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DIVERSITY OF DIATOM SILICON TRANSPORTER SEQUENCES AND THEIR EXPRESSIONS DURING A CHANGJIANG RIVER FLOODING EVENT IN THE EAST CHINA SEA

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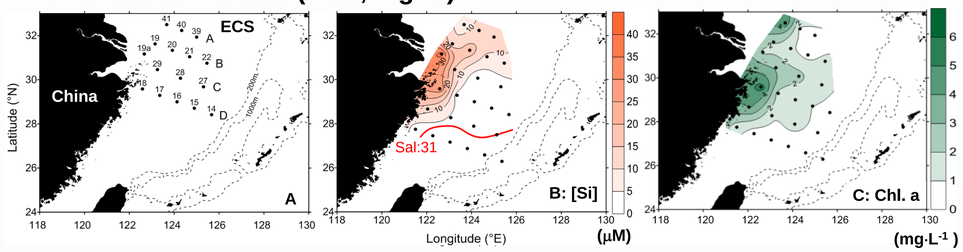
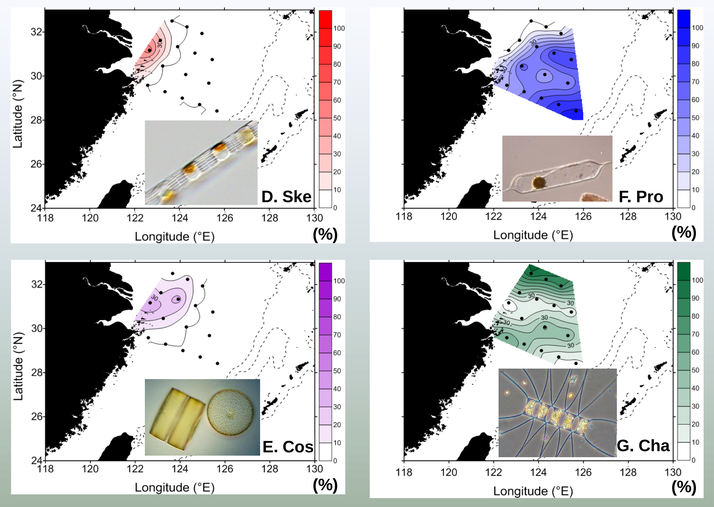
Introduction

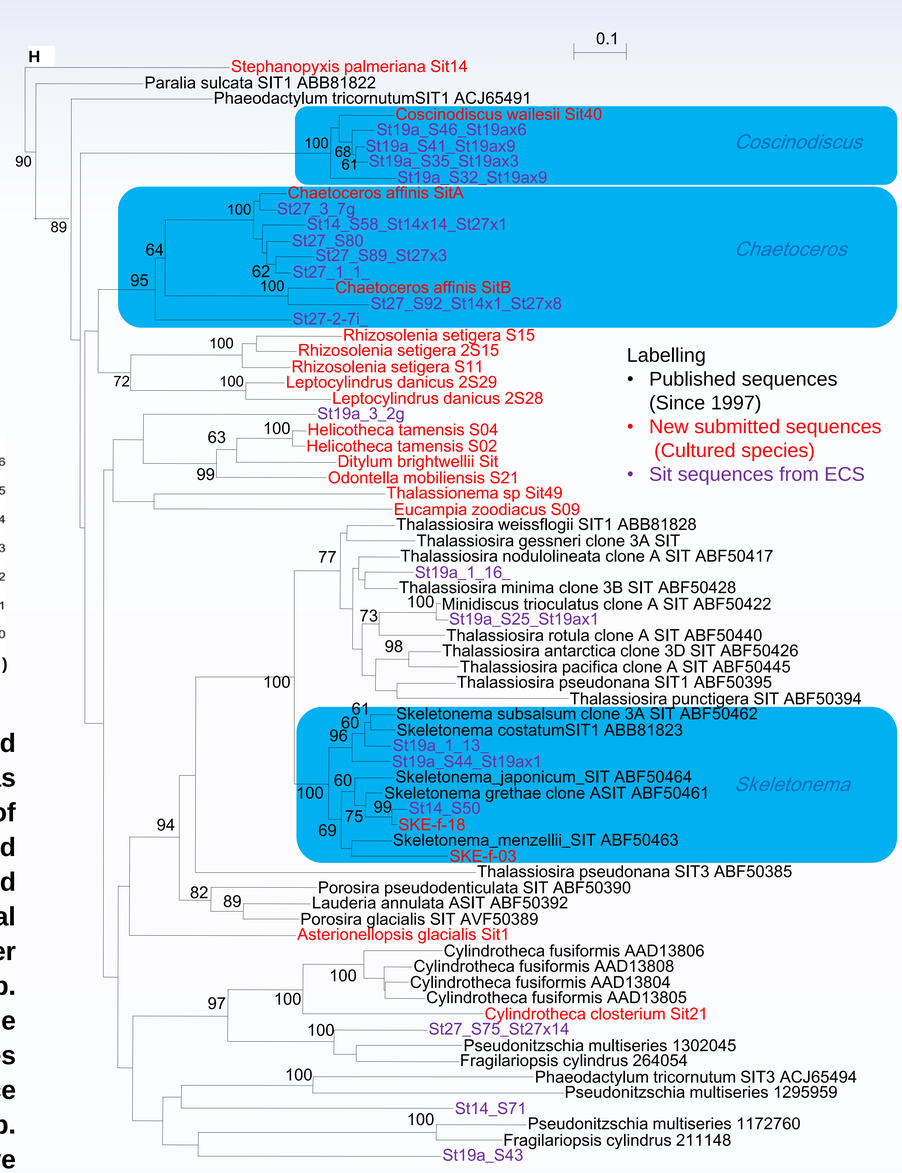
Marine diatoms are silicified phytoplankton responsible for about one fifth of global primary production. They tend to dominate phytoplankton communities in coastal and upwelling regions where sufficient light and nutrients are available to sustain their growth. Unlike other phytoplankton groups, diatoms require silica to construct their cell walls and are controlled by the availability and distribution of silicic acid. The most significant input (~80%) of silicic acid for the world ocean comes from rivers and the transport processes are influenced by climate and human perturbations. In this study, we investigated the genetic diversity of the silicon transporter genes (*Sit*) and measured *Sit* gene expression in one genus, *Chaetoceros*, as a molecular indicator of silicon deficiency in the East China Sea (ECS, Fig. A).



2010 summer cruise in the ECS

In a cruise conducted in July, 2010, the Changjiang plume was covered almost 2/3 area of the ECS, when the isohaline of salinity 31 was used as the outer boundary of the Changjiang dilute water. The distribution of silicate in the surface water was high of > 30 μM near the river mouth and decreased seaward toward the southeast (Fig. B). This pattern indicated that more than half of the surface water were enriched with terrestrial nutrients and subsequently led to elevated Chl. a concentration higher than 1 mg·L^{-1} (Fig. C). As to the diatom compositions, *Skeletonema* spp. and *Coscinodiscus* spp. were the major dominant species in the Changjiang mouth (Figs D & E). The relative abundance of these species gradually decreased seaward toward the southeast and the dominance were replaced by *Proboscia* spp. (Fig. F). In addition, *Chaetoceros* spp. displayed a wide distribution with an average over 30% in relative abundance throughout the surface water in the sampling area (Fig. G).



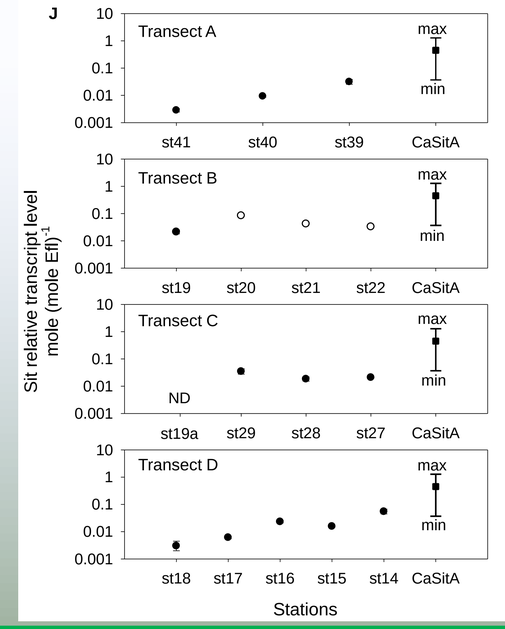


Sit diversity and phylotype distributions

To extend the *Sit* gene family in marine diatoms, *Sit* gene diversity was evaluated by sequencing new *Sit* fragments from cultured diatoms spanning 12 genera. With the aid of this expanded sequence database, the taxonomical affiliation of ECS *Sit* sequences from 3 stations (Sts. 19a, 14 and 27) were determined (Fig. H). *Sit* sequences similar to *Cosinodiscus* and *Skeletonema* were found in the Changjiang mouth (St. 19a). In contrast, *Sit* sequences similar to *Chaetoceros* were mainly found at Stations 14 and 27. According to the alignment of *Sit* sequences, *Chaetoceros*–specific primers were designed for detecting *Sit* mRNA levels by quantitative RT-PCR.

Chaetoceros SitA transcript levels in the ECS

During the cruise in 2010, the *Chaetoceros SitA* transcript levels were all lower than the threshold of silicate deficiency established from a cultured *C. affinis*, indicating *Chaetoceros* spp. were not limited by silicate in our study area (Fig, J). This could due to a devastating flood event of the Changjiang River in 2010 that exported large amounts of silicic acid to the ECS.



Sit gene expressions in *C. affinis*

Since 2 distinct forms of *Sit* gene sharing 75% identities were cloned in *C. affinis* and both homologs were obtained in ECS samples (Fig. H), the expression patterns of these 2 forms were confirmed in the Lab experiments (Fig. I). Our results showed that only the transcript levels of *CaSitA* increased responding to silicate deficiency. Therefore, *SitA* was chosen as a silicate marker gene in the ECS samples, and the silicate deficiency threshold was temporarily set at 450 mmole (mole Efl)$^{-1}$ according to the day5 value in the Low-Si experiments.