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1 # Alkaline phosphatase activity in the phytoplankton communities of
2 # Monterey Bay and San Francisco Bay

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Abstract

Enzyme Labeled Fluorescence (ELF) and bulk Alkaline phosphatase (AP) activity enzyme assays were used to evaluate the phosphorus status of phytoplankton communities in San Francisco and Monterey Bays. Both regions show spatial and temporal variability in bulk AP activity with maximum activities during the early spring and summer periods of high biological activity. ELF analysis revealed pronounced differences in the makeup of organisms responsible for AP activity in these two environments. In Monterey Bay dinoflagellates are responsible for the bulk of the AP activity. Diatoms infrequently exhibited AP activity. Dinoflagellates that comprised only 14% of all cells counted in Monterey Bay accounted for 78% of AP producing cells examined. The presence of AP activity in this group suggests that changes in P sources, concentrations, and bioavailability could disproportionably influence this group relative to diatoms in Monterey Bay. In San Francisco Bay, AP production, indicated by ELF, was associated primarily with bacteria attached to suspended particles, potentially used to hydrolyze organic compounds for carbon not P requirements. Our results highlight the importance of organic P as a bioavailable nutrient source in marine ecosystems and as a component of the marine P cycle.

Introduction

Phosphorus (P) is required by all organisms for energy transport and growth (Benitez-Nelson 2000) and may limit primary production in some oceanic systems (Karl et al. 1995; Wu et al. 2000). Phosphorus may be the ultimate limiting nutrient for primary production over geological time scales (Tyrrell 1999). Therefore, understanding the P

48 cycle is essential in determining the coupling between marine primary productivity and
49 the global carbon cycle.
50 Phytoplankton typically utilize dissolved orthophosphate in order to satisfy
51 cellular P requirements (Cembella et al. 1984). However, many phytoplankton taxa have
52 the capability to utilize dissolved organic P (DOP) forms (Cotner and Wetzel 1991;
53 Björkman and Karl 1994; Lobban 1994) as well as other inorganic P compounds, such as
54 polyphosphate (Palenik and Dyhrman 1998; Scanlan and Wilson 1999). In order for
55 uptake of most organic P compounds to occur, they must be first converted into
56 orthophosphate. The primary means by which marine phytoplankton can convert organic
57 P to bioavailable orthophosphate is induction of alkaline phosphatase (AP) (Cotner and
58 Wetzel 1991), an enzyme which has broad substrate specificity and hydrolyses ester
59 bonds between phosphate and organic molecules, making organic-P available for cellular
60 assimilation by converting it to phosphate (Cembella et al. 1984).
61 Traditionally, an ecosystem is described as P or nitrogen (N) limited based on the
62 relative N:P ratios dissolved in the water. Marine phytoplankton, on average, require N
63 and P at a ratio of 16:1 (Redfield Ratio). If the ratio of dissolved inorganic nitrogen
64 (DIN) to soluble reactive phosphate (SRP) in a marine system is greater than 16:1 the
65 system is considered P-limited. However, such characterization may not necessarily
66 represent real P stress of the whole phytoplankton community for several reasons: (a)
67 different phytoplankton species may have nutrient requirements different from the
68 Redfield Ratio (Geider and La Roche 2002; Guildford and Hecky 2000; Quigg et al.
69 2003), (b) the analytical determination of SRP and DIN do not account for other available
70 forms of P and N, such as organic sources, or include compounds that are not bio-

71 available (Baldwin, 1998; Benitz-Nelson 2000; Karl and Tien 1997), and (c) low
72 concentrations of a nutrient may not necessarily indicate deficiency, but rather represent
73 efficient recycling and utilization (Cañellas et al. 2000; Hudson et al. 2000; Karl and
74 Björkman 2002).

75 To more directly determine community P-status in natural aquatic ecosystems it
76 has been suggested that because in many phytoplankton species AP is regulated (induced
77 or repressed) by ambient inorganic P concentrations or by intracellular concentrations
78 (Vargo and Shanley 1985; Chrost and Overbeck 1987), AP activity can be used as an
79 index of P-status. Indeed, AP activity has been used to indicate P stress in fresh water
80 plankton communities (Chrost 1991; Hernandez et al. 1996) and in the marine
81 environment as well (Cotner et al. 2000; Stihl and Sommer 2001; Vidal et al. 2003).

82 AP activity in marine ecosystems has been studied using two primary techniques.
83 The first is the use of a quantitative enzyme assay of bulk AP activity of a given water
84 sample or cell mass (Koike and Nagata 1997, Li et al. 1998, Thingstad et al. 1998) in
85 which a specific phosphomonester substrate (e.g., *p*-nitrophenyl phosphate or 4-
86 methylumbelliferyl phosphate) is added to a water sample and is stoichiometrically
87 hydrolyzed in the presence of the enzyme AP. The second method is a qualitative, cell-
88 specific assay using enzyme labeled fluorescence (ELF) (Gonzalez-Gil et al. 1998;
89 Dyhrman and Palenik 1999; Rengefors et al. 2003). This method also involves the
90 addition of a phosphomonoester substrate (ELF-97 phosphatase substrate – Molecular
91 Probes) to the sample; however, instead of a soluble product that is released to the
92 medium, a fluorescent precipitate forms at the site of AP hydrolysis, thus, fluorescently
93 tagging cells that exhibit AP activity.

94 Much previous research on marine AP activity utilized the bulk enzyme assay
95 method, and focused on areas that have low SRP concentrations or elevated N:P ratios
96 such as freshwater-influenced marine systems (Cotner et al. 2000; Nausch 1998) or
97 oligotrophic settings such as the Red Sea, Mediterranean, Sargasso Sea, and central
98 Pacific (Ammerman et al. 2003; Li et al. 1998; Van Wambeke et al. 2002).
99 The ELF method has recently been applied to study P-status in laboratory cultures
100 (Gonzalez-Gil, 1998; Dyhrman and Palenik 1999) and for examining natural marine
101 populations (Carlsson and Caron, 2001; Dyhrman and Palenik 2001, Lomas et al., 2004).
102 Results from laboratory cultures typically, but not always, show a dependence of AP
103 activity on orthophosphate concentration. For example, dinoflagellates grown in P replete
104 conditions did not exhibit fluorescence indicative of AP activity (ELF labeling), while
105 cultures in orthophosphate depleted media showed over 90% of cells to be ELF labeled
106 (Dyhrman and Palenik 1999). Furthermore, this study reports a 50% decrease in cells
107 with AP activity following orthophosphate addition to P-depleted cultures. In field
108 populations, ELF labeling has shown variability in AP activity between a wide range of
109 species in waters of Narragansett Bay and the Sargasso Sea (Dyhrman and Palenik 1999;
110 Lomas et al. 2004).
111 While most oceanographic research has focused on AP activity as a proxy for P-
112 status in marine phytoplankton, AP is also produced by bacteria (Sebastián et al. 2004;
113 Sundareshwar et al. 2003). It has been suggested that AP activity is induced by bacteria
114 to hydrolyze organic P molecules to access reduced carbon, P or both (Kirchman et al.
115 2000; Van Wambeke et al. 2002). Heterotrophic bacteria, in particular, are likely to be P
116 limited as they have higher P requirements than phytoplankton (Cotner and Wetzel 1991,

117 Pomeroy et al. 1995). Using bulk AP activity estimates it is not possible to distinguish
118 between activity attributed to phytoplankton or heterotrophic bacteria, thus potentially
119 overestimating the activity attributed to phytoplankton.

120 This study utilizes a combination of the two methods, bulk enzyme assays and
121 ELF-labeling, to investigate AP dynamics in Monterey Bay and San Francisco Bay.
122 While bulk AP activity assays provide estimates for the total amount of enzyme being
123 produced by all organisms in a sample, it reveals nothing about which species in a diverse
124 field population contribute to this production. To better understand the nutrition role of
125 organic P compounds and their utilization by natural communities, ELF labeled
126 fluorescence was used to investigate species-specific AP activity.

127

128 **Methods**

129 *Sampling sites-*

130 Surface water samples were collected in San Francisco Bay, from the upper few
131 meters of the water column, along a 10-station north-south transect using a flow-through
132 system (Figure 1A). This was done on 12 cruises from November 2001 through April
133 2003. South San Francisco Bay is characterized by higher turbidity, high levels of
134 wastewater input from several municipal water treatment plants, and longer water
135 residence times than the northern and central parts of the bay (Walters, et al. 1985). The
136 northern bay is primarily influenced by influx of water from the Sacramento River
137 (Walters, et al. 1985). The middle of this transect (station 21) close to the Golden Gate
138 Bridge, represents the greatest input from open ocean water. During the cruises,
139 temperature, salinity, chlorophyll *a* (Chl *a*), turbidity and dissolved inorganic nutrient

140 concentrations (SRP, nitrate, nitrite, ammonia, silica) were determined. More details
141 about the sampling sites, instrumentation used, and analytical procedures can be found
142 through the US Geological Survey (USGS) web site (www.sfbay.wr.usgs.gov).
143 In Monterey Bay, samples were collected during eight cruises from December
144 2001 to April 2003 at three stations located about 2, 25, and 50 km from shore (C1, M1,
145 and M2 respectively) (Figure 1B). Samples were taken from the surface, 20 meters and
146 60 meters depth. Temperature, salinity, oxygen, chlorophyll *a*, phytoplankton species
147 abundance, and dissolved inorganic nutrient concentrations were determined as part of
148 ongoing time series data collected by Monterey Bay Aquarium Research Institute
149 (MBARI). More information about these sites, instrumentation used and analytical
150 procedures can be obtained at the MBARI web site (www.mbari.org).
151
152 *ELF-97 cell specific assay -*
153 Water samples (1 L) were processed as described in Dyhrman and Palenik (1999).
154 This involved collecting plankton samples on a 0.45 µm filter (by low vacuum filtration),
155 resuspending the sample in an ethanol solution adding the label (ELF-97) and transferring
156 the sample to an epitube. Samples were stored in the dark at 4º C until analysis. Cell
157 counts were preformed using a Nikon epifluorescent microscope using a 100-W mercury
158 lamp (DAPI filter set, excitation at 350 nm and maximum ELF emission at 520 nm) for
159 ELF activity as well as with standard illumination. Slides were scanned, and each
160 identifiable cell was tallied as either positive or negative for ELF labeling, indicating AP
161 activity (Figure 2). A positive tally was given to any cell that had a considerable amount
162 of fluorescent ELF labeling visible.

163 ELF analysis was performed on surface water samples from San Francisco Bay
164 collected during June and August 2002 at stations 36, 21, and 9, to represent different
165 regions in the Bay. An abundance of suspended sediment, however, precluded the ability
166 to identify and count cells, thus only qualitative observations were recorded. ELF
167 analysis was also performed on surface water samples from 03 June 2002, 08 August
168 2002 and 26 August 2002 for Monterey Bay stations M1, M2, and C1. Fifteen of the
169 most commonly observed diatom and dinoflagellate taxa were monitored for each
170 sample. Species composition varied greatly from cruise to cruise, and from station to
171 station. Because species often were present at one time or site and not at the next and
172 other species had very few individuals in any one sample, it was difficult to track
173 temporal or spatial changes for each of the individual species identified here. Because of
174 this variance, we evaluated the combined data for all diatom species and all
175 dinoflagellates from each sample as well as the data for all three cruises (Tables 1 and 2).
176
177 *Bulk enzyme assays -*
178 Samples for AP assays were collected in 125 mL polyethylene Nalgene bottles
179 and refrigerated until analysis (within 24 hours of collection). Activities in sample splits
180 analyzed upon collection or after refrigeration for 24 hours were identical within
181 analytical error suggesting that this storage did not influence the results. AP activity was
182 measured using a *p*-nitrophenyl phosphate (PNP) substrate (Fisher Scientific). Activity
183 was measured on both filtered (0.2 μm polycarbonate filter) and unfiltered fractions of
184 the samples where unfiltered samples represent the total activity and filtered samples

185 account for activity of enzyme that is released to the water. The difference between the
186 two, total activity minus dissolved activity, represents particle associated AP activity.
187 For each sample, 2.5 mL of sample water and 2.5 mL of pH 8.5 Modified
188 Universal Buffer were mixed in 15 mL glass centrifuge tubes and 1 mL of 25 mmol L^{-1}
189 PNP-phosphate substrate was added to yield a final substrate concentration of ~4 mmol
190 L^{-1} during incubation. After incubation and before spectrophotometric measurement 4 ml
191 of NaOH (0.1 N) was added to the solution (resulting in concentrations of 2.5 mmol L^{-1}).
192 These treatments are consistent with previously published protocols. Samples were
193 covered and incubated for 24 hours at room temperature on an orbital shaker at 50 rpm.
194 Incubations of this period are necessary due to relatively low AP activity in the samples
195 and are consistent with other marine studies. This time course was observed to be within
196 the linear range of response as determined by hourly analyses of both standards and
197 natural samples. All samples were done in duplicate along with controls using autoclaved
198 deionized water. Absorbance at 410 nm was measured to determine PNP concentrations.
199 Standards made using p-nitrophenol at concentrations ranging from 0 to 10 μmol L^{-1}
200 were used for calibration. Because other studies have used different substrate
201 concentrations and equilibration temperatures, it is difficult to directly compare levels of
202 activity between studies, but the method used is internally consistent and could be used to
203 demonstrate the temporal and spatial variability of AP in the study area.
204
205 *Ancillary data -*
206 Phytoplankton species abundance was determined from HPLC pigment analyses
207 and a set of equations that convert the HPLC data to the chlorophyll concentration (μg L^{-}

208 ^{1}) associated with various taxa as described in Andersen et al., (1996); HPLC data are
209 from Chavez (unpublished). Chlorophyll *a* concentration (mg chl m^{-3}) was measured by
210 fluorometry after water samples were filtered onto 25 mm Whatmann GF/F filters and
211 extracted in acetone. Approximately 10 mL of water was collected for dissolved nutrient
212 concentrations from each depth, frozen, and analyzed on a nutrient autoanalyzer.
213
214 **Results**
215 The relative abundance of the 15 most common diatom and dinoflagellate species
216 and the fraction of cells for each species which was labeled by cell-specific (ELF) AP
217 activity for the three cruises and from the three sites at Monterey Bay are given in Table
218 1. The percent ELF labeling for all diatoms and for all dinoflagellates for each sampling
219 period is summarized in Table 2 and shows the preferential labeling of dinoflagellates at
220 all sites throughout the year. Figure 2 shows ELF labeling as observed under microscopy
221 for a few representative samples. In San Francisco Bay, ELF analysis was complicated
222 by high amounts of suspended sediment. Because the size of suspended particles was
223 similar to the size of phytoplankton, it could not be separated by filtration. This resulted
224 in large amounts of particulate matter, and relatively few phytoplankton cells on the
225 filters. Because not enough phytoplankton cells were present to get statistically
226 significant results, species data were not statistically analyzed. We note, however, that
227 most phytoplankton (regardless of species) seen in San Francisco Bay during our
228 sampling period were not ELF labeled. The particulate matter in San Francisco Bay,
229 however, was laced with ELF-labeled microorganisms, indicating substantial amounts of
230 AP activity associated with bacteria attached to the particulate matter (Fig. 2C).

231 Bulk AP activity exhibited spatial and temporal variability in both San Francisco
232 Bay (Fig. 3) and Monterey Bay (Fig. 4). Typically, the activity was much greater in the
233 unfiltered fraction, suggesting that the activity in most samples was particle associated,
234 although on occasion, the dissolved portion (data not shown) accounted for a substantial
235 fraction of the activity observed at a station (e.g., Station C1 in April of 2002). AP
236 activities in Monterey Bay were typically higher at stations closer to shore (C1 and M1)
237 and in the upper water column (0 and 20 m) compared to the 60 m samples (Fig. 4).
238 Maximum activities tend to occur in the early spring and summer period. In San
239 Francisco Bay stations 32 and 36, in South Bay, exhibited the highest activity through
240 much of the year (Fig. 3). While the temporal distribution of activity was quite variable,
241 the highest levels tended to occur between February and May in both 2002 and 2003, a
242 period in the year when phytoplankton blooms are common (Cloern, pers. comm., 2005).
243 This also corresponds to periods of high turbidity and large amounts of suspended
244 sediments (Hollibaugh, 1996). The maximum activities observed were148 μmol PNP L^{-1}
245 h^{-1} and 30 μmol PNP L^{-1} h^{-1} in San Francisco Bay and Monterey Bay respectively. In San
246 Francisco Bay AP activity showed a weak but statistically significant ($p < 0.05$) positive
247 correlation with chlorophyll *a*, and weaker but still significant positive correlation with
248 SRP (Fig. 5). In Monterey Bay a similar weak positive correlation with chlorophyll *a*, is
249 evident but no correlation with SRP is observed. The bulk AP activity in Monterey bay
250 is plotted against dinoflagellate abundance determined from HPLC pigment analyses
251 (Fig. 6). The positive correlation suggests that this group is responsible for the bulk of
252 the AP activity.
253

254 **Discussion**

255 The results of ELF analysis revealed different phosphorus regimes in Monterey

256 and San Francisco Bays. Above background AP activity (background is defined here as 3

257 μmol PNP L^{-1} h^{-1} based on maximum levels observed in blanks) was measured

258 throughout the year in San Francisco Bay and in Monterey Bay, both systems which,

259 based on nutrient analysis, have high P (always measurable), and Redfield ratios which

260 do not indicate P-deficiency (on average 12 in Monterey Bay and 7 in San Francisco Bay,

261 during our sampling period). AP activities were observed in other coastal high P systems

262 such as Tokyo Bay, some fjords and in an upwelling system off North West Africa

263 (Kobori and Taga, 1979; Sebastián et al. 2004). When the taxa responsible for the AP

264 activity, in our study, are examined (using ELF; Tables 1 and 2) it is clear that the

265 activity is associated with specific components in the community and does not indicate

266 that the phytoplankton as a whole are P-deficient.

267 The dominant phytoplankton in most of the samples from Monterey Bay were the

268 diatom genera *Pseudonitzschia*, *Chaetoceros,* and *Eucampia* (accounting for 72% of all

269 cells observed for the combined data). Diatoms (all species) comprised 82% of the cells

270 counted in the samples and dinoflagellates accounted for the remainder (18%). On

271 average seventy-six percent of dinoflagellates exhibited ELF labeling (range from 33% to

272 100% for various species). In comparison, on average only 1.0% of diatoms were labeled

273 (range 0-12%). Dinoflagellates overall accounted for over 90% of all ELF labeled cells,

274 despite their low relative abundance in the phytoplankton population (Table 1, Fig. 2).

275 Our results are consistent with other studies (Kobori and Taga, 1979; Sebastián et al.

276 2004) that suggest that interpretation of bulk AP activity as a whole phytoplankton

277 community P-deficiency indicator cannot be applied indiscriminately. Moreover, our
278 results imply that high SRP (typically higher than 0.5 μmol L^{-1} in surface waters in our
279 study) and low N:P ratios (consistently lower than 16) cannot be used to suggest that the
280 phytoplankton community as a whole is not P-deficient. For example, although nutrient
281 enrichment, grow-out experiments in Monterey Bay indicated that additions of nitrate
282 provided the most potential for growth and biomass accumulation and P additions did not
283 have any measurable effects (Kudela and Dugdale, 2000), our data indicate that the
284 dinoflagellates may be P-deficient, this is despite measurable SRP concentrations and low
285 N:P ratios. It is possible that the P demands by dinoflagellates are higher than those of
286 diatoms or that some P component that is included in the SRP analysis is not accessible to
287 dinoflagellates. Our work emphasizes the importance of identifying specific organisms
288 within a community which exhibit AP activity. Moreover, data from San Francisco Bay
289 where AP activity is primarily associated with heterotrophic bacteria stresses that when
290 studying phytoplankton nutrition status it is crucial to assess the contribution of
291 heterotrophic bacteria to the bulk AP activity. If this for example was not done in San
292 Francisco Bay the observed AP activity could be wrongly interpreted as reflecting
293 phytoplankton P-deficiency.

294 In Monterey Bay, diatoms, the dominant class of phytoplankton in the bay waters,
295 generally did not exhibit much AP activity. The percent labeling for different diatoms
296 species ranged from 0-12% with the most abundant species (*Pseudonitzschia* and
297 *Chaetoceros*) exhibiting the least AP activity as determined by ELF labeling. Diatoms do
298 have the ability to synthesize AP as was shown in culture experiments (Lomas, pers.
299 comm., 2004) and by instances were individual diatoms were observed to be ELF labeled

300 in our and other field samples (Lomas et al., 2004; Nicholson 2003; Fig. 2, Table 1). In
301 contrast, a large fraction of the dinoflagellate taxa in our samples exhibited AP activity;
302 33-100% of the cells for various species were labeled throughout the year. Interestingly
303 some variability between dinoflagellate taxa tendency to be labeled is observed; with
304 *Dissodinium*, when found in the water column, exhibiting labeling for 100% of the
305 observed cells. The more abundant dinoflagellate groups, *Prorocentrum minimum* and
306 *Gymnodinium*, typically showed less labeling (74% and 81% of cells labeled
307 respectively). The only dinoflagellate that exhibited labeling of less than 50% of the cells
308 was *Ceratium lineatum*. As expected from the above results, the bulk AP activity in
309 Monterey bay is positively correlated with dinoflagellate abundance ($r^2 = 0.39$) (Fig. 6A).
310 The correlation is even better (Fig. 6B, $r^2 = 0.89$) when only the ELF labeled fraction of
311 dinoflagellates is considered (e.g., dinoflagellate abundance multiplied by the percent of
312 dinoflagellates exhibiting ELF labeling) confirming that this group is responsible for the
313 bulk of the AP activity.
314 AP is P-regulated in some dinoflagellates (Dyhrman and Palenik, 1999), if indeed
315 this AP induction is universal among dinoflagellates, our results indicate that despite
316 presumably replete P conditions (high SRP concentrations) and relatively low N:P ratios,
317 bio-available inorganic P did not fulfill P-demand by the dinoflagellates. However, it is
318 possibility that AP is not regulated by P availability for all dinoflagellate taxa (Gonzalez-
319 Gill 1998); culture enrichment experiments should be conducted to establish taxa specific
320 regulation. Preliminary incubation experiments (Nicholson, 2003) of a natural sample
321 (mixed taxa collected on May 2003) from Monterey Bay (station M1) with
322 orthophosphate, nitrate, and glucophosphate (organic P) additions, show lowering of bulk

323 AP activity and activity associated with dinoflagellates (determined using ELF) when
324 inorganic P is added (compared to the control and other additions), suggesting that the
325 AP activity indeed reflects P-status (Fig. 7).

326 The observed difference in AP activity in diatoms and dinoflagellates in Monterey
327 Bay (e.g., Table 1) suggests that changes in nutrient input, and particularly P loading,
328 may not only affect overall phytoplankton abundance, but could also influence species
329 composition. Indeed, the positive correlation between SRP concentrations and
330 dinoflagellet chlorophyll concentrations (which is expected to be proportional to their
331 abundance) during some months of the year in Monterey Bay (Fig. 8) suggests that this
332 group may become more abundant under higher SRP conditions at least under conditions
333 characteristic of these months/cruises (mostly winter months and non-upwelling
334 conditions). Additional research needs to be done to clearly describe and understand the
335 specific interactions between SRP, other environmental parameters and dinoflagellates
336 abundance in Monterey Bay.

337 AP activities in Monterey Bay were typically higher in the upper 20 m of the
338 water column compared to the 60 m samples (Fig. 4). Although we did not observe a
339 strong correlation between AP activity and SRP we would expect lower activity in deeper
340 samples where SRP concentrations are typically higher. The AP activity is also higher
341 closer to shore (C1); this may be due to the typically higher dinoflagellate abundances
342 closer to shore in Monterey Bay (e.g., Station C1, Fig. 9). This is evident from both the
343 higher absolute dinoflagellate chlorophyll concentrations and in the fraction of
344 dinoflagellate chlorophyll to total chlorophyll at this site (data not shown). Maximum
345 activities tend to occur in the spring and summer period, it is possible that during this

346 high productivity season the phytoplankton demand for SRP is high compared to the
347 bioavailable pool resulting in higher utilization of DOP and thus higher AP activities.
348 This is also the time of year when a peak in dinoflagellate abundance is observed
349 (Chavez, pers. comm., 2005) which may in turn account for the higher activities.
350 In San Francisco Bay ELF assays show that practically all of the AP activity is
351 associated with bacteria and not phytoplankton. The highest activity through much of the
352 year was recorded in stations 32 and 36 in the South Bay (Fig. 3). The South Bay is
353 characterized by abundant suspended organic matter and high labile particulate and
354 dissolved organic carbon concentrations (Conomos et al., 1979) that could provide
355 substrates for bacterial growth. Indeed bacterial counts tend to be higher in the South
356 Bay particularly near station 36, which is the closest to both land runoff and sewage
357 inputs (Hollibaugh, 1996). It is not clear why these bacteria are synthesizing AP
358 however; since SRP concentrations in the water column are high (between 1.5 and 15
359 μmol L^{-1}) it is unlikely that this is used to access P. In addition, the absence of
360 relationship between SRP and AP activity in San Francisco Bay questions the use of AP
361 as a proxy for P-deficiency. It is most likely that AP is used for hydrolysis of dissolved
362 organic matter for carbon utilization as has been suggested for other places (Kirchman et
363 al. 2000; Van Wambeke et al. 2002). Indeed, the highest activity levels are observed in
364 the South Bay (stations 32 and 36) where DOC and POC concentrations are higher as
365 mentioned above. These results from San Francisco Bay further support our conclusion
366 that SRP concentrations, N:P ratios, or bulk AP activities alone cannot be used to
367 determine P-status of natural eukaryotic phytoplankton populations and that field and
368 culture based ELF assays may enhance our understanding of ecosystem response to P

369 availability. The results also suggest that organic P compounds may have an important
370 role in the environment as potential sources for both P and C to various organisms.
371 It is important to further examine the differences in P demand and utilization
372 between species in order to understand how eukaryotic phytoplankton respond to nutrient
373 availability in coastal environments and their ability to utilize various pools of nutrients.
374 Our results imply that organic P compound may have an important role in the P cycle and
375 the availability of this P pool as a nutrient source that supports primary production and C
376 uptake should not be ignored.
377
378
379

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493 **Table 1:** Cell-specific AP activity from Monterey Bay. The results include summed data
494 from three cruises from June 2002 through August 2002, and from three sites (see Fig.
495 1B).

Genera	Group	Positive	Negative	Total	Labeled (%)	Relative abundance
Asterionellopsis glacialis	diatom	1	41	42	2.4%	0.88%
Chaetoceros	diatom	6	675	681	0.9%	14.28%
Eucampia zodiacus	diatom	2	397	399	0.5%	8.36%
Pseudonitzschia	diatom	2	2352	2354	0.1%	49.35%
round diatom	diatom	29	219	248	11.7%	5.20%
Thalsasiosira	diatom	0	202	202	0.0%	4.23%
Ceratium	dinoflagellate	76	1	77	98.7%	1.61%
Ceratium lineatum	dinoflagellate	24	47	71	33.8%	1.49%
Dinophysis	dinoflagellate	13	1	14	92.9%	0.29%
Dissodinium pseudolunula	dinoflagellate	14	0	14	100.0%	0.29%
Gymnodinium	dinoflagellate	109	26	135	80.7%	2.83%
Procentrum rostratum	dinoflagellate	31	4	35	88.6%	0.73%
Prorocentrum minimum	dinoflagellate	310	111	421	73.6%	8.83%
Protoperidinium	dinoflagellate	45	11	56	80.4%	1.17%
unidentified nonthecate	dinoflagellate	20	1	21	95.2%	0.44%
Totals		682	4088	4770	14.30%	
	diatoms	40	3886	3926	1.0%	82.31%
	dinoflagellates	642	202	844	76.1%	17.69%

496

497 **Table 2:** ELF labeling of diatoms versus dinoflagellates from three cruises from June

498 2002 through August 2002, in Monterey Bay.

499

500

Date	Station	Diatoms			Dinoflagellates		
		Positive	Negative	Percent labeled	Positive	Negative	Percent labeled
03 Jun 02	C1	5	513	1.0%	75	16	82.4%
03 Jun 02	M1	8	480	1.6%	77	31	71.3%
03 Jun 02	M2	0	202	0.0%	231	73	76.0%
08 Aug 02	C1	0	205	0.0%	20	8	71.4%
08 Aug 02	M1	27	564	4.6%	102	8	92.7%
08 Aug 02	M2	0	582	0.0%	11	4	73.3%
26 Aug 02	C1	0	760	0.0%	4	14	22.2%
26 Aug 02	M1	0	580	0.0%	122	48	71.8%

501 **FIGURE LEGENDS**

502

503 **Figure 1: (A)** Sampling stations for San Francisco Bay cruises. Samples were collected

504 from Stations 9, 13, 15, 18, 21, 24, 27, 30, 32, and 36 from the surface layer. **(B)**

505 Sampling stations for Monterey Bay cruises. Samples were collected at stations C1, M1,

506 and M2 at depths of 60 meters, 20 meters, and at the surface.

507

508 **Figure 2:** Examples of ELF fluorescence samples. Left panels show bright field images,

509 while the right panels show the corresponding epifluorescence image with a DAPI long-

510 pass filter set. Top pair: ELF labeled *Prorocentrum rostratum.* Middle pair: Example of

511 ELF labeled diatom (*Pseudonitzschia*) and unlabeled diatoms (*Pseudonitzschia and*

512 *Chaetoceros*). Bottom pair: bacteria on sediment particles and aggregates showing ELF

513 labeling in San Francisco Bay.

514

515 **Figure 3:** Bulk AP activity in San Francisco Bay from November 2001 through April

516 2003. Station 9 is at Suisun Bay in the North SF Bay and station 36 is at the most

517 southern station (see Fig. 1A).

518

519 **Figures 4:** Bulk AP activity for stations M2, M1 and C1 in Monterey Bay from

520 December 2001 through April 2003. Stations show seasonal increases in AP activity

521 during spring phytoplankton blooms and higher activities in stations closer to shore.

522

523 **Figure 5:** Bulk AP activity plotted against chl *a* and SRP for (A, B) San Francisco Bay
524 and (C, D) Monterey Bay. **(A, C)** Both San Francisco and Monterey Bays show
525 significant weak positive correlations between AP activity and chl *a* concentration. (r^2 =
526 0.283 and 0.113 respectively) **(B, D)** San Francisco Bay samples show a slight positive
527 correlation between SRP and AP activity; no correlation was observed in Monterey Bay
528 (r^2 = 0.084 and 0.038 respectively). Significant correlations were calculated based on
529 linear regressions (least square method) using the *f* observed value, the relevant degrees
530 of freedom, and a 0.05 confidence level.
531
532 **Figure 6: (A)** Bulk AP activity is correlated with dinoflagellate abundance as estimated
533 from the dinoflagellate chlorophyll concentration (r^2 = 0.39), and **(B)** more strongly
534 correlated with dinoflagellate abundance multiplied by the percent of dinoflagellates
535 exhibiting ELF labeling, which represents the abundance of ELF labeled dinoflagellates
536 (r^2 = 0.89).
537
538 **Figure 7:** Bulk AP activity in nutrient addition incubation experiments with Monterey
539 Bay samples. SRP addition treatment has lower AP activity as compared to control,
540 nitrate, or organic phosphate additions. Most of the activity observed was associated with
541 dinoflagellates in the samples as determined by ELF labeling.
542
543 **Figure 8:** Dinoflagellate pigment concentrations which are proportional to dinoflagellate
544 abundance in relation to SRP concentrations in Monterey Bay.
545

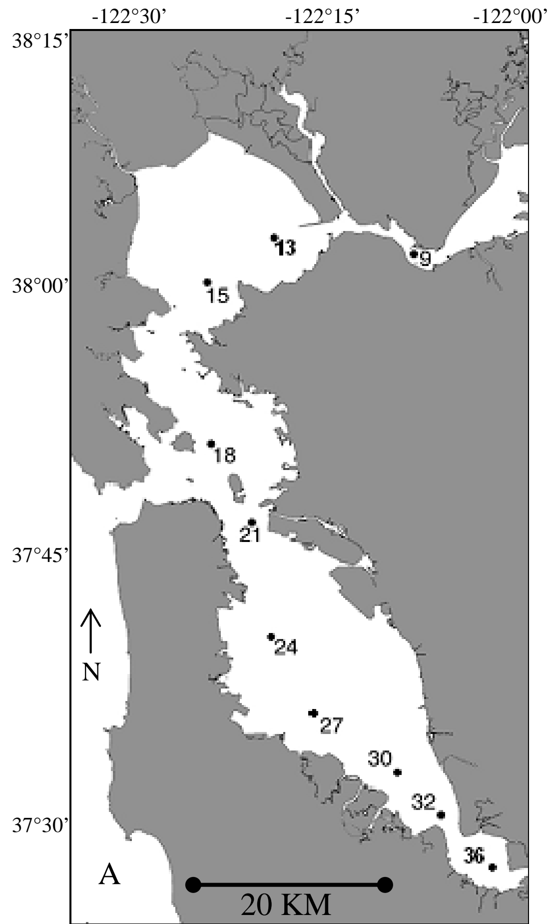
546 **Figure 9**:

547 The seasonal cycle for dinoflagellates chlorophyll concentrations at time series stations

548 C1, M1, and M2 in Monterey Bay. Monthly averages for the years 1989-2000 are shown.

549

550



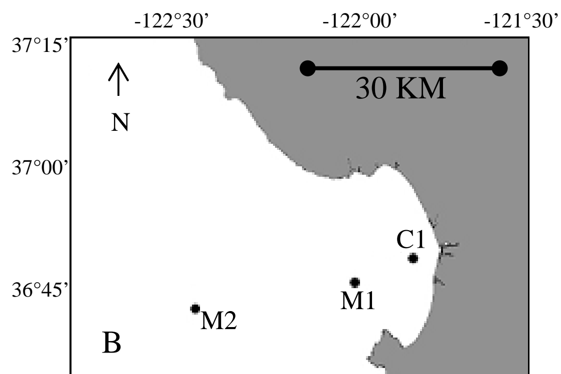
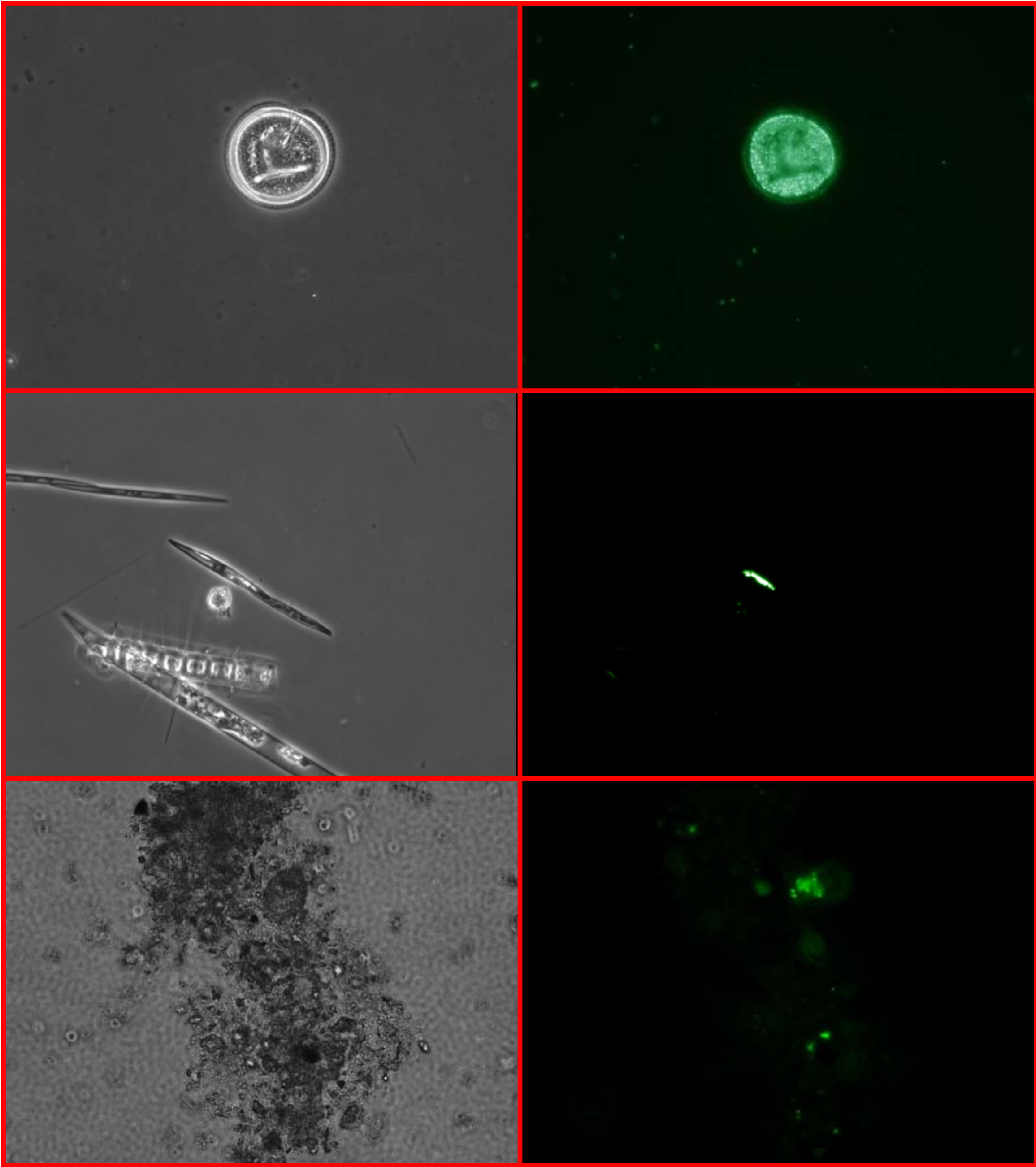


Fig. 1



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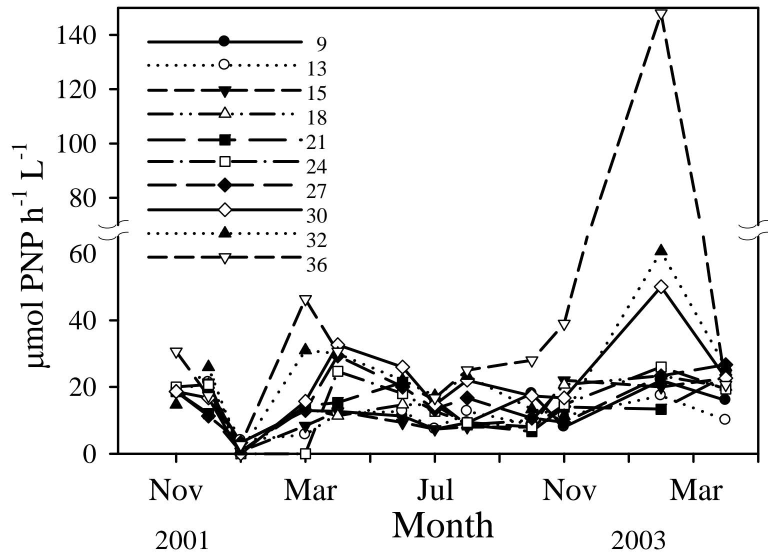

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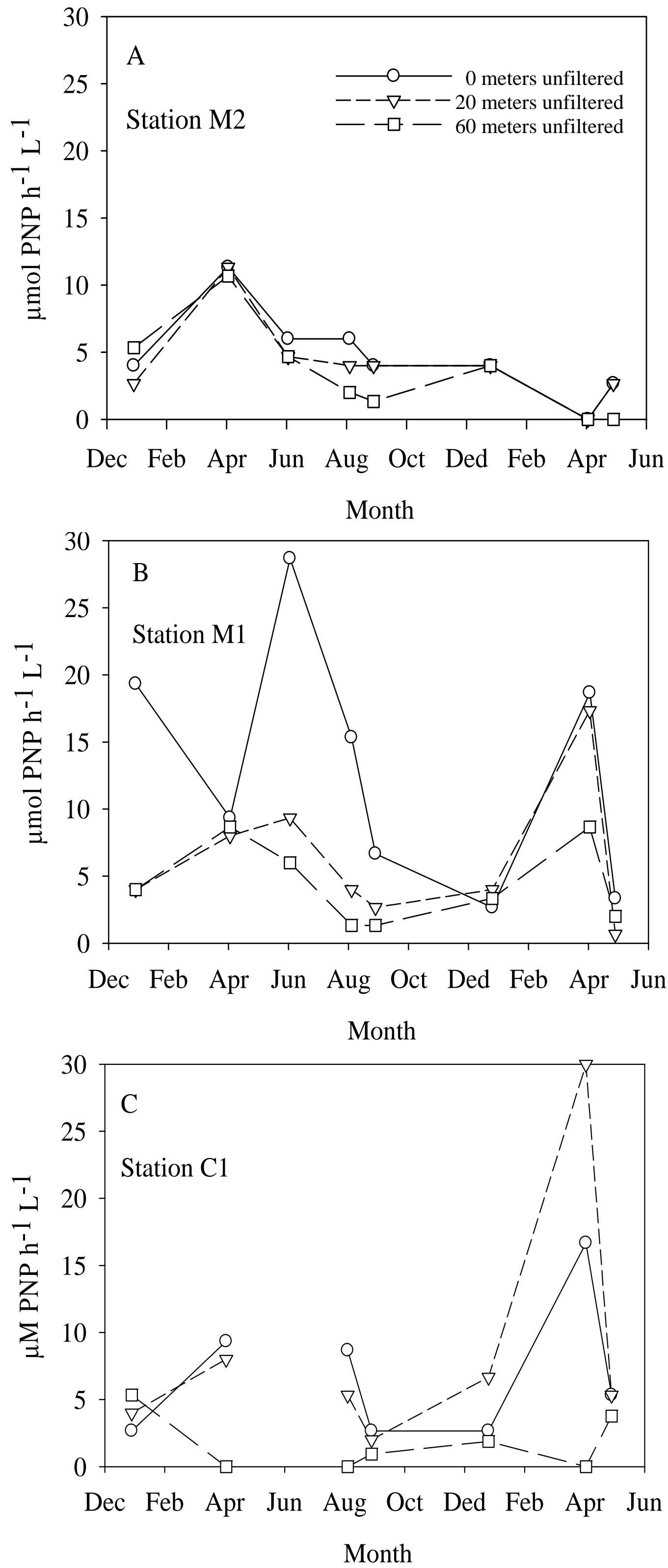

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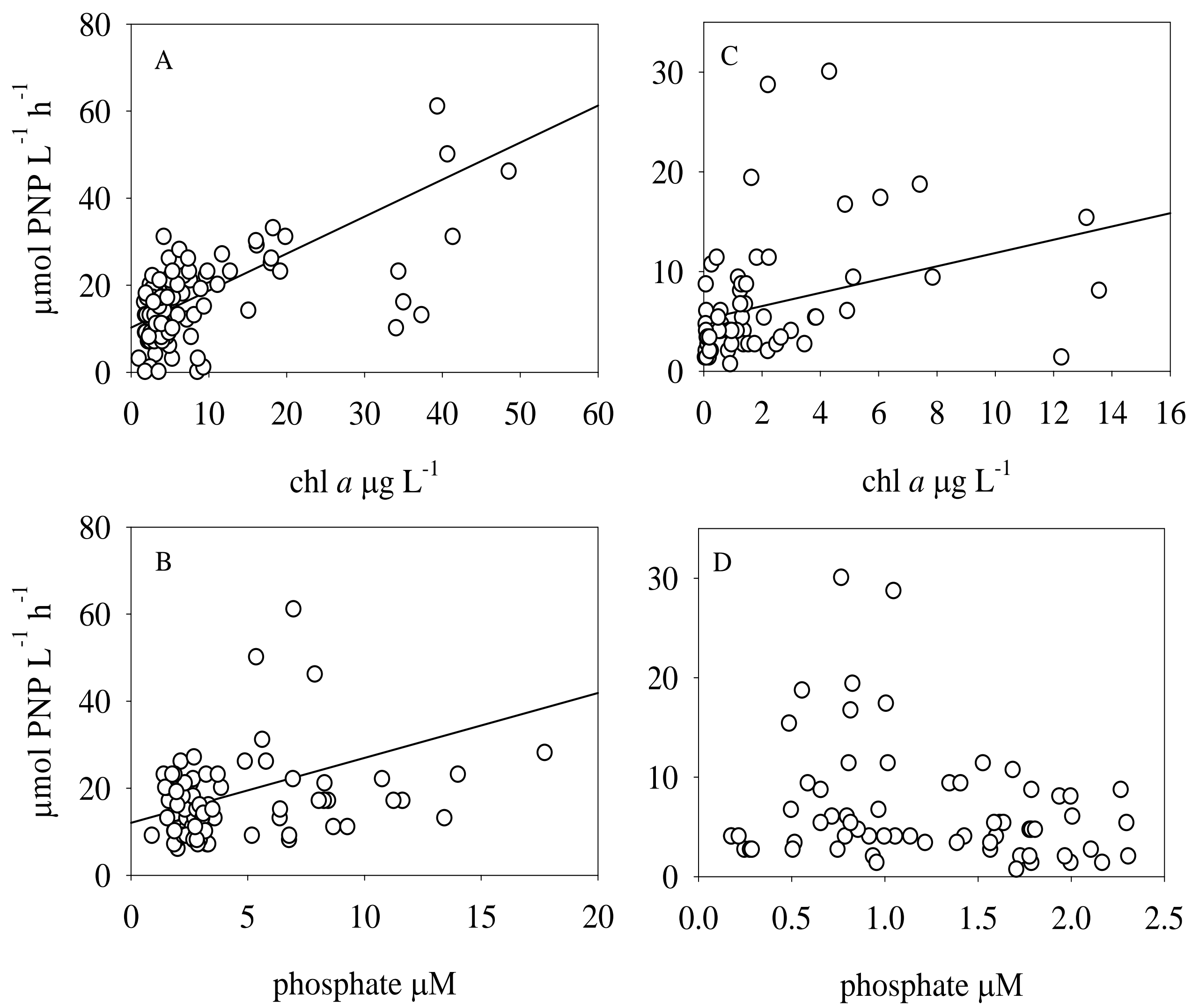

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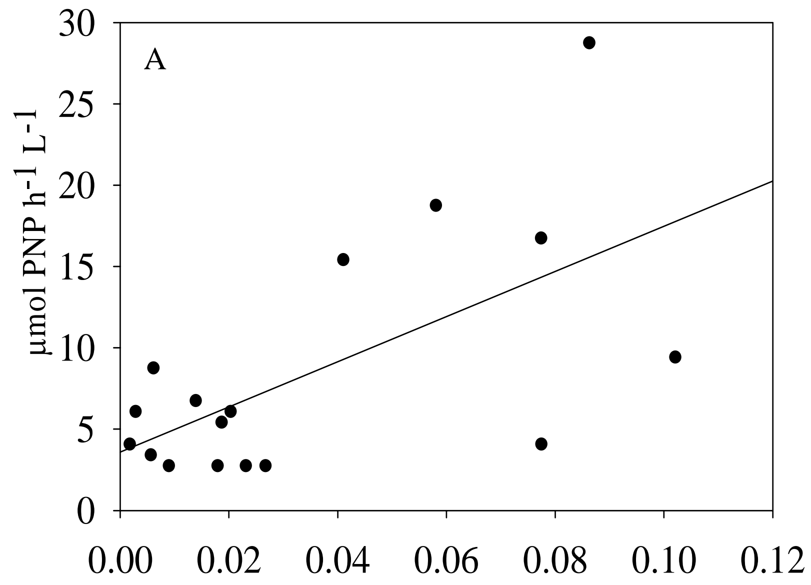

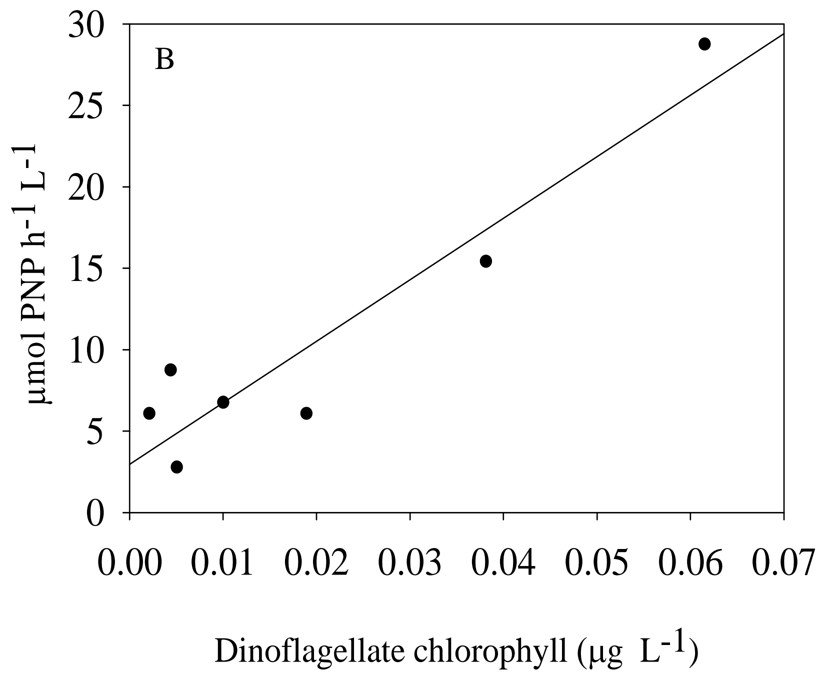

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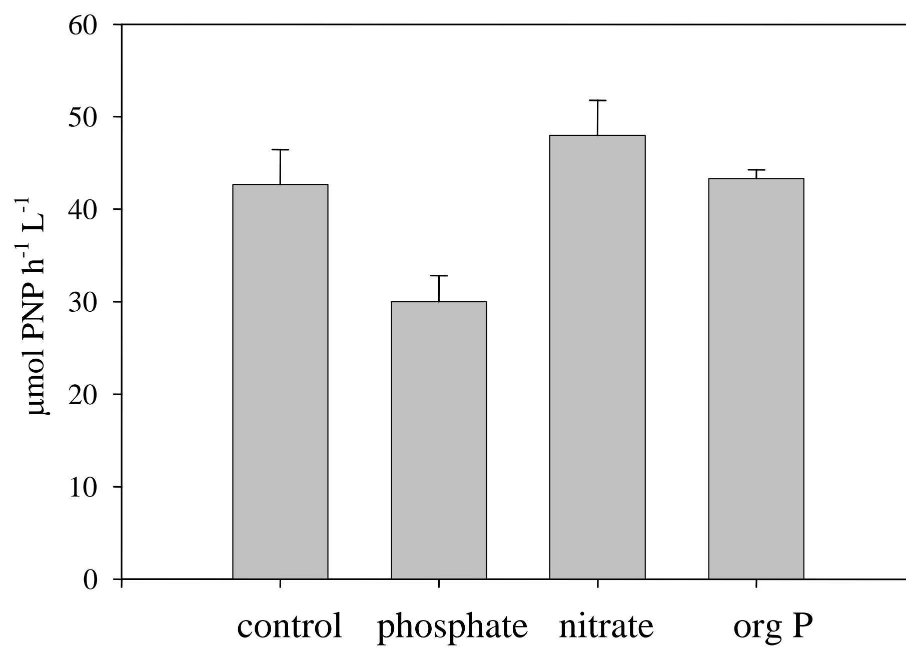

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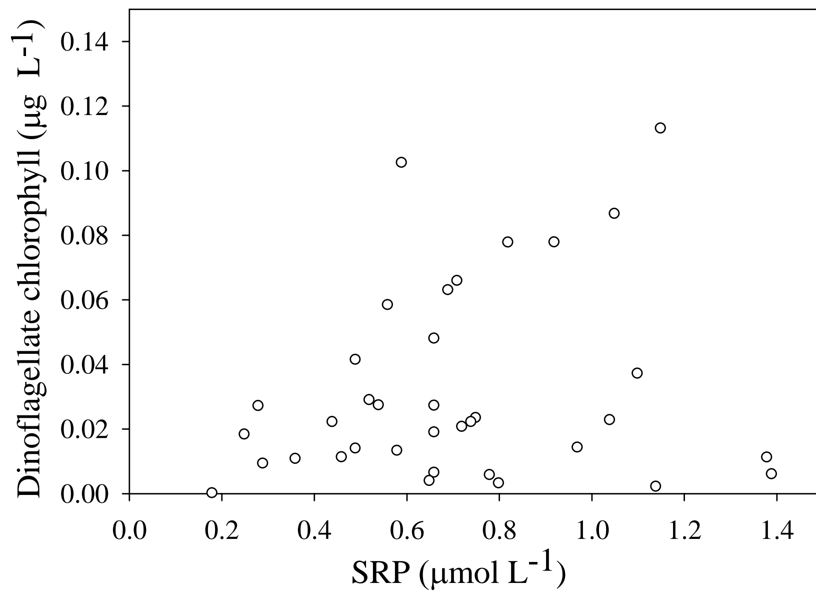

Fig. 8

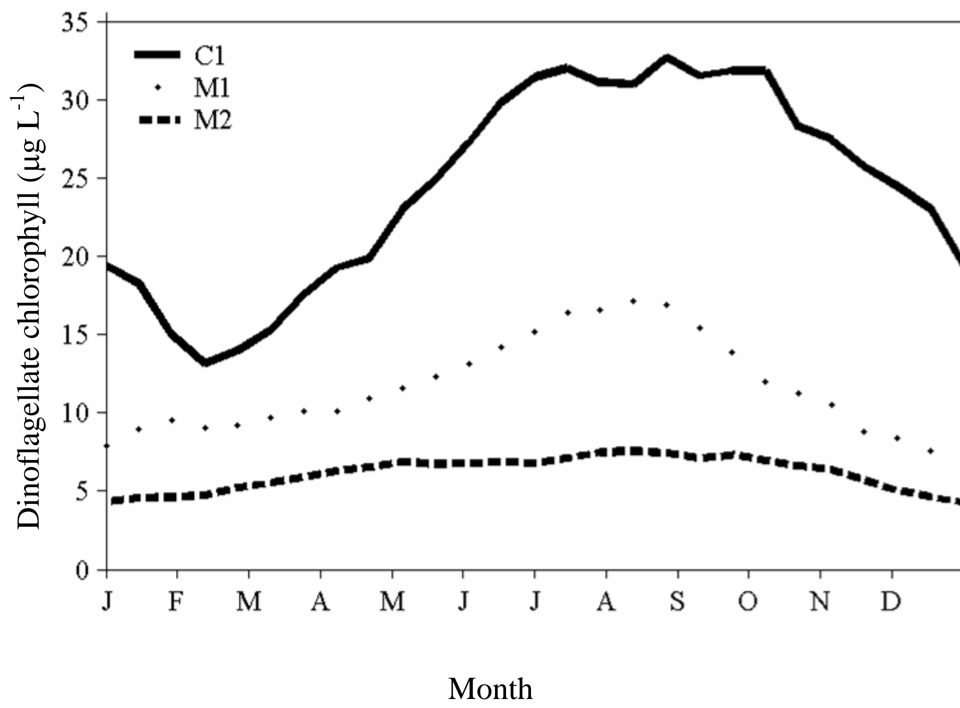

Fig. 9