney).

122

123 **Results:**

124

125 **Structure of the zooplankton community in Toulon Bay:**

126 In June 2005, the zooplankton community averaged 19000 individuals per m<sup>3</sup>. Nearly 89 % of  
127 the zooplankton were crustaceans (Fig. 1). Copepoda was the largest group. Cyclopoids,  
128 particularly *Oithona nana*, were the most numerous Copepoda . Several developmental stages  
129 were found: only 34 % of the zooplankton community and 39% of the Copepoda group was  
130 adult; 43 % of total zooplankton and 48 % of Copepoda were copepodites, 23% of total  
131 zooplankton and 13 % of Copepoda were larvae. The Cirriped larvae were almost all nauplii and  
132 made up only 1 % of the zooplankton community. Eleven % of the community were larvae that  
133 were not Crustacea. Less than 1% of total zooplankton were Decapodes zoe.

134

135 **Interspecies comparisons:** Phosphatase activities with high and low affinities were found for all  
136 zooplankton groups. The Km for the low affinity activity was between 0.3 and 1 mM. For the  
137 high affinity activity, it was below 0.02 mM. At least 70 % of the phosphatase activity (expressed  
138 as Vmax) was supported by low affinity enzymes (Fig. 2). The contribution of this low affinity  
139 component to the to overall phosphatase activity (expressed as Vmax) was more important in  
140 Zoe (Decapods: ZDec) and nauplii (Cyclopoids: Ncycl and Cirripeds: NCir), than in adults  
141 (Cyclopoids: ACycl and Calanoids: ACal) and copepodites (Calanoids: CCala). For Cirriped  
142 nauplii (NCir), more than 99 % of the phosphatase activity was due to low affinity enzyme.  
143 Conversely, the contribution of high affinity activity was predominant in Copepod adults  
144 (Cyclopoids: ACycl, and Calanoids: ACal) and copepodites (Calanoids: CCala). The relative  
145 proportion of the phosphatase activity due to the high affinity enzymes was substantially lower in  
146 Branchiopods (Cladocera) than in Copepods.

147

148 **The Vmax of the low affinity enzyme** showed considerable differences between developmental  
149 stages (Fig. 3). In Copepods (Cyclopoids and Calanoids), Vmax values were relatively low for  
150 adults and copepodites (ACycl, ACal and CCala), but were approximately 50 times higher for  
151 nauplii (NCycl) than for adults. However, there was substantial variability between batches for  
152 this group. Significantly higher activities existed in Branchiopod (Cladocera) adults than in  
153 Copepoda (p<0.05). But the most exceptional activities were found for Cirriped nauplii. Their

154 specific phosphatase activity was approximately 70 times higher than for Copepoda nauplii and  
155 more than 1000 times higher than for adult Copepoda. The differences with the other taxonomic  
156 groups were highly significant ( $p < 0.05$ ).

157  
158 The  $V_{max}$  of the high affinity enzyme was always lower than the  $V_{max}$  of the low affinity  
159 component (Fig. 4). Copepod larvae and copepodites (Calanoid copepodites: CCal and  
160 Cyclopid nauplii: NCycl) in addition to Cirriped nauplii (Cirriped nauplii: NCir) had the  
161 highest activities (not statistically significant except for Cirriped nauplii with  $p < 0.05$ ). However,  
162 for Cirriped, the high values of the low affinity activity made the accurate determination of the  
163 high affinity activity more difficult.

164  
165 Analysis of the phosphatase activity of Cirriped nauplii: We characterised the phosphatase  
166 activity of Cirriped nauplii, including its dependence on pH, cations and phosphorus, and its  
167 cellular localisation..

168  
169 - pH dependence: pNPP hydrolysis by the larvae had a pH optimum of 8.4, close to that of  
170 sea water (Fig. 5). At pH 7 this activity was still significant since it accounted for approximately  
171 40 % of its maximum value. This is also the case at pH 9.5 where this percentage was 27%,  
172 whereas, the activity was completely inhibited at pH 10.

173  
174 - Role of cations: Phosphatase activity was inhibited by almost 90 % when the substrate  
175 was prepared in fresh water instead of in seawater. In synthetic water made of 50 mM  $Mg^{2+}$ , 20  
176 mM  $Ca^{2+}$ , 500 mM  $Na^+$  and 50 mM  $K^+$ , phosphatase activity was nearly 70 % of its seawater  
177 value. Individually, these ions stimulated the activity unequally. With magnesium (up to 50 mM)  
178 the activity reached nearly 40 % of its level in sea water. Sodium also stimulated the activity, but  
179 to a lesser extent. For 500 mM Na the activity was only 20 % of its level in sea water. The other  
180 ions like calcium and potassium had more limited effects (Fig. 6).

181  
182 - Role of phosphorus: In the synthetic medium, the  $V_{max}$  of Cirriped larvae phosphatase  
183 activity was approximately 25 % lower than in sea water without any significant change in its  
184  $K_m$ . The  $K_m$  was typically 0.3 mM in the absence of phosphate and 0.6 mM in its presence; the



185  $V_{max}$  remained unchanged (Fig. 7). This  $K_m$  effect indicates that phosphorus competitively  
186 inhibited the Cirriped larvae activity.

187  
188 -Localization of phosphatase activity: Very high phosphatase activities were found when  
189 larvae homogenates were used (Fig. 8), whereas intact larvae gave very low pNP concentrations.  
190 Very low phosphatase activities were found in the water where larvae remained for 24 hours.  
191 These results indicate that phosphatase activity associated with Cirriped larvae was essentially  
192 intracellular.

193

194 **Discussion:**

195  
196 We investigated the role of marine zooplankton in the production of enzymes involved in the  
197 metabolism of phosphorylated compounds in Toulon Bay on the French Mediterranean Coast.  
198 These compounds are of major importance for plankton growth and for the control of cellular  
199 activities.

200 We confirmed that in size fractionation experiments (Jean et al. 2003, Bogé et al. 2006, Gambin  
201 et al. 1999, Jamet and Bogé, 1998) the hydrolysis of phosphate esters by zooplankton could be  
202 described by two Michaelian mechanisms with distinct  $K_m$  and  $V_{max}$ . This work specifies that,  
203 on the basis of their  $V_{max}$ , the contribution of the low affinity activity was always more  
204 significant than that of the high affinity activity, particularly in larvae. The high affinity activities  
205 have been revealed with adults as well as larvae. They could be active in the local hydrolysis of  
206 low phosphoric ester concentrations, as found in the natural medium. Previous results have  
207 suggested that bacteria may contribute to the phosphatase activity associated with the  $>90 \mu m$   
208 size class fraction, which is mostly composed of zooplankton. But they cannot explain the high  
209 levels of the low affinity activity (Gambin et al., 1999; Jean et al., 2002).

210 The low affinity activity is probably responsible for the hydrolysis of intracellular substrates.  
211 Indeed, this activity cannot be detected extracellularly with intact Cirriped nauplii as the enzyme  
212 source (Figure 8). We also showed that this low affinity activity fluctuated greatly according to  
213 the developmental stages of the zooplankton. In adults and juveniles (copepodites), phosphatase  
214 levels were generally low, notably in Cyclopoids, Cladocera and Calanoids. Phosphatase activity  
215 in larvae was more variable, but was generally higher as observed for Copepoda nauplii (Figure  
216 3). In Toulon Bay, nauplii were only approximately 13 % of the total number of Copepods during  
217 the sampling period (Figure 1).

218 Since the phosphatases are internal activities it is thus possible that zooplankton lose a major part  
219 of their enzyme activity after the freezing treatment. To test this possibility additional  
220 experiments have been carried out. Copepods were isolated from fresh and pre-frozen  
221 zooplankton. The results indicated that the activities of individuals isolated from pre-frozen  
222 zooplankton were lower, but the differences with fresh zooplankton were not significant due to  
223 natural intra-species variation. Cirriped nauplii are much more tolerant to freezing, and their cell  
224 membranes are probably better at withstanding freezing or thawing. The possible under estimate

225 of the activity of pre-frozen copepod does not change the conclusion that the activity of the  
226 cirriped larvae was considerably higher.

227 The role of these high phosphatase activities remains obscure. Physiologically, phosphatases  
228 could contribute to the synthesis or to the use of nutritional or energy reserves. In nauplii the  
229 mean P content is higher than in adults which could be related with higher phosphatase activities  
230 (Carillo et al., 2001). Nauplii have several stages during which molting and growth take place.  
231 Naupliar stage I derives energy from remaining egg yolk reserves, while stages II through VI  
232 derive their energy from consumed phytoplankton. In egg yolk, glycolipoproteins are an  
233 important source of proteins, lipids, and carbohydrates (De Chaffoy de Courelles and Kondo,  
234 1980). Phosphate has been found covalently bound on these proteins in insects and crustaceans  
235 (Allerton and Perlmann, 1965; Fialho et al., 2002; De Chaffoy de Courelles and Kondo; 1980). In  
236 Crustacea, the degradation of these reserves is greatest during the nauplii stage due to enhanced  
237 lysosomal hydrolytic activities (Perona and Vallejo; 1985). In Artemia, these activities include  
238 cathepsin B acid ribonuclease, acid deoxyribonuclease, acid phosphatase, acid phosphodiesterase,  
239  $\beta$ -glucosidase,  $\beta$ -N-acetylgalactosaminidase and acid lipase (Perona and Vallejo, 1985). Nucleic  
240 acids, mono and poly-phosphate esters are also possible sources of organic phosphorus for  
241 plankton (Benitez-Nelson, 2000). Phosphatases (acid and alkaline), and nucleotidases catalyze  
242 the hydrolysis of these compounds (Hope, 2003). These activities catalyze also pNPP hydrolysis.  
243 The most striking observation of this work was the high specific phosphatase activity associated  
244 with Cirriped larvae, several hundred times higher than for other taxons. In Toulon Bay, Cirriped  
245 nauplii accounted for less than 1 % of the total zooplankton community. Higher abundances were  
246 generally found in May, June and August, but they never exceed 400 individuals per m<sup>3</sup> (Jean et  
247 al. 2003). During these periods the phosphatase activity of zooplankton homogenates peaks such  
248 that the contribution of zooplankton to the total particulate activity reached 80% or more  
249 (Gambin et al., 1999). On the other hand when these larvae are absent, the activity of the  
250 zooplankton falls by more than 90 % (Jean et al.2003).

251 The fertilized eggs of Cirripeds are brooded within the shell of adults. When they change into  
252 nauplius larvae, they are released into the water as free-swimming plankton. After several moult  
253 stages, they become cyprid larvae. These larvae are enclosed within a carapace and possess  
254 antennae and numerous appendages. They also have detectors that can recognise suitable solid  
255 substrata. During settlement, glands of the antennae secrete a special cement to attach the larvae

256 to a rock (Anderson, 1994; Foster, 1987). During these larvae nauplii stages, reserves, consisting  
257 of lipids and proteins, are also synthesized in abundance. These reserves are necessary for later  
258 stages, particularly for the cyprid stage which does not feed during settlement. In Balanus  
259 amphitrite, the concentration of a specific protein, the cyprid major protein (CMP), accumulates  
260 in the haemocoel, increases during the naupliar stages and decreases with aging of cyprids and  
261 during the early juvenile period (Satuito et al., 1996; Shimizu et al., 1996). This protein appears to  
262 function as a storage protein during settlement of cyprids as well as during metamorphosis to  
263 juveniles. It shares similarities with the egg-yolk phosphoprotein, vitellin. Incidentally, this  
264 protein may explain the unusual freeze resistance of these larvae, which can survive several hours  
265 at  $-20\text{ }^{\circ}\text{C}$  (personal observation). The high phosphatase activities found in Cirriped larvae of  
266 Toulon Bay could thus play an important role in the dephosphorylation of this protein or of other  
267 phosphorylated compounds during the fasting period.

268 These internal activities can also influence the regeneration of phosphate. It is generally accepted  
269 that less than 20 % of ingested phosphorus is used for growth. The remainder is excreted in the  
270 medium as soluble reactive phosphorus and organic phosphorus (Valiela, 1995). In Swedish west  
271 coast, Bamstedt (1985) showed, that excretion rates of inorganic phosphate and dissolved organic  
272 phosphorus by 19 zooplanktonic species averaged respectively  $2.22\text{ nmol mg protein}^{-1}\cdot\text{h}^{-1}$  and  
273  $0.8\text{ nmol mg protein}^{-1}\cdot\text{h}^{-1}$  in spring. Taking into account the protein contents of barnacle nauplii,  
274 the quantities rejected per individual nauplius would approximate respectively  $0.01\text{ nmol}\cdot\text{h}^{-1}$  and  
275  $0.0036\text{ nmol}\cdot\text{h}^{-1}$ . But due to the very high specific phosphatase activities, the excretion of  
276 inorganic phosphate is probably higher, except in cypris which do not feed. These intracellular  
277 activities could also contribute to the hydrolysis of phosphorylated compounds of sea water when  
278 enzymes are released in sea water from dead cells (Jansson et al., 1988). In the case of Cirriped  
279 nauplii, the activity was approximately  $200\text{ nmol}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$  in May-June and the dissolved activity  
280 was  $450\text{ nmol}\cdot\text{l}^{-1}\cdot\text{h}^{-1}$  during the same period (Bogé et al., 2006). So it is thus possible that part of  
281 this dissolved activity comes from dead larvae.

282 Some characteristics of this activity have been also specified in this work. This activity had a pH  
283 optimum close to 8.4. It was strongly stimulated by the sea water components (Figure 6) and it  
284 was inhibited by high orthophosphate concentrations (Figure 7). These results are in agreement  
285 with previous data obtained on homogenates from particulate material of the  $> 90\text{ }\mu\text{m}$  size class  
286 (Gambin et al. 1999). This work brings additional information concerning the role of sea water

287 cations. Magnesium was most effective, which suggests that the enzyme could be alkaline  
288 phosphatase, its activity being generally controlled by this cation (McComb et al., 1979, Jansson  
289 and Al, 1988). Sodium also stimulated the phosphatase activity of the larvae (Figure 6) but high  
290 concentrations were required (up to 500 mM). So it is possible that this effect comes from the  
291 high tonicity that these high concentrations generate (McComb and al.1979).

292 In conclusion, this work showed that zooplankton of Toulon bay has phosphatase activities with  
293 low and high affinities; that these activities are higher in larvae and in particular in Cirriped  
294 larvae where they could be of great importance for their metabolism and for the regeneration of  
295 phosphate.

296

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298

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## Figure legends

Fig. 1 : Structure of the zooplankton community in Toulon Bay (Mean abundances in June 2005).

Fig. 2 : Contribution, in percent of the total activity, of the high (V2) and low (V1) affinity components to the phosphatase activity of isolated larvae (N: nauplii, Z : zoe), copepodites (C) and adults (A) from Cladocera (Clad), Cyclopoids (Cycl), Calanoids (Cal), Cirripeds (Cir) and Decapods (Dec) originated from Toulon Bay

Fig. 3 : The specific activities (in  $\text{nmol h}^{-1} \mu\text{g}^{-1}$  protein, logarithmic scale) of the low affinity component of the phosphatase activity of isolated larvae (N: nauplii, Z : zoe), copepodites (C) and adults (A) from Cladocera (Clad), Cyclopoids (Cycl), Calanoids (Cal), Cirripeds (Cir) and Decapods (Dec) originated from Toulon Bay (Mean  $\pm$  Standard error).

Fig. 4 : The specific activities (in  $\text{nmol h}^{-1} \mu\text{g}^{-1}$  protein) of the high affinity component of the phosphatase activity of isolated larvae (N: nauplii, Z : zoe), copepodites (C) and adults (A) from Cladocera (Clad), Cyclopoids (Cycl), Calanoids (Cal), Cirripeds (Cir) and Decapods (Dec) originated from Toulon Bay (Mean  $\pm$  Standard error).

Fig. 5 : The pH dependence of the phosphatase activity of the Cirriped larvae (Specific activities are expressed in  $\text{nmol h}^{-1} \mu\text{g}^{-1}$  protein) (Mean  $\pm$  Standard error).

Fig. 6 : The role of cations on the of the phosphatase activity of the Cirriped larvae (Synth water : synthetic water made of 50 mM  $\text{Mg}^{2+}$ , 20 mM  $\text{Ca}^{2+}$ , 500 mM  $\text{Na}^+$  and 50 mM  $\text{K}^+$  ).

Fig. 7 : Effect of 5 mM  $\text{K}_2\text{HPO}_4$  on the phosphatase activity of Cirriped larvae (Eadie Hofstee plots, and  $K_m$  and  $V_{max}$  values, SW- $\text{PO}_4$  : Sea Water without  $\text{PO}_4$ , SyW- $\text{PO}_4$  : Synthetic Water without  $\text{PO}_4$ , SyW+ $\text{PO}_4$  : Synthetic Water with  $\text{PO}_4$ , Specific activities are expressed in  $\text{nmol h}^{-1} \mu\text{g}^{-1}$  protein with standard errors).

Fig. 8 : Contribution, in per cent of the total activity, of external, internal and secreted activities

to the Cirriped larvae phosphatase.

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## Figures

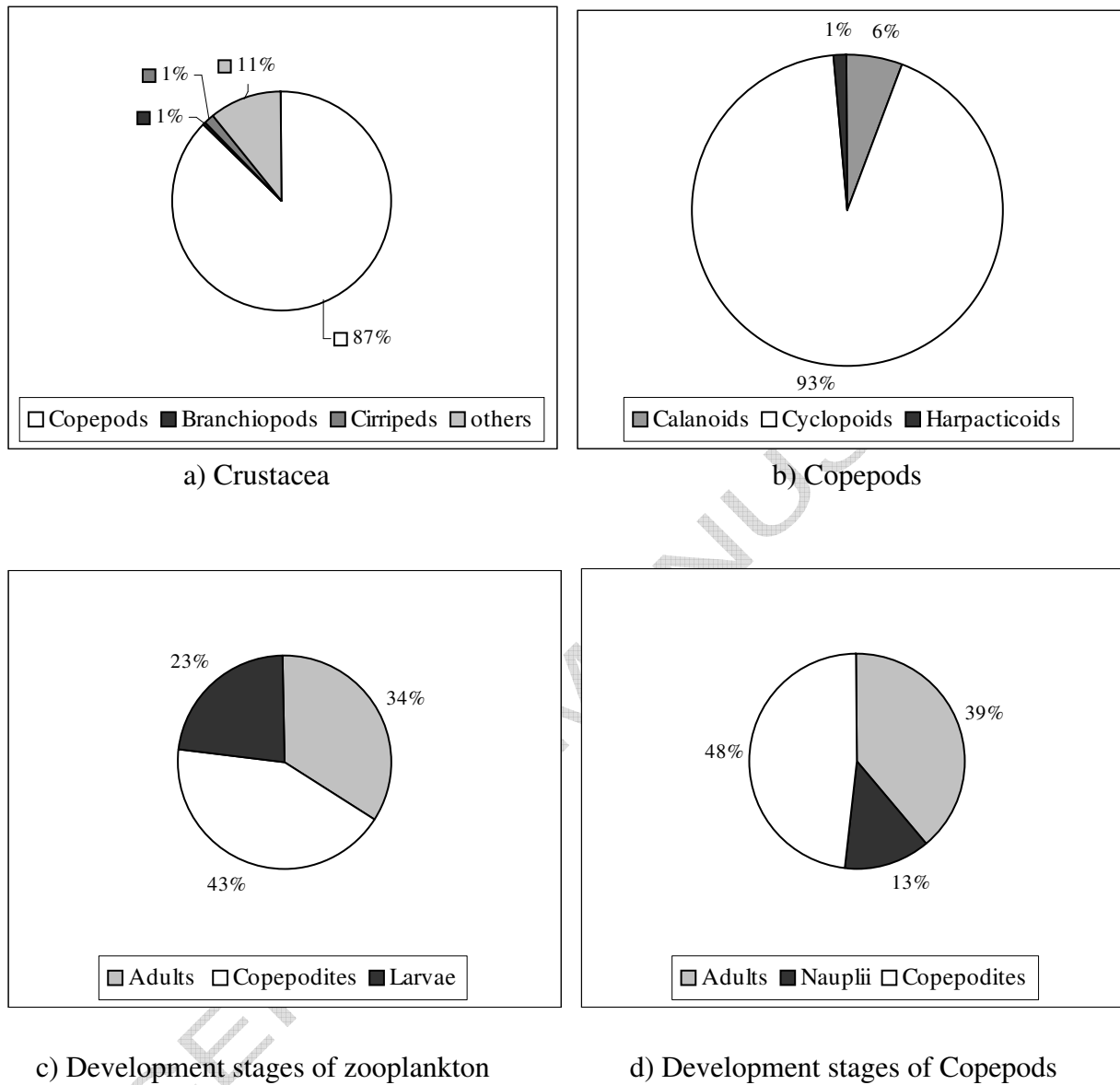


Fig. 1

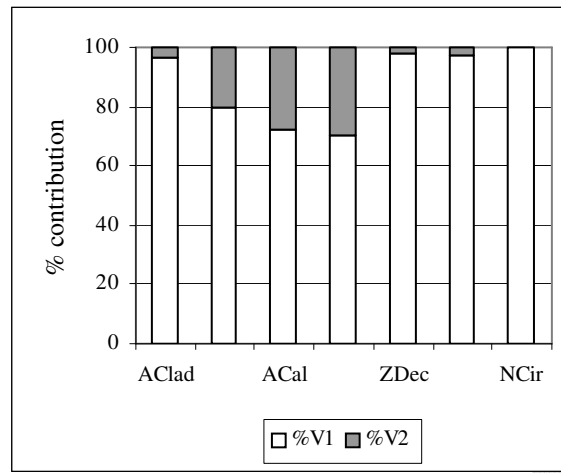


Fig. 2

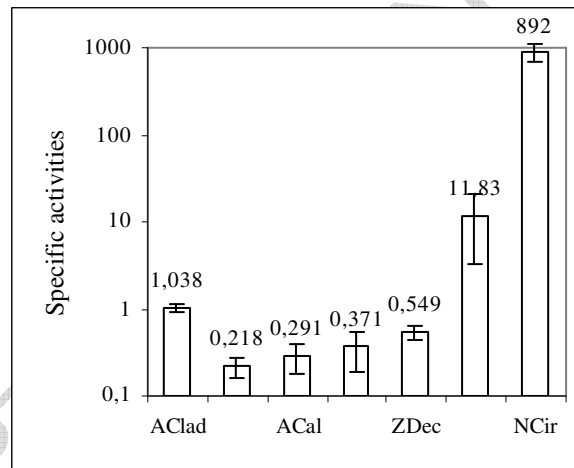


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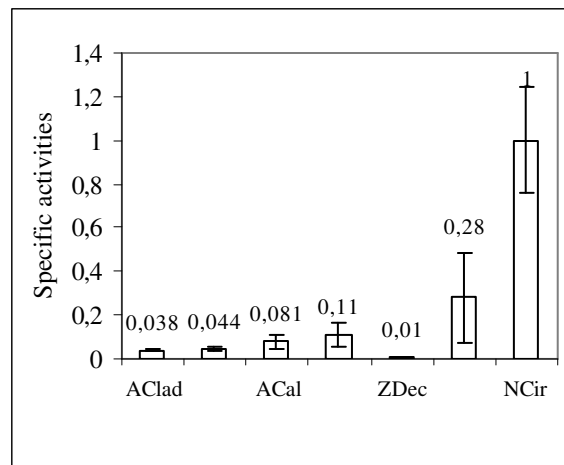


Fig. 4

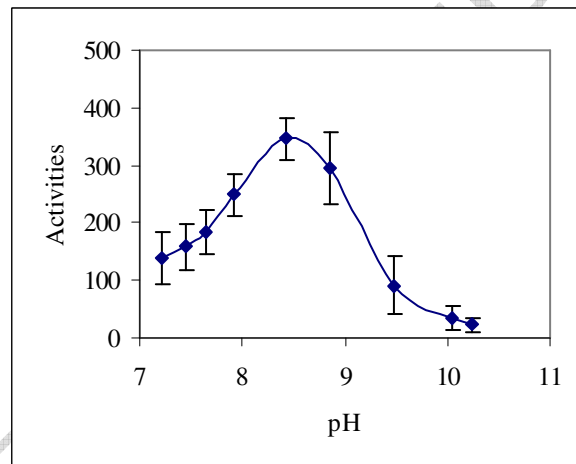


Fig.5

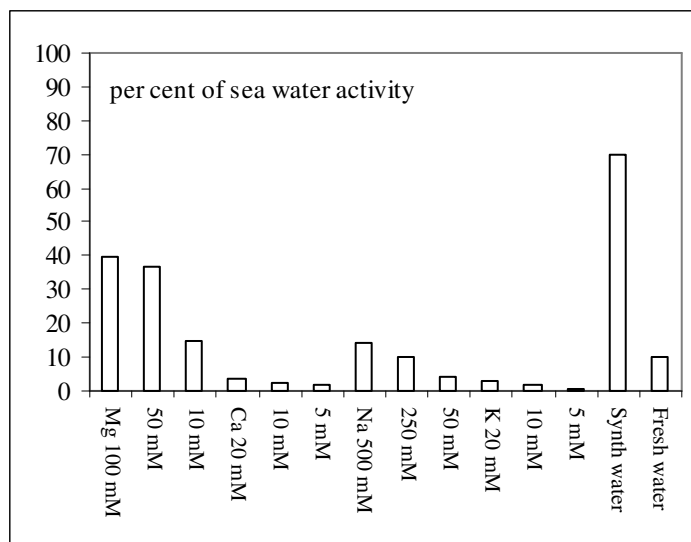
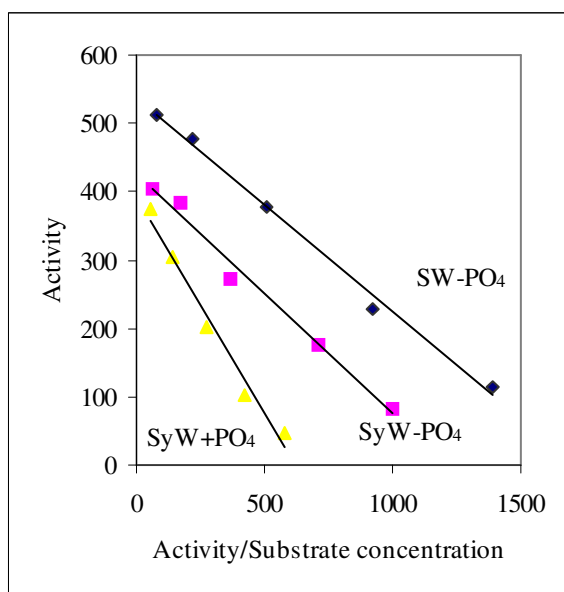


Fig. 6



	K <sub>m</sub>	V <sub>m</sub>
SW-PO <sub>4</sub>	0,31 (0,02)	535 (15)
SyW-PO <sub>4</sub>	0,35 (0,02)	425 (15)
Sy+PO <sub>4</sub>	0,64 (0,054)	392 (30)

Fig. 7

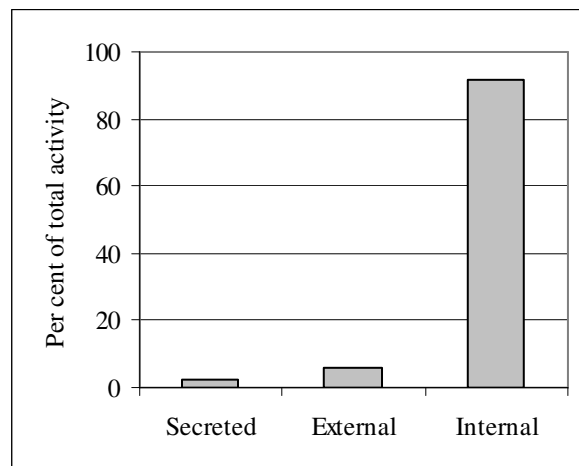


Fig. 8