Pervasive Iron Limitation at Subsurface Chlorophyll Maxima of the California Current

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Subsurface chlorophyll maximum layers (SCMLs) are nearly ubiquitous in stratified water columns and exist at horizontal scales ranging from the submesoscale to the extent of oligotrophic gyres. These layers of heightened chlorophyll and/or phytoplankton cell counts are generally thought to be a consequence of a balance between light energy from above and a limiting nutrient flux from below, typically nitrate (NO\textsubscript{3}). Here we present multiple lines of evidence demonstrating that iron (Fe) limits or with light colimits phytoplankton communities in SCMLs along a primary productivity gradient from coastal to oligotrophic offshore waters in the southern California Current ecosystem. SCML phytoplankton responded markedly to added Fe or Fe/light in experimental incubations and transcripts of diatom and picoeukaryote Fe stress genes were strikingly abundant in SCML metatranscriptomes. Using a biogeochemical proxy with data from a 40-yr time series, we find that diatoms growing in California Current SCMLs are persistently Fe deficient during the spring and summer growing season. We also find that the spatial extent of Fe deficiency within California Current SCMLs has significantly increased over the last 25 y in line with a regional climate index. Finally, we show that diatom Fe deficiency may be common in the subsurface of major upwelling zones worldwide. Our results have important implications for our understanding of the biogeochemical consequences of marine SCML formation and maintenance.

Fe and light are essential for phytoplankton photosynthesis, but both resources are scarce in much of the ocean. Surface ocean primary productivity is limited by the availability of Fe in some regions (1), and mesoscale Fe fertilization experiments now firmly demonstrate that Fe availability controls phytoplankton biomass and growth rates in the Southern, equatorial Pacific, and subtropical Pacific oceans (2). In addition, phytoplankton Fe limitation has been observed in midlatitude coastal upwelling zones (3, 4), throughout mesoscale circulation features (5, 6), and at the edge of subtropical gyres (7). In the surface ocean light attenuates rapidly to less than 1% of incident photosynthetically available radiation (z\textsubscript{1%}) at depths from 50 m to 200 m, depending on turbidity. However, many diverse phytoplankton groups have adapted to growth at depths approaching z\textsubscript{1%} despite the challenging low-light conditions. Prior studies noting the overlapping scarcity of Fe and light in much of the ocean predicted that these two resources synergistically colimit phytoplankton growth (8), particularly in subsurface chlorophyll maximum layers (SCMLs) (9). Indeed, work with cultured phytoplankton demonstrates that Fe/light colimitation can arise when demand for Fe-rich photosynthetic redox proteins increases under low-light conditions (9, 10). However, the potential for phytoplankton Fe or Fe/light (co)limitation in SCMLs has been explored only in a handful of field studies despite the significant feedbacks linking Fe/light (co)limitation, dust deposition, and oceanic CO\textsubscript{2} uptake in global biogeochemical models (11). Although Fe/light colimitation has been observed in some high-latitude SCMLs (12, 13), mid-/low-latitude SCMLs from both coastal and pelagic zones remain understudied. Dissolved Fe minima at SCMLs from the subtropical North Pacific gyre (14) and the Sargasso Sea (15) may be a consequence of intense biological demand, even Fe limitation, during summer months. One study documented phytoplankton Fe/light colimitation from mesotrophic and oligotrophic SCMLs in the California Bight and the eastern tropical North Pacific (16), while another found SCMLs in the oligotrophic Western Pacific to be mostly light limited with some groups of microbial eukaryotes potentially

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exhibiting Fe/light colimitation (17). However, studies using multiple complementing experimental approaches at varied scales are needed to establish the prevalence of phytoplankton Fe limitation or Fe/light colimitation within SCMLs worldwide. High primary productivity in the southern California Current (CC) and other Eastern Boundary Current systems is supported by intense upwelling of macronutrient-rich waters (18). However, local variation in Fe concentrations and other biogeochemical factors can generate Fe-limited phytoplankton communities in the CC (4, 19). During July 2007 we investigated the role of Fe and light as (co)limiting factors along a transect of California Cooperative Oceanic Fisheries Investigations (CalCOFI) line 93.3 (SI Appendix, Fig. S1), which spans the inshore (~150 km offshore), transition (~150–450 km offshore), and offshore (~450 km offshore) zones of the southern CC (20). In addition to sampling all standard CalCOFI stations on this transect, we intensively sampled three stations (93.3/40, 93.3/80, and 93.3/120) representing the inshore, transition, and offshore zones, respectively. At these three stations we measured total dissolved Fe (dFe) concentrations and the concentrations of strong, organic Fe-binding ligands (L1). L1 concentrations are positively correlated with Fe-limited phytoplankton growth in incubation studies, suggesting that phytoplankton and/or associated bacteria preferentially produce L1 as an adaptation to Fe limitation (21, 22). We also determined two other biogeochemical proxies for Fe deficiency: the N03 to dFe ratio (N:dFe) and S1ex. Fe is likely to be a proximate limiting or colimiting nutrient in waters where the N:dFe ratio is greater than ~8 µmol/nmol (4, 19). At or above this threshold residual N03 accumulation in the water column can be attributed to the direct or indirect suppression of phytoplankton N03 utilization by low Fe availability (SI Appendix, section 4). S1ex (µmol L−1) is a modified form of the Sθ proxy (23) and traces shifts in the elemental composition of diatoms as a result of Fe deficiency (24) (SI Appendix, section 13). Negative S1ex values in the water column indicate preferential diatom uptake of H2SO4 relative to N03 due to Fe deficiency. S1ex and the N:dFe ratio were negatively correlated across all samples (ρ = −0.75, P = 2.4e-7), indicating that low Si and high N03 waters generally also had low dFe concentrations, consistent with Fe-limiting conditions for diatoms (SI Appendix, Fig. S2). S1ex increased substantially upon addition of Fe and Fe + light to inshore and transition zone SCML incubations (SI Appendix, Fig. S3), further emphasizing the proxy’s responsiveness to the Fe status of the aggregate phytoplankton community.

At each of the intensively sampled stations we conducted incubation experiments with factorial resource additions of Fe, light, and combined Fe + light to SCML water. We measured chlorophyll a concentrations, primary production rates, the photochemical quantum efficiency of photosystem II (Fv/Fm), N03 consumption over the course of the incubations, and phytoplankton community composition by microscopy. Using these measurements we then classified incubation responses as simultaneous colimitation, independent colimitation, or serial limitation (25) (SI Appendix, section 11). Community transcriptomes were collected and sequenced from SCML and surface waters and were introduced in a prior publication (26). Here we focus on expression patterns of phytotransferrin (pTF), formerly known as ISIP2A (27, 28), and of iron stress-inducible protein 3 (ISIP3), which are diagnostic biosignatures of algal Fe stress in culture (29, 30) and in the field (31, 32).

Results and Discussion

Fe Limitation at SCMLs of the Inshore CC Sector. Biogeochemical proxies (S1ex and N:dFe ratio), community transcriptomes, and incubation experiments consistently pointed to significant diatom Fe limitation at inshore SCMLs and were indicative of serial or single Fe limitation rather than colimitation (25). Fe concentrations were low in the surface mixed layer (mean of 0.10 nmol L−1) and the depths of the ferricline (74 m) and nitracline (31 m) were strongly decoupled at station 93.3/40 (Fig. L4 and SI Appendix, Table S1). L1 concentrations peaked at the SCML, N:dFe ratios were strikingly elevated (N:dFe max = 120 µmol/nmol) within the SCML, and the S1ex profile mirrored that of N:dFe (Fig. 1B). The SCML incubation experiments displayed a five- to sevenfold increase in total chlorophyll a (Chl a), an ~2.5-fold increase in bulk N03 consumption, and an approximately twofold increase in Fv/Fm over the control in response to added Fe and Fe + light. The control and added light conditions were statistically indistinguishable (Fig. 2 and SI Appendix, Fig. S3). Primary production rates increased the most (2.5- to fivefold over control) with added Fe + light and Fe, while light alone had no effect. Large chain-forming diatoms dominated in added Fe and Fe + light incubation conditions (SI Appendix, Fig. S4). ISIP3 and pTF transcripts were strikingly abundant relative to other protein functional families in community transcriptomes from the SCML at station 93.3/40 with transcripts from four taxonomic groups exceeding the 95th rank percentile of all taxonomically aggregated community transcripts (Fig. 3). Phaeocystis and pelagophytes were two of the most abundant taxonomic groups detected at 93.3/40 (SI Appendix, Fig. S5), and pTFs belonging to these groups were in the top 1% of all identifiable Phaeocystis and pelagophyte transcripts/isoforms as well as the top 1% of all taxonomically aggregated community transcripts. Indeed, the expression of pTF/ISIP3 by diatoms, dinoflagellates, pelagophytes, and Phaeocystis often rivaled or exceeded that of highly expressed genes for essential cellular functions from each taxonomic group including ribosomal assembly proteins, N03 transporters, heat-shock–like proteins, and photosynthesis-related proteins (Fig. 3). Proteorhodopsin-like genes, which have recently been proposed as an alternative, Fe-independent energy acquisition strategy for iron-limited diatoms (33), were some of the most highly expressed diatom transcripts at the SCML of 93.3/40—perhaps also due to Fe stress. These expression patterns suggest that inshore SCML phytoplankton were investing significant resources into cellular pathways for managing Fe deficiency.

Fe/Light Colimitation at the CC Transition and Offshore Zones. At the oligotrophic edge of the transition zone and in the offshore zone we observed a deepening of the nitracline and SCML toward the base of the euphotic zone where low light levels may have increased photosynthetic Fe demand. Phytoplankton Fe limitation is evident in offshore waters where the N:dFe ratio is greater than 8 µmol/nmol (4, 19) and the depths of the ferricline and nitracline were strongly decoupled at station 93.3/120 and moderately offset at station 93.3/80 (Fig. L4). We observed a localized Fe depletion at the outer transition zone SCML, potentially due to enhanced localized biological Fe uptake. We also measured a localized L1 increase at the offshore zone SCML, consistent with enhanced biological production of Fe acquisition molecules at this depth. N:dFe ratios greater than 8 µmol/nmol and negative S1ex values highlighted the base of the SCML as a region of potential diatom Fe deficiency in most of the transition zone (Fig. 1B). In the offshore zone, negative S1ex tracked the σθ = 26 kg m−3 isopycnal to depths 100 m below the euphotic zone (Fig. 1C), indicating that the signal may not have been from local diatom Fe deficiency and potentially Fe-limited waters advected from elsewhere (SI Appendix, section 13C.2). In this sector mesoscale circulation features (6, 34) and wind-stress curl upwelling (35) are likely important sources of new N to the euphotic zone but may be too infrequent to generate chronic Fe-limited diatom growth and a persistent S1ex signal at the SCML.

The outer transition zone incubation responses at 93.3/80 were most consistent with independent Fe/light colimitation (SI Appendix, section 11). Fe + light stimulated the greatest N03 drawdown (fourfold over control), primary production (15-fold}
over control), and total Chl \(a\) (5.5-fold over control) increases, while responses to Fe or light alone were similar to each other and significantly smaller than for Fe + light. However, the single addition of iron or light significantly enhanced NO\(_3\) drawdown (Fe, 1.7-fold over control; light, 1.5-fold over control) and the addition of iron significantly increased the Chl \(a\) concentration (2.5-fold over control). Large chain-forming diatoms dominated incubation responses to added Fe + light (SI Appendix, Fig. S4), while iron-stress transcripts were in the top 1% of all expressed transcripts in the metatranscriptome and in the top 0.1% of pelagophyte transcripts. In all test cases and datasets we examined \(\text{SiO}_3\) as a biogeochemical proxy for Fe deficiency because \(\text{H}_3\text{SiO}_4\) and NO\(_3\) measurements are readily available in the CalCOFI dataset and there is a strong correlation between negative \(\text{SiO}_3\) and experimentally determined Fe limitation in our results (Figs. 1 and 2 and SI Appendix, Fig. S3) and the results of others (5, 6, 24). The \(\text{SiO}_3\) tracer assumes minimal effects from horizontal mixing/advection, shifts in upwelling source depth, and other processes (nitrification, denitrification, variability in SCN remineralization ratios) that may integrate non-specific biogeochemical signals. In most settings, such as in the euphotic zone of the CC, these assumptions appear to be valid, but we note that in other biogeochemical regimes they may not be. In all test cases and datasets we examined \(\text{SiO}_3\) performed as a robust tracer (SI Appendix, section 13A).

During the spring and summer months over the last 40 y at least 30% of all SCML samples in the southern CC were Fe deficient to some degree (\(\text{SiO}_3 < 0\)). Fe deficiency is disproportionately concentrated in SCMLs from the inshore (43% negative) and transition zones (25% negative) compared with the offshore zone (7% negative) (Fig. 4 and SI Appendix, Fig. S6). On average \(\text{SiO}_3\) from inshore and transition zone SCMLs has steadily become more negative since 2000 in contrast to the general increase in the 1990s and most of the 1980s (SI Appendix, Fig. S7). We also find that the total spatial area of Fe-deficient SCMLs has significantly increased for the inshore
sector over the last 40 y (Fig. 4E). These trends have occurred in conjunction with a general shoaling of the SCML, the nitracline, and the euphotic zone in the southern CC (SI Appendix, Fig. S8) (20, 35, 36), particularly for the offshore and transition zones during the early 2000s. Negative Si\textsubscript{ex} values often coincided with the highest observed Chl \textsubscript{a} concentrations in the inshore zone (SI Appendix, Fig. S6), suggesting a major contribution of Fe-deficient diatoms to coastal primary production. Negative Si\textsubscript{ex} values in the offshore zone generally tracked the base of the euphotic zone (SI Appendix, Fig. S9) or extended below it (Fig. 1A). This signal may be caused by chronic in situ diatom Fe deficiency at the deepest parts of the offshore euphotic zone and/or may reflect along-isopycnal propagation of a negative Si\textsubscript{ex} signal generated inshore as has been observed for other tracers (37, 38).

Patterns of Negative Si\textsubscript{ex}, CC Winds, the North Pacific Gyre Oscillation, and Biogeochemical Variables. SCML Fe deficiency may track decadal modes of climate variability in the southern CC. The North Pacific Gyre Oscillation (NPGO) mode is a regional climate index that is significantly correlated with fluctuations in the biogeochemistry and hydrography of the southern CC (39). Positive NPGO intervals (SI Appendix, Fig. S10) are associated with wind shifts that result in upwelling-favorable conditions in the CC (39) and coincide with the most extreme negative Si\textsubscript{ex} events from the last 40 y (Fig. 4 and SI Appendix, Fig. S7). The strengthening of the NPGO amplitude since 1993 (39) also corresponds with a significant increase in the inshore spatial extent of diatom Fe deficiency at the SCML (Fig. 4E), potentially indicating shared forcing. However, the time-lagged correlations between Si\textsubscript{ex} extent and wind-stress curl or Ekman-driven coastal upwelling rates are quantitatively weak (\(p < 0.2\), SI Appendix, Fig. S11). This apparent weak correlation may actually reflect nonlinear (40) or cumulatively integrative (41) responses and does not necessarily exclude a mechanistic relationship between regional Si\textsubscript{ex} trends at the SCML and decadal patterns of atmospheric forcing. Si\textsubscript{ex} signals at southern CC SCMLs were moderately correlated with the difference between the nitracline and SCML depths (\(p = 0.53, P < 1e-200\)) and the potential density at the SCML (\(p = -0.49, P < 1e-200\)) (SI Appendix, Fig. S11). The positive correlation between Si\textsubscript{ex} and the nitracline/SCML depth offset suggests that diatom Fe deficiency is associated with SCMLs that form below the top of the nitracline, which may reflect a mismatched stoichiometric supply of NO\textsubscript{3} and Fe from below. The negative correlation between Si\textsubscript{ex} and SCML potential density may reflect an association with diatom Fe deficiency and increased isopycnal shoaling or upwelling strength in general.

**Si\textsubscript{ex} from a Global Perspective.** To contextualize the pervasive Fe deficiency we observed at SCMLs from the southern CC, we
also determined Si$_{ex}$ at a global scale at 50 m depth using data from the 2013 World Ocean Atlas (42) (SI Appendix, section 6). Our results highlight the subsurface of coastal upwelling zones as potential hotspots of diatom Fe deficiency (Fig. 5). The CC (Figs. 4 and 5A) and the Humboldt/Peru Current (Fig. 5B) system show the largest negative Si$_{ex}$ signals at 50-m depth, while the Benguela and Canary Currents (Fig. 5 C and D) display negative signals to a lesser extent. The Oman upwelling zone and the North Arabian Sea also appear to have notably Fe-deficient (Fig. 5E) Si$_{ex}$ values in the subsurface. The greatest spatial extent of subsurface diatom Fe deficiency appears to overlap with well-known high-nutrient low-chlorophyll regions, such as the Subarctic North Pacific (43) and the Pacific equatorial upwelling zone (44). It is worth noting that many regions with negative Si$_{ex}$ values identified in our analysis also overlap or potentially overlap with oxygen minimum zones (OMZs) and/or anoxic OMZs (45) where denitrification and anammox can decouple the cycling of H$_2$SiO$_4$ from NO$_3$ and may impart a nonspecific signal to Si$_{ex}$. However, the occurrence of denitrification/anammox above the upwelling source isopycnal would counter any signal of iron-limited diatom uptake and push Si$_{ex}$ in a positive direction. Phytodinplankton iron (co)limitation has been experimentally identified in surface waters from many of the negative Si$_{ex}$ regions identified here (7, 43, 44, 46, 47), but few studies have investigated the potential for iron limitation at the depth ranges of SCMLs in these regions. We find that subsurface waters of many major oceanic upwelling regions display a biogeochemical imprint of diatom iron deficiency (negative Si$_{ex}$), which is consistent with our experimental and time series results from SCMLs in the CC.

Conclusions

The diagnosis of nutrient limitation in situ is a critical step toward better understanding fluxes of energy and matter in marine ecosystems. Our results suggest a strong coastal to offshore gradient in the combined effects of iron and light on SCML phytodinplankton of the southern CC, a highly productive eastern boundary upwelling regime, and potentially upwelling zones worldwide (Fig. 5). The shallower inshore and inner transition zone SCML communities, which represent maxima in both diatom biomass and productivity, appear particularly susceptible to Fe limitation or Fe/light colimitation. It is less obvious whether deeper SCML communities from the oligotrophic offshore zones can be Fe deficient, but we do observe a significant effect of Fe + light on total Chl a concentrations and notably high expression of phytoplankton Fe-stress genes in situ. Based on our results we speculate that oligotrophic SCMLs may experience periods of Fe/light colimitation or Fe serial/single limitation, but further direct observations in the oligotrophic ocean are needed to confirm this hypothesis.

Persistent diatom Fe (co)limitation at SCMLs likely has downstream consequences for the carbon cycle. For example, increased diatom silification may enhance particulate carbon export efficiency by increasing sinking rates and shielding cells from grazing in productive upwelling zones (5, 6). Furthermore, biogeochemical patterns of diatom iron deficiency at the CC subsurface appear to track dominant modes of climate variability in the North Pacific, which may be due to regional atmospheric patterns that decouple the nitracline, the ferricline, and the depth of the SCML. Biogeochemical models predict increased upwelling and NO$_3$ fluxes to the southern

**Fig. 4.** Time series (1977–2017) of Si$_{ex}$ (µmol·L$^{-1}$) at the SCML in the CalCOFI sampling area. (A and B) Interpolated values of Si$_{ex}$ at the depth of the SCML for (A) June/July 2004 (season with most negative SCML Si$_{ex}$ values) and (B) June/July 2007 (approximate time of cruise). Small circles depict CalCOFI sampling stations, while large circles display locations of incubations and metatranscriptomes. Dashed lines separate inshore–transition–offshore zones. (C–E) Average spring–summer (April–September) fraction of SCMLs with negative Si$_{ex}$ for each zone. A and B time points in C–E correspond to the areal contour plots in A and B. Red and blue series show two different Si$_{ex}$ estimates using a source upwelling isopycnal of 26.5 kg·m$^{-3}$ and 25.8 kg·m$^{-3}$, respectively. (E) There is a significant monotonic increasing trend (nonparametric Mann–Kendall test P < 0.002) in the extent of negative Si$_{ex}$ for the inshore region since 1990 (dashed lines are linear regressions for each source isopycnal). Shaded regions show when the NPGO index is positive.

**Fig. 5.** Global distribution of Si$_{ex}$ (µmol·L$^{-1}$) at 50 m depth relative to an upwelling source isopycnal of $\sigma_\theta = 26.5$ kg·m$^{-3}$. Data are from the 1st–resolved annual (1955–2012) mean isosurface 2013 World Ocean Atlas. A–E highlight mean negative Si$_{ex}$ upwelling regions. The solid black line in the Southern Ocean shows the outcropping of the $\sigma_\theta = 26.5$ kg·m$^{-3}$ isopycnal.
CC under anthropogenic climate change (48, 49), which may drive diatom communities at the SCML toward Fe limitation if the associated Fe fluxes do not increase proportionally. This potential atmospheric–biogeochemical linkage demonstrates a connection mediated by iron, by which the changing climate may influence carbon cycling and primary productivity in SCLMs of the CC and potentially other eastern boundary currents.

Materials and Methods

Method details are available in SI Appendix. Samples for total dissolved iron concentrations, iron-binding ligand concentrations, and incubation experiments were collected and processed as described in refs. 4 and 24. Dissolved iron was measured as in ref. 4 and Fe-binding ligands were measured as in ref. 50. The transcriptomic data (including all relevant methods) were introduced in a prior publication (26). Macronutrients were sampled using a rosette sampler and analyzed following the standard operating procedures from the California Current Ecosystem Long-Term Ecological Research program. Triplicate or duplicate 4-L incubaitions were conducted in acid-cleaned polycarbonate bottles, housed in a Peridial incubator at 16°C under high-light treatment and added Fe (SI Appendix, Table S3). Primary productivity and photosynthesis were measured as described in refs. 17 and 26. CalCOFI and World Ocean Atlas hydrographic and nutrient data were downloaded from new.data.calcofi.org and https://www.nodc.noaa.gov. The $\Delta$Si$_4$ proxy (Si/Fe ratio, section 13A) at SCML depths was calculated as $\Delta$Si$_4 = (\mu$mol $\text{H}_{2}\text{SiO}_3\text{-L}^{-1}) - (\mu$mol NO$_3\text{-L}^{-1}) \times R_{\text{NO}_3}$, where $R_{\text{NO}_3}$ is the micromolar ratio of $\text{H}_2\text{SiO}_3$ to NO$_3$ at $\sigma_T = 25.8$ kg m$^{-2}$ or 26.5 kg m$^{-3}$.

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