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Original Article

Estimating concentrations of essential omega-3 fatty acids in the ocean: supply and demand

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Vertebrates have a universal requirement for essential fatty acids (FAs), but in the ocean these FAs are synthesized only by phytoplankton. All other marine organisms must source their essential FA directly from phytoplankton or indirectly through the food web. Thus, the growth and abundance of all organisms in the marine ecosystem is constrained not just by the rate of carbon fixation in photosynthesis but also by the rate of synthesis of essential FAs. Despite the significance of this controlling step, we have had until now only very limited knowledge of the amount, distribution and rate of synthesis of essential FAs in the sea. Here, we report results on the quantity of a specific essential omega-3 FA, eicosapentaenoic acid (EPA) in the ocean, obtained with a novel application of ocean-colour data collected by remote sensing. Using *in situ* samples collected in the Northwest Atlantic, we developed a simple model to describe the relationship between total FAs and total chlorophyll-*a*. We refined these by examining the relationships of FAs produced predominantly by diatoms with the fraction of total chlorophyll-*a* derived from diatoms. These models were then applied to satellite data to map the distribution of EPA relative to diatom carbon in the Northwest Atlantic. With extrapolation to the global oceans, we were able to provide a first estimate of annual production of EPA, which demonstrated that the supply was barely sufficient to meet the nutritional demand of the world population in the present day; as the world population increases, this resource may become inadequate to meet those demands. This approach will allow us to begin to address issues such as the budget of essential FAs in the ocean and the maximum sustainable rate at which these FAs could be harvested from the ocean without compromising the integrity of the marine ecosystem.

Keywords: Lipid, phytoplankton, remote sensing.

Introduction

Essential fatty acids (FAs) are critical nutrients required for growth and survival by all organisms (Holman, 1998). In the marine food web, they are synthesized only by phytoplankton—all other marine organisms derive their essential FAs directly or indirectly from phytoplankton (Iverson, 2009; Lavie *et al.*, 2009). Many essential FAs are members of the family of omega-3 FAs that have carbon–carbon double bonds at specific positions in their carbon chains. Eicosapentaenoic (EPA) is an essential omega-3 FA that serves as a precursor for a number of eicosanoids (Simopoulos, 1999; Simopoulos, 2002). These are biologically active substances, similar to hormones, that directly control many important cellular functions, including inflammatory responses. For example, they govern important basic physiological processes such as the dilation of blood vessels and disaggregation of platelets (Simpolous, 2002). Omega-3 FAs also influence gene expression regulating the synthesis

of eicosanoids and other hormones (Kersten, 2008), and thus also have an indirect role in controlling inflammatory responses. In fish, essential FAs are necessary in specific amounts to ensure larval survival and, in many species, have integral roles in regulating membrane fluidity in response to temperature fluctuations (Tocher, 2003; Arts and Kohler, 2009).

Because these FAs are absolutely required by vertebrates for normal, healthy survival, they may limit production at higher trophic levels. For instance, it has been postulated that climate-induced fish community transitions are linked to changes in the availability of essential FAs in the ocean (Litzow *et al.*, 2006). The conservation of essential FAs from phytoplankton in food webs also makes them excellent candidates for modelling fluxes through the marine environment using data assimilation techniques. Despite their importance, methods of estimating their abundance in the ocean at global scales are lacking. Only *in situ* methods are currently available, and these require costly ship-based collection of water samples and extensive lab preparation and analysis; as a result, they are limited in spatial coverage. The ability to map the distribution of essential FAs on synoptic scales using remotely sensed ocean-colour data would represent a major advancement.

A significant relationship has been established between *in situ* phytoplankton carbon and chlorophyll-*a* in the ocean (Sathyendranath *et al.*, 2009). If FAs represented a specific fraction of phytoplankton carbon, we would expect FAs also to vary predictably with chlorophyll-*a* concentrations; thus, this could provide a means of estimating FA concentrations in the ocean from satellite-derived chlorophyll-*a* data. Although there are limited data available in the literature, measurements of the ratio of total FA:C production, or concentration, in laboratory cultures of phytoplankton and *in situ* samples range from 0.08–0.2 for a variety of phytoplankton classes, including diatoms, dinoflagellates and prymnesiophytes (Marañón and Gonzáles, 1997; Riebesell *et al.*, 2000; Hamanaka *et al.*, 2002), suggesting that FA concentrations may indeed be related to chlorophyll-*a* concentrations.

Different classes of phytoplankton produce FAs with differing structures, so that specific FAs are derived predominantly from, and may serve as biomarkers for, specific phytoplankton classes (reviewed in Dalsgaard *et al.*, 2003). For instance, 16:4n-1 (see Material and methods for naming conventions) is synthesized almost exclusively by diatoms, whereas 18:5n-3 is produced predominantly by flagellates (Viso and Marty, 1993; Dunstan *et al.*, 1994; Hamm and Rousseau, 2003). Diatoms are also the major producers of EPA, while flagellates have elevated proportions of docosahexaenoic acid (DHA), another essential FA (Dalsgaard *et al.*, 2003). Using remote sensing of ocean colour, Sathyendranath *et al.* (2004) developed an algorithm to estimate the proportion of satellite-derived chlorophyll-*a* concentration associated with diatoms. Thus, examining the correlation of diatom marker FAs with diatom-associated chlorophyll-*a* could further refine the relationship of total FA:C. Similarly, if chlorophyll-*a* that was not associated with diatoms could be assumed to be derived from flagellates, then the concentrations of flagellate markers, 18:5n-3 and DHA, could be expected to be correlated with non-diatom chlorophyll-*a* concentrations.

Here, we test the hypothesis that *in situ* total FA concentration, as well as concentrations of specific FAs, correlates with *in situ* total and fractional chlorophyll-*a* concentration in the Northwest Atlantic. The goal of this work is to establish methods that will allow the concentrations of essential FAs in nature to be estimated on local and global scales. With these new methods, we can begin to study the dependence of higher trophic levels, such as commercial fish and invertebrates, on these essential FAs produced by phytoplankton in natural settings.

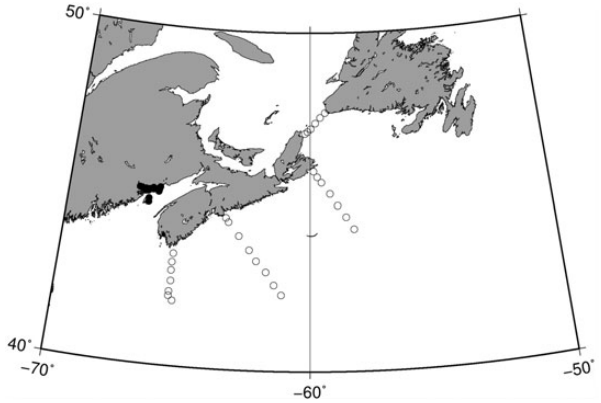

Figure 1. Sites of sample collection. Water samples were collected from the Bay of Fundy near St Andrews, New Brunswick, in July and August, 2008, and from the Scotian Shelf off the southeastern and eastern coast of Nova Scotia in April, 2011.

Material and methods

In situ pigment and FA analyses

Water samples were collected from the Bay of Fundy (BF), near St Andrews, New Brunswick (Canada), between 7 July and 26 August 2008, and from the Scotian Shelf (SS), off the southeastern and eastern coast of Nova Scotia, between 7 April and 17 April 2011 (Figure 1). In BF, samples ($n = 56$) were collected from 13 locations during the first sampling period of 7–9 July, with one sample taken at each location from the top 1 m of the water column. Subsequently, sampling was carried out at 4–6 stations each week, depending on weather conditions, with one sample collected at each station. BF samples were collected in relatively close proximity each week, compared with the SS sampling. On the SS, one sample was collected at each of 23 stations, spread along four transects with collection depths ranging from 3–10 m ($n = 21$). In BF, 250 and 500 ml of water were filtered for pigment and FA analyses, respectively; on the SS, the corresponding volumes were 500–750 ml and 1000–1500 ml. Pigment samples were filtered through Whatman GF/F filters and stored in cryovials in liquid nitrogen until analysis. Pigment concentrations were quantified using reverse-phase high performance liquid chromatography (HPLC) at Horn Point Laboratory (Cambridge, MD, USA) as described in Van Heukelem and Thomas (2001). Pigments were identified by retention time and comparison of absorbance spectra with spectra from commercially available calibration pigment standards or those isolated from naturally occurring sources. *In situ* total chlorophyll-*a* concentration (mg m^{-3}) represented the sum of chlorophyll-*a* (including chlorophyllide-*a*, plus chlorophyll-*a* epimers and allomers). The proportion of *in situ* chlorophyll-*a* (mg m^{-3}) associated with diatoms was estimated based on specific biomarker HPLC pigments using the method of Uitz *et al.* (2006) as modified by Devred *et al.* (2011). Non-diatom chlorophyll-*a* represented total chlorophyll-*a* minus diatom chlorophyll-*a*.

Samples for FA analysis were filtered through precombusted Whatman GF/C filters and stored in chloroform. Cholestane was added as an internal standard and lipids were extracted with chloroform and methanol (Parrish, 1999). FA methyl esters (FAMEs) were formed using sulphuric acid-catalyzed transesterification (Budge *et al.*, 2006) and analysed by gas chromatography–flame ionization detection with splitless injection

(Budge *et al.*, 2006) using a flexible fused silica column (30 m × 0.25 mm ID) coated with 50% cyanopropyl polysiloxane (0.25 μm film thickness; Agilent DB-23). Approximately 70 FAMEs were identified by comparison of retention times with known standards (Nu Check Prep, Elysian, MN, USA), or using GC-mass spectrometry. Each fatty acid was described using a shorthand nomenclature of A:Bn-X, where A represents the number of carbon atoms, B the number of double bonds and X the position of the double bond closest to the terminal methyl group. Eicosapentaenoic acid and docosahexaenoic acid are more widely recognized by their systematic names of EPA and DHA than the shorthand notation of 20:5n-3 and 22:6n-3, so the former nomenclature was adopted for those two FAs. All FAs were quantified as both proportions (% total FA mass) and concentrations (mg m^{-3}). Proportional data were used to evaluate marker FAs and FA ratios. Diatoms typically have higher proportions of 16:1n-7 than 16:0, and greater levels of FAs with 16 carbon atoms than of those with 18 carbon atoms, allowing the ratios of 16:1n-7/16:0 and ΣC16 FA/ΣC18 FA to be used to determine the relative importance of diatoms (Claustre *et al.*, 1989; Dalsgaard *et al.*, 2003). FA concentration data was used to investigate relationships with chlorophyll-*a*. Data were log-transformed, and a linear equation was fitted to the log-transformed data using a model I regression (Figure 2).

Satellite images

A total of 57 daily images (Level-2) of remote sensing reflectances from the European Space Agency's MERIS (Medium Resolution Imaging Spectrometer) sensor were downloaded from the NASA Ocean Color website (http://oceandata.sci.gsfc.nasa.gov/, last accessed 1 June 2013) for the month of May 2010, chosen to represent an intermediate month between the months in which *in situ* samples were collected (April, July and August). Daily chlorophyll-*a* concentrations were calculated from the remote-sensing reflectances (Sathyendranath *et al.*, 2004), and a monthly composite image was generated for the month of May by taking the mean of all the available daily values (Figure 3a). Phytoplankton C was then estimated from the satellite-derived chlorophyll-*a* concentrations by relating phytoplankton C to HPLC-derived chlorophyll-*a* concentrations (Sathyendranath *et al.*, 2009). Total FAs (mg m^{-3}) were derived from the regression results of Figure 2a using the remotely sensed chlorophyll-*a* concentration. The ratio of total FAs to phytoplankton carbon was then calculated, and daily images were composited to obtain monthly values (Figure 3b). It was anticipated that FAs would represent a predictable fraction of total phytoplankton C; the composite image of total FAs to phytoplankton C thus provides a means of studying the variation in this ratio over large scales in the natural environment.

For each daily image used to derive the monthly composite above, the chlorophyll-*a* attributed to diatoms was calculated on a pixel-by-pixel basis (Sathyendranath *et al.*, 2004). The concentration of EPA (mg m^{-3}) was calculated from the regression results of Figure 2c. Diatom chlorophyll-*a* was multiplied by a factor of 56, following Sathyendranath *et al.* (2009), to yield diatom carbon. EPA concentration was then divided by this diatom carbon concentration to generate the ratio for all available pixels for all the images. The images of ratios were averaged over the month of May 2010 to generate Figure 3c. When applied to production estimates of diatom C from the literature (Denman *et al.*, 2007; Uitz *et al.*, 2010), the mean ratio of EPA:diatom C allowed EPA production to be estimated (see Discussion).

Results

FA data

The FA proportional data for samples from both sites were generally similar with 16:0, 16:1n-7, EPA and DHA dominating the profile and comprising ~50% of the total mass of FAs identified (Table 1). However, proportions of specific FAs did vary, with BF samples having lower levels of 16:1n-7 and higher levels of 18 carbon FAs, specifically 18:1n-9, 18:1n-7, 18:2n-6 and 18:3n-3. Mean proportions of EPA in the SS samples were twice that of BF samples.

The relationships of marker FAs and FA ratios revealed trends across the individual samples from the two regions. For instance, the diatom marker ratios of 16:1n-7/16:0 and ΣC16/ΣC18 (Figure 4a) were quite low and constant in BF samples with the exception of ~12 samples collected in August. For the SS, diatom marker levels were generally higher, pointing to a greater contribution of diatom biomass in those samples. There was also a strong positive correlation between the two marker ratios in all samples (Spearman's Rank Correlation: $\rho = 0.927$, $p < 0.001$). We found a similar positive correlation of EPA with both diatom markers (Figure 4b; $\rho > 0.72$ for both; $p < 0.001$), but not with DHA, the flagellate marker. A significant negative correlation of the diatom marker, 16:4n-1, with the flagellate marker, 18:5n-3 (Figure 4c), was also noted ($\rho = -0.60$; $p < 0.001$).

FA and chlorophyll-*a* concentrations

Total FA and chlorophyll-*a* concentrations ranged from 7.9–220 mg m^{-3} and 0.6–9.4 mg m^{-3}, respectively. BF samples spanned these ranges but all SS samples had FA concentrations <75 mg m^{-3} and thus served to strengthen the correlation at low FA concentrations. The relationship between total FAs and total chlorophyll-*a* was significant (Figure 2a; $p < 0.01$; $r^2 = 0.52$; $n = 79$):

$$(total\ fatty\ acid) = 15^{*}(total\ chlorophyll\text{-}a)^{1.0} \quad (1)$$

Correlations were improved when the diatom markers, 16:4n-1 and EPA, were regressed with diatom-associated chlorophyll-*a* (Figure 2b and c; $p < 0.01$ for both; $r^2 = 0.67$ for 16:4n-1 and $r^2 = 0.57$ for EPA):

$$(16{:}4n\text{-}1) = 0.47^{*}(diatom\ chlorophyll\text{-}a)^{1.1} \quad (2)$$

$$(EPA) = 2.2^{*}(diatom\ chlorophyll\text{-}a)^{0.95} \quad (3)$$

The dinoflagellate marker, 18:5n-3, had a significant correlation with non-diatom chlorophyll-*a* (Figure 2d; $p < 0.01$; $r^2 = 0.73$):

$$(18{:}5n\text{-}3) = 2.1^{*}(non\text{-}diatom\ chlorophyll\text{-}a)^{1.0} \quad (4)$$

The correlation of DHA with non-diatom chlorophyll-*a* was significant but weak (Figure 2e; $p < 0.01$; $r^2 = 0.38$):

$$(DHA) = 3.6^{*}(non\text{-}diatom\ chlorophyll\text{-}a)^{0.69} \quad (5)$$

There was no correlation of the diatom markers, 16:4n-1 and EPA, with non-diatom chlorophyll-*a* (Figure 2f and g).

Satellite images

Composite images of total or specific FAs (data not shown) mirrored that of chlorophyll-*a* (Figure 3a) because of the correlation between these properties (FA concentrations were derived from the total or fractional chlorophyll-*a* concentrations using the

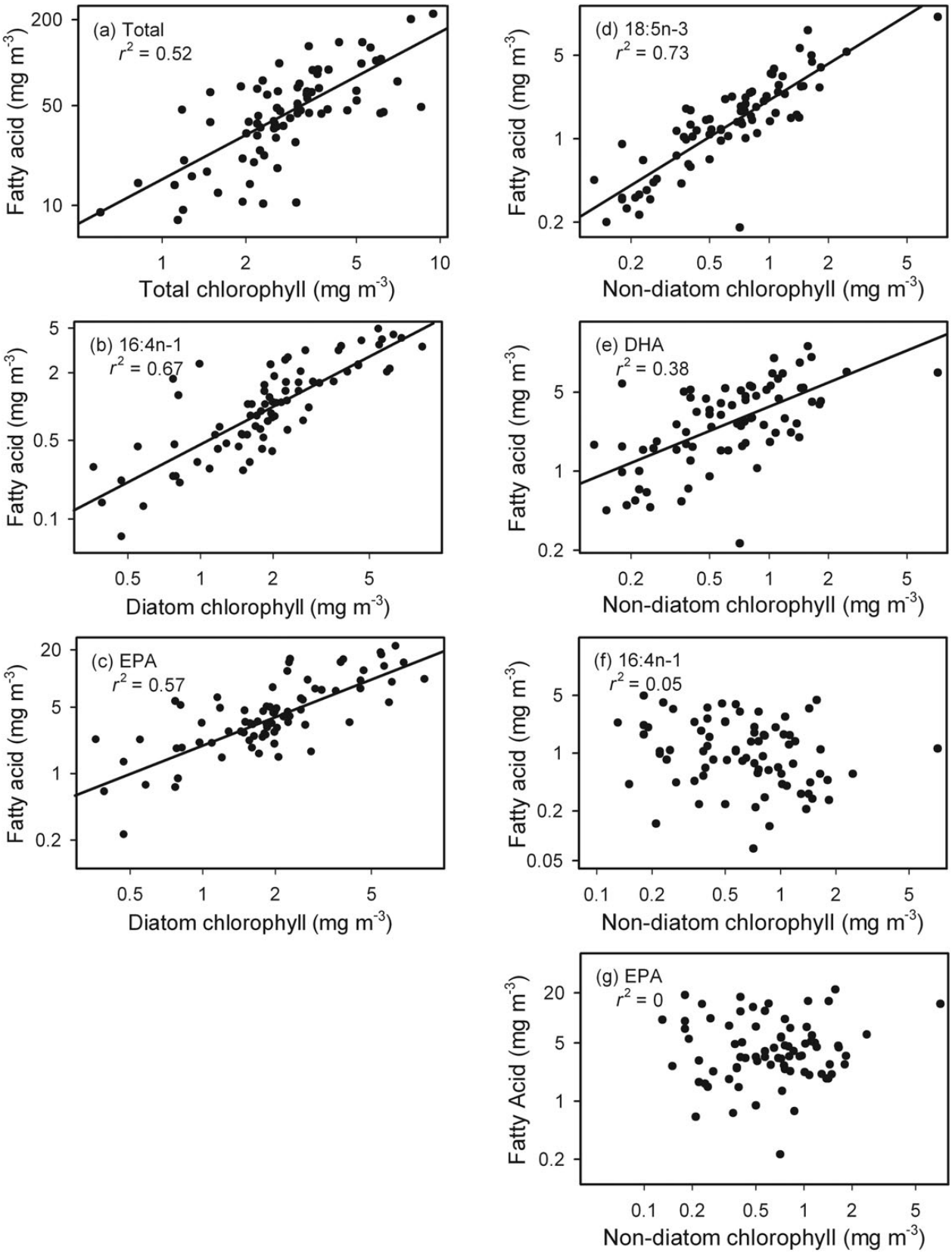

Figure 2. *In situ* concentrations of FAs derived from phytoplankton plotted against *in situ* chlorophyll-*a* concentration in samples ($n = 77$) collected in July and August 2008 in the Bay of Fundy, NS, and April 2011 on the Scotian Shelf, NS. (a) total FAs vs. total chlorophyll-*a*; (b) 16:4n-1 vs. diatom chlorophyll-*a*; (c) EPA vs. diatom chlorophyll-*a*; (d) 18:5n-3 vs. non-diatom chlorophyll-*a*; (e) log DHA vs. non-diatom chlorophyll-*a*; (f) 16:4n-1 vs. non-diatom chlorophyll-*a*. (g) EPA vs. non-diatom chlorophyll-*a*.

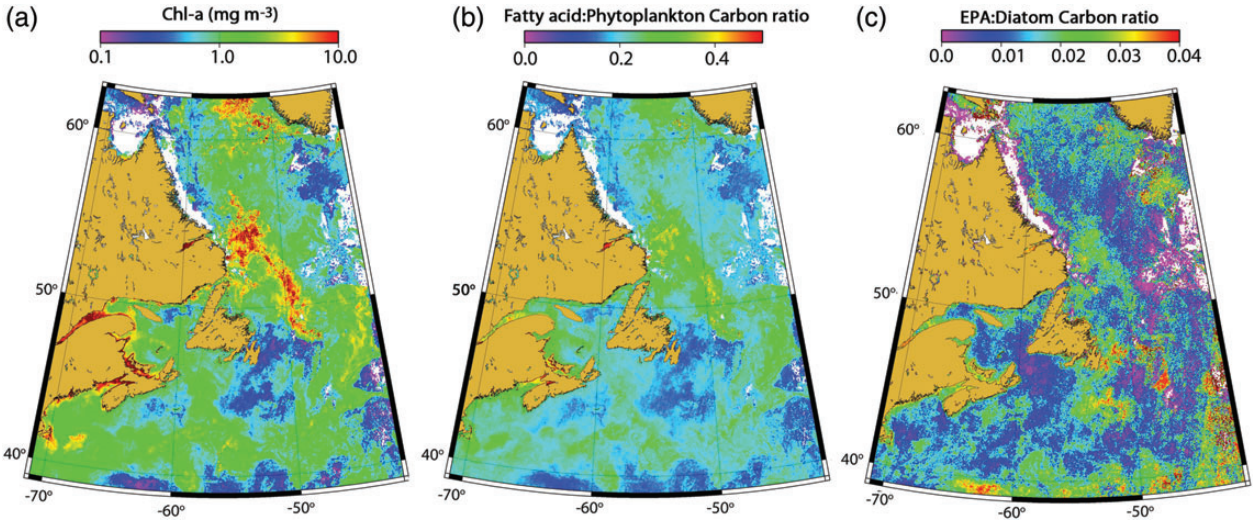

Figure 3. Composite images for May 2010. (a) Total chlorophyll-*a* (mg m^{-3}). (b) Ratio of total FAs (mg m^{-3}) to total phytoplankton carbon (mg m^{-3}). (c) Ratio of EPA (mg m^{-3}) to diatom carbon (mg m^{-3}).

linear relationships shown in Figure 2). Similarly, the composite image of the ratio of total FAs to phytoplankton C also paralleled that of total chlorophyll-*a* distribution, although the range of values was much smaller (0.1–0.5; Figure 3b). In contrast, the ratio of EPA to diatom carbon (Figure 3c) relies on the chlorophyll concentration derived from diatoms, so the patterns are different from those in the other two maps. Areas in the southeast had lowest chlorophyll-*a* concentrations (~1 mg m^{-3}; Figure 3a) but were among the areas of highest EPA:diatom C (Figure 3c), while areas of moderate chlorophyll-*a* (~1 mg m^{-3}; Figure 3a) corresponded to areas of either moderate or low EPA:diatom C.

Discussion

Method development

Our goal in this work was to develop a technique to allow the estimation of total or specific FAs using satellite ocean colour data. Because phytoplankton produce class-specific FAs, it is important to sample from as diverse a community as possible. We used phytoplankton biomarkers to assess the major phytoplankton classes present. For instance, in BF, the toxic dinoflagellate, *Alexandrium* spp., is known to make a substantial contribution to biomass in summer, occasionally being responsible for harmful algal blooms (Martin *et al.*, 2009). The flagellate markers, 18:5n-3 and DHA, support this, with the mean values being greater in BF than SS samples, suggesting a greater contribution of flagellates to the FAs recovered. The flagellate markers are also present in SS samples in spring and are much more likely derived from prymnesiophytes, such as *Phaeocystis* sp. (Gieskes *et al.*, 2007), rather than from dinoflagellates. In contrast, diatom markers dominated SS samples, as well as those collected in August in BF (Figure 4). The spring bloom typically occurs in late March to April on the SS (Song *et al.*, 2011; Zhai *et al.*, 2011), and total chlorophyll-*a* concentrations of ~3 mg m^{-3} suggest that these samples represent the spring diatom bloom. Thus, the biomarker data indicates that we have captured a range of FAs and chlorophyll-*a* concentrations derived from several classes of phytoplankton, likely representing typical communities in both shelf and near-shore oceanographic regions in the Northeast Atlantic.

Sathyendranath *et al.* (2009) developed a model to infer phytoplankton carbon as a function of chlorophyll-*a* concentration, leading to our hypothesis that FA concentrations are also correlated with chlorophyll-*a* (Figure 2a). This hypothesis was based on the assumption that FAs would be related to total phytoplankton carbon in a predictable manner. Our composite image of total FAs to total phytoplankton C (Figure 3b) yielded a five-fold range in ratios from 0.1–0.5 in the North Atlantic. Since the equation relating total FAs to total chlorophyll-*a* concentration (Equation 1) is linear, variability in the ratio of FAs to phytoplankton C is arising solely from the non-linearity in the relationship between chlorophyll-*a* and total phytoplankton C (Sathyendranath *et al.*, 2009). This relatively small variation in total FAs:phytoplankton C compared with that seen in total chlorophyll-*a* in the same area suggests that, as a first approximation, total FAs may be expressed as a specific fraction of total phytoplankton C. These FAs:phytoplankton C ratios (0.1–0.5) are somewhat larger than those found with nutrient-replete algal cultures of 0.08–0.2 (Marañón and Gonzáles, 1997; Riebesell *et al.*, 2000; Hamanaka *et al.*, 2002). However, studies have demonstrated that algae living in natural conditions usually experience nitrogen limitation when compared with laboratory cultures, which has been shown to lead to an increased production of lipids and FAs relative to biomass (Mock and Kroon, 2002; Hu *et al.*, 2008; Jiang *et al.*, 2012). In addition to nitrogen availability, FA concentrations in algal cultures have been shown to vary with phosphorus and silicate levels (Lombardi and Wangersky, 1991; Reitan *et al.*, 1994; Klein Breteler *et al.*, 2005; Adams and Bugbee, 2013), as well as temperature and light intensity (Roessler, 1990; Renaud *et al.*, 1995; Hu *et al.*, 2008). Total chlorophyll-*a* concentrations are also known to decrease with nutrient depletion (Riemann *et al.*, 1989), while adaptation to low light levels leads to an increase in the intracellular chlorophyll-*a* concentration (Cullen, 1982). Thus, the correlation established between total FAs and chlorophyll-*a* (Figure 2a) may be modulated by changes in the light and nutrient environments. Data from more diverse regimes are required to understand how the relationships between FAs and chlorophyll-*a* reported in this work may be modulated by environmental conditions such as temperature, nutrients and light.

Correlations between FAs and phytoplankton pigments were improved when chlorophyll-*a* was partitioned into a diatom fraction and regressed with the diatom markers, 16:4n-1 and EPA (Figure 2b and c). The FA 16:4n-1 is almost exclusively derived

Table 1. FA composition (weight percentage of total; mean ± sd) of *in situ* samples collected in the Bay of Fundy ($n = 56$) and on the Scotian Shelf ($n = 21$).

Fatty acid	Bay of Fundy	Scotian Shelf
Saturated		
i-14:0	0.33 ± 0.12	0.46 ± 0.16
14:0	7.94 ± 2.04	7.79 ± 1.48
i-15:0	0.50 ± 0.22	0.36 ± 0.11
ai-15:0	0.34 ± 0.14	0.28 ± 0.10
15:0	0.77 ± 0.22	0.68 ± 0.19
i-16:0	0.51 ± 0.52	0.42 ± 0.38
16:0	19.73 ± 2.72	14.41 ± 3.20
17:0	0.35 ± 0.13	0.31 ± 0.16
18:0	3.49 ± 1.42	2.71 ± 1.79
Subtotal	33.97 ± 3.71	27.42 ± 5.77
Monounsaturated		
14:1n-5	0.41 ± 0.22	0.95 ± 0.36
16:1n-11	1.07 ± 0.57	0.12 ± 0.07
16:1n-9	0.89 ± 0.41	0.65 ± 0.34
16:1n-7	10.96 ± 5.74	17.23 ± 11.56
16:1n-5	0.43 ± 0.11	0.78 ± 0.24
18:1n-9	4.42 ± 1.51	2.92 ± 1.60
18:1n-7	2.51 ± 0.94	1.20 ± 0.35
20:1n-9	0.44 ± 0.50	0.15 ± 0.23
20:1n-7	0.45 ± 0.32	1.89 ± 1.83
22:1n-11	0.45 ± 0.73	0.18 ± 0.28
Subtotal	22.04 ± 4.48	26.06 ± 8.77
Polyunsaturated		
16:2n-4	1.17 ± 0.65	1.50 ± 0.75
16:3n-6	0.71 ± 0.47	0.00 ± 0.00
16:3n-4	0.87 ± 0.55	1.53 ± 0.96
16:4n-3	0.76 ± 0.52	0.12 ± 0.07
16:4n-1	2.08 ± 1.13	5.09 ± 2.44
18:2n-6	2.72 ± 1.21	1.59 ± 0.64
18:3n-3	2.80 ± 1.38	1.10 ± 0.72
18:4n-3	6.52 ± 1.58	5.34 ± 0.94
18:5n-3	3.83 ± 1.63	3.06 ± 2.00
20:2n-6	0.25 ± 0.14	0.20 ± 0.07
20:4n-6	0.30 ± 0.15	0.13 ± 0.06
20:4n-3	0.31 ± 0.07	0.33 ± 0.10
EPA	8.44 ± 2.97	15.39 ± 4.43
DHA	7.06 ± 2.80	5.80 ± 2.96
Subtotal	37.83 ± 5.39	41.19 ± 6.96
Total	93.83 ± 1.42	94.67 ± 2.19

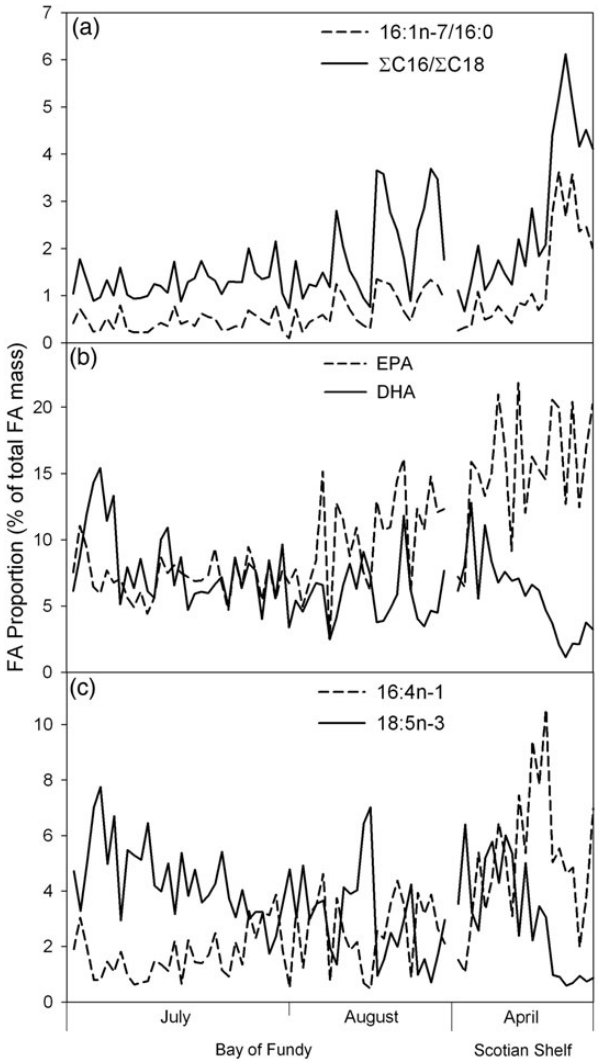

Figure 4. FA markers and marker ratios for samples collected in the Bay of Fundy in July and August of 2008 and on the Scotian Shelf in April of 2011. (a) Both ratios are markers of diatoms. (b) EPA is a diatom marker, DHA is a flagellate marker. (c) 16:4n-1 is a diatom marker, 18:5n-3 is a flagellate marker.

from diatoms, and its strong correlation with diatom-associated chlorophyll-*a* offers independent support that, in these *in situ* samples, estimated diatom chlorophyll-*a* (as derived from HPLC accessory pigments) was truly diatom-derived. Similarly, the complete lack of correlation of the diatom markers, 16:4n-1 and EPA, with non-diatom chlorophyll-*a* (Figure 2f and g) implies once again that these FAs were derived principally from diatoms. The significant relationship between EPA and diatom-chlorophyll-*a* is of particular importance because it allows us, for the first time, to derive estimates of basin- to global-scale concentrations of essential EPA from remotely sensed chlorophyll-*a* data.

Analogous to the diatom marker 16:4n-1, the relationship between the flagellate marker 18:5n-3 and non-diatom chlorophyll-*a* (Figure 2d) suggests that flagellates were the source of the non-diatom fraction of chlorophyll-*a*. One would thus anticipate a strong relationship between the flagellate marker DHA and non-diatom chlorophyll-*a*. This was not the case, however: the relationship explained only 30% of the variation in the data (note that the correlation was significant, but the fit was much poorer than other comparisons). In our study area, it is likely that DHA was not synthesized primarily by flagellates and is thus not a good indicator of flagellate abundance. There are reports that some species of diatoms can synthesize DHA (Kayama *et al.*, 1989; Viso and Marty, 1993; Dalsgaard *et al.*, 2003) at low levels, but it is unlikely that they could be responsible for producing DHA at proportions >5% of total FAs as seen here. It is more probable that an algal source other than diatoms or flagellates was making a substantial contribution to DHA.

In this study, flagellates in general were considered as the source of non-diatom chlorophyll; in the BF, flagellates are primarily represented by dinoflagellates, but on the SS in spring, they are much more likely to be comprised of prymnesiophytes, specifically *Phaeocystis* sp. (Gieskes *et al.*, 2007). It may be that these two classes are not synthesizing DHA in the same proportion as they synthesize chlorophyll-*a*, thus contributing to the poor fit of DHA to

non-diatom chlorophyll-*a* concentrations. If non-diatom chlorophyll-*a* could be better apportioned to specific algal classes, the fit would be expected to improve.

Applications

Sathyendranath *et al.* (2009) developed a simple conceptual model to infer *in situ* phytoplankton carbon as a function of chlorophyll-*a*, allowing indirect estimates of the carbon:chlorophyll ratio of phytoplankton in the sea. This method can be applied to satellite data to map phytoplankton carbon:chlorophyll ratios on synoptic scales. By combining this carbon:chlorophyll-*a* ratio with the relationships established here for FAs and chlorophyll-*a*, and using global primary production estimates from the literature, in units of C, we can begin to estimate the production of essential FAs in the ocean. This is an important extension because it provides us with additional measures to explore the drivers of well-known phenomena. For instance, correlations of growth rate, hatching time and recruitment of shrimp and haddock with the timing of the spring bloom (Platt *et al.*, 2003; Fuentes-Yaco *et al.*, 2007; Koeller *et al.*, 2009) have been used to support the match–mismatch hypothesis. With methods such as those developed here, we can begin to examine the role of essential, and potentially limiting, FAs in these relationships.

The strong correlation between chlorophyll-*a* and total FAs yields satellite maps of FA distribution which are very similar to those of chlorophyll-*a* distributions. More interesting are the maps of the ratios of total FAs:phytoplankton C and EPA:diatom carbon (Figure 3b and c). As noted above, the composite image of FAs:phytoplankton C was similar to the chlorophyll map but had less variation. This can be attributed to the fact that total FAs display a linear relationship with chlorophyll-*a*, while phytoplankton C varies non-linearly with chlorophyll-*a* concentration (Sathyendranath *et al.*, 2009), according to a power law with the power being significantly < 1. Thus, the increase in both FAs and phytoplankton C with increasing chlorophyll-*a* results in much less variation in the FAs:C ratio than in chlorophyll-*a* itself in the same region.

In contrast, the map of EPA:diatom C showed a markedly different distribution to that of chlorophyll-*a*. Notably, the highest EPA:diatom C ratios correspond to the areas of lowest chlorophyll-*a* concentration, such as those found in the nutrient-poor oligotrophic Gulf Stream (lower portion of the image, Figure 3c). Like total FAs, diatom cultures experiencing nutrient limitation also show increased EPA concentrations relative to biomass (Harrison *et al.*, 1990; Reitan *et al.*, 1994), although the effect is less distinct than for total FA. This will lead to high ratios of EPA:diatom C in nutrient-limited areas, as was observed in our study. On the other hand, it may also be that the relationship we have reported for EPA as a function of diatom chlorophyll-*a* does not hold at very low chlorophyll concentrations, since our lowest *in situ* chlorophyll-*a* concentrations were $\sim$0.6 mg m^{-3}, while the lowest satellite-derived concentrations were in the order of 0.1 mg m^{-3}. Sathyendranath *et al.* (2009) also noted greater scatter in their relationship between particulate C and chlorophyll-*a* at low chlorophyll-*a* concentrations. Since diatom C is derived indirectly from total particulate C, it is likely that uncertainty in that relationship is also contributing to the unexpectedly high EPA:diatom C values in certain oligotrophic conditions.

Estimates of global EPA production

Using published estimates of global primary production in units of carbon and the mean mass ratio of EPA:diatom C from the images here (0.015), we can derive a first estimate of total global EPA production by diatoms in the ocean as follows:

$$\textit{Total marine EPA production} = C_G{}^{*}P_D{}^{*}P_{EPA}$$

where C_G is global primary production (50 Gt C year^{-1}; Denman *et al.*, 2007), P_D is the proportion of primary production derived from diatoms (0.32; Uitz *et al.*, 2010), and P_{EPA} is the proportion of EPA relative to carbon in diatoms (0.015; Figure 3c). This sets the maximum global production of EPA at 240 Mt year^{-1}.

Since EPA is an essential FA for human health, an important quantity is the proportion of global EPA production necessary to meet human nutritional requirements. The estimates for recommended daily intake (RDI) of EPA fall in the range of 100–305 mg (see Supplementary data). With a global population of seven billion and applying a median RDI of 250 mg, a minimum of 0.64 Mt year^{-1} of EPA is required to meet human health requirements alone. Since dietary EPA is sourced almost entirely from fish or extracted fish oils, we must therefore also consider the efficiency of transfer of EPA through the food web. Assuming transfer efficiencies of essential FA are equivalent to those of carbon at 10% (Lindeman, 1942), in a presumed three-trophic-level foodweb, only 1% of the 240 Mt year^{-1} EPA production can be expected to be available for consumption by higher trophic level consumers, representing 2.4 Mt year^{-1}. Humans alone require 0.64 Mt year^{-1}, or $\sim$25% of the global EPA production available at that trophic level, leaving the remainder to support the entire marine ecosystem at the third or higher trophic level.

With increasing world population, we expect that the proportion of EPA production required for human nutrition will only grow over time. Perhaps most concerning is that with this estimate, only $\sim$1.8 Mt EPA (or $\sim$75% of production at the third trophic level) remains in the ecosystem to support all other vertebrates in the marine environment from carnivorous fish to marine mammals. In teleost fish, essential FAs are necessary in specific proportions to ensure larval survival, and have integral roles in regulating immune response and membrane fluidity (Tocher, 2003; Arts and Kohler, 2009). On a global basis, inadequate supply of EPA may be one of the factors contributing to the recruitment failure of fish stocks.

We stress that the value put forward here for annual global EPA production is a preliminary estimate, based on extrapolation of data from the Northwest Atlantic Ocean to the global oceans, and merely represents an initial attempt to calculate the order of magnitude of oceanic production of an essential FA. We acknowledge that there are many limitations and sources of error in this type of global extrapolation, including the fact that EPA concentrations may vary depending on growth phase, light regime and local nutrient conditions. Nevertheless, it is useful as a proof-of-concept of the technique, and in the future may be refined further to minimize the numerous potential sources of error.

Improving the accuracy of our EPA production estimates requires a better understanding of the variations in the relationship of EPA to diatom C. For instance, collecting data from areas with different nutrient and light regimes would help to define the relationship at the extreme high and low values of chlorophyll-*a* concentrations. Greater temporal range in sample collection would also allow us to address seasonality in the relationship.

Another critical assumption in our calculations is the proportional contribution of diatoms to total global marine primary production. We used a value of 32% for all microphytoplankton (cells > 20 μm, including diatoms) but this is likely an overestimate

since it also includes a contribution from dinoflagellates (Uitz *et al.*, 2010). These authors assumed that fucoxanthin was a marker for diatoms alone, but there is also a contribution of this pigment from prymnesiophytes (see for example, Devred *et al.*, 2011). This could lead to an overestimation of the global EPA production.

Conversely, the availability of EPA at the third trophic level may be underestimated. We used a trophic transfer efficiency of 10% (Lindeman, 1942; Pauly and Christensen, 1995), but there is evidence to suggest that FAs may be transferred more efficiently than bulk carbon. In a shallow reservoir, Gladyshev *et al.* (2011) found that specific groups of polyunsaturated FAs, including EPA, were transferred approximately twice as efficiently as bulk carbon. Application of a 20% transfer efficiency to our data from the marine environment would yield an estimate of ~9.6 Mt year^{-1} EPA at the third trophic level, with human requirements therefore only representing ~7% of total available EPA. Certainly, reliable estimates of trophic transfer efficiencies are key in accurately estimating the oceanic supply of EPA available to consumers.

We have demonstrated here the use of remote sensing to monitor the regional fluctuations in an essential nutrient. We have also presented a first step towards estimating global marine production of EPA by diatoms, and the extent to which it is available for human consumption. Further pursuit of this approach, with additional data from across the globe under various natural environmental conditions, will allow us to refine the estimate of EPA production and, ultimately, address concerns about food security for a growing human population in a changing climate. It is now apparent that to better understand ecosystem function and its ability to meet human nutrition requirements, we must measure the flow of essential FAs in the ocean.

Supplementary data

A description of current recommended daily intake (RDI) of EPA (based on six national and international organizations) and the rationale for the mean value used in this work are available at *ICES Journal of Marine Science* online.

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