

Viewpoint

Deuterium in marine organic biomarkers: toward a new tool for quantifying aquatic mixotrophy

Summary

The traditional separation between primary producers (autotrophs) and consumers (heterotrophs) at the base of the marine food web is being increasingly replaced by the paradigm that mixoplankton, planktonic protists with the nutritional ability to use both phago (hetero)trophy and photo(auto)trophy to access energy are widespread globally. Thus, many 'phytoplankton' eat, while 50% of 'protozooplankton' also perform photosynthesis. Mixotrophy may enhance primary production, biomass transfer to higher trophic levels and the efficiency of the biological pump to sequester atmospheric CO₂ into the deep ocean. Although this view is gaining traction, science lacks a tool to quantify the relative contributions of autotrophy and heterotrophy in planktonic protists. This hinders our understanding of their impacts on carbon cycling within marine pelagic ecosystems. It has been shown that the hydrogen (H) isotopic signature of lipids is uniquely sensitive to heterotrophy relative to autotrophy in plants and bacteria. Here, we explored whether it is also sensitive to the trophic status in protists. The new understanding of H isotope signature of lipid biomarkers suggests it offers great potential as a novel tool for quantifying the prevalence of mixotrophy in diverse marine microorganisms and thus for investigating the implications of the 'mixoplankton' paradigm.

Introduction

Marine ecosystems play a pivotal role in global photosynthetic carbon fixation (Field *et al.*, 1998; Falkowski *et al.*, 2000). Their activity contributes to maintaining the balance between O₂ and CO₂ in the atmosphere and consequently to keeping climate relatively stable. The biological gravitational pump exports between *c.* 4.0 and 9.1 Pg of particulate organic carbon from surface waters annually (Boyd *et al.*, 2019). Most current marine biogeochemical models assume that the plankton community is clearly divided into autotrophic phytoplankton and heterotrophic zooplankton (Duarte *et al.*, 2013; Flynn *et al.*, 2013; Williams *et al.*, 2013; Leles *et al.*, 2018). Increasingly, it is recognised that there is no strict separation between producers and consumers (Fig. 1) and that photo(auto)trophic and

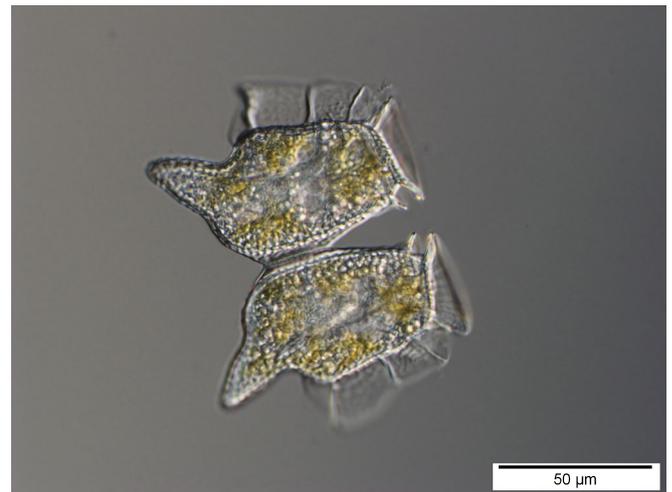


Fig. 1 Mixoplanktonic dinoflagellate *Dinophysis caudata*. The concept of animal and vegetable gets blurred when applied to unicellular organisms. Dinoflagellates can perform photosynthesis while devouring prey by phagocytosis and consuming dissolved organic molecules via osmotrophy. This latter mechanism is ubiquitous in phytoplankton. Photo by Nicolas Chomérat & Véronique Séchet (Ifremer).

phago(hetero)trophic behaviours are not mutually exclusive. Indeed, most of the protist unicellular organisms at the base of the plankton food web cannot be regarded strictly as producers or consumers (Flynn *et al.*, 2013, 2019; Mitra *et al.*, 2014). Modelling suggests that mixoplanktonic activity (i.e. nutrition involving both autotrophy and phagotrophy) enhances primary production, biomass transfer to higher trophic levels and the biological carbon pump by up to 35% (Mitra *et al.*, 2014; Ward & Follows, 2016; Leles *et al.*, 2021). To this, we add the renewed interest in mixotrophy supported by a combination of phototrophy and osmotrophy in diatoms and also in oceanic prokaryote phytoplankton (e.g. Yelton *et al.*, 2016; Benavides *et al.*, 2017; Muñoz-Marín *et al.*, 2020). The mixoplanktonic behaviour of many toxic protists also likely explains their ecological success and the occurrence of harmful algal blooms (HABs) (Burkholder *et al.*, 2008) that can severely affect coastal ecosystems and their services. Changing the prevalence of mixotrophic behaviours (involving osmotrophy and/or phagotrophy) within the plankton community could have a large impact on the global carbon cycle and thus on the climate. For example, a large >50-yr time series analysis suggests an increase in the relative abundance of diatoms vs dinoflagellates (Hinder *et al.*, 2012). Another example, using quantitative niche models, suggests that oceanic cyanobacterial communities will experience complex changes as a result of projected future climate conditions (Flombaum *et al.*, 2013). These changes may result in different mixotrophic activities in the surface ocean and affect the efficiency of the biological pump. Thus, there are a number of reasons for having a better understanding of the role of mixotrophy in marine microbial ecology.

Even though mixoplankton (i.e. photosynthetic microbes capable of phagotrophy or 'photo-osmo-phago-trophic' plankton) are a key component of marine ecosystems (Leles *et al.*, 2017, 2019), with all other phytoplankton capable of mixotrophy via osmotrophy (Flynn *et al.*, 2019), no tool currently exists to infer, both spatially and temporally, the trophic mode and the level of heterotrophic growth within different plankton groups. Tracking protist trophic modes, without an appropriate tool, in modern oceans is challenging as their species-specific metabolism and trophic adaptability remain largely unknown. Tracking their behaviour over geological time presents even greater challenges. For example, it was recently suggested that protists, mainly haptophytes, 'turned to hunting' in order to survive the end-Cretaceous impact (Gibbs *et al.*, 2020). However, due to the absence of any direct proxy, only indirect lines of evidence, such as eco-evolutionary modelling and microfossils, are available to support this hypothesis. Only a fraction of protist taxa produce an observable microfossil in sediments (e.g. Radi *et al.*, 2013; Cormier *et al.*, 2016; Sophie *et al.*, 2021). Such difficulties, combined with the need for data to better constrain biogeochemical models, drive the need for a means of tracking protists' metabolic behaviour (particularly the prevalence of mixoplanktonic lifestyle) in response to global environmental changes (Chavez *et al.*, 2011). This would facilitate the incorporation of mixotrophy into marine ecosystem models that are used to examine the future effects of anthropogenic perturbation on biogeochemical cycling in the global ocean.

Stable isotope ratios

Variations in the relative abundance of stable isotopes of diverse elements (e.g. hydrogen, carbon, nitrogen and oxygen) give important information about (bio)geochemistry and palaeoclimate. The measurement of their relative abundance, as preserved in geological and biological archives (e.g. sediment cores, tree rings, plankton and herbaria), is ubiquitous over a broad range of earth science studies. Generally expressed with a delta notation (Werner & Cormier, 2022), much of what we know about the past functioning of the biosphere comes from these measurements.

Isotopes of an element have identical chemical properties because the chemical reactivity is determined by the valence electrons. However, they do not behave like identical twins due to the differences in atomic mass, which lead to isotopic fractionation in their abundance during chemical and/or biological reactions (often termed α or ϵ). The rate of kinetic reactions and the position of chemical equilibria can be different when different isotopes are involved. Different nuclear masses will influence the intramolecular bond energies due to the lower vibrational energy when a heavier isotope is substituted on the bond. Molecules in which positions are substituted with heavy isotopes are more stable, and more energy is needed to cleave a covalent bond between atoms when heavier isotopes are involved. Correspondingly, a greater amount of energy is released during bond formation with heavier isotopes at equilibrium (Werner & Cormier, 2022). Light isotopes react more quickly than heavy isotopes due to their lower activation energy during reactions influenced by kinetics.

There are indications that the relative abundance of hydrogen (H) isotopes in lipids (i.e. deuterium (^2H) and protium (^1H), expressed as $\delta^2\text{H}$ values) is highly sensitive to the metabolism of bacteria (Zhang *et al.*, 2009; Wijker *et al.*, 2019), vascular plants (Luo & Sternberg, 1992; Cormier *et al.*, 2018), in particular orchids (Gebauer *et al.*, 2016; Schiebold *et al.*, 2018), and parasitic plants (Cormier *et al.*, 2019). Despite these indications, the application of the ^2H content in organic biomarkers has been largely limited to (palaeo)hydrological or salinity studies. Indeed, there have been various studies focused on using $\delta^2\text{H}$ values of organic material from terrestrial plants to reconstruct hydrology (Sachse *et al.*, 2012), and a handful of studies on marine phototrophs, with a focus almost entirely on the effects of salinity on lipid $\delta^2\text{H}$ values (Kasper *et al.*, 2014). Nonetheless, the effect of metabolism on $\delta^2\text{H}$ values provides evidence that $\delta^2\text{H}$ values can offer other valuable biogeochemical information (Estep & Hoering, 1980; Ziegler, 1989; Luo & Sternberg, 1992; Zhang *et al.*, 2009; Meer *et al.*, 2015; Gebauer *et al.*, 2016; Cormier *et al.*, 2018, 2019). Here, we suggest that $\delta^2\text{H}$ values of marine organic material could become a useful tool to study the role of mixotrophy in marine ecosystems.

Two main factors determine the $\delta^2\text{H}$ values of organic compounds produced by aquatic organisms: (1) the $\delta^2\text{H}$ value of the source water (Chikaraishi & Naraoka, 2003); and (2) the biosynthetic ^2H fractionation ($^2\text{H}-\epsilon_{\text{bio}}$) (Sternberg *et al.*, 1984; Yakir & DeNiro, 1990; Luo *et al.*, 1991; Zhang & Sachs, 2007). The latter involves several biochemical pathways and is calculated as the ^2H fractionation between water and the synthesised organic compounds. Because of the strength of the C-H bond in lipids, $\delta^2\text{H}$ values of lipids are relatively stable over time. Isotopic exchanges of C-bound H can nevertheless slowly occur over geological timescales. Such exchange can be recognised (e.g. via comparison of coeval n-alkyl and isoprenoid hydrocarbons) and does not preclude the valuable use of $\delta^2\text{H}$ values in geological studies (Sessions, 2016). Most palaeostudies using $\delta^2\text{H}$ derived from terrestrial plant biomarkers have considered $^2\text{H}-\epsilon_{\text{bio}}$ to be constant within a species (Sachse *et al.*, 2006) simplifying the application of biomarker $\delta^2\text{H}$ values as a (palaeo)hydrological proxy (Rach *et al.*, 2014, 2017; Ladd, 2021). In plankton, however, salinity and growth rate are also known to influence $^2\text{H}-\epsilon_{\text{bio}}$ during lipid biosynthesis (Englebrecht & Sachs, 2005; Schouten *et al.*, 2006). It is mainly because of the influence of salinity on $^2\text{H}-\epsilon_{\text{bio}}$ that $\delta^2\text{H}$ values can be used to derive palaeosalinity in aquatic basins (Ladd & Sachs, 2015). Evaporation in (semi-)closed aquatic basins also influences both salinity and source water $\delta^2\text{H}$ values, which is correspondingly recorded in the $\delta^2\text{H}$ values of the biomarkers produced in those basins (Nelson & Sachs, 2016).

Most models of $\delta^2\text{H}$ values in organic compounds have endeavoured to minimise the impact of metabolism in order to explain the variability in $\delta^2\text{H}$ values as a result of a specific environmental parameter. Roden *et al.* (2000) suggested that in plants, ^2H -depleting photosynthetic fractionation pathways and ^2H -enriching post-photosynthetic fractionation pathways determine a constant value for $^2\text{H}-\epsilon_{\text{bio}}$. While this approach is very useful for interpreting hydrological conditions from cellulose in tree rings, it does not capture the effects of environmental change on the

balance between photosynthetic and post-photosynthetic processes and their impact on $\delta^2\text{H}$ values. Additionally, the approach has not yet been extended to compounds other than cellulose or to taxa other than angiosperms.

A new conceptual view of H isotope ratios

To fill these gaps, a conceptual biochemical model has been proposed to describe how post-photosynthetic processes imprint a strong metabolic signal in $\delta^2\text{H}$ values of plant-derived organic

compounds (i.e. up to 60‰) in response to environmental changes (Cormier *et al.*, 2018, 2019). These biochemical pathways leave a metabolic signal on the $\delta^2\text{H}$ values because they induce a different isotopic fractionation. The model expresses that, overall, photosynthetic pathways deplete organic compounds in ^2H , while post-photosynthetic (or heterotrophic) pathways enrich compounds in ^2H .

Specifically, in Cormier's model, the photosynthetic carbohydrate supply rate affects $^2\text{H}\text{-}\epsilon_{\text{bio}}$ for carbohydrates and lipids (Fig. 2). This pattern is mostly driven by the carbohydrate pool size, the cycling rates of individual organic molecules in their respective

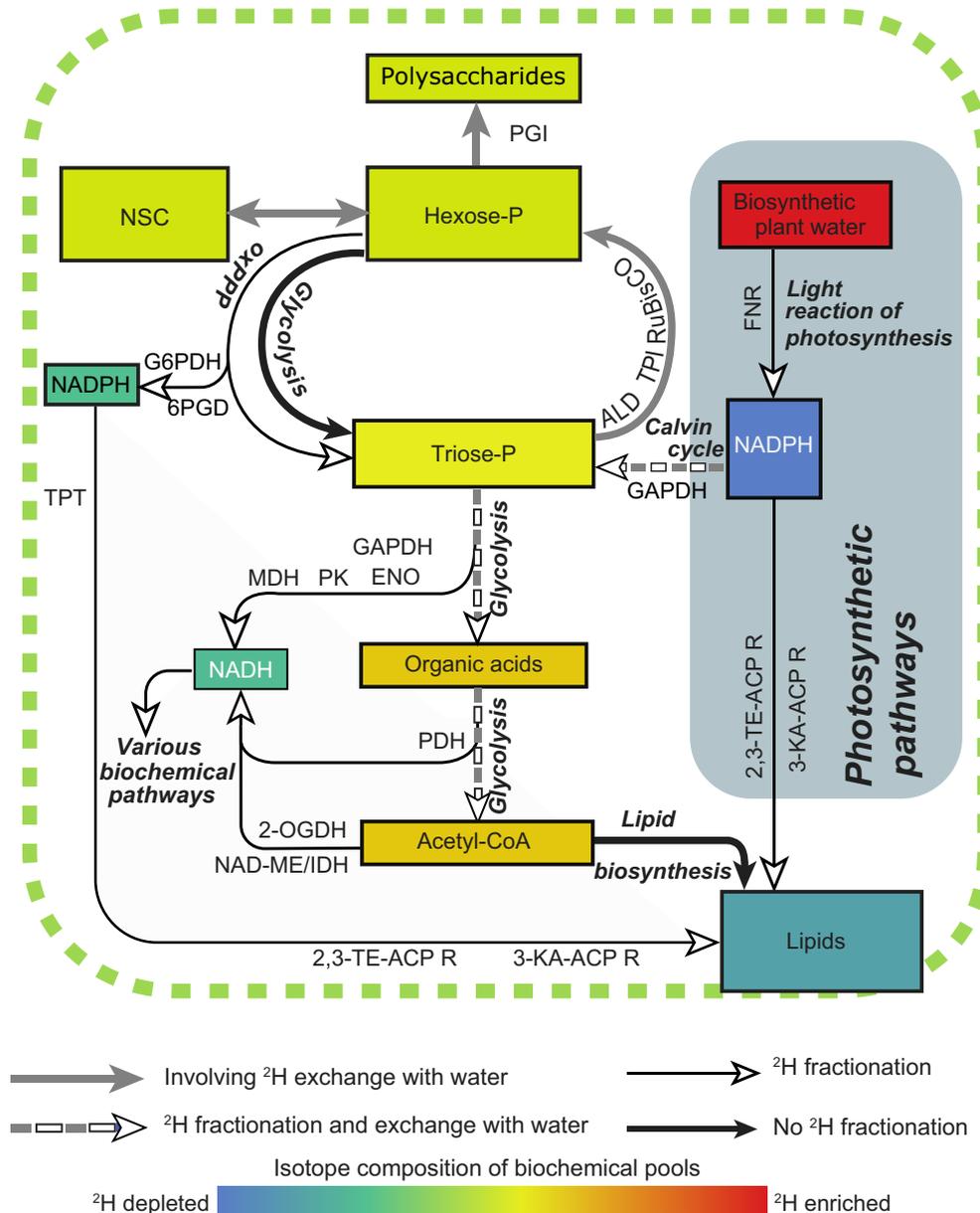


Fig. 2 Schematic view of H flow during processes leading to lipids, polysaccharides and nonstructural carbohydrates (NSC) $^2\text{H}\text{-}\epsilon_{\text{bio}}$. The key enzymes and pathways responsible for H flow are indicated by their following abbreviations and are based on known biochemical pathways: 2-OGDH, 2-oxoglutarate dehydrogenase; 6PGD, 6-phosphogluconate dehydrogenase; ACP, acyl carrier protein; ALD, aldolase; ENO, enolase; FNR, ferredoxin-NADP⁺ reductase; G6PDH, glucose-6-phosphate dehydrogenase; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; KA, ketoacyl; ME, malic enzyme; NADP, nicotinamide adenine dinucleotide; NADPH, nicotinamide adenine dinucleotide phosphate; oxPPP, oxidative pentose phosphate pathway; PDH, pyruvate dehydrogenase; PGI, phosphoglucose isomerase; PK, pyruvate kinase; R, reductase; RuBisCO, ribulose-1,5-bisphosphate carboxylase/oxygenase; TE, *trans*-enoyl; TPI, triosephosphate isomerase; and TPT, triose phosphate translocator. Simplified from Cormier *et al.* (2018) and references therein.

pools, the associated exchange of C-bound H with ^2H -enriched cellular water and preferential removal of light ^1H via the oxidative pentose phosphate pathway (oxPPP) (Cormier *et al.*, 2018). Consequently, a high photosynthetic (e.g. autotrophically dominated) carbon supply results in ^2H -depleted organic molecules, reflecting the ^2H -depleted signal of NADPH generated by the light reaction of photosynthesis (i.e. grey zone; Fig. 2). By contrast, a low photosynthetic (e.g. heterotrophically dominated) carbon supply leads to ^2H -enriched lipids, where higher cycling rates of individual

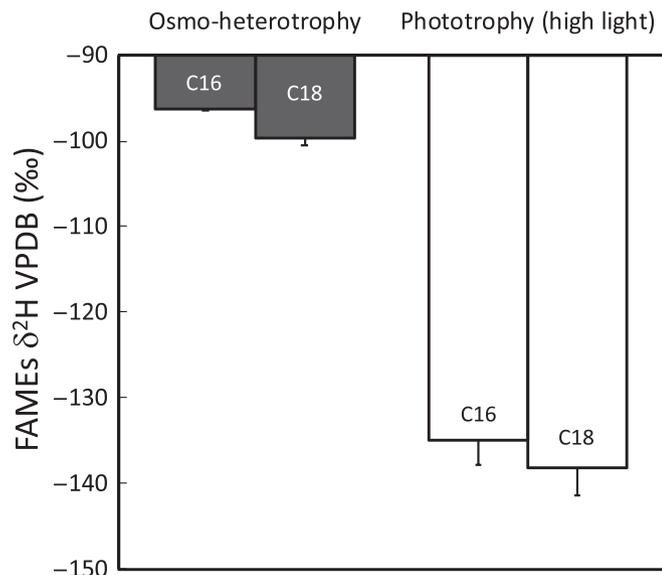


Fig. 3 Average $\delta^2\text{H}$ values of fatty acids (methyl esterified) produced by *Chlorella sorokiniana* under different metabolic conditions. For the heterotrophic mode, *C. sorokiniana* was fed with glucose in the dark. C16 and C18 correspond to palmitic and stearic acid, respectively. Error bars correspond to \pm SD ($n = 3$).

organic molecules are associated with an increasing exchange of C-bound H with ^2H -enriched cellular water and removal of ^1H from the C-skeletons via the oxPPP. While such a model highlights the likely considerable effect of fluxes through metabolic networks on H isotopic fractionation (Kruger & Ratcliffe, 2015), it also explains why a metabolic signal in H isotopic fractionation due to a shift in trophic behaviour is discernible in diverse biological organisms. As such, Gebauer *et al.* (2016) recently adopted this rationale successfully for analysis of environmental samples to study orchid ecology and the prevalence of heterotrophic and myco-heterotrophic orchid taxa in Europe. Similar to most marine protists, orchids behave auto-, mixo- and heterotrophically. The successful application of $\delta^2\text{H}$ values to study orchid ecology underlines the potential of compound-specific $\delta^2\text{H}$ values as a metabolic proxy in diverse environments and biological systems. In plants, studying such biochemical effects is greatly complicated by the spatial and temporal variation of the isotopic composition of water, the H source (i.e. within soil–water gradients and throughout plant stems and leaves). By contrast, sea water has an essentially constant $\delta^2\text{H}$ value in comparison with the scale of the biological fractionations, allowing a primary focus on biochemical variations in plankton. In preliminary work (Supporting Information Notes S1), we have observed that hydrogen isotopic fractionation during fatty acid biosynthesis in the green algae *Chlorella sorokiniana* is also sensitive to heterotrophy (Fig. 3). This metabolic sensitivity represents a fundamentally different philosophical approach to most previous studies of ^2H fractionation in photo(auto)trophs, which have assumed near-constant biochemistry and sought to reconstruct environmental variables.

The way towards the new tool

Laboratory-based calibrations are essential for *in situ* monitoring of protist trophic behaviours (including phago(hetero)trophic vs

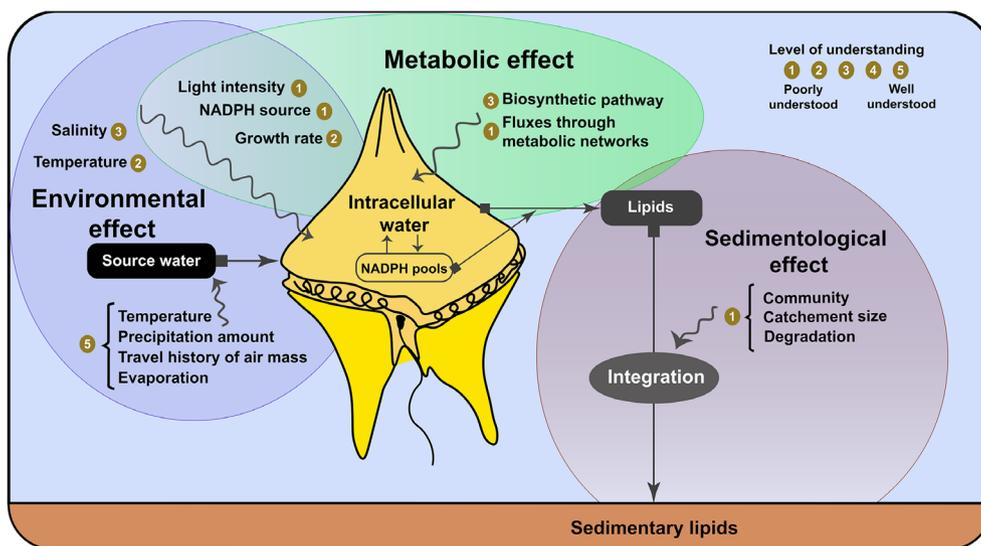


Fig. 4 Overview of the processes affecting $\delta^2\text{H}$ values of lipid biomarkers in protists, where they take place (wiggly arrows), and their current level of understanding (inspired by Sachse *et al.*, 2012). To effectively use $\delta^2\text{H}$ values as a tool to quantify mixotrophic processes, key limitations in their understanding need to be resolved.

photo(auto)trophic growth) using isotopic measurements of lipid biomarkers from environmental samples. For instance, the magnitude of the photosynthetic ^2H fractionation, which occurs during the light reaction of the photosynthesis (Fig. 2, grey zone), has only been estimated by Yakir & DeNiro (1990) who reported a fractionation of -171‰ for cellulose in the multicellular aquatic plant *Lemna gibba*. This value, although widely utilised, most likely varies appreciably between organisms and trophic behaviours. Moreover, even if some variables are known to influence post-photosynthetic ^2H fractionation during lipid biosynthesis (e.g. temperature, salinity, light intensity, growth rate, biosynthetic pathway, metabolic network and metabolic source of NADPH), a comprehensive understanding of how these variables impact, individually and synergistically, on ^2H fractionation in marine microbes is still lacking (Fig. 4).

With the appropriate investigations targeting these variables, compound-specific isotope analysis has the potential to provide a valuable research tool: a metabolic proxy for the quantitative assessment of the ratio between autotrophic and heterotrophic metabolism in diverse marine microorganisms and their contribution to the global carbon cycle in modern and, potentially, palaeoenvironmental contexts (i.e. assuming that the metabolic effects can be deconvoluted from the environmental and sedimentological effects using other proxies). Moreover, defining the metabolic influences on H isotope fractionation during biomarker synthesis will provide a much better understanding of their $\delta^2\text{H}$ values in response to changes in environmental conditions and will improve their utility as versatile palaeoecological proxies for, *inter alia*, temperature (Feng & Epstein, 1994), hydrological conditions (Sachse *et al.*, 2012) and sea surface salinity (Kasper *et al.*, 2014).

Acknowledgements

M-AC, KJF, REMR, DJM and RSL received funding from the UK Biotechnology and Biological Sciences Research Council for the DINTROPHY project (award references BB/V00994X/1 and BB/V010492/1, respectively). M-AC also received funding from Campus France while hosted at the Ifremer of Nantes (France). The authors are thankful to Dr Francis Mairet, Dr Matthieu Garnier, Nathalie Schreiber and Virginie Raimbault for their help in performing the experiment in the PBA laboratory and to Dr Maya Al-Sid-Cheikh (Surrey) for her proofreading and the scientific support. The authors also acknowledge Dr Daniel B. Nelson and the Plant Physiological Ecology group at the University of Basel for their help with compound-specific isotope analyses (Supporting Information Notes S1).

Author contributions

M-AC, REMR and NJK planned and designed the research. M-AC, J-BB and GB designed and performed the experiment and analysed the data. M-AC and J-BB performed chemical measurements. M-AC, KJF and REMR wrote the manuscript with contributions from CNT, DJM and RSL.

ORCID

Jean-Baptiste Berard  <https://orcid.org/0000-0001-7350-8006>
 Gaël Bougaran  <https://orcid.org/0000-0002-3463-7590>
 Marc-André Cormier  <https://orcid.org/0000-0001-7645-1375>
 Kevin J. Flynn  <https://orcid.org/0000-0001-6913-5884>
 Nicholas J. Kruger  <https://orcid.org/0000-0003-0492-3542>
 Daniel J. Mayor  <https://orcid.org/0000-0002-1295-0041>
 Rosalind E. M. Rickaby  <https://orcid.org/0000-0002-6095-8419>
 Clive N. Trueman  <https://orcid.org/0000-0002-4995-736X>

Data availability

The data that support the findings of this study are available in Supporting Information Notes S1.

Marc-André Cormier^{1*} , **Jean-Baptiste Berard**² ,
Gaël Bougaran² , **Clive N. Trueman**³ ,
Daniel J. Mayor⁴ , **Richard S. Lampitt**⁴,
Nicholas J. Kruger⁵ , **Kevin J. Flynn**⁶  and
Rosalind E. M. Rickaby¹ 

¹ Department of Earth Sciences, University of Oxford, South Parks Road, Oxford, OX1 3AN, UK;

²IFREMER, Physiology and Biotechnology of Algae (PBA) Laboratory, rue de l'Île d'Yeu, BP 21105, Nantes Cedex 3, 44311 France;

³Ocean and Earth Science, National Oceanography Centre Southampton, University of Southampton, Southampton, SO14 3ZH, UK;

⁴Ocean Biogeosciences, National Oceanography Centre, Southampton, SO14 3ZH, UK;

⁵Department of Plant Sciences, University of Oxford, South Parks Road, Oxford, OX1 3RB, UK;

⁶Plymouth Marine Laboratory, Prospect Place, Plymouth, PL1 3DH, UK

(*Author for correspondence: email ma_cormier@alumni.ethz.ch)

References

- Benavides M, Berthelot H, Duhamel S, Raimbault P, Bonnet S. 2017. Dissolved organic matter uptake by *Trichodesmium* in the Southwest Pacific. *Scientific Reports* 7: 41315.
- Boyd PW, Claustre H, Levy M, Siegel DA, Weber T. 2019. Multi-faceted particle pumps drive carbon sequestration in the ocean. *Nature* 568: 327–335.
- Burkholder JM, Glibert PM, Skelton HM. 2008. Mixotrophy, a major mode of nutrition for harmful algal species in eutrophic waters. *Harmful Algae* 8: 77–93.
- Chavez FP, Messié M, Pennington JT. 2011. Marine primary production in relation to climate variability and change. *Annual Review of Marine Science* 3: 260.
- Chikaraishi Y, Naraoka H. 2003. Compound-specific δD – $\delta^{13}\text{C}$ analyses of n-alkanes extracted from terrestrial and aquatic plants. *Phytochemistry* 63: 371.
- Cormier M-A, Rochon A, de Vernal A, Gélinas Y. 2016. Multi-proxy study of primary production and paleoceanographical conditions in northern Baffin Bay during the last centuries. *Marine Micropaleontology* 127: 10.
- Cormier M-A, Werner RA, Leuenberger MC, Kahmen A. 2019. ^2H -enrichment of cellulose and n-alkanes in heterotrophic plants. *Oecologia* 189: 373.
- Cormier M-A, Werner RA, Sauer PE, Gröcke DR, Leuenberger MC, Wieloch T, Schleucher J, Kahmen A. 2018. ^2H -fractionations during the biosynthesis of

- carbohydrates and lipids imprint a metabolic signal on the $\delta^2\text{H}$ values of plant organic compounds. *New Phytologist* 218: 491.
- Duarte CM, Regaudie-de-Gioux A, Arrieta JM, Delgado-Huertas A, Agustí S. 2013. The oligotrophic ocean is heterotrophic*. *Marine Science* 5: 551–569.
- Englebrecht AC, Sachs JP. 2005. Determination of sediment provenance at drift sites using hydrogen isotopes and unsaturation ratios in alkenones. *Geochimica et Cosmochimica Acta* 69: 4253–4265.
- Estep MF, Hoering TC. 1980. Biogeochemistry of the stable hydrogen isotopes. *Geochimica et Cosmochimica Acta* 44: 1206.
- Falkowski P, Scholes RJ, Boyle E, Canadell J, Canfield D, Elser J, Gruber N, Hibbard K, Höglberg P, Linder S *et al.* 2000. The global carbon cycle: a test of our knowledge of Earth as a system. *Science* 290: 296.
- Feng X, Epstein S. 1994. Climate implications of an 8000-year hydrogen isotope time series from bristlecone pine trees. *Science* 265: 1081.
- Field CB, Behrenfeld MJ, Randerson JT, Falkowski P. 1998. Primary production of the biosphere: integrating terrestrial and oceanic components. *Science* 281: 237–240.
- Flombaum P, Gallegos JL, Gordillo RA, Rincon J, Zabala LL, Jiao N, Karl DM, Li WKW, Lomas MW, Veneziano D *et al.* 2013. Present and future global distributions of the marine Cyanobacteria *Prochlorococcus* and *Synechococcus*. *Proceedings of the National Academy of Sciences, USA* 110: 9824–9829.
- Flynn KJ, Mitra A, Anestis K, Anschutz AA, Calbet A, Ferreira GD, Gypens N, Hansen PJ, John U, Martin JL *et al.* 2019. Mixotrophic protists and a new paradigm for marine ecology: where does plankton research go now? *Journal of Plankton Research* 41: 375–391.
- Flynn KJ, Stoecker DK, Mitra A, Raven JA, Glibert PM, Hansen PJ, Granéli E, Burkholder JM. 2013. Misuse of the phytoplankton–zooplankton dichotomy: the need to assign organisms as mixotrophs within plankton functional types. *Journal of Plankton Research* 35: 11.
- Gebauer G, Preiss K, Gebauer AC. 2016. Partial mycoheterotrophy is more widespread among orchids than previously assumed. *New Phytologist* 211: 11–15.
- Gibbs SJ, Bown PR, Ward BA, Alvarez SA, Kim H, Archontikis OA, Sauterey B, Poulton AJ, Wilson J, Ridgwell A. 2020. Algal plankton turn to hunting to survive and recover from end-Cretaceous impact darkness. *Science Advances* 6: eabc9123.
- Hinder SL, Hays GC, Edwards M, Roberts EC, Walne AW, Gravenor MB. 2012. Changes in marine dinoflagellate and diatom abundance under climate change. *Nature Climate Change* 2: 275.
- Kasper S, van der Meer MTJ, Mets A, Zahn R, Damsté JSS, Schouten S. 2014. Salinity changes in the Agulhas leakage area recorded by stable hydrogen isotopes of C_{37} alkenones during termination I and II. *Climate of the Past* 10: 260.
- Kruger NJ, Ratcliffe RG. 2015. Fluxes through plant metabolic networks: measurements, predictions, insights and challenges. *Biochemical Journal* 465: 38.
- Ladd SN, Maloney AE, Nelson DB, Prebble M, Camperio G, Sear DA, Hassall JD, Langdon PG, Sachs JP, Dubois N. 2021. Leaf wax hydrogen isotopes as a hydroclimate proxy in the tropical Pacific. *Journal of Geophysical Research Biogeosciences* 126: e2020JG005891.
- Ladd SN, Sachs JP. 2015. Influence of salinity on hydrogen isotope fractionation in *Rhizophora* mangroves from Micronesia. *Geochimica et Cosmochimica Acta* 168: 206–221.
- Leles SG, Bruggeman J, Polimene L, Blackford J, Flynn KJ, Mitra A. 2021. Differences in physiology explain succession of mixoplankton functional types and affect carbon fluxes in temperate seas. *Progress in Oceanography* 190: 102481.
- Leles SG, Mitra A, Flynn KJ, Stoecker DK, Hansen PJ, Calbet A, McManus GB, Sanders RW, Caron DA, Not F *et al.* 2017. Oceanic protists with different forms of acquired phototrophy display contrasting biogeographies and abundance. *Proceedings of the Royal Society B: Biological Sciences* 284: 20170664.
- Leles SG, Mitra A, Flynn KJ, Tillmann U, Stoecker D, Jeong HJ, Burkholder J, Hansen PJ, Caron DA, Glibert PM *et al.* 2019. Sampling bias misrepresents the biogeographical significance of constitutive mixotrophs across global oceans. *Global Ecology and Biogeography* 28: 418–428.
- Leles SG, Polimene L, Bruggeman J, Blackford J, Ciavatta S, Mitra A, Flynn KJ. 2018. Modelling mixotrophic functional diversity and implications for ecosystem function. *Journal of Plankton Research* 40: 627–642.
- Luo Y-H, Steinberg L, Suda S, Kumazawa S, Mitsui A. 1991. Extremely low D/H ratios of photoproduced hydrogen by cyanobacteria. *Plant and Cell Physiology* 32: 900.
- Luo Y-H, Sternberg LSL. 1992. Hydrogen and oxygen isotopic fractionation during heterotrophic cellulose synthesis. *Journal of Experimental Botany* 43: 50.
- Mitra A, Flynn KJ, Burkholder JM, Berge T, Calbet A, Raven JA, Granéli E, Glibert PM, Hansen PJ, Stoecker DK *et al.* 2014. The role of mixotrophic protists in the biological carbon pump. *Biogeosciences* 11: 1005.
- Muñoz-Marín MC, Gómez-Baena G, López-Lozano A, Moreno-Cabeuelo JA, Díez J, García-Fernández JM. 2020. Mixotrophy in marine picocyanobacteria: use of organic compounds by *Prochlorococcus* and *Synechococcus*. *The ISME Journal* 14: 1065–1073.
- Nelson DB, Sachs JP. 2016. Galápagos hydroclimate of the common era from paired microalgal and mangrove biomarker $^2\text{H}/^1\text{H}$ values. *Proceedings of the National Academy of Sciences, USA* 113: 3481.
- Rach O, Brauer A, Wilkes H, Sachse D. 2014. Delayed hydrological response to Greenland cooling at the onset of the Younger Dryas in western Europe. *Nature Geoscience* 7: 4.
- Rach O, Kahmen A, Brauer A, Sachse D. 2017. A dual-biomarker approach for quantification of changes in relative humidity from sedimentary lipid D/H ratios. *Climate of the Past* 13: 757.
- Radi T, Bonnet S, Cormier M-A, de Vernal A, Durantou L, Faubert É, Head MJ, Henry M, Pospelova V, Rochon A *et al.* 2013. Operational taxonomy and (paleo-)autecology of round, brown, spiny dinoflagellate cysts from the Quaternary of high northern latitudes. *Marine Micropaleontology* 98: 57.
- Roden JS, Lin GG, Ehleringer JR. 2000. A mechanistic model for interpretation of hydrogen and oxygen isotope ratios in tree-ring cellulose. *Geochimica et Cosmochimica Acta* 64: 35.
- Sachse D, Billault I, Bowen GJ, Chikaraishi Y, Dawson TE, Feakins SJ, Freeman KH, Magill CR, McInerney FA, van der Meer MTJ *et al.* 2012. Molecular paleohydrology: interpreting the hydrogen-isotopic composition of lipid biomarkers from photosynthesizing organisms. *Annual Review of Earth and Planetary Sciences* 40: 221–249.
- Sachse D, Radke J, Gleixner G. 2006. δD values of individual *n*-alkanes from terrestrial plants along a climatic gradient – implications for the sedimentary biomarker record. *Organic Geochemistry* 37: 483.
- Schiebold JM-I, Bidartondo MI, Lenhard F, Makiola A, Gebauer G. 2018. Exploiting mycorrhizas in broad daylight: partial mycoheterotrophy is a common nutritional strategy in meadow orchids. *Journal of Ecology* 106: 168–178.
- Schouten S, Ossebaer J, Schreiber K, Kienhuis MVM, Langer G, Benthien A, Bijma J. 2006. The effect of temperature, salinity and growth rate on the stable hydrogen isotopic composition of long chain alkenones produced by *Emiliania huxleyi* and *Gephyrocapsa oceanica*. *Biogeosciences* 3: 113–119.
- Sessions AL. 2016. Review: factors controlling the deuterium contents of sedimentary hydrocarbons. *Organic Geochemistry* 96: 43–64.
- Sophie W, Noah JP, Ming-Yu Z, Pincelli MH. 2021. Revisiting the sedimentary record of the rise of diatoms. *Proceedings of the National Academy of Sciences, USA* 118: e2103517118.
- Sternberg LO, Deniro MJ, Ting IP. 1984. Carbon, hydrogen, and oxygen isotope ratios of cellulose from plants having intermediary photosynthetic modes. *Plant Physiology* 74: 107.
- van der Meer MTJ, Benthien A, French KL, Epping E, Zondervan I, Reichart G-J, Bijma J, Sinninghe Damsté JS, Schouten S. 2015. Large effect of irradiance on hydrogen isotope fractionation of alkenones in *Emiliania huxleyi*. *Geochimica et Cosmochimica Acta* 160: 16–24.
- Ward BA, Follows MJ. 2016. Marine mixotrophy increases trophic transfer efficiency, mean organism size, and vertical carbon flux. *Proceedings of the National Academy of Sciences, USA* 113: 2963.
- Werner RA, Cormier M-A. 2022. Isotope terminology, definitions and nomenclature in stable isotopes. In: Siegwolf RTW, Brooks JR, Roden J, Saurer M, eds. *Stable isotopes in tree rings: inferring physiological, climatic and environmental responses*. Cham, Switzerland: Springer Nature Switzerland AG.
- Wijker RS, Sessions AL, Fuhrer T, Phan M. 2019. $^2\text{H}/^1\text{H}$ variation in microbial lipids is controlled by NADPH metabolism. *Proceedings of the National Academy of Sciences, USA* 116: 12173–12182.
- Williams PJLB, Quay PD, Westberry TK, Behrenfeld MJ. 2013. The oligotrophic ocean is autotrophic. *Annual Review of Marine Science* 5: 535–549.
- Yakir D, DeNiro MJ. 1990. Oxygen and hydrogen isotope fractionation during cellulose metabolism in *Lemma gibba* L. *Plant Physiology* 93: 332.

- Yelton AP, Acinas SG, Sunagawa S, Bork P, Pedrós-Alió C, Chisholm SW. 2016. Global genetic capacity for mixotrophy in marine picocyanobacteria. *The ISME Journal* **10**: 2946–2957.
- Zhang X, Gillespie AL, Sessions AL. 2009. Large D/H variations in bacterial lipids reflect central metabolic pathways. *Proceedings of the National Academy of Sciences, USA* **106**: 12586.
- Zhang Z, Sachs JP. 2007. Hydrogen isotope fractionation in freshwater algae: I. Variations among lipids and species. *Organic Geochemistry* **38**: 582–608.
- Ziegler H. 1989. Hydrogen isotope fractionation in plant tissues. In: Rundel PW, Ehleringer JR, Nagy KA, eds. *Stable isotopes in ecological research*. New York, NY, USA: Springer-Verlag, 105–123.

Supporting Information

Additional Supporting Information may be found online in the Supporting Information section at the end of the article.

Notes S1 Cultures of *Chlorella sorokiniana* and compound-specific isotope analyses.

Please note: Wiley-Blackwell are not responsible for the content or functionality of any Supporting Information supplied by the authors. Any queries (other than missing material) should be directed to the *New Phytologist* Central Office.

Key words: biomarker, carbon cycle, hydrogen, isotope, mixoplankton, mixotrophy, protist.

Received, 15 September 2021; accepted, 3 February 2022.



About *New Phytologist*

- *New Phytologist* is an electronic (online-only) journal owned by the New Phytologist Foundation, a **not-for-profit organization** dedicated to the promotion of plant science, facilitating projects from symposia to free access for our Tansley reviews and Tansley insights.
- Regular papers, Letters, Viewpoints, Research reviews, Rapid reports and both Modelling/Theory and Methods papers are encouraged. We are committed to rapid processing, from online submission through to publication 'as ready' via *Early View* – our average time to decision is <23 days. There are **no page or colour charges** and a PDF version will be provided for each article.
- The journal is available online at Wiley Online Library. Visit **www.newphytologist.com** to search the articles and register for table of contents email alerts.
- If you have any questions, do get in touch with Central Office (np-centraloffice@lancaster.ac.uk) or, if it is more convenient, our USA Office (np-usaoffice@lancaster.ac.uk)
- For submission instructions, subscription and **all the latest information** visit **www.newphytologist.com**