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MICROALGAL ASSEMBLAGES IN A POIKILOHALINE POND[1]

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Microalgal strains for algal biofuels production in outdoor ponds will need to have high net growth rates under diverse environmental conditions. A small, variable salinity pond in the San Elijo Lagoon estuary in southern California was chosen to serve as a model pond due to its routinely high chlorophyll content. Profiles of microalgal assemblages from water samples collected from April 2011 to January 2012 were obtained by constructing 18S rDNA environmental clone libraries. Pond assemblages were found to be dominated by green algae *Picochlorum* sp. and *Picocystis* sp. throughout the year. Pigment analysis suggested that the two species contributed most of the chlorophyll *a* of the pond, which ranged from 21.9 to 664.3 $\mu g \cdot L^{-1}$ with the *Picocystis* contribution increasing at higher salinities. However, changes of temperature, salinity or irradiance may have enabled a bloom of the diatom *Chaetoceros* sp. in June 2011. Isolates of these microalgae were obtained and their growth rates characterized as a function of temperature and salinity. *Chaetoceros* sp. had the highest growth rate over the temperature test range while it showed the most sensitivity to high salinity. All three strains showed the presence of lipid bodies during nitrogen starvation, suggesting they have potential as future biofuels strains.

Key index words: **18S rDNA libraries; algal biofuels; *Chaetoceros*; *Picochlorum*; *Picocystis*; poikilohaline pond**

Algae are a potential alternative renewable fuel feedstock that could end the food versus fuel debate as they can be grown on less arable land and without using potable water. Generally, two types of algal cultivation are used commercially: open ponds and photo bioreactors. As the oldest and simplest system for algal production, open ponds are easier to construct and operate than most closed systems. Low construction and operating costs make them popular especially for large-scale commercial production (Shen et al. 2009). The major limitations of open ponds are poor light utilization by cells, evaporative losses, loss of CO_2, if added, to the atmosphere, and the requirement of large areas of land. Furthermore, contamination by predators and other fast growing competitors can also restrict the commercial production of algae in open culture systems (Pienkos and Darzins 2009).

Although open ponds are more vulnerable to various environmental factors compared to closed reactors, temperature, light, salinity, and nutrients together may result in a relatively simple ecological system with some dominant strains. In particular, extreme conditions may select for only a few species while excluding common predators and competitors. For example, brackish habitats have been reported to form assemblage characteristics with strong dominance by a few species and thus relatively low diversity (Reizopoulou et al. 1996, Mistri et al. 2001). With this concept in mind, we characterized the phytoplankton communities in a high chlorophyll, variable salinity (poikilohaline) pond in the San Elijo Lagoon in San Diego, California as a source of potential algal biofuel strains.

MATERIALS AND METHODS

Sample collection. San Elijo Lagoon is one of the largest coastal wetlands in San Diego County, California, USA. The lagoon is primarily a shallow-water estuary. Its watershed is 77 square miles, feeding two main creeks, Escondido Creek and Orilla Creek. Samples were collected from a small permanent pond that is part of the lagoon system (~33.00°N, 117.27°W) but separated from the lagoon by land. It has a surface area of 160 m^2 (0.04 acres). This pond is a typical poikilohaline pond. The height of the pond is relatively low to mean high tide and thus seawater enters the pond occasionally. Constant evaporation in the dry season (July to September) raises the salinity, while rains in the winter reduce the salinity (Fig. 1). Samples were collected on April 26, June 6, August 8, and November 7 in 2011, and January 5 in 2012 between 10 and 11 am. The pond's temperature ranged from 9°C to 24°C during sampling (Table 1).

Water samples (500–1000 mL) were taken from the surface of the pond and poured into glass bottles. The samples were filtered within 1 h of collection. Samples were first filtered through 225 μm mesh to remove large particles such as leaf detritus. The pretreated samples were then filtered through 47 mm diameter Pall Supor®-200 (0.2 μm pore size) using 3 psi vacuum to collect microbial biomass, and frozen at −80°C until subsequent DNA extraction. Quantities of 50 mL pretreated water samples were frozen at −20°C for nutrient analysis. Samples of 5–40 mL volume were collected on 25 mm Whatman GF/F filters and kept in −80°C for pigment analysis.

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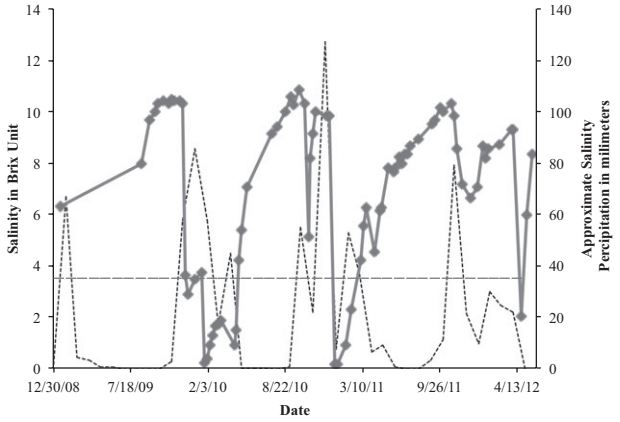

FIG. 1. Salinity change in San Elijo Lagoon pond. Dashed line shows average seawater salinity. Dotted line shows precipitation records in mm (Source: Western Regional Climate Center website http://www.wrcc.dri.edu/). Surface salinities were typically lower during the southern California rain season.

Salinity, nutrient, and pigment analysis. Salinity was measured using a handheld refractometer in Brix units (Reichert Technologies, Depew, NY, USA).

Approximate salinity in part per thousand was calculated using the formula: Salinity = 8.33 × Salinity (Brix unit). Nutrient analyses (phosphate, silicate, nitrate + nitrite, nitrite, and ammonia) were performed on a Seal Analytical continuous-flow AutoAnalyzer 3. The methods used are described by Hager et al.(1972) and Gordon et al.(1992).

Pigments were analyzed on an Agilent 1100 series HPLC system using a method adapted from (Zapata et al. 2000): column Waters Symmetry C8 column, 3.5 μm particle size, 4.6 × 150 mm, silica, reverse-phase; solvents A methanol, acetonitrile, and an aqueous pyridine solution (0.25 M, pH = 5; 50:25:25 v:v:v) solvent B methanol, acetonitrile, and acetone (20:60:20 v:v:v) and the gradient (time, %A, %B) 0, 100, 0; 12, 60, 40; 36, 0, 100; 38, 0, 100; 40, 100, 0. Samples were extracted in 1.6 mL acetone doped with canthaxanthin as an internal standard, ground, and centrifuged for 5 min prior to injection. The percentage of Chlorophyll *a* (Chl *a*) associated with the three groups was calculated using pigment ratios (Diatoms: Fucoxanthin/Chl *a*; *Picochlorum*: Chl *b*/Chl *a*; *Picocystis*: Diatoxanthin/Chl *a,* with corrections applied to *Picochlorum*-Chl *b* due to *Picocystis* and *Picocystis*-Diatoxanthin due to diatom Diatoxanthin.). For sample SE lagoon 9/4, pigment ratio neoxanthin/violaxanthin was used instead. The method is described by Goericke and Montoya (1998).

DNA extraction and amplification. DNA was extracted from the filters and used as the template for PCR amplification of an ~1800-bp region of the 18S rRNA gene. The procedures used were as previously described (Palenik et al. 2009). Briefly, microbial biomass retained by filters was digested with lysozyme, proteinase K, and 10% sodium dodecyl sulfate at 56°C. The crude extract was purified by phenol/chloroform extraction and DNeasy Blood & Tissue Kits (Qiagen, Germantown, MD, USA). PCR used 1 μL of purified DNA as template and primer set 18sMoon (Moon-van der Staay et al. 2000). Amplifications in 25 μL volumes were run for 35 cycles (94°C for 0.5 min, 52°C for 2 min and 72°C for 3 min) with 3-min 94°C initial denaturation and 10-min 72°C final elongation. Additional PCR amplifications are as noted below. Fluorescence microscopy of benthic samples showed the presence of cyanobacteria but these were not quantified. Using the same approach, cyanobacteria did not appear to be significant members of the plankton, so 16S rRNA or cyanobacterial-specific 16S rRNA analyses were not done.

Clone libraries and sequencing. PCR products from these reactions were separately cloned into the pCR 2.1 cloning vector (TOPO-TA, Invitrogen, Life Technologies, Carlsbad, CA, USA) by using recommended instructions. Plasmids were isolated from randomly picked colonies using the QIAprep spin miniprep Kit (Qiagen). Clones were screened by restriction enzyme digestion using EcoRV (New England Biolabs Inc., Ipswich, MA, USA) and agarose gel electrophoresis for the correct insert size.

For the first library (April 26, 2011), 19 clones were sequenced by standard sequencing (Retrogen Inc., San Diego, CA, USA) using PCR forward primer 18sMoonA (Moon-van der Staay et al. 2000). A convenient internal sequencing primer based on a region highly conserved in 18S rDNA fragments was used as well for longer length coverage (Worden 2006): 502F 5′-GAGCARATYGTYTAYTTYAA-3′, which starts at position 502 of the 1800-bp fragment.

Because the whole clone library was dominated by a single species, *Picochlorum,* as described by Henley (Henley et al. 2004), the library was reconstructed by first treating the PCR product with restriction enzyme PstI (New England Biolabs Inc.). This cuts the DNA of the dominant strain once and following gel electrophoresis the remaining 1800 bp product was extracted from the gel and cloned. Nine clones were sequenced using the 18sMoonA primer. To obtain a more diverse library, two more sets of 18S rRNA primers, 18s P45/47 (Dorigo et al. 2002) and 18s COM (Zhang and Lin 2002)were used. In addition, nested PCR with primers 1F/1528R and 82F/1055R were performed to amplify diatom DNA as described (Metfies et al. 2007). Twenty-four, 21, and 20 clones were sequenced, respectively.

For libraries on June 2, August 8, and January 5, 18sMoon primers were used and additional restriction enzyme digestions with PstI were performed to analyze the band patterns for screening purpose. Twenty-seven, 48, and 48 plasmids were prescreened for the above libraries, respectively. Colonies with PCR products cut by PstI with the same pattern as before were counted as *Picochlorum.* Fifteen, 13, and 10 clones, respectively, were finally sequenced.

Sequence data were assembled and edited using the software CLC Genomic Workbench 6.0 and characterized by BLAST searching the database of GenBank nr/nt for highly similar sequences (megablast) program. Sequences were aligned by the SINA alignment service using the SILVA SSU

TABLE 1. Sampling conditions and nutrients of samples.

Nutrient (unit)	Temp. (°C)	Salinity (Brix)	NO_3 (μmol · L^{-1})	PO_4 (μmol · L^{-1})	Si (μmol · L^{-1})	NO_2 (μmol · L^{-1})	NH_4 (μmol · L^{-1})
4/26/2011	–	7.5	14.69	1.02	21	0.39	0.32
6/2/2011	–	9.4	0	6.18	21.5	1.49	3.79
8/8/2011	24	11.1	0.6	8.56	207.1	4.53	8.51
11/7/2011	9	9.2	2.76	8.08	184.9	3.59	6.06
1/5/2012	9.5	9.3	0.36	1.89	120.5	0.87	1.01

(Pruesse et al. 2012). All sequences were checked for chimeras using Bellerophon (Huber et al. 2004). Phylogenetic analyses were conducted with maximum likelihood (ML) methods including bootstrapping 100 data sets by CLC Genomic Workbench 6.0 and RAxMLGUI1.3. The clone libraries showed some polymorphic sites: *Picocystis* (4/1470), *Picochlorum* (22/1208) and *Chaetoceros* (1/1497). For the phylogenetic tree, we used their consensus and listed clone numbers in the tree (Fig. 2).

Algal isolation and culture. Two strains, SENEW1 and SENEW3, were isolated from the poikilohaline pond sample on April 26, 2011. A diatom isolate was obtained from the sample on June 2, 2011. Isolation involved a combination of streaking on agar plates or pour-plating in 0.4% agarose, and culturing in liquid medium. Liquid and solid media were both enriched in f/2 nutrient (Guillard and Ryther 1962). Maintenance cultures in liquid media were grown at 21°C and constant cool white fluorescent light at intensity of 30–50 μmol photons $\cdot m^{-2} \cdot s^{-1}$. 18s rRNA sequences of the strains were obtained as above.

Temperature studies. The growth rates of strains isolated from the poikilohaline pond were measured at different temperatures. Volumes of 500 mL of f/2 medium cultures were transferred to tubes (20 mL each). Tubes were placed into a temperature block ranging from 10°C to 33°C under constant light. Light intensity inside the block is difficult to measure. Light intensity was 355–435 μmol photons $\cdot m^{-2} \cdot s^{-1}$ above the temperature blocks and 6 μmol photons $\cdot m^{-2} \cdot s^{-1}$ underneath. Growth was measured noninvasively using chlorophyll fluorescence with a 10-AU™ fluorometer (Turner Designs, Sunnyvale, CA, USA). The salinity of the medium was ~35. Duplicates were applied for each treatment.

Salinity studies. The growth rates of isolates were tested with 10%, 1×, 2×, and 3× artificial seawater (salts from Red Sea, Houston, TX, USA) based f/2 medium, equivalent to salinity 3.5, 35, 70, and 105, respectively. 20 mL of medium were inoculated with 50 μL stock and placed under cool white fluorescent light at intensity of 30–50 μmol photons $\cdot m^{-2} \cdot s^{-1}$ at 21°C. All treatments were tested by duplicates. Growth was measured using a 10-AU™ fluorometer (Turner Designs, Sunnyvale, CA, USA). Growth was separately tested on the freshwater medium BG11 (Stanier et al. 1971).

Nitrogen limitation and cell staining. Isolates were grown in f/2 medium with reduced (50 μM) sodium nitrate. Stationary phase cells were mixed with 1 mM BODIPY 493/503 (Invitrogen) in 10% DMSO in a ratio of 500:1 and incubated in the dark for 15 min. Stained cells were observed and imaged using a Zeiss Axioskop fluorescence microscope (Carl Zeiss, Oberkochen, Germany) and SPOT Pursuit Camera (Spot Imagining Solutions, Sterling, MI, USA).

RESULTS

Salinity and nutrients. Surface salinities in the pond ranged from 0.2 to 13 Brix units (~1.7–108.3), showing reduced salinities due to rain events occurring in the winter in southern California followed by evaporation during the rest of the year (Fig. 1). This type of pattern is termed mixohaline or poikilohaline by the Venice Symposium (Anonymous 1958). This cyclic pattern with return to the same salinity each year was also reported by Carpelan (1978) for other California lagoons (1978). Nutrient levels of the samples obtained from different dates varied greatly as well (Table 1). Samples on August 8, 2011 have the highest salinity and concentrations of phosphate, silicate, nitrite, and ammonia. However, the highest nitrate concentration was observed on April 26, 2011, when the nitrite and ammonia reached the lowest concentrations. The consistently high ammonia concentrations suggest that growth of algae in the pond is not nutrient limited, with ammonia concentrations likely driven by either water column or sedimentary remineralization.

Phylogenetic analysis. The ML phylogenetic tree of all pond sequences revealed two main clusters (Fig. 2). The first cluster consisted of 38 sequences, most closely related to *Picochlorum* sp. (UTEX 2491) with very high identities (>99%; Henley et al. 2004). The second cluster of eight sequences was most closely related at 97%–99% identity to sequences of *Picocystis salinarum* as described by Lewin et al. (Lewin et al. 2000) including strain SSFB (Hollibaugh et al. 2001). Sequences from these two clusters were found in all samples (April 26, June 2, August 8, and January 5) indicating that the two species dominate the pond most of the year. Three sequences (SE0602-3, SE0602-17, and SE0602-10)

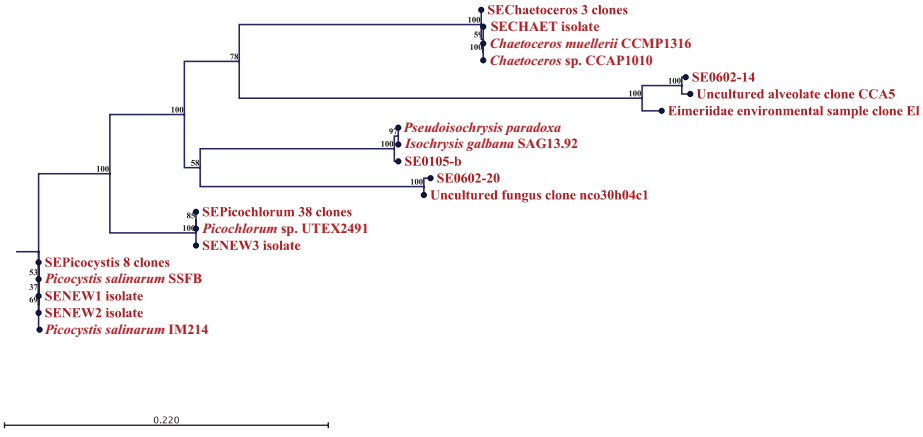

FIG. 2. Phylogenetic analysis of San Elijo Lagoon environmental 18S rRNA clone libraries and isolates. This maximum likelihood phylogenetic tree demonstrates that the pond is dominated by *Picochlorum* sp. and *Picocystis* sp. *Chaetoceros* sp. are also detected in one sample in June. Data names are composed of sample date and number. Numbers at nodes are bootstrap values.

were observed to form a small cluster, which was most closely related to *Chaetoceros muelleri* (Theriot et al. 2010). One sequence (SE105-b) was most closely related to *Isochrysis* sp. or *Pseudoisochrysis* sp. sequences deposited in Genbank.

Since the April sample was dominated by *Picochlorum* sp., additional 18S rRNA primers, 18sP45/47 and 18sCOM were used to ensure this was not a result of PCR bias. As shown in Table 2, both primer sets reveal very similar results showing that the community in the April sample was dominated by *Picochlorum* sp. and *Picocystis* sp. Another additional effort to obtain more species diversity information was to use the PstI restriction enzyme digestion before the clone library construction to remove *Picochlorum* sp. PCR products. All sequenced clones (nine clones) were *Picocystis* sp., which corresponded to previous results.

Isolate identification and characterization. Cells of isolate SENEW3 are slightly oval, nonflagellated with an approximate mean size of 2 μm. Pigment analysis shows that it contains chlorophylls *a* and *b*, violaxanthin, and zeaxanthin (Table S1 in the Supporting Information). The pigment profile is very similar to the *Picochlorum* isolate reported by de la Vega et al. (2011), which contains lutein, neoxanthin, violaxanthin, and beta-carotene. Other reported isolates such as *Picochlorum Oklahomesis* SPNWR (Henley et al. 2004) and *Picochlorum* RCC237 (Dimier et al. 2007) have similar pigment contents. It is broadly halotolerant and can grow in the freshwater medium BG11 or the seawater medium f/2 in both solid and liquid culture. The 18S rRNA sequence of the isolate showed very high percentage (99%) identity with a published 18S rDNA sequence of *Picochlorum* sp., which is classified as belonging to the Domain Eukarya, Kingdom Protista, Division Chlorophyta, and Class Trebouxiophyceae (Graham and Wilcox 2000, Henley et al. 2004). Thus, SENEW3 has all the characteristics of a *Picochlorum* sp. isolate.

The cells of isolate SENEW1 are green, spherical or ovoid, with a diameter of 2–3 μm.

The pigments of this alga are chlorophylls *a* and *b*, neoxanthin, alloxanthin, diadinoxanthin, diatoxanthin, and zeaxanthin (Table S1). The result is very similar to the pigment profile of *P. salinarum* gen. et sp. reported by Lewin et al. (2000). It can be grown in seawater f/2 media or a 10% seawater f/2 medium, but we were not able to grow it in the freshwater medium BG11. This isolate has an 18S rRNA sequence 99% identical to *P. salinarum* (Hollibaugh et al. 2001) again showing this is a *Picocystis* sp. isolate (Fig. 2).

The diatom isolate is a *Chaetoceros* isolate with a diameter of 5–10 μm and spines of 20 μm. Based on the pigment analysis, it contains Chlorophyll *a*, *b*, *c*, fucoxanthin, violaxanthin, diadinoxanthin, diatoxanthin, and zeaxanthin (Table S1). The 18S rRNA sequences of this isolate is 99% identical to *Chaetoceros muellerii* (Theriot et al. 2010). It grows in seawater f/2 or a 10% seawater f/2 medium, but we were not able to grow it in the freshwater medium BG11. The medium preference of this isolate corresponds to the report that *Chaetoceros muellerii* requires at least 10 mM sodium for growth (Fujii et al. 1995).

Taxon-specific pigments. Pigment analysis was performed for the three isolates (*Picochlorum*, *Picocystis*, and *Chaetoceros*) and pond samples collected on April 24, June 2, August 8, and September 4 in 2011 (Fig. 3 and Table S1). Overall, the concentration of total Chlorophyll *a* and *b* increased from April to September, which suggests the overall increase in

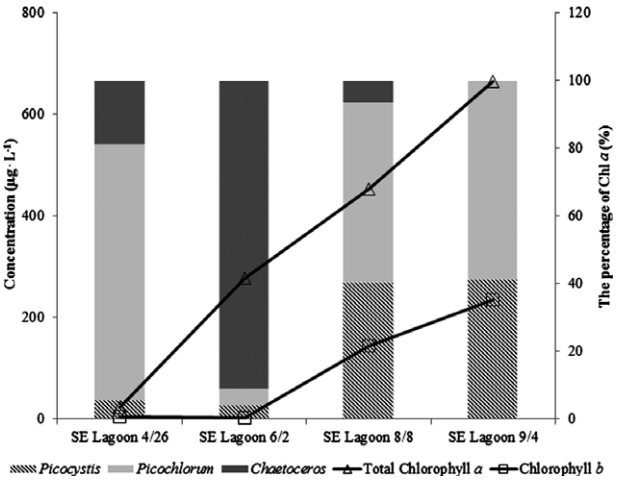

FIG. 3. Total Chlorophyll *a* and Chlorophyll *b* of SE lagoon samples. Stacked columns indicate the calculated contributions of the three species to the total Chlorophyll *a* calculated using accessory pigment measurements as described in methods.

TABLE 2. Sequenced clones of San Elijo Lagoon 18S rRNA clone libraries.

Date	Primers	*Picochlorum*-like	*Picocystis*-like	*Chaetoceros*-like	Other	Total
4/26/2011	18sMoon	19	0	0	0	19
4/26/2011	18sP45/P47	19	3	0	2	24
4/26/2011	18sCOM	21	0	0	0	21
6/2/2011	18sMoon	5(+2*)	1	3	5	15
8/8/2011	18sMoon	8(+9*)	4(+2*)	0	1	13
1/5/2012	18sMoon	6	3	0	1	10

*Additional clones showing the same digestion pattern as sequenced clones, which are treated as the same species.

algal and green algal biomass. This may result from (i) algal growth, (ii) the evaporation of pond, which concentrates salt, nutrient, and algae biomass, and (iii) reduced grazing due to high salinities. The contributions of different isolates to total Chl *a* in the pond were calculated from isolate pigment ratios (Fig. 3). Diatom pigment biomass peaked in June but was low in other samples reflecting the high diatom abundance in June but lower abundance in other months. The percentage of *Picocystis* increased from 7.1% in April to 48.2% in September.

Temperature studies. As shown in Figure 4, the growth rates of all three isolates increased with temperature and reached a plateau above 24°C (*Chaetoceros* and *Picocystis*) or 30°C (*Picochlorum*). *Picochlorum* (strain SENEW3) did not grow at ≤16°C. When the temperature was higher than 32°C, the growth rate of *Picochlorum* decreased slightly as well. This trend is consistent with the findings reported by de la Vega et al. (2011) that the optimal growth temperature of *Picochlorum* sp. HM1 is 30°C while growth at 15°C was extremely slow. Among the three isolates, the diatom isolate *Chaetoceros* sp. has the highest growth rates for the tested temperature span. In the literature, the growth rates of *Chaetoceros muelleri* ranged from 1 to 2 d^{-1}(Blinn 1984, McGinnis et al. 1997) and are reported to be higher than the growth rates of *Picochlorum* sp. and *Picocystis* sp. ranging from 0.5 to 1 d^{-1}(Lewin et al. 2000, Hollibaugh et al. 2001, Henley et al. 2004, de la Vega et al. 2011).

Salinity studies. *Picochlorum* (strain SENEW3) and *Picocystis* (strain SENEW1) grew with 10% ASW medium (~3.5 salinity) as did *Chaetoceros* after a long acclimation. When the salinity was higher than seawater, the growth rate of *Picochlorum* decreased while *Picocystis* increased. Compared to *Picochlorum*, *Chaetoceros* seems more sensitive to salinity changes. Its growth rate drops as the salinity is increased or decreased (Fig. 5). In addition, it acclimated very poorly to salinity changes increased or decreased relative to seawater with an immediate lack of growth (data not shown). These results and the previous temperature results may help explain how *Picochlorum* and *Picocystis* dominate the pond at high salinity and after rapid salinity changes.

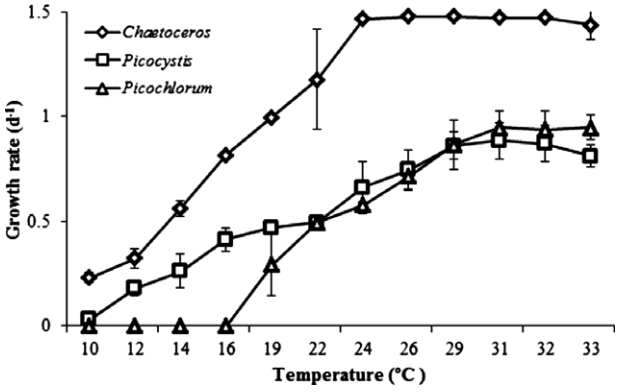

FIG. 4. Temperature effect on growth rate of three San Elijo pond isolates. Cultures in fresh seawater based f/2 were grown at constant cool white fluorescent light at intensity of 30–50 μmol photons · m^{-2} · s^{-1}.

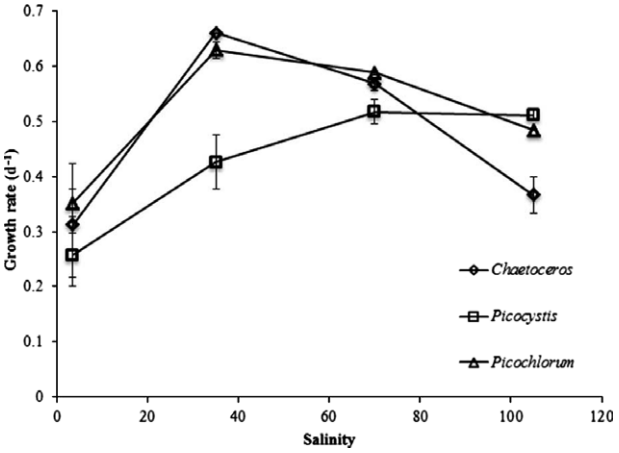

FIG. 5. Salinity effect on the growth rate of the three pond isolates. Cultures in f/2 based medium were grown at 21°C and constant cool white fluorescent light at intensity of 30–50 μmol photons · m^{-2} · s^{-1}.

Lipid droplet formation. Interestingly, we found that all isolates *Picochlorum* (strain SENEW3) and *Picocystis* (strain SENEW1) and *Chaetoceros* (strain SECHAET) show significant lipid drop accumulation under nitrogen limitation when stained with BODIPY 493 (Fig. 6). This capability for enhanced lipid synthesis suggests these strains could be promising algal biofuel candidates.

DISCUSSION

Ponds in the San Elijo Lagoon system exhibit salinity fluctuations over time due to rain events or rare seawater flushing. They are also subject to evaporation, which results in higher salinities during the dry season. Nutrients vary substantially as well. Nutrient concentrations (except nitrate) were highest on August 8, 2011 (Table 1). However, the highest nitrate concentration was observed on April 26, 2011 when the salinity dropped to 7.5 from the highest 11.1 Brix unit.

Organisms living in poikilohaline ponds are exposed to large salinity variations and may have developed ways to cope with these changes. Our 18S rDNA library results showed that the algal community was dominated by *Picochlorum* sp. *Picocystis* sp. were detected in all samples but accounted for a smaller fraction of the clone libraries. We also found a *Chaetoceros* sp. in June samples. The clone library data were also supported by the pigment data. One of the reasons that the two picoautotrophs could survive and thrive in such environments is that they are adapted to variable salinity environments.

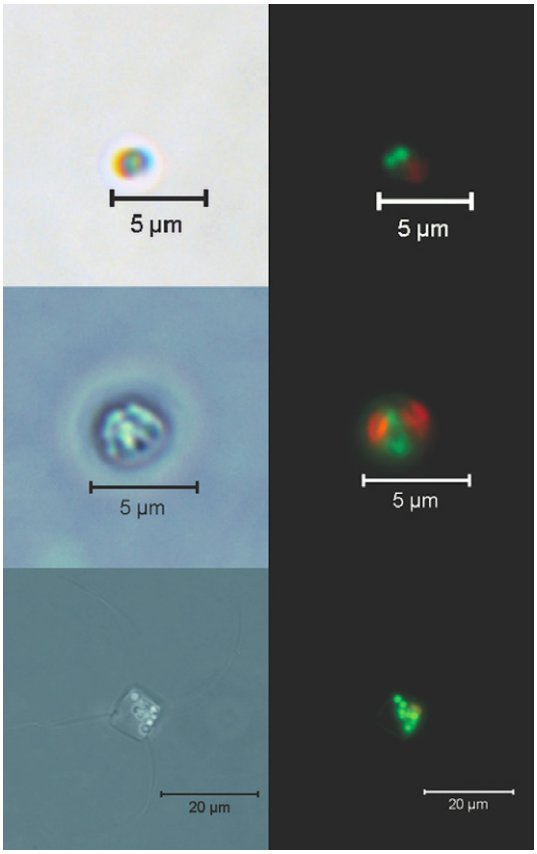

FIG. 6. Bright field and UV images of *Picochlorum* (SENEW3), *Picocystis* (SENEW1) and *Chaetoceros* (SECHAET; from the top to the bottom) stained with BODIPY. Green fluorescence indicates neutral lipids.

As reported by Cognetti and Maltagliati (2000), brackish habitats, which communicate directly with the sea, are usually colonized by euryhaline marine species, whose populations have developed a tolerance to unpredictable changes in habitat. Assemblage characteristics are quite similar in brackish habitats: a limited number of species, a strong dominance in abundance, and relatively low diversity (e.g., Reizopoulou et al. 1996, Mistri et al. 2001). Although these studies focused on zoobenthos, the same phenomenon may occur for microalgae in poikilohaline environments. In general, the three species we found are not common in local coastal seawater (Blinn 1984, Annan 2008, Krienitz et al. 2012).

Mono Lake in northern California is permanently hypersaline, and *Picocystis* is dominant. *Picocystis* is thought to prefer high salinity environments. In natural water bodies, the highest abundance of *Picocystis* was reported to occur at salinities of between 50.7 and 61.6 (Krienitz et al. 2012). Given the high salinities reached by our pond, it is not surprising that *Picocystis* would be a major species present. The variability in pond salinity (and pond chemistry), however, seems to have opened a niche for other species. *Picochlorum* is capable of growth at a wide range of salinities and while the growth rate of *Picochlorum okalahomensis* was decreased when salinity increased beyond 50 (Annan 2008) similar to our strain, *Picochlorum* is still capable of robust growth rates at very high salinities.

As shown in Figure 4, *Chaetoceros* (SECHAET) has the highest growth rate for the whole temperature span compared to *Picochlorum* (SENEW3) and *Picocystis* (SENEW1). However, it is not the dominant strain in the poikilohaline pond but only appeared in June samples. Other factors such as salinity changes (Fig. 5), nutrient availability, and grazers (top down control) thus are likely to have significant impacts on its growth and abundance.

As shown in Figure 6, *Picochlorum*, *Picocystis*, and *Chaetoceros* have significant lipid globules. Tests in outdoor open ponds also showed that all three strains have high growth rates and significant oil contents under local environmental conditions (manuscript in preparation). Given their relative ease of growth in lab conditions and outdoor environments as well, these three species merit further attention as future algal biofuels strains. It may also be useful to develop algal ponds with cocultures of these species as they appear to occupy slightly different ecological niches.

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Supporting Information

Additional Supporting Information may be found in the online version of this article at the publisher's web site:

Table S1. Pigment content of samples (in $\mu g \cdot L^{-1}$). Abbreviations used for pigment names are: Chl *a*1 – chlorophyll *a*; Chl *a*2 – divinyl-chlorophyll *a*; Chlide *a*1 – chlorophyllide *a*.