Vol. 85: 211–218, 2020
https://doi.org/10.3354/ame01951

AQUATIC MICROBIAL ECOLOGY
Aquat Microb Ecol

Published online December 17

Phosphate diester utilization by marine diazotrophs *Trichodesmium erythraeum* and *Crocosphaera watsonii*

Tamaha Yamaguchi[1,2,*], Mitsuhide Sato[2,3], Natsuki Gonda[2], Kazutaka Takahashi[2], Ken Furuya[2,4]

[1]Fisheries Resources Institute, Japan Fisheries Research and Education Agency, Kanagawa 236-8648, Japan
[2]Department of Aquatic Bioscience, The University of Tokyo, Tokyo 113-8657, Japan
[3]Graduate School of Fisheries and Environmental Sciences, Nagasaki University, Nagasaki 852-8521, Japan
[4]Graduate School of Science and Engineering, Soka University, Tokyo 192-8577, Japan

ABSTRACT: In the phosphate-depleted oligotrophic ocean, microbes utilize various dissolved organic phosphorus (P) compounds as alternative P sources, using enzymes such as alkaline phosphatases. However, knowledge of such P acquisition mechanisms is limited, especially in association with the physiology of nitrogen-fixing organisms, which play a substantial role in marine biogeochemical cycling. We show that nonaxenic clonal cultures of 2 oceanic diazotrophs, *Trichodesmium erythraeum* and *Crocosphaera watsonii*, have the ability to utilize phosphate diester as their sole P source, using a model artificial compound — bis-*p*-nitrophenyl phosphate (bisNPP). Although both diazotroph cultures likely preferred phosphate monoester to diester, the expressed diesterase activity was theoretically sufficient to fulfill their P demands, and they showed significant growth in bisNPP-added media. Interestingly, a distinct difference in their growth trends was observed, with faster onset of growth by *C. watsonii* and delayed onset of growth by *T. erythraeum*. This indicates that the *C. watsonii* consortium can effectively and rapidly assimilate *in situ* diesters as alternative P sources in the field. Nonetheless, when considering the poor bisNPP utilization reported from other marine phytoplankton taxa, our results indicate that the utilization of particular diester compounds is a notable and advantageous strategy for both diazotroph consortia to alleviate P limitation in the oligotrophic ocean.

KEY WORDS: *Trichodesmium* · *Crocosphaera* · Phosphate diester · Alkaline phosphatase activity

1. INTRODUCTION

Nitrogen-fixing organisms, or diazotrophs, play a crucial role in marine ecosystems by fixing almost half of the total nitrogen input to the ocean (Gruber & Sarmiento 1997). In particular, the new nitrogen fixed by diazotrophs comprises almost 40% of the primary production in the subtropical oligotrophic ocean (Zehr et al. 2000, Shiozaki et al. 2009). Marine diazotrophs are classified into 4 major groups: the non-heterocystous filamentous cyanobacterium *Trichodesmium*; the heterocystous filamentous cyanobacteria, including symbiotic forms such as *Richelia*; unicellular cyanobacteria (Groups A, B, and C); and heterotrophic bacteria (Sohm et al. 2011).

Among these groups, *Trichodesmium* and the Group B cyanobacterium *Crocosphaera* occasionally predominate the diazotrophic communities in the subtropical ocean (Church et al. 2008, 2009, Shiozaki et al. 2017). While the 2 diazotroph groups often coexist in the surface layer of the subtropical ocean, they are occasionally distributed in a slightly different manner. For example, *Trichodesmium* often forms a massive bloom at the very surface (Capone et

*Corresponding author: yamaguchit@affrc.go.jp

al. 1998, Moisander et al. 2010), whereas no such phenomenon has been reported for *Crocosphaera* to date. Depths at which their maximum abundances were observed were occasionally deeper for *Crocosphaera* than for *Trichodesmium* (Shiozaki et al. 2017), and these diazotrophs were not always concurrently detected (Church et al. 2008). Such distribution patterns indicate differences in their ecological niches, which are likely to result from various individual physiological requirements.

Since diazotrophs possess the unique ability to fix nitrogen gas into ammonium, the major limiting factors for their growth are phosphorus (P) and iron (Mills et al. 2004). However, compared to other oceanic regions, the iron:nitrogen supply ratio is relatively high in the western part of the North Pacific subtropical gyre (Ward et al. 2013). Thus, P, rather than iron, is likely to become limiting for the biological activities of the diazotrophs in this region (Weber & Deutsch 2014). The scarce amount of P in this region can also be inferred from the low levels of P^*, an indicator of excess P relative to nitrogen (Deutsch et al. 2007).

Despite such P-limiting conditions, the occurrence of *Trichodesmium* and *Crocosphaera* has been reported in the western North Pacific (Kitajima et al. 2009, Shiozaki et al. 2010), which demonstrates their ability to acclimate to P shortage. In general, microbes alleviate P stress under phosphate-depleted conditions in several ways, including the use of high-affinity phosphate transporters (Dyhrman & Haley 2006) and the utilization of dissolved organic phosphorus (DOP) (Yentsch et al. 1972). Some proportion of DOP serves as an alternative P source after decomposition by microbially derived enzymes, such as nucleotidases, alkaline phosphatases, and C-P lyases.

However, these strategies are not necessarily universal among microbes. For example, the C-P lyase pathway for phosphonate utilization has been found only in *Trichodesmium* and not in *Crocosphaera* (Dyhrman et al. 2006). Therefore, examining the biological responses of the diazotrophs to different alternative P sources provides insights into how they cope with P stress. Such a variety of physiological responses for P acquisition among diazotrophs may contribute to the construction of their respective ecological niches, and help maintain the diversity and co-occurrence of different diazotroph groups in the subtropical ocean (Zehr 2011 and references therein).

In this context, most of the previous studies on diazotrophs have focused only on phosphate monoesters as their alternative P source, among the various DOP components (Mulholland et al. 2002, Dyhrman & Haley 2006, White et al. 2010). Another phosphate ester class — diesters — has remained unstudied despite its generality as a vital component of living organisms, such as nucleic acids and phospholipids. Diester concentrations are occasionally reported to be comparable to monoester concentrations in various oceanic regions (Suzumura et al. 1998, Monbet et al. 2009, Yamaguchi et al. 2019), indicating the significance of the diester pool in the marine environment. Moreover, the positive correlation between nitrogen fixation and phosphodiesterase activity (DEA) found in the central North Pacific emphasizes the possible utilization of diesters by diazotrophs (Yamaguchi et al. 2019). Therefore, we aimed to elucidate the phosphate diester utilization ability of 2 oceanic diazotrophs, *T. erythraeum* and *C. watsonii* strains isolated from the western North Pacific, to provide a better understanding of their responses to P stress in the oligotrophic ocean.

2. MATERIALS AND METHODS

Nonaxenic clonal culture strains of *Trichodesmium erythraeum* ECS0305 and *Crocosphaera watsonii* were used. Both strains were isolated from the subtropical western North Pacific (Kitajima 2009). *T. erythraeum* was maintained in a TMV culture medium (Prufert-Bebout et al. 1993) at 26°C and 100 µmol quanta m^{-2} s^{-1}. The TMV culture medium initially contained 50 µM of phosphate, whereas no nitrogenous nutrients were added. *C. watsonii* was cultured in a modified TMV culture medium, with no addition of Mg^{2+} and Ca^{2+} salts, at 28°C and 200 µmol quanta m^{-2} s^{-1}. All media were prepared with subtropical oligotrophic seawater collected from the surface of the western North Pacific, where nitrogenous nutrients and phosphate were not detectable at the nanomolar level. Both diazotroph cultures were grown in 28 ml polycarbonate centrifuge tubes (Nalgene) under a 12L:12D light cycle. Growth curves were obtained by measuring *in vivo* chlorophyll (chl) *a* fluorescence with a fluorometer (TD-700; Turner Designs).

Firstly, cultures in the stationary phase were inoculated with a sterilized glass pipette into a phosphate-deficient TMV medium for P starvation. This phosphate-deficient medium was prepared without the addition of phosphate, although it initially contained ~5 nM of phosphate (Yamaguchi et al. 2016). Concurrently, control groups were inoculated into a normal phosphate-added TMV medium with 50 µM of phosphate. When the experimental groups reached the

stationary phase in the phosphate-deficient medium, 4, 8, and 4 ml of each culture suspension were collected to determine phosphomonoesterase activity (MEA), DEA, and chl *a* concentration, respectively.

To exclude most free-living bacteria, culture cells were collected on 25 mm GF/F filters (Whatman) for MEA and DEA measurements. These filters were immersed in a working solution, prepared in 15 ml conical tubes, containing 6 ml of sterilized seawater filtered through a 0.2 µm membrane filter, 0.81 ml of 50 mM tris-glycine buffer (pH 8.5), 0.081 ml of 1 mM $MgCl_2$, and 0.3 ml of either 8 mM *p*-nitrophenyl phosphate or bis-*p*-nitrophenyl phosphate (bisNPP) as substrates (modified from Fu & Bell 2003). The final substrate concentrations of ca. 0.33 mM were assumed to be at saturating levels based on previous studies (Stihl et al. 2001, Fu & Bell 2003). Thereafter, the samples were incubated for 4–7 h under the same temperature and light conditions as the respective culture strains. MEA and DEA were calculated from the hydrolysis rates of the substrates by measuring the absorbance of released *p*-nitrophenol into the working solution. In DEA calculation, the released product concentration was divided by 2 when considering the 2 *p*-nitrophenol moieties in bisNPP. Linearity in the increasing *p*-nitrophenol absorbance during the incubation period was preliminarily verified for both cultures (data not shown). Blank samples were prepared and incubated in the same manner as above, without sample filters.

In parallel, equal volumes of residual culture suspensions in the phosphate-deficient media were re-inoculated into 3 different media: (1) phosphate-added medium, (2) medium with bisNPP added as a sole P source, and (3) phosphate-deficient medium. The final concentration of added phosphate or bisNPP was set at 50 µM. After re-inoculation, *in vivo* chl *a* fluorescence was monitored for several weeks until the cultures were considered to be in the death phase. All incubation experiments were conducted in triplicate.

Here, the same diester compound for DEA measurements — bisNPP — was selected to facilitate a consistent interpretation between enzyme activity and culture growth, regardless of the substrate specificities of the expressed enzymes. Additionally, the bisNPP medium has been used for culturing other marine phytoplankton in several previous studies (Grainger 1989, Yamaguchi et al. 2005, Richardson & Corcoran 2015). The hydrolysis of bisNPP releases a toxic product, *p*-nitrophenol, into the media; however, we considered the toxicity of *p*-nitrophenol to be negligible, as phytoplankton growth was not likely inhibited at the maximum possible concentration of *p*-nitrophenol in this study (Megharaj et al. 1991).

3. RESULTS

MEA of *Trichodesmium erythraeum* and *Crocosphaera watsonii* cultures was 7.6 and 5.5 times higher in the phosphate-deficient medium than in the control medium (Fig. 1), respectively, indicating a P deficiency during the starvation process. Both MEA and DEA of *T. erythraeum* culture were significantly higher than those of *C. watsonii* culture regardless of the media types ($p < 0.05$, Mann-Whitney test). In contrast, a significant increase of DEA in the phosphate-deficient medium was observed only for *C. watsonii* culture and not for *T. erythraeum* culture (Fig. 1). These results show that *C. watsonii* culture stimulated DEA as a corresponding alternative to alleviate P stress. Here, MEA was constantly higher than DEA regardless of the experimental conditions or species ($p < 0.05$, Wilcoxon signed-rank test), which indicates that both diazotroph cultures prefer monoesters to diesters under phosphate-limited conditions.

After each culture was re-inoculated into 3 different media, both diazotrophs showed immediate onset of growth in the phosphate-added medium (Fig. 2). In contrast, no growth was observed in the phosphate-deficient medium, indicating successful P starvation before re-inoculation. There was a distinct trend in the growth onset in the bisNPP medium

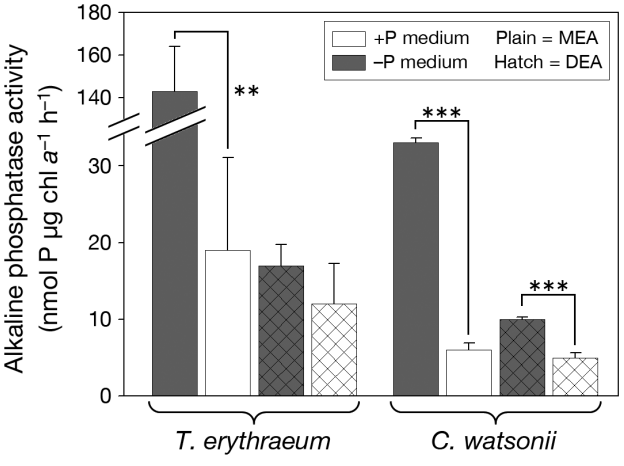

Fig. 1. Alkaline phosphatase activities of *Trichodesmium erythraeum* and *Crocosphaera watsonii* cultured in either phosphate-added (+P) or phosphate-deficient medium (–P), examined on Day 0 (see Fig. 2). Error bars represent the standard deviation. MEA: phosphomonoesterase activity, shown as plain bars; DEA: phosphodiesterase activity, shown as hatched bars; **$p < 0.01$; ***$p < 0.001$

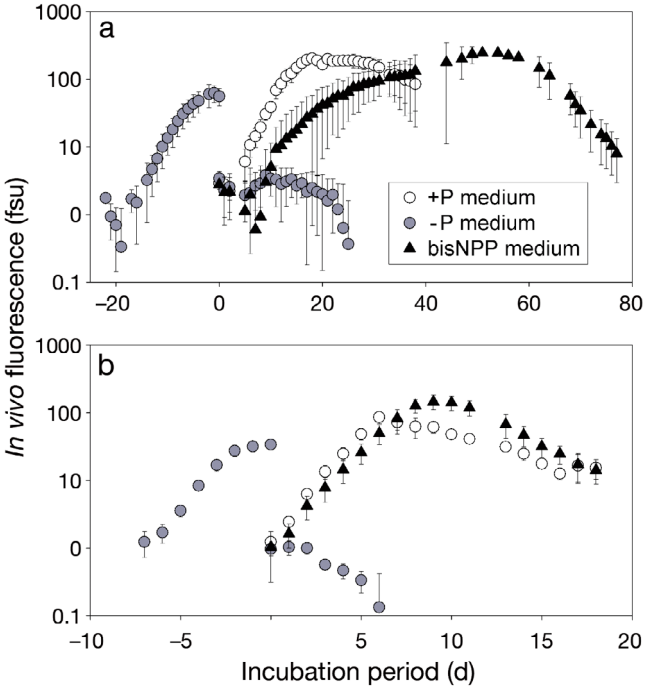

Fig. 2. Growth curves of (a) *Trichodesmium erythraeum* and (b) *Crocosphaera watsonii* shown in fluorescent signal units (fsu). Error bars represent the standard deviation. Both cultures were inoculated on Day 0 from a phosphate-deficient medium (–P) to either the phosphate-added medium (+P), –P medium, or bis-*p*-nitrophenyl phosphate (bisNPP)-added medium. Note that *T. erythraeum* growth in the +P medium was only observed to Day 38 for operational reasons

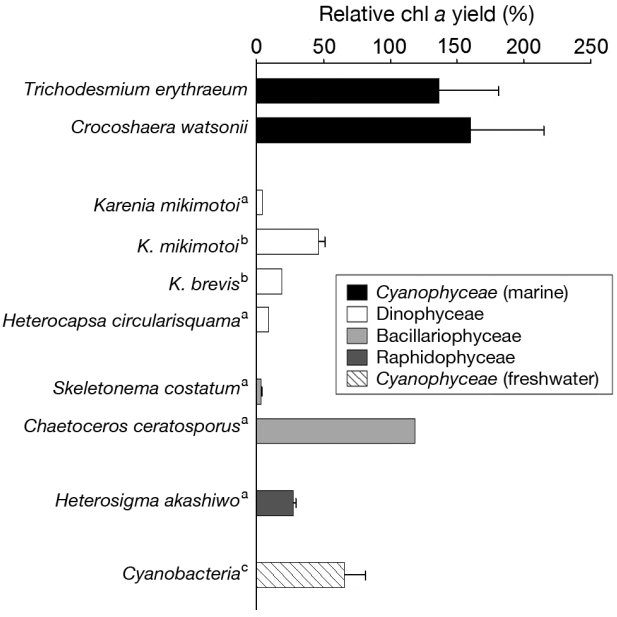

Fig. 3. Growth efficiency of phytoplankton in the bis-*p*-nitrophenyl phosphate (bisNPP) medium. Relative chlorophyll (chl) *a* yield was calculated as a relative percentage of the maximum *in vivo* fluorescence value in the bisNPP medium to that in the phosphate-added medium. Error bars represent the standard deviation. [a]Yamaguchi et al. (2005); [b]Richardson & Corcoran (2015); [c]Grainger (1989), exceptionally shown in relative dry weight yield

(Fig. 2); while *C. watsonii* growth onset was observed immediately after re-inoculation, *T. erythraeum* required an average of 12 d before the onset of its regrowth. The specific growth rates of *T. erythraeum* and *C. watsonii* were 0.37 ± 0.06 (SD) and 0.62 ± 0.05 d^{-1} in the bisNPP medium, respectively, and 0.35 ± 0.04 and 0.68 ± 0.01 d^{-1} in the phosphate-added medium, respectively; no significant difference was observed between the 2 media ($p > 0.05$, Mann-Whitney test). From the blank results obtained during the enzyme assays, the abiotic autohydrolysis of bisNPP in the medium was considered to be negligible against microbial bisNPP hydrolysis.

Notably, for both diazotroph cultures, the maximum *in vivo* fluorescence values in the bisNPP medium were as high as those in control cultures grown with phosphate ($p > 0.05$, Wilcoxon signed-rank test). When compared with other phytoplankton grown on bisNPP, relative chl *a* yields, i.e. the relative percentage of maximum *in vivo* fluorescence value in the bisNPP medium to that in the phosphate-added medium, were higher for these 2 diazotroph cultures (Fig. 3). Thus, both diazotroph cultures demonstrated their ability for full growth on this model diester compound as the sole P source.

4. DISCUSSION

The present study used nonaxenic cultures of 2 marine diazotrophs, *Trichodesmium erythraeum* and *Crocosphaera watsonii*; therefore, our results should be evaluated in consideration of co-associated heterotrophic bacterial activities. Filtration by GF/F filters before enzyme assays cannot fully exclude the contribution of such bacteria, because *Trichodesmium* spp. are known to possess tightly associated epibionts (Nausch 1996), and the interactions between the epibionts are reported to regulate their P acquisition (Van Mooy et al. 2012). In addition, the *C. watsonii* strain used in the present study formed a viscous culture (data not shown), which can entangle ambient bacteria inside this mucilaginous colony. Since the co-associated bacteria in both cultures could not be removed, regardless of our repeated axenization over 10 yr of culture maintenance, we considered this microbial consortium as an inseparable unit of naturally occurring *T. erythraeum* and *C. watsonii*.

Although we cannot exclude the contribution from the epibionts, the presence of particulate-associated DEA in both diazotroph cultures is consistent with a previous genetic analysis revealing that *T. erythraeum* and Pacific *C. watsonii* strains possess either the *phoX* or *phoD* gene (Orchard et al. 2009, Bench et al. 2013), which encode phosphatases that can hydrolyze bisNPP (Wu et al. 2007, Kageyama et al. 2011). However, the commonly used enzyme assays should be carefully interpreted since they incorporate operational constraints in terms of substrate specificity. For example, bisNPP cannot be hydrolyzed by all phosphodiesterases associated with biological processes, such as glycerophosphodiesterase (Larson et al. 1983). Thus, our discussion below does not apply to all diester compounds.

The immediate growth recovery observed for both diazotrophs in the phosphate-added medium (Fig. 2) showed that the culture conditions prior to re-inoculation and the inoculated volume were both suitable for achieving regrowth when a sufficient amount of phosphate was available. Therefore, the stimulated DEA of the *C. watsonii* consortium at the time of re-inoculation (Fig. 1) was likely to be sufficiently high, and thus facilitated its immediate growth onset in the bisNPP medium. Moreover, the shorter time lag observed for *C. watsonii* after re-inoculation into the bisNPP medium indicates its rapid adaptation to utilize bisNPP as a P source when compared with *T. erythraeum*. Such distinct growth trends between these diazotrophs in the bisNPP medium indicate putative differences in their responses against encountering diesters, and that at least reactive diesters — such as bisNPP — are more accessible P compounds for *C. watsonii* than for *T. erythraeum*.

However, the delayed growth onset of *T. erythraeum* in the bisNPP medium seems somewhat contradictory because their DEA under P deficiency was substantially higher than that of the *C. watsonii* consortium, although there was no increase from the phosphate-replete condition (Fig. 1). Here, the P requirement of *T. erythraeum* growth onset in the bisNPP medium was estimated as 0.23–3.1 nmol P µg chl a^{-1} h^{-1} from the specific growth rate observed in this study and the P:chl *a* ratio (nmol:µg) of natural *Trichodesmium* colonies, which ranges from 15 to 206 (Mulholland et al. 2002). Notably, this estimated P requirement range of *T. erythraeum* was lower than that of *C. watsonii*, which was estimated as 2.6–3.8 nmol P µg chl a^{-1} h^{-1}, based on the chl *a*:C (µmol:mol) and C:P (mol:mol) ratios of *C. watsonii* WH8501 as 58–83 and 130, respectively (Fu et al. 2008, Jacq et al. 2014). When considering our results (Fig. 1), the DEA of the *T. erythraeum* consortium at the time of re-inoculation cannot be lower than this estimated P requirement range, even taking into account the low bisNPP concentration in the medium. Thus, not only *C. watsonii* but also the *T. erythraeum* consortium was inferred to possess the potential ability to sufficiently fulfill its P requirement at the time of re-inoculation.

Nevertheless, *T. erythraeum* did not realize an immediate growth onset in the bisNPP medium and required a lag period in advance. A similar lag period has been reported for nonaxenic dinoflagellates grown on various P compounds, including bisNPP (Richardson & Corcoran 2015). The authors proposed a potential role of co-associated heterotrophic bacteria supporting the phytoplankton growth by transforming P compounds during this lag period. The present study did not measure the DEA in bacteria-enriched filtrates; thus, we cannot exclude the possibility that the P acquisition by diazotrophs was partly indirect. However, such undetermined bacterial activities do not fundamentally affect the discussion herein when considering the above estimations. Rather, heterotrophic bacteria may have competed against *T. erythraeum* for the phosphate released by enzymes, delaying its growth onset.

Moreover, the lag period of *T. erythraeum* is perhaps related to the 'luxury uptake' of phosphate after experiencing P deficiency. This phenomenon was previously reported for *T. erythraeum* IMS101, which demonstrated a faster increase of cellular P quota relative to its cellular carbon, nitrogen, and chl *a* content, after inoculating the culture to fresh P-rich media (White et al. 2006, 2010). The rapid P uptake and subsequent utilization of internal P pools likely fueled its growth (White et al. 2006, 2010). Therefore, we considered that the P requirement of *T. erythraeum* after re-inoculation was temporarily higher than our theoretical estimation, and thus the notable increase in *in vivo* fluorescence was delayed until sufficient P was accumulated.

When considering the *in situ* distribution features of hydrolyzable diesters in the pelagic ocean, the immediate growth using diesters under phosphate-limited conditions is likely more advantageous for *C. watsonii* than for *T. erythraeum*. The concentration of hydrolyzable diesters in the subtropical North Pacific has been revealed to be low at nanomolar levels (Yamaguchi et al. 2019), which was determined from the hydrolytically released amount of phosphate by addition of a venom-derived enzyme (Suzumura et al. 1998). Within its narrow concentration range, hydrolyzable diesters present a rather patchy distri-

bution pattern compared to those of phosphate and hydrolyzable monoesters in the euphotic zone, showing randomly localized patches regardless of depth (Yamaguchi et al. 2019). In addition, the turnover time of dissolved DNA, a typical diester compound, is <1 d (Brum 2005), and its diel variability has been previously reported in the coastal regions (Paul et al. 1988, Weinbauer et al. 1995). Due to such a sparse distribution and putative temporal variability, hydrolyzable diesters may be utilized on a 'first-come-first-served' basis in the pelagic ocean.

Here, the venom-derived enzyme Phosphodiesterase I applied to determine *in situ* diesters (Yamaguchi et al. 2019) hydrolyzes not only nucleic acids but also bisNPP (Suzumura et al. 1998, Turner et al. 2002). This implies that the phosphodiesterases expressed by *T. erythraeum* and *C. watsonii* consortia partly possess common features with Phosphodiesterase I, and that they are able to utilize the *in situ* diester pool. Active utilization of *in situ* diesters by diazotroph consortia is also supported by the significant positive correlation between DEA and nitrogen fixation in the central North Pacific (Yamaguchi et al. 2019). Therefore, it is likely that the *C. watsonii* consortium can effectively utilize the opportunity of encountering the sparse *in situ* diester patch to a larger extent than the *T. erythraeum* consortium.

Although there is a possible advantage for the *C. watsonii* consortium in diester utilization, the opposite can be said for phosphonate utilization because *T. erythraeum* possesses C-P lyase encoding genes, while *C. watsonii* does not (Dyhrman et al. 2006). Therefore, such a discrepancy in the utilization of alternative P sources can be interpreted as one of their strategies to survive in oligotrophic conditions. Since both diazotrophs are occasionally reported to be distributed in a slightly different manner in the pelagic ocean (Capone et al. 1998, Church et al. 2008, Moisander et al. 2010, Shiozaki et al. 2017), the indicated differences in the utility of alternative P sources may contribute to constructing their respective ecological niches.

In comparison with other marine phytoplankton taxa, the ability of both diazotroph cultures to grow solely on diesters was generally high because no other species, except for *Chaetoceros ceratosporus*, presented high relative chl *a* yields when grown in the bisNPP medium (Fig. 3) (Yamaguchi et al. 2005, Richardson & Corcoran 2015). This indicates that the wider variety of P compounds utilized by both diazotroph consortia become favorable to their P acquisition among natural microbial communities. In contrast, 84% of a total of 51 freshwater cyanobacteria species showed rather high relative chl *a* yields in the bisNPP medium (Fig. 3) (>50%, Grainger 1989), indicating that the ability of diester utilization is likely a typical characteristic among the taxon *Cyanophyceae*. Further evaluation is needed to reveal whether other phytoplankton, particularly non-diazotrophic marine cyanobacteria, can live on diesters, as well as the utility of various DOP — including other diester compounds — and the kinetics of phosphatase activities. Nevertheless, although diesters are most likely a subsidiary P source of monoesters, they likely have a certain contribution to the P acquisition of diazotroph consortia, and thus benefit their growth in the oligotrophic ocean.

5. CONCLUSION

This study demonstrated the effective utilization of the model diester compound — bisNPP — by the 2 nonaxenic diazotroph cultures, *Trichodesmium erythraeum* and *Crocosphaera watsonii*. Since P is an essential nutrient for diazotrophs, the uncommon ability to utilize diesters among marine phytoplankton is likely an advantageous strategy to meet the P demand of these diazotrophs and also their co-associated bacteria in oligotrophic oceans. However, *T. erythraeum* required a longer acclimation period for growth onset in the bisNPP medium compared to *C. watsonii* despite its higher DEA. This notable growth discrepancy suggests that the *C. watsonii* consortium can assimilate hydrolyzable diesters more effectively and grow faster than the *T. erythraeum* consortium under low phosphate availability. Although our findings are reported under limited conditions, they will facilitate understanding of the responses by these species to P limitation and the construction of the ecological niches in the oligotrophic ocean.

Acknowledgements. We thank Dr. Satoshi Kitajima for the establishment of the *Trichodesmium* and *Crocosphaera* strains. This research was financially supported by MEXT Grants-in-Aid (24121005, 24121006) and JSPS Grants-in-Aid (16J08143, 19K24396).

LITERATURE CITED

Bench SR, Heller P, Frank I, Arciniega M, Shilova IN, Zehr JP (2013) Whole genome comparison of six *Crocosphaera watsonii* strains with differing phenotypes. J Phycol 49:786–801

Brum JR (2005) Concentration, production and turnover of viruses and dissolved DNA pools at Stn ALOHA, North Pacific Subtropical Gyre. Aquat Microb Ecol 41:103–113

Capone DG, Subramaniam A, Montoya JP, Voss M and others (1998) An extensive bloom of the N_2-fixing cyanobacterium *Trichodesmium erythraeum* in the central Arabian Sea. Mar Ecol Prog Ser 172:281–292

Church MJ, Björkman KM, Karl DM, Saito MA, Zehr JP (2008) Regional distributions of nitrogen-fixing bacteria in the Pacific Ocean. Limnol Oceanogr 53:63–77

Church MJ, Mahaffey C, Letelier RM, Lukas R, Zehr JP, Karl DM (2009) Physical forcing of nitrogen fixation and diazotroph community structure in the North Pacific subtropical gyre. Glob Biogeochem Cycles 23:GB2020

Deutsch C, Sarmiento JL, Sigman DM, Gruber N, Dunne JP (2007) Spatial coupling of nitrogen inputs and losses in the ocean. Nature 445:163–167

Dyhrman ST, Haley ST (2006) Phosphorus scavenging in the unicellular marine diazotroph *Crocosphaera watsonii.* Appl Environ Microbiol 72:1452–1458

Dyhrman ST, Chappell PD, Haley ST, Moffett JW, Orchard ED, Waterbury JB, Webb EA (2006) Phosphonate utilization by the globally important marine diazotroph *Trichodesmium*. Nature 439:68–71

Fu FX, Bell PRF (2003) Effect of salinity on growth, pigmentation, N_2 fixation and alkaline phosphatase activity of cultured *Trichodesmium* sp. Mar Ecol Prog Ser 257: 69–76

Fu FX, Mulholland MR, Garcia NS, Beck A and others (2008) Interactions between changing pCO_2, N_2 fixation, and Fe limitation in the marine unicellular cyanobacterium *Crocosphaera*. Limnol Oceanogr 53:2472–2484

Grainger SLJ (1989) Filament structure and phosphatase activity in the Rivulariaceae. PhD dissertation, Durham University

Gruber N, Sarmiento JL (1997) Global patterns of marine nitrogen fixation. Global Biogeochem Cycles 11:235–266

Jacq V, Ridame C, L'Helguen S, Kaczmar F, Saliot A (2014) Response of the unicellular diazotrophic cyanobacterium *Crocosphaera watsonii* to iron limitation. PLOS ONE 9: e86749

Kageyama H, Tripathi K, Rai AK, Cha-Um S, Waditee-Sirisattha R, Takabe T (2011) An alkaline phosphatase/phosphodiesterase, PhoD, induced by salt stress and secreted out of the cells of *Aphanothece halophytica*, a halotolerant cyanobacterium. Appl Environ Microbiol 77:5178–5183

Kitajima S (2009) Research on biology of nitrogen fixing cyanobacteria in tropical and subtropical Pacific (in Japanese). PhD dissertation, The University of Tokyo

Kitajima S, Furuya K, Hashihama F, Takeda S, Kanda J (2009) Latitudinal distribution of diazotrophs and their nitrogen fixation in the tropical and subtropical western North Pacific. Limnol Oceanogr 54:537–547

Larson TJ, Ehrmann M, Boos W (1983) Periplasmic glycerophosphodiester phosphodiesterase of *Escherichia coli*, a new enzyme of the *glp* regulon. J Biol Chem 258: 5428–5432

Megharaj M, Pearson HW, Venkateswarlu K (1991) Toxicity of phenol and three nitrophenols towards growth and metabolic activities of *Nostoc linckia*, isolated from soil. Arch Environ Contam Toxicol 21:578–584

Mills MM, Ridame C, Davey M, La Roche J, Geider RJ (2004) Iron and phosphorus co-limit nitrogen fixation in the eastern tropical North Atlantic. Nature 429:292–294

Moisander PH, Beinart RA, Hewson I, White AE and others (2010) Unicellular cyanobacterial distributions broaden the ocean N_2 fixation domain. Science 327:1512–1514

Monbet P, McKelvie ID, Worsfold PJ (2009) Dissolved organic phosphorus speciation in the waters of the Tamar estuary (SW England). Geochim Cosmochim Acta 73: 1027–1038

Mulholland MR, Floge S, Carpenter EJ, Capone DG (2002) Phosphorus dynamics in cultures and natural populations of *Trichodesmium* spp. Mar Ecol Prog Ser 239: 45–55

Nausch M (1996) Microbial activities on *Trichodesmium* colonies. Mar Ecol Prog Ser 141:173–181

Orchard ED, Webb EA, Dyhrman ST (2009) Molecular analysis of the phosphorus starvation response in *Trichodesmium* spp. Environ Microbiol 11:2400–2411

Paul JH, DeFlaun MF, Jeffrey WH, David AW (1988) Seasonal and diel variability in dissolved DNA and in microbial biomass and activity in a subtropical estuary. Appl Environ Microbiol 54:718–727

Prufert-Bebout L, Paerl HW, Lassen C (1993) Growth, nitrogen fixation, and spectral attenuation in cultivated *Trichodesmium* species. Appl Environ Microbiol 59: 1367–1375

Richardson B, Corcoran AA (2015) Use of dissolved inorganic and organic phosphorus by axenic and nonaxenic clones of *Karenia brevis* and *Karenia mikimotoi.* Harmful Algae 48:30–36

Shiozaki T, Furuya K, Kodama T, Takeda S (2009) Contribution of N_2 fixation to new production in the western North Pacific Ocean along 155°E. Mar Ecol Prog Ser 377: 19–32

Shiozaki T, Furuya K, Kodama T, Kitajima S, Takeda S, Takemura T, Kanda J (2010) New estimation of N_2 fixation in the western and central Pacific Ocean and its marginal seas. Global Biogeochem Cycles 24:GB1015

Shiozaki T, Bombar D, Riemann L, Hashihama F and others (2017) Basin scale variability of active diazotrophs and nitrogen fixation in the North Pacific, from the tropics to the subarctic Bering Sea. Global Biogeochem Cycles 31: 996–1009

Sohm JA, Webb EA, Capone DG (2011) Emerging patterns of marine nitrogen fixation. Nat Rev Microbiol 9: 499–508

Stihl A, Sommer U, Post AF (2001) Alkaline phosphatase activities among populations of the colony-forming diazotrophic cyanobacterium *Trichodesmium* spp. (Cyanobacteria) in the Red Sea. J Phycol 37:310–317

Suzumura M, Ishikawa K, Ogawa H (1998) Characterization of dissolved organic phosphorus in coastal seawater using ultrafiltration and phosphohydrolytic enzymes. Limnol Oceanogr 43:1553–1564

Turner BL, McKelvie ID, Haygarth PM (2002) Characterisation of water-extractable soil organic phosphorus by phosphatase hydrolysis. Soil Biol Biochem 34:27–35

Van Mooy BAS, Hmelo LR, Sofen LE, Campagna SR and others (2012) Quorum sensing control of phosphorus acquisition in *Trichodesmium* consortia. ISME J 6: 422–429

Ward BA, Dutkiewicz S, Moore CM, Follows MJ (2013) Iron, phosphorus, and nitrogen supply ratios define the biogeography of nitrogen fixation. Limnol Oceanogr 58: 2059–2075

Weber T, Deutsch C (2014) Local versus basin-scale limitation of marine nitrogen fixation. Proc Natl Acad Sci USA 111:8741–8746

Weinbauer MG, Fuks D, Puskaric S, Peduzzi P (1995) Diel, seasonal, and depth-related variability of viruses and

dissolved DNA in the northern Adriatic Sea. Microb Ecol 30:25–41

White AE, Spitz YH, Karl DM, Letelier RM (2006) Flexible elemental stoichiometry in *Trichodesmium* spp. and its ecological implications. Limnol Oceanogr 51:1777–1790

White AE, Karl DM, Björkman KM, Beversdorf LJ, Leteliera RM (2010) Production of organic matter by *Trichodesmium* IMS101 as a function of phosphorus source. Limnol Oceanogr 55:1755–1767

Wu JR, Shien JH, Shieh HK, Hu CC, Gong SR, Chen LY, Chang PC (2007) Cloning of the gene and characterization of the enzymatic properties of the monomeric alkaline phosphatase (PhoX) from *Pasteurella multocida* strain X-73. FEMS Microbiol Lett 267:113–120

Yamaguchi H, Yamaguchi M, Fukami K, Adachi M, Nishijima T (2005) Utilization of phosphate diester by the marine diatom *Chaetoceros ceratosporus.* J Plankton Res 27: 603–606

Yamaguchi T, Furuya K, Sato M, Takahashi K (2016) Phosphate release due to excess alkaline phosphatase activity in *Trichodesmium erythraeum.* Plankton Benthos Res 11: 29–36

Yamaguchi T, Sato M, Hashihama F, Ehama M, Shiozaki T, Takahashi K, Furuya K (2019) Basin-scale variations in labile dissolved phosphoric monoesters and diesters in the central North Pacific Ocean. J Geophys Res Oceans 124:3058–3072

Yentsch CM, Yentsch CS, Perras JP (1972) Alkaline phosphatase activity in the tropical marine blue-green alga, *Oscillatoria erythraea* ('*Trichodesmium*'). Limnol Oceanogr 17:772–774

Zehr JP (2011) Nitrogen fixation by marine *cyanobacteria*. Trends Microbiol 19:162–173

Zehr JP, Carpenter EJ, Villareal TA (2000) New perspectives on nitrogen-fixing microorganisms in tropical and subtropical oceans. Trends Microbiol 8:68–73

Editorial responsibility: Douglas Capone, Los Angeles, California, USA

Submitted: April 7, 2020; Accepted: September 17, 2020
Proofs received from author(s): December 1, 2020