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Source identification of the Arctic sea ice proxy IP₂₅

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Analysis of the organic geochemical biomarker IP₂₅ in marine sediments is an established method for carrying out palaeo sea ice reconstructions for the Arctic. Such reconstructions cover timescales from decades back to the early Pleistocene, and are critical for understanding past climate conditions on Earth and for informing climate prediction models. Key attributes of IP₂₅ include its strict association with Arctic sea ice together with its ubiquity and stability in underlying marine sediments; however, the sources of IP₂₅ have remained undetermined. Here we report the identification of IP₂₅ in three (or four) relatively minor (<5%) sea ice diatoms isolated from mixed assemblages collected from the Canadian Arctic. In contrast, IP₂₅ was absent in the dominant taxa. Chemical and taxonomical investigations suggest that the IP₂₅-containing taxa represent the majority of producers and are distributed pan-Arctic, thus establishing the widespread applicability of the IP₂₅ proxy for palaeo Arctic sea ice reconstruction.

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The reconstruction of sea ice conditions in the polar regions represents a key objective within palaeoceanography and palaeoclimatology¹. This is largely owing to the major role that sea ice has in controlling the energy budget at the Earth's surface, due to its high albedo, and also since it limits heat, gas and moisture exchange between the oceans and the atmosphere^{2,3}. Further, sea ice contributes to ocean ventilation and circulation via brine rejection and freshwater input, following formation and melting, respectively².

Performing such reconstructions is challenging, however, since sea ice itself leaves no direct legacy signature in geological archives, necessitating the use of so-called proxy methods. A number of proxies for sea ice exist and these are both biogenic¹ and non-biogenic in origin^{4–6}. Within the former category, the recent discovery of the organic geochemical biomarker IP₂₅ (ref. 7) (a C₂₅ highly branched isoprenoid (HBI) lipid⁸) has attracted considerable interest, not least because it possesses the unique attribute of being produced within the sea ice itself. Consistent with this sea ice origin, the occurrence of IP₂₅ in marine sediments shows a strong correlation, spatially, to seasonally ice-covered Arctic waters⁹, and, from a temporal perspective, IP₂₅ appears to be stable in sediments for millions of years¹⁰. Combined, these attributes of IP₂₅ have provided the foundation for decadal to millennial-scale sea ice reconstructions across the Arctic^{9–15}.

Despite these interesting and valuable applications of the IP₂₅ sea ice proxy, the source organisms responsible for formation of this lipid in sea ice have remained elusive. A diatom source has been proposed^{7,16}, however, since IP₂₅ has been reported in Arctic sea ice biota dominated by diatoms^{16,17} and similar lipids occur in some non-sea ice algae^{18–22}. More specifically, it has been hypothesized that sea ice diatoms belonging to the *Haslea* genus are likely producers of IP₂₅ on the basis of biosynthesis of related biomarkers by such species^{7,16,17,23–25}. Nevertheless, IP₂₅ has never been reported in any cultures of diatoms, including some *Haslea* species isolated from Arctic sea ice⁹. Arguably, until the sources of IP₂₅ have been determined, together with information regarding their distributions across the Arctic, the potential for IP₂₅ as a palaeo sea ice proxy cannot be fully realized.

In the current study, we identify three (or four) sea ice endemic diatom species that are responsible for IP₂₅ production in Canadian Arctic sea ice during a spring bloom. We achieve this by isolation of individual cells from mixed sea ice diatom assemblages and analysis of their lipid composition by gas chromatography–mass spectrometry (GC–MS). The identified IP₂₅ producers (*Pleurosigma stuxbergii* var. *rhomboides* (Cleve in Cleve and Grunow) Cleve, *Haslea kjellmanii* (Cleve) Simonsen, *H. crucigeroides* (Hustedt) Simonsen and/or *H. spicula* (Hickie) Lange-Bertalot) constitute a relatively minor proportion of the ice taxa, but, significantly, they are nonetheless common pan-Arctic species that validate the notion that IP₂₅ represents a widespread proxy for palaeo Arctic sea ice.

Results

Identification of IP₂₅ producers. Taxonomic assessment of the diatom assemblage within several algal aggregates from Resolute Bay in the Canadian Arctic revealed a distinctive flora, characteristic of sea ice^{26–28}. Thus, the three most abundant species were *Navicula pelagica* (ca. 40–50%), *Nitzschia frigida* (ca. 10–20%) and *Pauliella taeniata* (ca. 10–20%) (Table 1), with a near absence of planktic cells (<0.5%). From one assemblage (SIA-1), we isolated and combined sufficient numbers of cells from (at least) six individual species to perform quantitative lipid analysis by GC–MS to show that IP₂₅ was present in *P. stuxbergii*

var. *rhomboides* and at least two species from the genus *Haslea* (*H. kjellmanii*, *H. crucigeroides* and/or *H. spicula*) (Fig. 1), but was absent in other important ice algal species; *N. pelagica*, *N. frigida*, and *Entomoneis paludosa* (Fig. 1 and Table 2).

The occurrence of IP₂₅ in *H. crucigeroides* (and/or *H. spicula*) and *H. kjellmanii* is consistent with the production of other HBIs by members of the genus *Haslea*^{18,19,25,29–31}, which led to the previous suggestion that *Haslea* was a likely source of IP₂₅ in Arctic sea ice^{7,16,23,24}, despite the failure for such species to produce IP₂₅ in culture²⁹. The identification of *P. stuxbergii* var. *rhomboides* as an IP₂₅ producer is also consistent with the formation of other HBIs by several *Pleurosigma* diatoms^{20,32}, although we are unaware of any reports describing the HBI content of *P. stuxbergii* var. *rhomboides*, per se. In contrast, IP₂₅ was absent in at least two of the typically abundant ice flora (viz, *N. pelagica* and *N. frigida*), supporting the notion that IP₂₅ is produced selectively by a limited number of Arctic sea ice diatom taxa^{7,16,23,24}. Indeed, the same conclusion was reached following taxonomic analysis and lipid characterization of mixed diatom assemblages in sectioned sea ice cores collected from the same location as the samples described here¹⁶.

Previously, it has been shown that, although the physiology of diatom genera can be important for production of HBIs, not all species within such genera are HBI producers. Thus, some species within the *Haslea*, *Pleurosigma* and *Navicula* genera are known HBI producers, but others are not. For example, species such as *H. ostrearia*¹⁸ and *P. intermedium*³² produce HBIs, but *H. wawriake* and *P. angulatum* do not²⁹. Within the current context, we note that IP₂₅ was identified in *H. kjellmanii*, but was absent in *H. vitrea* (Table 2). IP₂₅ was also found in *H. crucigeroides* and/or *H. spicula*; however, since *H. crucigeroides* and/or *H. spicula* could not be distinguished during the cell isolation (see Methods section), we are unable to conclude whether both (or only one) of these are IP₂₅ producers. IP₂₅ and other HBIs were absent in cells of *N. pelagica* (Table 2), despite the production of HBIs by some species of *Navicula*²². The absence of IP₂₅ in *N. frigida* is not surprising, however, since there have been no reports of HBI production in the genus *Nitzschia*.

In addition to IP₂₅, the structurally related di-unsaturated HBI biomarker C_{25:2}⁹ was also identified in each of the IP₂₅-producing species (Table 2). Previous studies based on the analysis of IP₂₅ in sea ice and sediments have shown a consistent abundance relationship between these two structural homologues indicating a common source at least within the Arctic^{12,24,33,34}. Our data not only confirm this source association but, the similarity of the C_{25:2}/IP₂₅ ratio in producers (2.3 ± 0.8) to that found in sea ice and sediments^{12,24,33,34}, implies a close link between the source and Arctic sedimentary signatures of these two biomarkers. As such, a significant formation of IP₂₅ over C_{25:2} (or vice versa) in sea ice or differential degradation of either biomarker *in situ*, seems unlikely. Finally, the ranges of intracellular concentrations of IP₂₅ and C_{25:2} in the isolated sea ice diatoms were similar between individual species (Table 2) and to those of HBIs measured in culture¹⁶.

Major IP₂₅-producing taxa. The species selected for lipid analysis corresponded to ~65% of the total taxa in SIA-1, with IP₂₅ producers representing only 1.8% of the total. As such, the absence of IP₂₅ in the abundant *N. pelagica* and *N. frigida* corresponded to ~63% of total cell numbers. Although not all species were investigated (mainly because of low cell numbers of many minor species), these data suggest that the majority of sea ice diatom cells do not synthesize IP₂₅ (Tables 2 and 3), which is consistent with previous lipid-based estimates that indicated only the minority of sea ice flora (1–5%) likely contribute to IP₂₅

Table 1 | Taxonomic composition of diatom aggregates.

	SIA-1	SIA-2	SIA-3	SIA-4
<i>Amphora laevisissima</i>	1.7	1.9	2.0	0.9
<i>Attheya septentrionalis</i>	0.7	1.3	0.4	0.9
<i>Bacillaria paxillifera</i>		1.1		0.5
<i>Coscinodiscus centralis</i>				0.1
<i>Cylindrotheca closterium</i>		0.1	0.2	
<i>Entomoneis gigantean</i>	0.4		0.2	
<i>Entomoneis kjellmanii</i>	0.4	0.4	0.4	1.0
* <i>Entomoneis paludosa</i>	2.0	2.0	3.8	2.2
<i>Fragilariopsis cylindrus</i>	3.9	8.4	7.2	1.3
<i>Gyrosigma tenuissimum</i>			0.7	
*<i>Haslea crucigeroides</i> (and/or <i>Haslea spicula</i>)	1.4	2.4	1.8	0.2
<i>Haslea crucigeroides</i> (and/or <i>Haslea spicula</i>) (empty frustule)		0.4		0.5
*<i>Haslea kjellmanii</i>		0.4	0.7	
<i>Haslea kjellmanii</i> (empty frustule)		0.1	0.2	
* <i>Haslea vitrea</i>		0.1		
<i>Melosira</i> sp.				0.2
<i>Navicula algida</i>	0.2	0.1		0.1
<i>Navicula cf. trigonocephala</i>				0.1
<i>Navicula directa</i>	3.0	3.3	0.4	2.4
<i>Navicula kariana</i>	0.6	0.1	0.2	0.3
<i>Navicula obtuse</i>	0.2	0.4	0.7	0.1
* <i>Navicula pelagica</i>	39.0	44.8		52.8
<i>Navicula septentrionalis</i>		0.4	0.4	0.2
<i>Navicula superba</i>			0.2	
<i>Navicula transitans</i>	0.6	0.8	2.7	0.6
<i>Navicula trigonocephala</i>	0.2		0.7	
<i>Nitzschia arctica</i>	3.1	2.5	6.7	
<i>Nitzschia brebissonii</i> var. <i>borealis</i>				0.1
* <i>Nitzschia frigida</i>	22.1	9.5	14.3	8.7
<i>Nitzschia laevisissima</i>	0.4	1.1	4.5	0.9
<i>Nitzschia neofrigida</i>		0.7	4.2	2.3
<i>Nitzschia pelagica</i>			14.3	
<i>Nitzschia promare</i>		0.1	1.1	
<i>Nitzschia seriata</i>		0.1		
<i>Nitzschia</i> sp.			0.4	
<i>Pauliella taeniata</i>	13.0	11.5	18.0	16.1
<i>Phaeocystis</i> sp.				0.6
<i>Pinnularia quadratarea</i>	1.5	1.1	4.0	2.0
<i>Pinnularia semiinflata</i>	0.2	0.1		
<i>Plagiotropis</i> sp.				0.1
<i>Pleurosigma</i> sp.				0.1
*<i>Pleurosigma stuxbergii</i> var. <i>rhomboides</i>	0.4	0.8	0.2	0.1
<i>Pseudogomphonema arcticum</i>	3.0	1.7	4.0	2.3
<i>Pseudogomphonema groenlandicum</i>	1.1	0.2	2.7	1.0
<i>Stenoneis inconspicua</i>	0.4		0.4	
<i>Synedropsis hyperborean</i>	0.6	2.1	1.8	0.5
<i>Thalassiosira</i> sp.			0.2	0.1
Other microorganisms	0.2	0.2		0.3
Total (%) <i>IP</i> ₂₅ -producing cells	1.8	3.6	2.7	0.3

Abundances (%) of diatoms identified in ice algal aggregates. *IP*₂₅-producing species are indicated in bold.
*Species analyzed for *IP*₂₅.

biosynthesis^{7,16}. Of course, it remains feasible that some other minor taxa may also be *IP*₂₅ producers but, given the low percentages of all unexamined species, together with the consistency in cellular abundances of those taxa that are producers, we suggest that their contributions are not significant. In support of this, we note that none of the unexamined genera (Table 1) are known HBI producers.

In order to confirm that *H. crucigeroides* (and/or *H. spicula*), *H. kjellmanii* and *P. stuxbergii* var. *rhomboides* indeed represented the majority (at least) of the *IP*₂₅-producing species, and

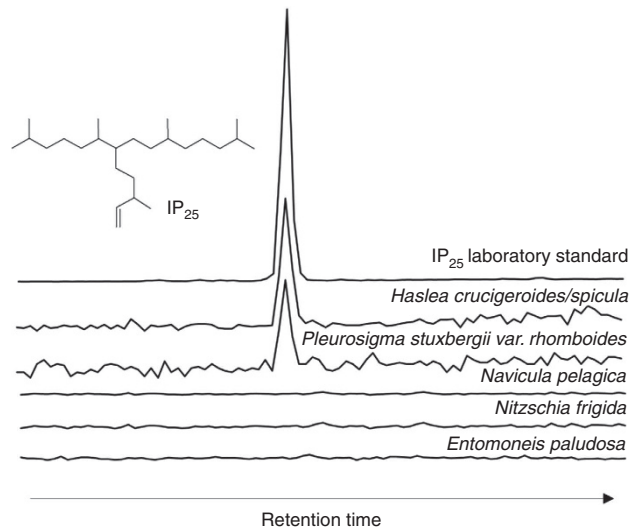


Figure 1 | Lipid extracts from isolated diatoms. Structure of *IP*₂₅ and partial GC-MS (selective ion monitoring; *m/z* 350.3) chromatograms of lipid extracts obtained from various diatoms isolated from the mixed assemblages together with that of an authentic sample of *IP*₂₅ (ref. 57).

that the unanalyzed cells were not significant contributors to the *IP*₂₅ budget, we calculated the HBI abundance of SIA-1 relative to the total organic carbon (TOC) content (HBI/TOC = 0.05%) and compared this ratio with the corresponding value obtained from a previously known HBI-producing species (*H. ostrearia*) in culture (HBI/TOC = 1.4%). The ~30 times lower contribution of HBIs to sea ice algal TOC compared with that found for cultured diatoms provides a reasonable estimate of the percentage of HBI-producing diatoms in the sea ice algal assemblage (~3.6%). This low percentage confirms the relatively small proportion of *IP*₂₅ producers in the total assemblage, while the close similarity of this estimate to the combined percentages of *H. crucigeroides* (and/or *H. spicula*), *H. kjellmanii* and *P. stuxbergii* var. *rhomboides* between SIA-1-4 (Table 2) indicates that these species represent the majority (if not all) of the *IP*₂₅ producers.

Discussion

Having identified the major contributors of *IP*₂₅ in sea ice from a single study location in the Canadian Arctic, we next aimed to address the significance of this discovery with respect to the wider scale applicability of the *IP*₂₅ biomarker for palaeo sea ice studies. To do this, we compiled literature accounts of *P. stuxbergii* var. *rhomboides*, *H. kjellmanii*, *H. crucigeroides* and *H. spicula* (note: the latter two as separate species), and compared findings with reports of *IP*₂₅ in sediments (and sea ice). A summary of the spatial relationship between the two is shown in Fig. 2.

According to Poulin³⁵, *Haslea* and *Pleurosigma* are typical of sea ice biota with five common taxa between them. *H. crucigeroides*, *H. kjellmanii* and *H. spicula* are identified as Arctic/sub-Arctic in distribution³⁵, while *H. crucigeroides* and *P. stuxbergii* var. *rhomboides* are the most commonly reported (Table 1). Owing to the close similarity between *H. crucigeroides* and *H. spicula*³⁶, their occurrences may have been combined in some investigations, as has been the case here for the cell isolation and extraction experiments (see Methods section). Despite their widespread occurrence, however, *H. crucigeroides*, *H. kjellmanii*, *H. spicula* and *P. stuxbergii* var. *rhomboides* are always relatively minor taxa, with abundances usually <5% (Table 3), as we found here (Table 1). Individual studies have reported these *IP*₂₅-producing species in first-year

Table 2 | HBI content of isolated cells.

Diatom species	Contribution to assemblage (%)	No. of cells isolated	IP ₂₅ (pg cell ⁻¹)	C _{25:2} (pg cell ⁻¹)
<i>Haslea crucigeroides</i> (and/or <i>Haslea spicula</i>)	1.4	200–264	0.6–3.4	1.5–10.3
<i>Haslea kjellmanii</i>	ND	40–49	1.9–3.3	4.7–9.0
<i>Haslea vitrea</i>	ND	25	—	—
<i>Pleurosigma stuxbergii</i> var. <i>rhomboides</i>	0.4	100–120	0.6–3.8	0.6–10.7
<i>Entomoneis paludosa</i>	2.0	201	—	—
<i>Navicula pelagica</i>	39.0	200	—	—
<i>Nitzschia frigida</i>	22.1	220	—	—

‘—’ indicates not detected. ND, not determined.

Table 3 | Reported presence of IP₂₅-producing diatoms in sea ice and IP₂₅ in Arctic sediments.

Study region	Year of study	Occurrence of IP ₂₅ -producing diatoms					Combined abundance (%)	Literature source	
		1	2	3	4	5		Species identification	IP ₂₅
Cape Eglinton	1890s	✓*	✓*	✓*		✓*	P	58	23
West Greenland	1890s	✓*	✓*	✓*		✓*	P	58	44
Canada Basin	1893	✓*	✓*	✓*		✓*	P	58	—
Makarov Basin	1894	✓*	✓*	✓*		✓*	P	58	—
Barrow	1964	✓*		✓*			P	59	23
Davis Strait	1978		✓	✓			P	37	23
Beaufort Sea	1980		✓*	✓*			1–5	60	61
Manitounuk Sound	~1980	✓				✓	P	62	63
Barents Sea	1985	✓					1–5	28	42,43
Resolute Bay	1989	✓		✓		✓	<1	28	7,40
Laptev Sea	1987–90	✓		✓		✓	P	38	24,34
East Siberian Sea	1987–90	✓		✓		✓	P	38	23
Chukchi Sea	1987–90	✓					P	38	23
N/E Svalbard	1991			✓			<1	28	64
Franz Josef Land	1991	✓		✓		✓	P	39	24
NE Water Polynya	1993	✓		✓		✓	1–5	65	44
West Greenland	1995				✓		<1	28	—
White Sea	2001–03	✓		✓		✓	P	66	—
McDougal Sound	2001	✓		✓		✓	0.6	67	7,16
McDougal Sound	2003	✓	✓	✓		✓	2.6	7	7,16
Franklin Bay	2004	✓				✓	0.9	68	69
Central Arctic	2004	✓		✓			1–3	49	—
Churchill	2005	✓					2.5	7	7
Resolute Bay	2011	✓	✓	✓		✓	6.1	70	16
Mean							2.2		

‘—’ No published data from this region; P, present but not quantified.

Occurrences of *H. crucigeroides* (1), *H. spicula* (2), *H. kjellmanii* (3), *H. spp* (4) and *P. stuxbergii* var. *rhomboides* (5); and combined percentages identified in taxonomic studies of Arctic sea ice. The approximate study locations are indicated in Fig. 2. Reports of occurrences of IP₂₅ from nearby locations are also indicated.

*Historical synonym.

sea ice for locations ranging from 58 to 87°N, including coastal, shelf and deep ocean environments. Further, such species have been reported in sea ice of varying thickness (for example, 4–300 cm)^{37,38} and type^{28,39}.

With respect to biomarker-based studies, IP₂₅ has also been reported in surface and downcore sediments from across the Arctic, as reviewed by Belt and Müller⁹, while here, we demonstrate the excellent spatial relationship between occurrences of IP₂₅ in Arctic surface sediments (and sea ice) and reports of the IP₂₅-producing diatoms identified in the current study (Table 3 and Fig. 2). For example, *H. crucigeroides*, *H. kjellmanii*, *H. spicula* and *P. stuxbergii* var. *rhomboides* have been identified in sea ice from across the Canadian Arctic and sub-Arctic (Table 1), and IP₂₅ has been identified as a common component in surface sediments from these regions, with downcore abundances providing the basis for Holocene sea ice reconstructions^{9,13,40,41}. Similarly, in the eastern Arctic, where *H. crucigeroides*, *H. kjellmanii*, *H. spicula* and *P. stuxbergii*

var. *rhomboides* are also common, IP₂₅ occurrence in recent sediments from the Kara, Laptev and Barents Sea shows a strong correlation with modern sea ice cover^{24,42}, and this has aided the reconstruction of Holocene (and older) sea ice records from these regions^{10,43}. Further, the observation of IP₂₅-producing diatoms in sea ice from around Svalbard and the North East Polynya (NE Greenland) is consistent with the occurrence of IP₂₅ in nearby surface sediments^{42,44}, and the longest IP₂₅-based palaeo sea ice records to date are also from this region^{10,45}. The majority of these IP₂₅ studies have been conducted in relatively shallow marine settings, proximal to continental shelves while, in contrast, there is currently a paucity of data from deeper oceanographic settings such as the Greenland Sea or the central Arctic Ocean. For the latter, in particular, it has been suggested previously that the combined presence of thick multi-year ice, likely unsuitable for diatom growth, and low sediment accumulation rates may somewhat limit the application of the IP₂₅ proxy method for palaeo sea ice reconstruction⁹. However,

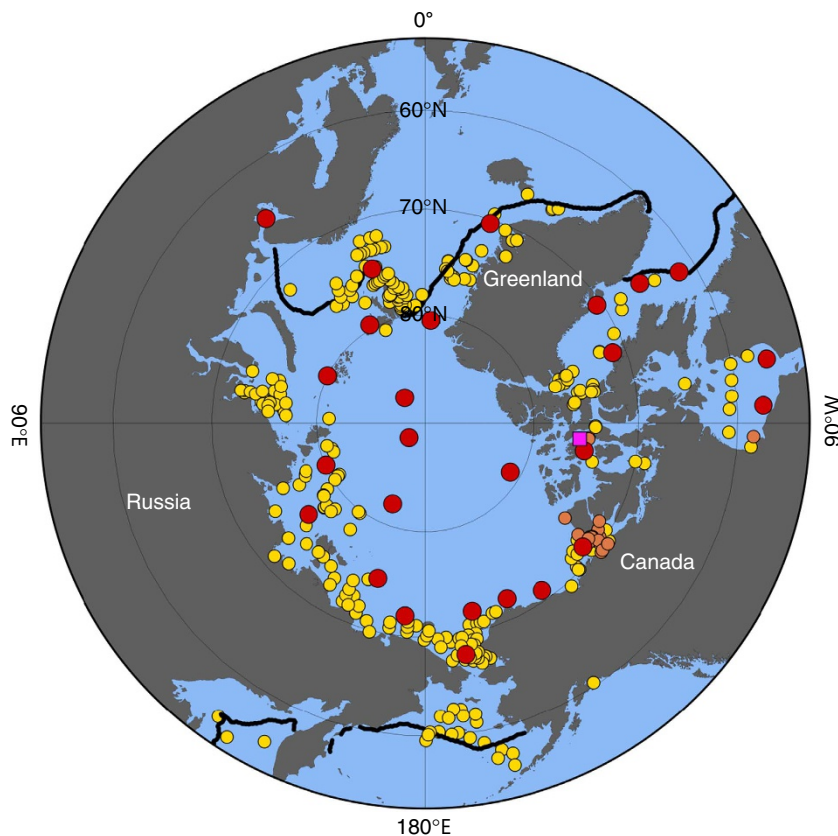


Figure 2 | Distribution of IP₂₅ producers and presence of IP₂₅ in sediments. Summary map showing locations where IP₂₅ has been identified in Arctic surface sediments (yellow circles) and sea ice (orange circles) (Supplementary Data 1). Red circles indicate the approximate locations of sea ice taxonomic studies reporting *Haslea* spp. and *Pleurosigma stuxbergii* var. *rhomboides* (summarized in Table 1). The pink square shows the collection location of the ice algal aggregates analyzed in the current study. The black line shows the median March sea ice extent (National Snow and Ice Data Center; 1981-2010).

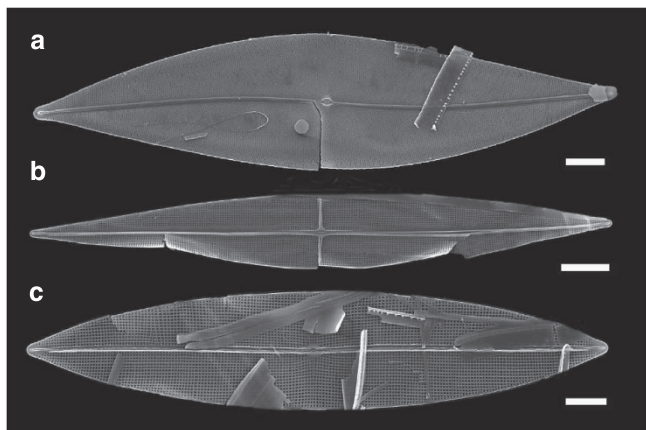


Figure 3 | IP₂₅-producing diatom species. Scanning electron micrographs of IP₂₅-producing diatoms identified in SIA-1. (a) *Pleurosigma stuxbergii* var. *rhomboides*; (b) *Haslea crucigeroides* (and/or *Haslea spicula*); and (c) *Haslea kjellmanii*. Scale bars, 10 μm.

within the context of recent climate change and a reduction in both Arctic sea ice extent and thickness^{46,47}, in particular, multi-year ice is becoming increasingly replaced by thinner first-year ice⁴⁸. Consistent with such a change, we note that some of the IP₂₅ producers identified here have, in fact, been reported in first-year ice within the central Arctic (87°N)⁴⁹, and IP₂₅ has recently been detected in surface sediments >80°N (X. Xiao

and R. Stein, personal communication). We suggest that such observations, in combination with our results here, will likely expand the potential for the IP₂₅ proxy to provide palaeo sea ice reconstruction data for the entire Arctic.

Previously, the identification of IP₂₅ in Arctic sediments has been interpreted as proxy evidence for past seasonal sea ice cover, with variations in sedimentary abundance attributed to corresponding changes in sea ice; an approach supported by a number of meaningful reconstructions⁹. In contrast, the possible influence of ecological controls over IP₂₅ production and their potential impact on its sedimentary abundance have been largely ignored, although its likely importance has been alluded to^{7,9,16}. Having now identified those species that are responsible for IP₂₅ formation, it should be possible, in the future, to not only discuss ecological factors when interpreting sedimentary IP₂₅ distributions, but also to test the significance of these experimentally, and in an informed manner.

Here, we suggest that the relatively consistent contribution of IP₂₅ producers to mixed Arctic sea ice diatom assemblages (Table 3) provides some evidence that the larger temporal changes in IP₂₅ concentration often seen in sediments^{9,12,13,40,45} are more likely attributable to sea ice variations than major modifications to diatom assemblage composition, although changes in overall production may still be important. On the other hand, relatively small or subtle variations in sedimentary abundance may, potentially, simply reflect minor changes in species composition or overall production rather than variations in sea ice cover. What is now clear, however, is that since IP₂₅ production is species specific and restricted to the minority

diatom taxa, conclusions from future investigations into environmental or phenotypic variables over IP₂₅ production will require investigations of specific species, probably via *in situ* measurements in the field, rather than whole assemblage or simple biomass determinations. In this respect, the discovery of the IP₂₅ producers represents a key step in determining the factors that control the production and fate of IP₂₅, which have been identified as important for its development as a palaeo sea ice proxy⁹.

Methods

Sample collection. Four floating ice algal aggregates (SIA-1–4) were collected from a sampling hole cut in first-year ice at Resolute Bay in association with the Arctic-ICE (Ice Covered Ecosystems) project (1–9 June 2012; 74°43.613'N; 95°33.496'W; Fig. 2). Aggregates consisted of sea ice algal assemblages recently sloughed from the underside interstitial channels of first-year ice and were stored in Whirl-Pak bags and frozen (–20 °C). A taxonomic description of the content of sectioned ice cores collected from the same location is given elsewhere¹⁶.

Species identification. Taxonomic identification of diatom species was carried out on each aggregate (Table 1). Sub-samples of each of SIA-1–4 were freeze-dried, and ~10 mg of dried material was re-suspended in 100 ml of artificial seawater. Aliquots (0.5 ml) were taken for cell enumeration using the Utermöhl method⁵⁰. Cell counts (400–600) were performed on parallel transects using an inverted microscope (Nikon Ti-S) at ×60 magnification^{51,52}.

More detailed examination of certain taxa was achieved by dry-mounting sub-samples of cleaned (10% HCl; 70 °C for 30 min and 3 × 10 ml Milli-Q washes) cells and examination using a JEOL 7001 F scanning electron microscope. Specifically, diatoms belonging to the genus *Haslea* (Fig. 3) were identified based upon general morphological dimensions in addition to features considered characteristic of the genus including, for example, the presence of external longitudinal strips over many areolae, with intervening continuous slits^{35,53,54}. Additional characteristic features included elongated helictoglossae, a well-defined accessory rib on the primary side of the raphe sternum and typically straight external raphe fissures with only slight terminal deflection/expansion⁵⁵. Although both *H. crucigeroides* and *H. spicula* were identified in the assemblages as part of the detailed taxonomic analysis (Tatarek, Poland), it was not possible to distinguish between these two species during the low magnification (×40) cell isolation stage (Brown, UK) because of the close similarities between them³⁶. For *P. stuxbergii* var. *rhomboides*, identification was confirmed from the characteristic sigmoidal valve displaying fine striae, with slightly denser oblique than transverse pattern⁵⁵ and equally thickened primary and secondary raphe sternum (cf. *Gyrosigma*³⁴; Fig. 3).

Total organic carbon. Sub-samples (~50 mg) of freeze-dried algae were decarbonated (10% HCl; 10 ml), washed (3 × 10 ml Milli-Q water) and freeze-dried (–80 °C; 0.001 mbar; 24 h) before analysis using a Thermoquest EA1110 CHN analyser. L-cystine was used as a calibration standard.

Extraction and analysis of lipids. Lipids were extracted from bulk algal aggregates and combined cells of individual species. For analysis of bulk aggregates, ~50 mg of each aggregate was first washed (3 × 10 ml Milli-Q water) to remove marine salts before being freeze-dried (–80 °C; 0.001 mbar; 24 h) and re-weighed before extraction. For extractions of individual diatom species, non-washed aggregate sub-samples were re-suspended in ~3 ml filtered (0.2 µm) artificial seawater (deionized water; 32 p.p.t. Tropic Marin salt) in a clean glass Petri dish. Individual diatom cells were identified using a Nikon TS2000 inverted light microscope (×10 and ×40 objectives) in phase contrast and isolated manually using a modified Pasteur pipette.

Following addition of an internal standard (9-octylheptadec-8-ene; 2 µg), bulk aggregates were saponified (20% KOH; 80 °C; 60 min) and extracted with hexane according to Brown *et al.*¹⁷, whereas for isolated cells a total hexane extract only was obtained (hexane; 3 × 1 ml, ultrasonication; 3 × 5 min). In each case, the resulting total hexane extract suspensions were filtered through pre-extracted (dichloromethane/methanol) cotton wool to remove cells before being partially dried (N₂ stream) and fractionated into non-polar lipids by column chromatography (hexane (3 ml)/SiO₂).

Analysis of partially purified non-polar lipids was carried out using GC–MS techniques⁵⁶ with minor modifications to increase instrument sensitivity. Identification of IP₂₅ was achieved by a characteristic mass spectral response (*m/z* 350.3; Fig. 1) using selective ion monitoring and co-injection of extracts with an authentic standard of IP₂₅ (ref. 57). Quantification was achieved by integrating the *m/z* 350.3 ion responses of IP₂₅ and the internal standard in selective ion monitoring mode, and normalizing the ratio between them using an instrumental response factor⁵⁶ and the number of cells extracted.

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Author contributions

T.A.B. designed and implemented the study, performed lipid analyses and data synthesis; A.T. carried out taxonomic assessments of samples; T.A.B. and S.T.B. wrote the manuscript with significant input from C.J.M.

Additional information

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