Phycological Research

*Phycological Research* 2023 doi: 10.1111/pre.12530

RESEARCH NOTE

# Composition of galactolipids, betaine lipids and triglyceride-associated fatty acids of the symbiotic dinoflagellate *Zooxanthella* (*Brandtodinium*) *nutricula*: A glimpse into polyunsaturated fatty acids available to its polycystine radiolarian host

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## SUMMARY

*Zooxanthella nutricula* is a photosynthetic dinoflagellate symbiont of polycystine radiolarians. As such, it is hypothesized to provide fixed organic carbon, including in the form of acylglycerolipids and sterols, to its non-photosynthetic host. We have previously characterized the sterols of *Z. nutricula* that may be transferred to its host and, in the present study, have turned our attention to three classes of fatty acid-containing lipids, chloroplast-associated galactolipids, betaine lipids, which are non-phosphorylated phospholipid analogs present in many eukaryotes, and triglycerides. *Zooxanthella nutricula* was observed using positive-ion electrospray/mass spectrometry (ESI/MS) and ESI/MS/MS to produce the galactolipids mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively) enriched in octadecapentaenoic (18:5(n-3)) and octadecatetraenoic (18:4(n-3)) acid to place it within a group of peridinin-containing dinoflagellates in a $C_{18}/C_{18}$ (*sn*-1/*sn*-2 fatty acid regiochemistry) cluster, as opposed to another cluster with $C_{20}/C_{18}$ MGDG and DGDG, where the $C_{20}$ fatty acid is eicosapentaenoic acid (20:5(n-3)) and the $C_{18}$ fatty acid is either 18:5(n-3) or 18:4(n-3). *Zooxanthella nutricula* was also observed to produce 38:10 (total number of fatty acid carbons:total number of double bonds), 38:6, and 44:7 diacylglycerylcarboxyhydroxymethylcholine (DGCC) as the sole type of betaine lipid. Although it is more difficult to determine which fatty acids are present in the *sn*-1 and *sn*-2 positions on the glycerol backbone of DGCC using ESI/MS/MS, gas chromatography/mass spectrometry (GC/MS)-based examination indicated the putatively DGCC-associated polyunsaturated fatty acid (PUFA) docosahexaenoic acid (22:6(n-3)). Coupled with the $C_{18}$ PUFAs of MGDG and DGDG, and fatty acids associated with triglycerides (also examined via GC/MS), *Z. nutricula* could serve as a rich source of PUFAs for its radiolarian host. These data demonstrate that *Z. nutricula* produces a similar set of PUFA-containing lipids as *Symbiodinium microadriaticum*, a photosynthetic dinoflagellate symbiont of cnidarians, indicating a metabolic commonality in these phylogenetically discrete dinoflagellate symbionts with unrelated host organisms.

Key words: *Brandtodinium*, chloroplast, dinoflagellate, Dinophyceae, lipid, *Zooxanthella*.

## INTRODUCTION

*Zooxanthella nutricula* K.Brandt, also incorrectly known as *Brandtodinium nutricula* (K. Brandt) Probert & Siano (Probert *et al.*, 2014; Krueger, 2016), is a photosynthetic symbiont of marine polycystine radiolarians (Probert *et al.*, 2014). As with the photosynthetic dinoflagellate symbionts of cnidarians, *Z. nutricula* is considered to provide fixed organic carbon, such as lipids, to its host in exchange for a 'safe' place to live (Yellowlees *et al.*, 2008). Lipids, which are generally non-polar organic macromolecules extracted from biological material using a non-polar solvent such as chloroform (recognizing that in reality lipids are diverse in structure and there is a range in polarity from charged, amphiphilic to neutral lipids), can be present as fatty acid-containing acylglycerolipids or ringed sterols, among other possible non-polar macromolecules such as photosynthetic pigments and lipophilic toxins. We have previously described the sterol composition of an *ex hospite* isolate of *Z. nutricula* as the first examination of its lipids, and have found it to produce 15 sterols, ranging from $C_{27}$ to $C_{30}$ (Graeff & Leblond, 2021), which could in theory be transferred to the host as part of an *in hospite* relationship, using the dinoflagellate symbionts of cnidarians as an example (Anderson, 2012; Hambleton *et al.*, 2019); note that some radiolarians have been observed to subsist on captured prey, whereas others appear to subsist on an established symbiont (Swanberg & Anderson, 1985; Matsuoka, 2007). Some of these sterols, such as 4α,23,24-trimethyl-5α-cholest-22E-en-3β-ol (dinosterol), are common dinoflagellate sterols (Volkman, 1986, 2003; Leblond *et al.*, 2010), which overlapped with those found in closely related *Heterocapsa* species (Probert *et al.*, 2014), whereas others, such as cholesta-5,22E-dien-3β-ol (22-dehydrocholesterol), have a more limited distribution in dinoflagellates (Leblond *et al.*, 2010).

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Received 2 March 2023; accepted 12 July 2023.

As photosynthetic organisms, dinoflagellates possess membranes comprised primarily of the fatty acid-containing galactolipids, mono- and digalactosyldiacylglycerol (MGDG and DGDG, respectively), which form the matrix within which photosynthetic pigments and enzymes are housed (Gao *et al.*, 2018; Hölzl & Dörmann, 2019; Hernández & Cejudo, 2021). Galactose, rather than glucose, as the hexose in these particular lipids was originally identified by Harrington *et al.* (1970) and has subsequently been noted in other studies (Bishop *et al.*, 1976; Parrish *et al.*, 1998). Peridinin-containing dinoflagellates, which represent the largest group of photosynthetic dinoflagellates (Zapata *et al.*, 2012), have been observed using positive-ion electrospray/mass spectrometry (ESI/MS) and ESI/MS/MS to cluster into two groups depending on the forms (i.e. species) of MGDG and DGDG produced (Gray *et al.*, 2009). Cluster 1 is characterized by taxa with a $C_{18}/C_{18}$ arrangement (*sn*-1/*sn*-2) of fatty acids, where the predominant fatty acids are polyunsaturated octadecapentaenoic (18:5(n-3)) and octadecatetraenoic (18:4(n-3)) acid (double bond positions omitted for simplification after first mention with a particular fatty acid). Cluster 2 is characterized by taxa with a $C_{20}/C_{18}$ arrangement of fatty acids, where the predominant fatty acids are polyunsaturated eicosapentaenoic (20:5(n-3)) and the same $C_{18}$ fatty acids as Cluster 1.

Non-phosphorylated analogs of phospholipids known as betaine lipids are important fatty acid-containing structural lipids that are assumed to be present in non-chloroplast membranes of certain protozoa and multicellular, non-animal eukaryotes (Kunzler & Eichenberger, 1997; Rozenstvet *et al.*, 2001; Roche & Leblond, 2010; Leblond *et al.*, 2013; Huang *et al.*, 2019). The few dinoflagellates (in comparison to the larger number of dinoflagellates that have had their galactolipids characterized) such as *Peridinium aciculiferum* Lemmermann and *Symbiodinium microadriaticum* (Freud.) Loeblich et Sherley that have had their betaine lipids characterized have been shown previously to be enriched in the betaine lipids diacylglycerylcarboxyhydroxymethylcholine (DGCC) and diacylglyceryl-*N*,*N*,*N*-trimethylhomoserine (DGTS) with polyunsaturated fatty acids (PUFAs) as inferred by the total number of fatty acid carbons and double bonds as observed using positive-ion ESI/MS and ESI/MS/MS (Kato *et al.*, 1996; Flaim *et al.*, 2014; Leblond *et al.*, 2015; Anesi *et al.*, 2016; Sikorskaya *et al.*, 2021). Note that it is analytically more difficult using MS to determine the regiochemistry of fatty acids associated with betaine lipids because the fragmentation pattern is not as well defined (Leblond *et al.*, 2015; Anesi *et al.*, 2016). As a result of fewer dinoflagellates having been examined, it is unknown whether dinoflagellates segregate into clusters according to betaine lipid composition, as has been observed with galactolipids in peridinin-containing dinoflagellates.

Triglycerides (triacylglycerols) are well known to be vehicles for storing excess carbon in many forms of life, including animals. Dinoflagellates are known to produce triglycerides, which have been shown to be comprised by a variety of fatty acids, including several PUFAs, ranging from $C_{16}$ to $C_{28}$ (Řezanka *et al.*, 2017). At the current time there is no knowledge on whether dinoflagellates cluster according to triglyceride composition.

*Zooxanthella nutricula* has been shown to be phylogenetically related to the genus *Heterocapsa* (Probert *et al.*, 2014). The galactolipid composition of *Heterocapsa* as a closely related genus has not been extensively examined, with only a single published observation of *Heterocapsa niei* (A.R. Loeblich) L.C. Morrill & A.R. Loeblich in the survey of peridinin-containing dinoflagellates by Gray *et al.* (2009) to place it firmly in Cluster 1. However, unpublished data show that *Heterocapsa pygmaea* Lobelich III, R.J. Schmidt & Sherley is also found in Cluster 1 (J. Leblond, unpublished data) and there is a published report on the lipids of *Heterocapsa circularisquama* Horiguchi (Hiraga *et al.*, 2002) also matching what has been observed in *H. niei* and *H. pygmaea*. Yamaoka *et al.* (1999) have also published a report on the fatty acids of *H. circularisquama*, and they are much more enriched in 18:4 than 20:5, possibly indicating that its galactolipids are of the $C_{18}/C_{18}$ type of Cluster 1. Conversely, there is a report of 20:5/$C_{18}$ fatty acid-containing DGDG in *Heterocapsa triquetra* (Ehrenberg) F.Stein by Said (2019); however, that study utilized one-dimensional thin-layer chromatography (TLC) separation of lipid classes, followed by gas chromatography/mass spectrometry (GC/MS) characterization of fatty acids liberated from TLC spots scraped off the TLC plate. In our opinion, this methodology lacks the specificity of the positive-ion ESI/MS and ESI/MS/MS approach of Gray *et al.* (2009) and it is possible that more than one lipid class could be present within a TLC spot identified as DGDG. Thus, there is a general expectation that other *Heterocapsa* species will also reside in Cluster 1.

Because of its phylogenetic closeness to *Heterocapsa*, we hypothesized that *Z. nutricula* would also reside in Cluster 1. In the present study, we report the MGDG and DGDG composition of *Z. nutricula* as it relates to *Heterocapsa* and other peridinin-containing dinoflagellates. We also present the betaine lipid and triglyceride-associated fatty acid composition of *Z. nutricula* and make comparisons with those dinoflagellates that have had their betaine lipid and triglyceride compositions published. To the greatest extent possible, these comparisons extend to dinoflagellates identified as cnidarian symbionts (e.g. *Symbiodinium*) to determine whether there is any lipid biochemistry overlap between these two symbiotic systems.

## MATERIALS AND METHODS

*Zooxanthella nutricula* CCMP 3427 was acquired from the National Center for Marine Algae and Microbiota (East Boothbay, ME, USA; previously known as the Center for the Culture of Marine Phytoplankton, hence the CCMP designation). This culture is the same as the RCC 3387 originally housed at the Roscoff Culture Collection (Roscoff, France). *Zooxanthella nutricula* was grown autotrophically in triplicate in 2 L of L1 medium (Guillard & Hargraves, 1993) at a salinity of 35 psu and 20°C under a 14:10 h light/dark photocycle at an irradiance of approximately 50 μmol photons $m^{-2}$ $s^{-1}$ using a combination of cool white fluorescent and LED lights. Cells were harvested via filtration onto Whatman 934-AH glass microfiber filters (GE Healthcare, Chicago, IL, USA) during the exponential phase of growth after approximately 1 month of growth when cells were in the range of $10^4$ cells $mL^{-1}$.

Total lipids were extracted and galactolipids, betaine lipids and triglycerides were separated from other lipid classes according to the techniques described by Leblond and Chapman (2000); in brief, in their methodology, galactolipids were present in glycolipid-containing fraction 4, betaine lipids in polar lipid-containing fraction 5 and triglycerides in fraction 2. Galactolipids were characterized as sodium adducts [M+Na$^+$] using positive-ion ESI/MS and ESI/MS/MS in accordance with the original description by Gray *et al.* (2009). The positions of the acyl chains (*sn*-1 or *sn*-2) were determined based on Gray *et al.* (2009) according to a variation of the procedure established by Guella *et al.* (2003). Betaine lipids that are already positively charged were also characterized by positive-ion ESI/MS using the same instrument conditions. Additional instrument details are provided by Leblond *et al.* (2019). The fatty acids associated with triglycerides, and the fatty acids derived from fractions 4 and 5, were examined via GC/MS as fatty acid methyl esters (FAMEs) and 4,4-dimethyloxazoline (DMOX) derivatives according to Leblond & Chapman (2000) and Leblond *et al.* (2019), the latter of which utilizes a newer instrument.

## RESULTS AND DISCUSSION

Three galactolipids produced by *Z. nutricula* were observed as sodium adducts via positive-ion ESI/MS and ESI/MS/MS. These were 18:5(n-3)/18:5 MGDG (*m/z* 789, relative percentage of 42.0 ± 1.1%;), 18:5/18:4(n-3) MGDG (*m/z* 791, 24.4 ± 2.5% after correction for *m/z* 789 natural abundance $^{13}$C isotope contribution) and 18:5/18:4 DGDG (*m/z* 953, 33.5 ± 3.3%). Figure 1a shows the full-scan positive-ion ESI/MS data with prominent galactolipid ions (peaks) at *m/z* 789, 791 and 953. There was a trace ion at *m/z* 951, which could be 18:5/18:5 DGDG, but it was so minimal that we could not confirm its presence via ESI/MS/MS. This particular galactolipid appears especially rare in peridinin-containing dinoflagellates (Gray *et al.*, 2009). Figure 1b–d display the positive-ion ESI/MS/MS spectra of these same three galactolipids, respectively. In Fig. 1b, the single ion fragment at *m/z* 515 represents the mass of 18:5/18:5 MGDG minus either of its 18:5 fatty acids. In Fig. 1c, the larger *m/z* 517 ion fragment represents the mass 18:5/18:4 MGDG minus 18:5 cleaved preferentially from the *sn*-1 position, whereas the smaller *m/z* 515 fragment represents the mass of the lipid minus the 18:4 fatty acid cleaved less preferentially from the *sn*-2 position. In Fig. 1d, the fragmentation pattern of 18:5/18:4 DGDG is the same as 18:5/18:4 MGDG with the exception that the ion fragments are each of a larger mass as a result of the presence of a second galactose. Thus, the *m/z* 679 ion fragment corresponds to the *m/z* 517 ion fragment and the *m/z* 677 ion fragment corresponds to the *m/z* 515 ion fragment in Fig. 1c. Although unlikely as based on these positive-ion/ESI/MS/MS data, we cannot rule out the presence of stereospecific isomers wherein the positions of 18:5 and 18:4 would be reversed. It should be mentioned, however, that Anesi *et al.* (2016) utilized reverse phase liquid chromatography (LC) as their separation technique immediately prior to ESI/MS and ESI/MS/MS analysis, rather than the 'shotgun' technique that we employed post-fractionation, and they did not report two stereoisomers of lipids, such as 36:9 MGDG, with the same mass.

The double bond positions of both 18:5 and 18:4 were confirmed to be n-3 as evidenced by the 12-amu mass differences in DMOX derivatives, as shown in the Supporting Information (Fig. S1). Analysis of FAMEs was carried out with the inclusion of tricosanoic acid (23:0) methyl ester as an internal standard to also allow for an interfraction comparison of fatty acid biomass (i.e. glycolipid- vs. betaine lipid- vs. triglyceride-containing fractions) per cell (see Supporting information, Table S1) as a means of indicating how much fatty acid material could be hypothetically available to a radiolarian host. The Supporting information (Table S1) shows that fatty acids associated with glycolipids, which could also include sulfoquinovosyldiacylglycerol (SQDG, not examined in this study) in addition to MGDG and DGDG, were more abundant at 14.4 ± 7.6 pg cell$^{-1}$ than those found in the betaine lipid-containing fraction (5.4 ± 2.5 pg cell$^{-1}$) or a triglyceride-containing fraction (2.6 ± 1.1 pg cell$^{-1}$). These values are in approximate agreement with other recent studies on fatty acid content per dinoflagellate cell (Mansour *et al.*, 1999; Yoon *et al.*, 2017). As another glycolipid considered to be found within plastid membranes (Hölzl & Dörmann, 2019), SQDG has received less attention in terms of characterization of its associated fatty acids. Anesi *et al.* (2016) observed a 34:1 form of SQDG in a number of dinoflagellates; however, they did not identify the associated fatty acids (although this total number of fatty acid carbons:total number of unsaturations indicates that PUFAs were not present). Rosset *et al.* (2019) examined two dinoflagellates within the Symbiodiniaceae and observed the presence of 30:1, 30:0 and 32:0 SQDG; although they did not identify the associated fatty acids, these total numbers of carbons:total numbers of unsaturations indicate some combination of 16:0 and 14:0 as the associated fatty acids. This agrees with an earlier study by Awai *et al.* (2012) who found SQDG in two *Symbiodinium* strains to be enriched in 16:0.

The galactolipid composition of *Z. nutricula* places it firmly within Cluster 1 per Gray *et al.* (2009). Although the existence of Clusters 1 and 2 for peridinin-containing dinoflagellates does not permit the same level of chemotaxonomic resolution as does sterols per Leblond *et al.* (2010), the $C_{18}/C_{18}$ galactolipids of *Z. nutricula* are in general agreement with those of its closest phylogenetic relative, members of the genus *Heterocapsa* (see above).

There is limited, equivocal evidence for one unidentified species of *Heterocapsa* to serve as a symbiont within a radiolarian host (Decelle *et al.*, 2012), and so perhaps a more relevant comparison in terms of host-symbiont physiology and biochemistry of *Z. nutricula* and its radiolarian host is to the galactolipids of those dinoflagellates, namely within the genus *Symbiodinium*, which have an important metabolic relationship with their cnidarian hosts (Gordon & Leggat, 2010). All *Symbiodinium* isolates examined *ex hospite* thus far at the same growth temperature as for *Z. nutricula* have been shown to produce $C_{18}/C_{18}$ galactolipids that fall within Cluster 1 (Gray *et al.*, 2009), and one, *Symbiodinium microadriaticum* LaJeunesse isolated from the jellyfish *Cassiopea xamachana*, was shown to modulate its galactolipid-associated fatty acids in response to a higher 30°C growth temperature by making the *sn*-2 $C_{18}$ fatty acid more saturated (Leblond *et al.*, 2015). We attempted to provide a similar 30°C growth temperature galactolipid comparison using

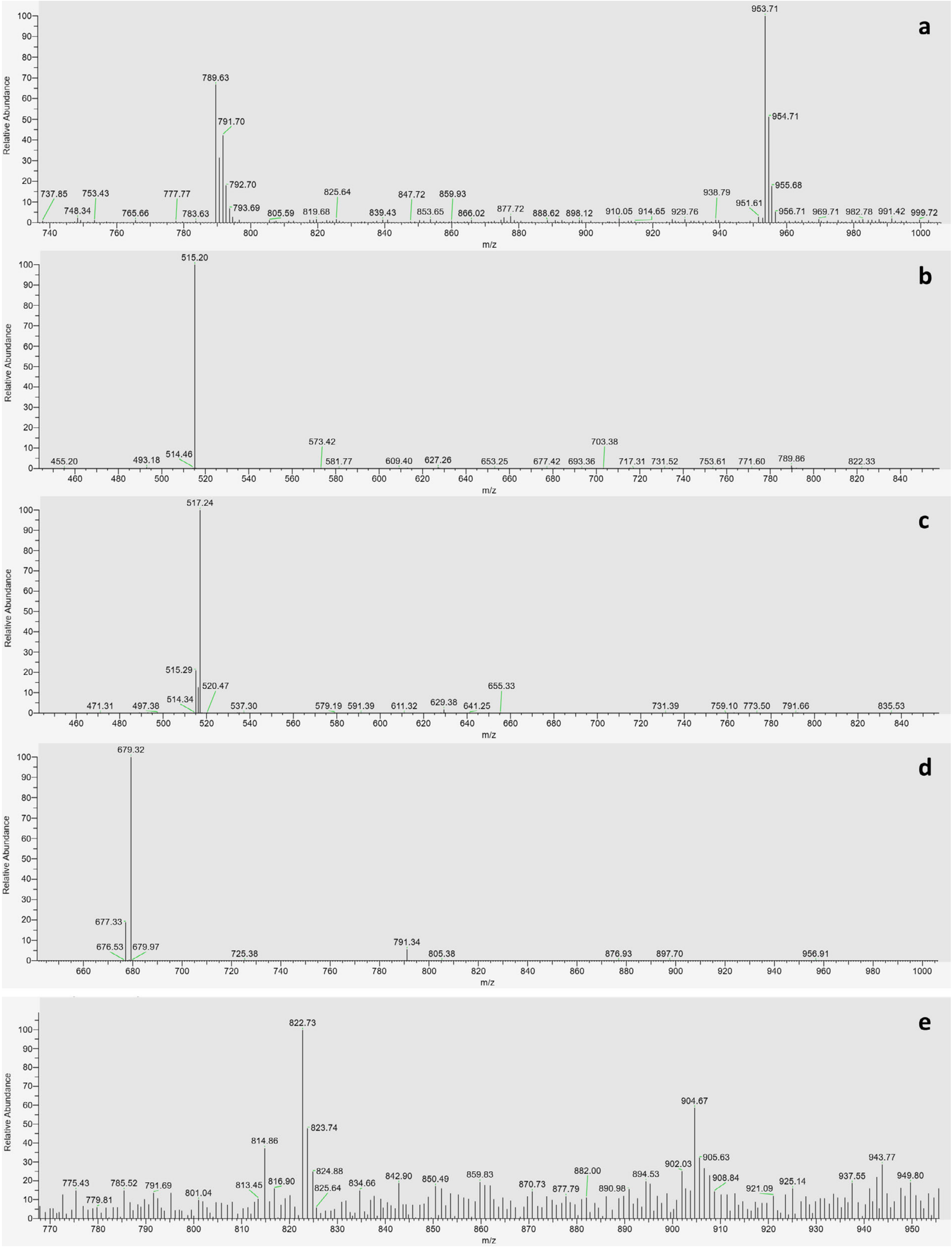

**Fig. 1.** (a) Close-up view of positive-ion electrospray ionization/mass spectrometry (ESI/MS) full-scan spectrum of sodium adducts of galactolipids from *Zooxanthella nutricula* CCMP 3427 where the following galactolipids are represented (with masses rounded down to the nearest whole number: 18:5/18:5 MGDG (*m/z* 789), 18:5/18:4 MGDG (*m/z* 791) and 18:5/18:4 DGDG (*m/z* 953). (b–d) ESI/MS/MS of these three galactolipids, respectively. In (e), the following betaine lipids are represented in the full-scan, close-up ESI/MS spectrum: 38:10 DGCC (*m/z* 814), 38:6 DGCC (*m/z* 822) and 44:7 DGCC (*m/z* 904).

*Z. nutricula* as a means of demonstrating how it may adapt to warmer seas, but were unable to acquire enough biomass to provide results. Nevertheless, based on our results, we can say that *ex hospite Z. nutricula*, when compared to *ex hospite Symbiodinium* at 20°C, possesses the same type of galactolipid matrix within which photosynthesis occurs.

Three betaine lipids were also observed in *Z. nutricula*: 38:10 DGCC (*m/z* 814, 15.6 $\pm$ 5.9%), 38:6 DGCC (*m/z* 822, 53.9 $\pm$ 2.9%) and 44:7 DGCC (*m/z* 904, 30.6 $\pm$ 6.6%) as shown in Fig. 1e. We are unaware of a commercial standard for DGCC, but its presence as the dominant betaine lipid in a dinoflagellate has been unambiguously identified by a combination of ESI/MS and nuclear magnetic resonance spectroscopy analyses by Flaim *et al.* (2014). Additionally, DGCC has been identified as the dominant betaine lipid by Řezanka *et al.* (2017). It should also be noted that the masses of the three forms of DGCC listed above do not conform to other possible betaine lipids, notably DGTS, previously observed in *S. microadriaticum* per Leblond *et al.* (2015). The numbers of total carbons and double bonds associated with these DGCC forms (i.e. lipid species) indicate the presence of PUFAs, possibly polyunsaturated $C_{18}$ to $C_{22}$ fatty acids, which may be available to the radiolarian host (discussed below). We were able to confirm the presence of docasahexaenoic acid (22:6 (n-3)) and hexadecenoic acid (16:0) via GC/MS analysis of FAMEs (see Supporting information, Table S1) and DMOX derivatives (data not shown) in a lipid fraction containing DGCC to show that 38:6 DGCC most likely contains 22:6(n-3) and 16:0 as the component fatty acids. Similarly, we observed small/trace amounts of 20:5 and 18:5 in this fraction that could explain the fatty acid composition of 38:10 DGCC; however, note that these two fatty acids were not the dominant fatty acids in this fraction (see Supporting information, Table S1). The 44:7 DGCC could contain octacosahepataenoic acid (28:7(*n*-6)), previously identified in dinoflagellates (Mansour *et al.*, 1999; Leblond & Chapman, 2000; Řezanka *et al.*, 2017) and 16:0, but we were unable to confirm the presence of 28:7(*n*-6) as either a FAME or DMOX derivative via GC/MS. Other fatty acids observed in this fraction could possibly be associated with anionic lipids, such as phosphatidic acid (Řezanka *et al.*, 2017), not detectable using positive-ion ESI/MS and ESI/MS/MS.

With both these betaine lipids, the overall number of forms was lower than the total number of betaine lipids and galactolipids in *S. microadriaticum* (Leblond *et al.*, 2015); however, in the present study, *Z. nutricula* was more difficult to grow even at 20°C to produce as much biomass (note cell concentration at low $10^4$ cells $mL^{-1}$), and we were unable to observe many of the more minor betaine lipids that would possibly be present at lower relative percentages (as observed by the ions listed above being close to the baseline in Fig. 1e). Nevertheless, the three betaine lipids observed in *Z. nutricula* were also observed in *ex hospite S. microadriaticum* and, in both species, 38:6 DGCC was the most abundant form (Leblond *et al.*, 2015).

We have also identified fatty acids associated with triglycerides (as the only acylglycerolipid class present in fraction 2); these fatty acids generally resembled those associated with betaine lipids and were present at approximately half the amount per cell (see Supporting information, Table S1). As a direct comparison with a study that used the same methodology, the fatty acids associated with triglycerides in *Z. nutricula* were in general agreement with the results of the GC/MS-based examination of *P. aciculiferum* and *Scrippsiella hangoei* (J. Schiller) J. Larsen (also known as *Apocalathium malmogiense* (G. Sjöstedt) Craveiro, Daugbjerg, Moestrup & Calado) by Leblond *et al.* (2006). With the exception of the $C_{28}$, triglyceride-associated fatty acids observed by Řezanka *et al.* (2017), the fatty acids observed in *Z. nutricula*'s triglyceride fraction also generally matched those in the Řezanka *et al.* (2017) study, which also examined *P. aciculiferum* as well as *Amphidinium carterae* Hulburt and *Cystodinium* sp. using high resolution Orbitrap LC/ESI/MS and LC/ESI/MS/MS to identify the three fatty acids attached to each glycerol backbone within an individual triglyceride (i.e. to identify each species of triglyceride).

In the present study, we have identified some of the major acylglycerolipids, such as MGDG and DGDG, and their associated fatty acids found within *Z. nutricula*. This work provides a baseline survey of the *ex hospite* galactolipid and betaine lipid biosynthetic capabilities of *Z. nutricula* to demonstrate that it produces a similar set of fatty acid-containing lipids as *S. microadriaticum*, one of the photosynthetic symbiont species of cnidarians. This indicates a metabolic overlap in these phylogenetically discrete dinoflagellates with unrelated host organisms, recognizing that the symbionts of cnidarians have been much better characterized (e.g. in terms of metabolic interplay between them and their hosts) over the past several decades. More specifically, we hypothesize that, if the radiolarian host ingests a portion of its *Z. nutricula* symbiont population (Anderson, 1976) and/or if there is some mechanism for direct transfer of fatty acid-containing lipids from *Z. nutricula* to its host, then the PUFAs associated with the galactolipids, betaine lipids and/or triglycerides of *Z. nutricula* may represent part of the pool of fatty acids available for uptake and metabolism by the host, as may occur in a dinoflagellate-cnidarian symbiosis (Garrett *et al.*, 2013). Future research should examine not only the *in hospite* galactolipid and betaine lipid composition of *Z. nutricula*, for example, but also the host-symbiont interplay in terms of possible lipid transfer, particularly PUFA-containing lipids, to confirm this hypothesis.

## ACKNOWLEDGMENTS

The helpful comments of three anonymous reviewers are greatly appreciated.

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## SUPPORTING INFORMATION

Additional Supporting Information may be found in the online version of this article at the publisher's web-site:

**Fig. S1.** (a) 4,4-Dimethyloxazoline (DMOX) derivative of octadecapentaenoic acid (18:5) and (b) DMOX derivative of 18:4 from the glycolipid fraction of *Zooxanthella nutricula*. In each panel, the bracket shows the same 12-amu mass difference to indicate the first unsaturation in the n-3 position.

**Table S1.** Relative percentage distributions (in terms of weight percentage) and interfraction comparison of fatty acid methyl esters (FAMEs) in *Zooxanthella nutricula*.