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Mélanie Gerphagnon <https://orcid.org/0000-0002-3363-4355>, Ramsy Agha <https://orcid.org/0000-0002-6109-4624>, Dominik Martin-Creuzburg <https://orcid.org/0000-0002-4248-0730>, Alexandre Bec <https://orcid.org/0000-0003-4353-3766>, Fanny Perriere, Cecilia Rad-Menéndez, Claire M. M. Gachon <https://orcid.org/0000-0002-3702-7472>, Justyna Wolinska <https://orcid.org/0000-0003-2913-2923>

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Comparison of sterol and fatty acid profiles of chytrids and their hosts reveals trophic upgrading of nutritionally inadequate phytoplankton by fungal parasites

Mélanie Gerphagnon^{1,a*}, Ramsy Agha^{1,a}, Dominik Martin-Creuzburg², Alexandre Bec³, Fanny Perriere³, Cecilia Rad-Menéndez⁴, Claire M.M. Gachon⁴, and Justyna Wolinska^{1,5}

(1) Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Germany

(2) Limnological Institute, University of Konstanz, Mainaustrasse 252, 78464 Konstanz, Germany

(3) Laboratoire Microorganismes : Génome et Environnement- Université Clermont Auvergne, France

(4) Culture Collection of Algae and Protozoa, Scottish Association for Marine Science, Scottish Marine Institute, UK

(5) Department of Biology, Chemistry, Pharmacy, Institute of Biology, Freie Universität, Germany

^a MG and RA contributed equally to this paper

*Corresponding author: Mélanie Gerphagnon; Leibniz-Institute of Freshwater Ecology and Inland Fisheries (IGB), Mueggelseedamm 301, 12587 Berlin, Germany; Tel: +33(0)142.759.006; melaniegerphagnon@hotmail.fr

Running title (not more than 50 characters): Trophic upgrading by chytrids

Originality-Significance Statement

Chytrids are important fungal parasites of phytoplankton that establish a trophic link between inedible or poorly nutritious phytoplankton taxa and zooplankton consumers (mycoloop). By characterizing the lipid profiles of two contrasting phytoplankton taxa and their respective chytrid parasites, this work reveals a novel qualitative facet of the mycoloop. In addition to rendering carbon and essential lipids from otherwise inedible phytoplankton hosts available to zooplankton, chytrids can upgrade the carbon of their hosts by *de novo* production of essential sterols. Evidence of trophic upgrading by chytrid parasites, together with their role as conveyors of carbon from inedible sources, emphasizes the importance of chytrid infection in enhancing carbon transfer in aquatic food webs.

Summary

Chytrids are ubiquitous fungal parasites in aquatic ecosystems, infecting representatives of all major phytoplankton groups. They repack carbon from inedible phytoplankton hosts into easily ingested chytrid propagules (zoospores), rendering this carbon accessible to zooplankton. Grazing on zoospores may circumvent bottlenecks in carbon transfer imposed by the dominance of inedible or poorly nutritious phytoplankton (mycocoloop). We explored qualitative aspects of the mycocoloop by analyzing lipid profiles (fatty acids, sterols) of two chytrids infecting two major bloom-forming phytoplankton taxa of contrasting nutritional value: the diatom *Asterionella formosa* and the filamentous cyanobacterium *Planktothrix agardhii*. The polyunsaturated fatty acid composition of chytrids largely reflected that of their hosts, highlighting their role as conveyors of otherwise inaccessible essential lipids to higher trophic levels. We also showed that chytrids are capable of synthesizing sterols, thus providing a source of these essential nutrients for grazers even when sterols are absent in their phytoplankton hosts. Our findings reveal novel qualitative facets of the mycocoloop, showing that parasitic chytrids, in addition to making carbon and essential lipids available from inedible sources, also upgrade their host's biochemical composition by producing sterols *de novo*, thereby enhancing carbon and energy fluxes in aquatic food webs.

Introduction

The trophic transfer efficiency across the autotroph–herbivore interface is crucial for carbon and energy fluxes in food webs. In aquatic ecosystems, phytoplankton communities are often dominated by species high resistance strategies to zooplankton grazing, such as large colonial diatoms or cyanobacteria (Ibelings *et al.*, 2011; Perga *et al.*, 2013; Rollwagen-Bollens *et al.*, 2013). Reduced grazing may result in trophic bottlenecks, limiting the efficient transfer of carbon and energy to higher trophic levels.

Several phytoplankton species, including large taxa, can be infected by fungal parasites belonging to the phylum Chytridiomycota (i.e. chytrids). These true fungi are commonly found in freshwater, brackish, and marine pelagic environments (Lepère *et al.*, 2016; Hassett *et al.*, 2017; Maier and Peterson, 2017). Chytrid fungi display a characteristic free-living life stage as

flagellated zoospores. Zoospores actively swim to find and infect new hosts. Upon encystment on a host, attached zoospores develop into a sporangium, a sessile reproductive structure which, once mature, releases new zoospores. In lakes, zoospore densities can reach more than 500 zoospores per ml (Jobard *et al.*, 2010) and the infection prevalence can be extremely high during chytrid epidemics (>90% of host cells are infected by at least one chytrid), as has been shown for filamentous cyanobacteria (Rasconi *et al.*, 2012) and diatom populations (Ibellings *et al.*, 2011). Zooplankton can take up chytrids by feeding on sporangia attached to edible hosts, i.e. concomitant predation. When phytoplankton assemblages are dominated by large inedible algae, zooplankton can still benefit from chytrid infections by efficiently feeding on zoospores. Zoospores have been proposed to constitute an alternative trophic link between primary and secondary production, the so-called mycoloop (Kagami *et al.*, 2014). Experimental evidence shows that the mycoloop can sustain zooplankton somatic growth and reproduction when large inedible diatoms or poorly edible filamentous cyanobacteria dominate phytoplankton communities (Kagami *et al.*, 2007; Agha *et al.*, 2016). Food web models support the importance of chytrid infections in enhancing carbon and energy flows; chytrid parasitism may convey as much as 20% of gross primary production (in terms of carbon) and provide up to 38% of zooplankton dietary requirements (Grami *et al.* 2011; Rasconi *et al.*, 2014; Haraldsson *et al.*, 2018).

In addition to the quantitative importance of the mycoloop, chytrids might constitute a high-quality food for zooplankton, being a source of essential lipids. Crustacean zooplankton does not synthesize polyunsaturated fatty acids (PUFA) and sterols *de novo* and thus need to acquire these essential lipids from their diet (Müller-Navarra *et al.*, 2000; Martin-Creuzburg *et al.*, 2008). PUFA are structural components of cell membranes and are involved in the modulation of a variety of vital membrane properties (Valentine and Valentine, 2004). Sterols, such as cholesterol, the predominant sterol in animals, are indispensable components of eukaryotic cell membranes and serve as precursors of steroid hormones (Goad, 1981). Due to the crucial importance of PUFA and sterols to consumers, *de novo* production of essential lipids by chytrid parasites might alleviate lipid deficiency in zooplankton, especially when phytoplankton communities are dominated by taxa lacking essential lipids. Cyanobacteria, as prokaryotes, differ from eukaryotic algae by lacking essential lipids, i.e. sterols and long-chain PUFA. Thus, during cyanobacterial blooms zooplankton may suffer from a deficiency in essential lipids, resulting in reduced growth and reproduction (Von Elert *et al.*, 2003; Martin-Creuzburg *et al.*, 2008). Chytrid infections might alleviate such deficiency if chytrid zoospores and/or sporangia

produce essential lipids *de novo*. This mechanism might enhance overall trophic transfer efficiency in the pelagic food web. Chytrids have been shown to upgrade allochthonous organic matter by increasing digestibility of pollen grains and facilitating the transfer of otherwise inaccessible PUFA from pollen to metazoans through chytrid zoospores (Masclaux *et al.*, 2013; Kagami *et al.*, 2017). However, it is unclear if a similar biochemical upgrading of autochthonous carbon sources, such as phytoplankton, occurs upon infection by chytrid parasites. Although the characterization of the single species *Zygorhizidium planktonicum*, infecting the diatom *Asterionella formosa*, revealed that zoospores contain significant amounts of sterols (Kagami *et al.*, 2007), it is still uncertain if chytrids incorporate sterols and other essential nutrients from their hosts, or if they synthesize them *de novo*.

The present study explores qualitative aspects of the mycoloop. Since phytoplankton taxa are highly diverse in terms of lipid composition, the influence of host lipid composition on chytrid zoospores might shape the functional importance of the mycoloop. We analyzed the lipid profiles of two phytoplankton hosts with contrasting nutritional quality for zooplankton (the long-chain PUFA- and sterol-rich diatom *Asterionella formosa* and the long-chain PUFA- and sterol-free cyanobacterium *Planktothrix agardhii*) and their respective chytrid parasites. We aimed to evaluate i) whether chytrids reflect the lipid composition of their hosts and thereby merely act as conveyors of inaccessible carbon and lipids to zooplankton consumers, and/or ii) if chytrids produce essential lipids *de novo* and therefore upgrade the carbon supplied by their hosts.

Results

Carbon content, infected and uninfected hosts and parasites densities

Carbon contents of uninfected host cells and zoospores were determined from carbon analyses and counts for each host-parasite system. Carbon contents were higher in uninfected *A. formosa* (40.1 ± 3.0 pg C cell⁻¹) than in *P. agardhii* (16.5 ± 4.9 pg C cell⁻¹). Zoospores infecting the diatom had three times lower carbon contents than zoospores infecting the cyanobacterium (19.4 ± 1.9 pg C cell⁻¹ and 49.6 ± 0.8 pg C cell⁻¹, respectively). Parasite-free host cultures had densities of $5.4 \pm 0.6 \times 10^5$ and $7.9 \pm 2.5 \times 10^5$ diatom and cyanobacteria cells ml⁻¹, respectively. Infected diatom and cyanobacteria cultures were composed of $2.9 \pm 0.8 \times 10^6$ and $9.5 \pm 0.5 \times 10^6$ host cells, $4.5 \pm 1.5 \times 10^5$ and $2.6 \pm 1.9 \times 10^5$ attached sporangia as well as $6.6 \pm 0.3 \times 10^3$ and $2.8 \pm 0.3 \times 10^4$ zoospores ml⁻¹, respectively.

Fatty acids

Uninfected hosts differed quantitatively and qualitatively in term of fatty acids (Fig. 2). While FA compositions of both hosts were similar in terms of major FA groups (Fig. 2B), they differed notably with regard to PUFA composition. The diatom displayed a diverse PUFA composition, characterized by the dominance of eicosapentaenoic acid (EPA; 20:5n-3; Fig. 2C; Table S1A), whereas the cyanobacterium did not contain any long-chain PUFA, but high concentrations of α -linolenic acid (ALA, 18:3n-3) and linoleic acid (18:2n-6) instead (Fig 2D; Table S1B).

Infection by chytrid parasites increased total FA content in the Host+Sporangia fraction of the diatom system (about 5-fold), but not in the cyanobacterium system (Fig. 2A). A comparison of uninfected hosts and zoospores across both systems revealed quantitative and qualitative changes in FA compositions. Overall, chytrid zoospores were especially rich in FA (Fig. 2A; Table S1). A marked reduction in the proportion of PUFA was evident in zoospores of both systems, together with a general increase in monounsaturated fatty acids (MUFA; Fig. 2B). The latter were dominated by 16:1n-7 and 18:1n-7 in zoospores of both chytrid species, although 18:1n-7 was found at very low proportions and concentrations in their respective hosts (Fig. 2C, D, Table S1). Also, zoospores contained significant amounts of 18:1n-9 (Fig 2C, 2D, Table S1). Zoospores of both systems presented similar proportions of FA groups and total PUFA contents (Fig. 2B, Table S1). However, PUFA compositions of zoospores of both systems partially reflected that of their respective hosts. Specifically, the PUFA composition of the diatom-infecting chytrid was dominated by EPA, although other PUFA detected in the uninfected diatom were not detected in the chytrid zoospores (Fig 2C, Table S1). The cyanobacterium-infecting chytrid contained ALA and 18:2n-6 as the major PUFA, together with stearidonic acid (18:4n-3). The latter was not detected in the cyanobacterium and was putatively produced *de novo* (Fig. 2D).

Both systems presented significant differences in FA diversity (assessed by the Shannon-Wiener Index) among the three fractions analyzed: Uninfected Host, Host+Sporangia and Zoospores (Fig. S1, Table 1). Zoospores of the diatom-infecting chytrid displayed a significantly less diverse FA composition than two other fractions (Fig. S1, Table 1). The opposite was found in the cyanobacterium system, zoospores had more diverse FA profiles (Fig. S1, Table 1).

Sterols

The diatom *A. formosa* was rich in sterols, consisting mainly of epibrassicasterol and 22-dehydrocholesterol (Fig. 3B), whereas the cyanobacterium *P. agardhii* lacked sterols completely (Fig. 3A).

Infection with chytrid parasites increased total sterol contents in both systems (Fig. 3A). In the diatom system, an increase in sterol diversity in the Host+Sporangia and Zoospores fractions was recorded, compared to the uninfected diatom (Fig. 3B, Fig S1). This was supported by significant differences in the Shannon-Wiener Index ($F_{2,3} = 145.6$; $p < 0.01$, Table 1). In the cyanobacterium system, the Host+Sporangia fractions presented cholesterol and dihydrocholesterol, indicating *de novo* production by the chytrid parasite (Fig. 3B).

Zoospore fractions from both systems were characterized by high sterol content (Fig. 3A), with cholesterol as the most prominent sterol. Yet, diatom-infecting chytrid zoospores displayed a more diverse sterol profile than the cyanobacterium-infecting chytrid zoospores (Fig. 3B). In both cases, *de novo* sterol production by chytrid parasites was indicated by the detection of individual sterols in the Zoospores and Host+Sporangia fractions that were otherwise not detected in their respective Uninfected Host.

Discussion

It has been proposed that chytrids can circumvent trophic bottlenecks by repacking otherwise inaccessible phytoplankton carbon into readily ingestible zoospores, rendering it available to zooplankton (Kagami *et al.*, 2007; Agha *et al.*, 2016). In the present study, we investigated whether chytrids reflect the lipid composition of their phytoplankton hosts, and/or produce lipids *de novo* thus upgrading phytoplankton diet.

Carbon contents of both chytrid species are within the range of those reported earlier for another chytrid species, *Zygorhizidium planktonicum* (Kagami *et al.*, 2007). We attribute differences in carbon contents between the two chytrid species used in our experiment to differences in the size of zoospores. Zoospores size has been shown to be a flexible parasite trait that varies with host genotype (Agha *et al.* 2018). As expected, zoospores of both chytrid species were rich in lipids, presenting a large lipid globule, a characteristic morphological feature of chytrids (Powell *et al.*, 2015; Van den Wyngaert *et al.*, 2018). Chytrids' high lipid contents could explain the observed increase in total FA contents in Host+Sporangia fraction compared to the uninfected diatom host. However, some fatty acids are known to exhibit anti-

fungus activity (Lim *et al.*, 2017) and this increase might also result from an immune response of host cells to infection.

Chytrids contained high proportions of MUFA, including 16:1n7, 18:1n7 and 18:1n9, which are common also in other fungi (Akinwale *et al.*, 2014; Arce Funck *et al.*, 2015). As MUFA are major substrates for β -oxidation (Bell *et al.*, 2002; Cruz *et al.*, 2018), they might be used by chytrid zoospores as endogenous lipid reserves to fuel swimming, as reported for aquatic zoosporic fungi belonging to the phylum Blastocladiomycota (Suberkropp & Cantino 1973; Mills & Cantino, 1977). Chytrid PUFA composition largely reflected that of their respective hosts. Like its host, the diatom-infecting chytrid contained almost exclusively EPA (20:5n-3), an important dietary constituent playing crucial role in zooplankton growth and reproduction (Müller-Navarra *et al.*, 2000; Wacker and von Elert, 2001; Martin-Creuzburg *et al.*, 2010). Also in line with its host, the cyanobacterium-infecting chytrid lacked EPA and contained linoleic acid (18:2n-6) and ALA (18:3n-3), in addition to stearidonic acid (18:4n-3) which was produced *de novo*. Despite the lack of EPA or other long-chain PUFA, this chytrid taxon arguably constitutes a source of essential PUFA for consumers, as zooplankton has been shown to use ALA and stearidonic acid to produce EPA, although this conversion is poorly efficient (Bec *et al.*, 2003; Taipale *et al.*, 2011). Marzetz *et al.* (2017) demonstrated the importance of dietary fatty acid diversity for *Daphnia* growth rates. Our results show that zoospores of the cyanobacterium-infecting chytrid presented the most diverse fatty acid profile, which suggests that this chytrid might biochemically upgrade the matter of its host. However, we found no evidence for a substantial qualitative or quantitative upgrading of host PUFA content or composition by chytrids.

The chytrids analyzed here largely reflected the FA compositions of their hosts. It is hence tempting to assume that contribution of chytrids to zooplankton PUFA demands will be determined by the phytoplankton species being infected. Yet, we cannot rule out that the observed differences in FA between two analysed chytrid species just reflect the phylogenetic distance (Akinwale *et al.*, 2014). Cross-infection experiments and/or isotopic analyses could unequivocally show to what extent the host's chemical composition determines the nutritional quality of their chytrids parasites. Nonetheless, our study supports the idea that readily ingestible zoospores facilitate the transfer of PUFA from inedible or poorly edible phytoplankton taxa to zooplankton. Thus, the qualitative contribution of chytrids to PUFA demands of zooplankton seems to depend on which phytoplankton species is primarily infected.

Arthropods are incapable of synthesizing sterols *de novo* and thus rely on dietary sources to cover their physiological demands (Von Elert *et al.*, 2003; Wacker and Martin-Creuzburg, 2012; Martin-Creuzburg *et al.*, 2014). Our analyses show that the diatom *A. formosa* constitutes a potential source of sterols (mainly epibrassicasterol). However, transfer of sterols from large diatoms to zooplankton, such as the cladoceran *Daphnia*, is hampered by their low-ingestibility (Kagami *et al.*, 2007). In contrast, no sterols were detected in *P. agardhii*, supporting previous findings showing that cyanobacteria constitute a sterol-deficient food source for zooplankton (Von Elert *et al.*, 2003; Martin-Creuzburg *et al.*, 2008). Analyses of zoospores demonstrated *de novo* sterol biosynthesis. Chytrids infecting the sterol-free cyanobacterium contained significant amounts of cholesterol and dihydrocholesterol. Chytrid infecting diatoms contained epibrassicasterol in concentrations similar to what was found in the uninfected host but, in addition, synthesized some sterols *de novo*. Zoospores of this species presented the highest sterol diversity. Although no study has yet investigated the putative role of sterol diversity in trophic interactions, a positive impact of sterol diversity on *Daphnia* physiology cannot be ruled out, similar to the one demonstrated for fatty acid diversity (Marzetz *et al.*, 2017). In spite of the phylogenetic distance, cholesterol was the predominant sterol in both chytrid species. The diatom-infecting chytrid, *Zygorhizidium affluens*, belongs to the order Lobulomycetales which is distantly related to other Chytridiomycota orders, including the here used cyanobacterium-infecting chytrid, *Rhizophydium megarrhizum* (order Rhizophydiales), (Rad-Menéndez *et al.*, 2018). Cholesterol is also the main sterol in *Zygorhizidium planktonicum* (Kagami *et al.*, 2007), another diatom-infecting chytrid belonging to the order Novel clade II (*sensu* Jobard *et al.*, 2012), and in four other species belonging to the order Chytridiales (Weete *et al.*, 2010). Taken together, cholesterol content seems to be a general feature of fungi of the phylum Chytridiomycota. Since cholesterol is the main sterol in most animals, and is often hardly represented in plants or algae (Martin-Creuzburg and Merkel, 2016), high cholesterol content in zoospores stresses the nutritional importance of chytrids for consumers. The qualitative contribution of the mycocoop to sustain secondary production would be even more significant when phytoplankton communities are dominated by inedible and/or sterol-deficient species, as chytrids produce sterols *de novo*, increase sterol diversity and concentrate existing sterols in readily ingestible zoospores.

Conclusion

Lipid analyses reported here uncover an additional facet of the mycoloop. The PUFA composition of chytrid parasites generally reflected that of their phytoplankton hosts. However, chytrids produced sterols *de novo* that are otherwise absent in their phytoplankton host. Therefore, in addition to conveying carbon from inedible or poorly edible sources in the form of readily ingestible zoospores, chytrid parasites i) convey essential fatty acids from their phytoplankton hosts to zooplankton and ii) upgrade the biochemical quality of host-derived organic matter by synthesizing sterols *de novo*. These findings stress the qualitative importance of the mycoloop as trophic link contributing to the coupling of primary and secondary production in pelagic food webs.

Experimental Procedures

Host and parasite cultures

Two host-parasite systems were used in this study: 1) the diatom *A. formosa*, strain CCAP 1005/23, with its chytrid parasite *Zygorhizidium affluens*, strain CCAP4086/1 (both isolated from Lake Pavin, France, in 2013) and 2) the cyanobacterium *P. agardhii*, strain NIVA-CYA630 (isolated from Lake Lyseren, Norway, in 2008) with its chytrid parasite *Rhizophydium megarrhizum*, strain Chy-Kol2008 (isolated from Lake Kolbotnvatnet, Norway, in 2008). The diatom host was maintained as non-axenic batch cultures in DM medium (Beakes *et al.*, 1988) at 20°C and a 12/12h light/dark regime ($64 \mu\text{mol m}^{-2} \text{s}^{-1}$). Its chytrid parasite was maintained on its diatom host at 16°C and the same light conditions, by transferring 20% (vol/vol) of a synchronized and highly infected culture into an uninfected host culture every weeks. The cyanobacterium host was maintained in Z8 medium (Kotai, 1972) as non-axenic batch cultures at 16°C and constant irradiance ($15 \mu\text{mol m}^{-2} \text{s}^{-1}$). The chytrid parasite was maintained on its cyanobacterium host cultivated at the same temperature and light conditions by transferring zoospore suspensions into uninfected cultures every two weeks (Sonstebo and Rohrlack, 2011). All culture transfers were performed aseptically. Cultures were mixed daily through gentle swirling by hand.

Experimental set-up

Uninfected cultures of *A. formosa* and *P. agardhii* were scaled up for 4 weeks and maintained as triplicate exponentially-growing semi-continuous cultures by weekly dilutions (1:2 in Z8 medium and 1:4 in DM medium, respectively). About 20% volume of each replicated culture

was separated whereas the remaining volume (80% vol) was inoculated with their respective chytrid parasites. Uninfected and infected cultures were further incubated under the above conditions. One *A. formosa* replicate (infected and uninfected) was lost due to handling error. Eleven days after the addition of chytrids, advanced infection and the presence of free-swimming zoospores were confirmed microscopically and cultures were size-fractionated for the analysis of carbon, sterols, and fatty acids (Fig. 1). Briefly, infected cultures containing a mixture of uninfected hosts, infected hosts (with attached sporangia), free-swimming zoospores, and heterotrophic bacteria, were filtered successively through 25 μm , 10 μm and 5 μm sterile nylon meshes and a 3 μm polycarbonate filter. The largest fraction (i.e. $>3 \mu\text{m}$), containing infected (incl. attached sporangia) and uninfected host cells was collected by washing the successive meshes with sterile DM or Z8 medium (depending on the system) and transferring the concentrate into sterile Erlenmeyer flasks. The filtrate, i.e. the $<3 \mu\text{m}$ fraction including zoospores and heterotrophic bacteria, was transferred to sterile glass Erlenmeyer flasks. Each size-fraction was then splitted into three sub-samples: one for particulate organic carbon (POC), one for fatty acid (FA), and one for sterol analyses (Fig.1). For FA and sterol analysis, each dedicated sub-sample was filtered on pre-combusted WhatmanTM GF/C glass-fiber filters ($\varnothing$ 47mm). Filters were kept at -80°C until lipid analyses. POC was determined from the sub-sample of each fraction, loaded onto pre-combusted and pre weighted WhatmanTM GF/C glass-fiber filters ($\varnothing$ 25mm). The filters were dried for 24 h, weighed, and analyzed by combustion elemental analysis using an Elementar Vario EL analyzer (Elementar, Germany).

Bacterial POC in the zoospore fraction ($<3\text{-}1.2\mu\text{m}$) was estimated from differences in bacterial counts (see below for counting methodology) before ($<3\text{-}1.2 \mu\text{m}$) and after filtration through GF/C glass-fiber filters ($<1.2 \mu\text{m}$; Fig.1), assuming an average carbon content of 20 fg cell⁻¹ (Ducklow & Carlson 1992).

Aliquots from each fraction of infected cultures were fixed i) in formaldehyde (2% final concentration) to determine zoospore and bacterial densities and ii) in Lugol's iodine solution to quantify host and sporangia densities (Fig.1). FA, sterols, and POC of the Uninfected hosts were analyzed accordingly, following the same procedures as above.

Fatty acid analysis

Filters containing biomass from the different fractions (1.78 - 2.1 mgC, 15.5 - 20.1 mgC and 4.9 - 7.5 mgC for Zoospore, Host+Sporangia and Uninfected host fractions, respectively) were extracted twice using chloroform:methanol (2/1), following the method of Folch *et al.* (1957). FA in the total lipid extract were converted into FA methyl-esters (FAME) by acid-catalyzed

transesterification after adding non-methylated 13:0 and 23:0 as internal standards according to a modified protocol of Christie (1982; 4% H₂SO₄ in methanol at 75°C for 2h). FAME were analyzed on a gas chromatograph (Agilent technologies 6850, Santa Clara, CA, USA) equipped with a DB-Wax column (J&W Scientific, 30 m × 0.25 mm ID × 0.25 mm film), and a flame ionization detector (250°C; split injection; carrier gas: helium; oven temperature ramp 150°C to 250°C at 3°C min⁻¹). FAME were quantified against internal standards (20 µg sample⁻¹ of 13:0 and 23:0) and identified by comparing retention times with those obtained from Supelco® standards (37 Component FAME Mix), Larodan FAME standards, and their mass spectra which were recorded using a gas chromatograph-mass spectrometer (GC-MS; Agilent Technologies, 5975B MSD). FAME concentrations were then normalized by POC contents in respective samples.

Sterol analysis

Filters prepared for sterol analysis were deposited in dichloromethane/methanol (2:1, v/v) and stored at -20°C overnight. Prior to extraction, 5 α -cholestane was added as internal standard (1 µg sample⁻¹). Total lipids were extracted twice with dichloromethane/methanol (2:1, v/v). The pooled cell-free lipid extracts were evaporated to dryness under N₂-atmosphere and saponified with methanolic KOH (0.2 mol L⁻¹, 70°C, 1 h). Subsequently, neutral lipids were partitioned into iso-hexane:diethylether (9:1, v/v), evaporated to dryness under N₂ and resuspended in iso-hexane (10–30 µl). Sterols were analyzed and quantified by gas chromatography (GC) using an HP 6890 gas chromatograph (Agilent Technologies, Santa Clara, CA, USA) equipped with a flame ionization detector (FID) and an HP-5 capillary column (Agilent Technologies, 30 m × 0.25 mm ID × 0.25 mm film). Additional GC configurations are given elsewhere (Martin-Creuzburg *et al.*, 2016). The injection volume was 1 µl. Sterols were quantified by comparison to 5 α -cholestane using multipoint calibration curves generated for a set of sterols that are available commercially. Sterols for which authentic standards were not available were quantified as cholesterol equivalents using the cholesterol calibration curve; C-24 epimers and cis-trans isomers were quantified using the calibration curve of the respective epimer/isomer available as authentic standard. The limit for quantification of sterols was 10 ng. Sterols were identified based on their relative retention times and their mass spectra, which were recorded using a gas chromatograph-mass spectrometer (GC-MS; Agilent Technologies, 5975 C inert MSD) equipped with a fused-silica capillary column (HP-5MS, Agilent; GC settings as described above for FID). Sterols were analyzed both in their free form and as their trimethylsilyl derivatives, which were prepared by incubating 20 µl of the iso-hexane sterol

extracts with 10 μl of N,O-bis(trimethylsilyl)trifluoroacetamide including 1 % trimethylchlorosilane for 1 h at room temperature. Mass spectra were recorded between m/z 50 and 600 in the electron ionization mode, and then compared with mass spectra of authentic standards and mass spectra found in a self-generated spectra library. The C-24 stereochemistry could not be identified with certainty and thus was not further specified or, if possible, derived from literature data. For instance, the 24-methylcholesta-5,22-dien-3 β -ol detected in *A. formosa* was assumed to show the 24 α configuration, thus representing epibrassicasterol rather than brassicasterol as has been proposed for other diatom species (Martin-Creuzburg and Merkel 2016). Sterol concentrations were related to the POC contents of their respective samples.

Host, sporangia, zoospore and free-living bacteria densities

Host and sporangia fractions (>3 μm). Host cells density (or filaments for cyanobacteria) and sporangia densities were estimated from 0.5 to 0.8 ml of Lugol-fixed samples using a Sedgewick-Rafter chamber and a Nikon Ti Eclipse inverted microscope. At least 400 host cells (or filaments) were investigated per sample.

Zoospores fraction (3-1.2 μm). Zoospore quantification was estimated from formaldehyde-fixed samples and analyzed using a Sedgewick-Rafter chamber. At least 20 μl (or 150 zoospores) were counted per sample. To rule out host contamination, the entire chamber was checked but no host cells or filaments were found. Bacterial densities were determined using a haemocytometer and a Zeiss Axioskop epifluorescence microscope after staining with 4', 6-diamidino-2-phenylindole (DAPI; 1 $\mu\text{g ml}^{-1}$ final conc.) followed by 1 h incubation in the dark. Bacterial POC in the zoospores fraction accounted for 6.5 ± 3.4 % and 3.4 ± 0.5 % of total POC in the diatom and the cyanobacterial systems, respectively. Lipid concentrations were related to the zoospore biomass only.

Data analysis

Fatty acid (H_{FA}) and sterol (H_{ST}) diversity within individual fractions was calculated using the Shannon-Wiener index. H_{FA} calculation included the 27 fatty acids representing at least 1% of total FA in at least one fraction in each system. Because sterol diversity was low, no pre-selection was made to calculate sterol H_{ST} . One-way ANOVAs were performed for the H_{FA} and H_{ST} of each system, including the different fractions (i.e. Uninfected Host, Host+Sporangia and Zoospores) as fixed factors, followed by Tukey's post hoc tests. Data were not normally distributed, even after transformation. ANOVA was still applied as the H_{FA} and H_{ST} of both systems showed equal variances of the residuals (Bartlett test; $\alpha=0.05$).

Q-q-plots of the residuals revealed no major deviations from normality. ANOVA, while very sensitive to lack of homocedasticity (which is not the case here) is very robust to deviations normality. Even moderate deviations from normality have little effect in false positive rates (Glass *et al.*, 1972; Harwell *et al.*, 1992; Lix *et al.*, 1996).

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Conflict of interest

The authors declare that they have no conflict of interest.

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Table and Figure legends

Table 1. Results of ANOVA followed by Tukey's post hoc test, testing the difference of Shannon-Wiener Index applied to fatty acids (H_{FA}) and to sterols (H_{ST}) between the different fractions: ZP (Zoospores), HS (Host+Sporangia) and H (Uninfected host) for diatom and cyanobacterium systems. ND: not determined, as cyanobacteria do not contain sterols. NSS: not statistically significant.

Fig. 1. Cultivation and sample fractionation procedure applied to acquire samples for subsequent fatty acid (FA), sterol (St), and particulate organic carbon (POC) analyses, and to determine prevalence of infection as well as host, bacteria (B), sporangia and zoospore (ZP) densities.

Fig. 2. Fatty acid content (A) and composition (B, C, D) of different fractions in the diatom and cyanobacterium systems. A: Values are given as means of replicates $\pm$ standard deviation (SD). B: Fatty acids are grouped into polyunsaturated (PUFA), monounsaturated (MUFA), saturated (SAFA), branched, and unidentified fatty acids. Numbers reported on the bars refer to mean of absolute concentrations of the respective fatty acid group ($\mu\text{g mgC}^{-1}$). C, D: Fatty acid composition of Zoospores and Uninfected hosts for the diatom (C) and cyanobacterium (D) systems. Values are given as means of replicates $\pm$ SD

Fig. 3. Sterol contents (A) and composition (B) of different fractions in the diatom and cyanobacterium systems. A: Values are given as means $\pm$ standard deviation of replicates. B: Other sterols: traces of lanosterol and three other unidentified sterols. Numbers refer to mean of absolute concentrations of each sterol (10^2 ng mgC^{-1}). ND: Not detected.

Supplemental Figures and Tables legends

FIGURE S1 Shannon-Wiener Index applied to fatty acids (H_{FA} , A) and to sterols (H_{ST} , B) for the different fractions of diatom and cyanobacterium systems. Values are given as means of replicates $\pm$ standard deviation. ND: Not detected

TABLE S1

Fatty acid composition and content of infected and uninfected host *A. formosa* (A) or *P. agardhii* (B) and of CCAP4086/1 (*A. formosa* –infecting chytrid) (A) and Chy-Kol2008 (*P. agardhii* –infecting chytrid) (B) zoospores. (SAFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acids). Values are given in percentages as means of replicates $\pm$ standard deviation. nd: not detected. The 27 fatty acids which represent at least 1% of total FA in at least one fraction of the two systems are colored in grey.

Fig1

Fig 2

Fig 3

Table 1

		Fractions		
		ZP vs HS	HS vs H	ZP vs H
H_{FA}	Diatom system			
	$F_{2,3} = 130.8, p < 0.001$	$p < 0.01$	NSS	$p < 0.01$
	Cyanobacteria system			
	$F_{2,6} = 820.8, p < 0.001$	$p < 0.01$	$p < 0.01$	$p < 0.01$
H_{ST}	Diatom system			
	$F_{2,3} = 145.6, p < 0.001$	NSS	$p < 0.01$	$p < 0.01$
	Cyanobacteria system			
	$F_{1,4} = 3.6, p=0.12$	NSS	ND	ND

Supporting Information

Comparison of sterol and fatty acid profiles of chytrids and their hosts reveals trophic upgrading of nutritionally inadequate phytoplankton by fungal parasites

Mélanie Gerphagnon^{1,a,*}, Ramsy Agha^{1a}, Dominik Martin-Creuzburg², Alexandre Bec³, Fanny Perriere³, Cecilia Rad-Menéndez⁴, Claire M.M. Gachon⁴, and Justyna Wolinska^{1,5}

FIGURE S1 Shannon-Wiener Index applied to fatty acids (H_{FA} , A) and to sterols (H_{ST} , B) for the different fractions of diatom and cyanobacterium systems. Values are given as means of replicates $\pm$ standard deviation. ND: Not detected

TABLE S1. Fatty acid composition and content of infected and uninfected host *A. formosa* (A) or *P. agardhii* (B) and of CCAP4086/1 (*A. formosa* – infecting chytrid) (A) and Chy-Kol2008 (*P. agardhii* – infecting chytrid) (B) zoospores. (SAFA: saturated fatty acid, MUFA: monounsaturated fatty acid, PUFA: polyunsaturated fatty acids). Values are given in percentages as means of replicates $\pm$ standard deviation. nd: not detected. The 27 fatty acids which represent at least 1% of total FA in at least one fraction of the two systems are colored in grey.

FIGURE S1

Table S1

A	<i>A. formosa</i>		<i>A. formosa</i> -CCAP4086/1			
	Uninfected Host		Host + Sporangia		Zoospores	
	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)
	Fatty acid					
12:0	0.5 ± 0.1	0.1 ± 0.0	n.d	n.d	0.3 ± 0.1	0.7 ± 0.1
14:0	16.4 ± 0.5	5.1 ± 1.4	12.8 ± 0.4	21.9 ± 5.5	2.4 ± 0.4	5.0 ± 0.2
15:0	0.4 ± 0.0	0.1 ± 0.0	0.5 ± 0.0	0.8 ± 0.2	0.9 ± 0.1	1.8 ± 0.1
16:0	4.8 ± 0.1	1.5 ± 0.4	4.9 ± 0.3	8.3 ± 1.4	19.7 ± 1.1	41.5 ± 6.9
17:0	n.d	n.d	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.7 ± 0.1
17:0 cyclic	n.d	n.d	n.d	n.d	1.1 ± 0.0	2.4 ± 0.3
18:0	2.5 ± 0.1	0.8 ± 0.3	1.1 ± 0.7	1.7 ± 0.8	10.3 ± 0.8	21.8 ± 4.0
20:0	n.d	n.d	0.1 ± 0.0	0.2 ± 0.1	0.4 ± 0.0	0.9 ± 0.0
22:0	0.1 ± 0.1	0.0 ± 0.1	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.1	0.7 ± 0.1
24:0	0.4 ± 0.0	0.1 ± 0.0	0.4 ± 0.0	0.7 ± 0.1	0.8 ± 0.2	1.6 ± 0.6
Σ SAFA	25.1 ± 0.4	7.9 ± 2.3	20.1	0.6	34.2	6.6
36.6 ± 1.3	77.0 ± 11.4					
20H 17:0	n.d	n.d	n.d	n.d	n.d	n.d
iso 17:1	n.d	n.d	n.d	n.d	n.d	n.d
20H 14:0	n.d	n.d	n.d	n.d	n.d	n.d
20H 15:0	n.d	n.d	n.d	n.d	0.8 ± 0.0	1.7 ± 0.2
20H 17:0	n.d	n.d	n.d	n.d	n.d	n.d
20XO 16:0	n.d	n.d	0.4 ± 0.1	0.8 ± 0.3	0.9 ± 0.1	1.9 ± 0.5
30H 16:0	n.d	n.d	n.d	n.d	n.d	n.d
anteiso 15:0	n.d	n.d	n.d	n.d	0.2 ± 0.0	0.5 ± 0.0
anteiso 15:1	n.d	n.d	n.d	n.d	n.d	n.d
anteiso 17:0	n.d	n.d	n.d	n.d	n.d	n.d
iso 14:0	n.d	n.d	n.d	n.d	n.d	n.d
iso 15:0	n.d	n.d	n.d	n.d	1.8 ± 0.2	3.7 ± 0.1
iso 15:1	n.d	n.d	n.d	n.d	0.9 ± 0.0	1.8 ± 0.1
iso 16:0	n.d	n.d	n.d	n.d	n.d	n.d
iso 17:0	n.d	n.d	n.d	n.d	n.d	n.d
Σ Branched	n.d	n.d	0.4 ± 0.1	0.8 ± 0.3	4.6 ± 0.2	9.6 ± 0.7
16:1(n-5)	0.3 ± 0.0	0.1 ± 0.0	0.3 ± 0.0	0.5 ± 0.1	0.4 ± 0.1	0.8 ± 0.0
16:1(n-7)	17.1 ± 0.3	5.4 ± 1.5	20.1 ± 1.1	34.2 ± 5.7	24.8 ± 0.7	52.0 ± 7.4
16:1(n-9)	n.d 0.0	n.d	n.d	n.d	0.5 ± 0.0	1.0 ± 0.0
18:1(n-7)	0.6 ± 0.0	0.2 ± 0.0	3.2 ± 0.1	5.5 ± 1.4	23.2 ± 0.4	48.6 ± 4.7
18:1(n-9)	1.1 ± 0.0	0.3 ± 0.1	0.8 ± 0.1	1.4 ± 0.2	2.9 ± 0.1	6.0 ± 0.5
22:1(n-9)	n.d 0.0	n.d	n.d	n.d	0.6 ± 0.1	1.2 ± 0.1
24:1(n-9)	1.7 ± 0.0	0.5 ± 0.2	n.d	n.d	n.d	n.d
Σ MUFA	20.8 ± 0.4	6.5 ± 1.9	25.5 ± 1.3	43.3 ± 7.3	52.3 ± 0.0	109.7 ± 12.4
16:2(n-4)	2.8 ± 0.3	0.9 ± 0.2	1.9 ± 0.2	3.2 ± 1.0	n.d	n.d
16:2(n-7)	6.6 ± 0.2	2.1 ± 0.6	4.1 ± 0.0	7.0 ± 1.6	n.d	n.d
16:3(n-4)	12.6 ± 0.4	3.9 ± 1.1	8.7 ± 0.6	15.0 ± 4.3	n.d	n.d
16:4(n-1)	5.1 ± 0.1	1.6 ± 0.4	3.3 ± 0.1	5.7 ± 1.1	n.d	n.d
18:2(n-6)	0.7 ± 0.0	0.2 ± 0.1	1.1 ± 0.1	1.8 ± 0.2	0.5 ± 0.1	1.1 ± 0.2
18:3(n-3)	n.d	n.d	0.2 ± 0.0	0.3 ± 0.0	n.d	n.d
18:3(n-6)	n.d	n.d	0.1 ± 0.0	0.2 ± 0.1	n.d	n.d
18:4(n-1)	n.d	n.d	0.1 ± 0.0	0.1 ± 0.0	n.d	n.d
18:4(n-3)	0.4 ± 0.5	0.1 ± 0.2	0.4 ± 0.0	0.7 ± 0.1	n.d	n.d
18:4(n-4)	3.2 ± 0.0	1.0 ± 0.3	2.1 ± 0.2	3.6 ± 1.1	n.d	n.d
20:2(n-9)	n.d	n.d	n.d	n.d	n.d	n.d
20:3(n-3)	n.d	n.d	n.d	n.d	n.d	n.d
20:4(n-3)	n.d	n.d	0.2 ± 0.0	0.4 ± 0.1	n.d	n.d
20:4(n-6)	0.7 ± 0.0	0.2 ± 0.1	0.8 ± 0.0	1.4 ± 0.4	n.d	n.d
20:5(n-3)	17.3 ± 1.9	5.5 ± 2.2	27.2 ± 0.9	46.6 ± 11.7	4.9 ± 1.1	10.1 ± 1.1
22:6(n-3)	n.d	n.d	0.2 ± 0.0	0.4 ± 0.1	n.d	n.d
Σ (n-3)	17.7 ± 2.4	5.7 ± 2.4	28.2 ± 0.9	48.4 ± 12.1	4.9 ± 1.1	10.1 ± 1.1
Σ (n-6)	1.4 ± 0.0	0.4 ± 0.1	2.0 ± 0.1	3.3 ± 0.6	0.5 ± 0.1	1.1 ± 0.2
Σ PUFA	49.2 ± 1.3	15.6 ± 5.1	50.4 ± 1.7	86.4 ± 21.8	5.4 ± 1.2	11.2 ± 1.3
Σ Unidentified	4.8	0.5	3.7	0.1	6.3	1.6
1.0	0.0	2.1	0.5			
B	<i>P. agardhii</i>		<i>P. agardhii</i> - <i>Rhizopidium megarhizium</i>			
	Uninfected Host		Host and Sporangia		Zoospores	
	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)	Fatty acid composition (%)	Fatty acid content (µg mgC ⁻¹)
	Fatty acid					
12:0	0.2 ± 0.0	0.2 ± 0.0	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.0	0.2 ± 0.1
14:0	n.d	n.d	n.d	n.d	1.0 ± 0.2	1.2 ± 0.3
15:0	n.d	n.d	0.1 ± 0.1	0.1 ± 0.1	0.9 ± 0.2	1.1 ± 0.3
16:0	27.6 ± 0.2	33.5 ± 3.4	21.7 ± 1.0	17.3 ± 4.0	10.6 ± 1.5	12.7 ± 2.8
17:0	n.d	n.d	0.3 ± 0.0	0.3 ± 0.1	0.5 ± 0.1	0.6 ± 0.1
17:0 cyclic	0.2 ± 0.1	0.3 ± 0.0	0.6 ± 0.0	0.5 ± 0.1	0.7 ± 0.2	0.5 ± 0.2
18:0	3.4 ± 0.0	4.2 ± 0.5	2.8 ± 0.2	2.2 ± 0.5	3.6 ± 0.6	4.3 ± 1.1
20:0	n.d	n.d	n.d	n.d	n.d	n.d
22:0	n.d	n.d	n.d	n.d	n.d	n.d
24:0	n.d	n.d	n.d	n.d	n.d	n.d
Σ SAFA	31.4 ± 0.1	38.1 ± 4.1	25.6 ± 0.9	20.4 ± 4.8	17.3 ± 2.2	20.7 ± 4.4
20H 17:0	n.d	n.d	n.d	n.d	n.d	n.d
iso 17:1	n.d	n.d	n.d	n.d	1.8 ± 0.1	2.1 ± 0.3
20H 14:0	n.d	n.d	n.d	n.d	0.4 ± 0.0	0.5 ± 0.1
20H 15:0	n.d	n.d	n.d	n.d	0.7 ± 0.1	0.9 ± 0.1
20H 17:0	1.0 ± 0.0	1.2 ± 0.1	0.9 ± 0.0	0.7 ± 0.2	0.4 ± 0.3	0.4 ± 0.4
20XO 16:0	n.d	n.d	n.d	n.d	0.0 n.d 0.0	n.d
30H 16:0	n.d	n.d	n.d	n.d	0.4 ± 0.4	0.5 ± 0.4
anteiso 15:0	n.d	n.d	n.d	n.d	1.0 ± 0.0	1.2 ± 0.1
anteiso 15:1	n.d	n.d	n.d	n.d	0.2 ± 0.2	0.2 ± 0.2
anteiso 17:0	0.5 ± 0.0	0.6 ± 0.0	1.1 ± 0.0	0.9 ± 0.2	0.6 ± 0.0	0.7 ± 0.1
iso 14:0	n.d	n.d	n.d	n.d	0.2 ± 0.2	0.3 ± 0.2
iso 15:0	n.d	n.d	n.d	n.d	9.7 ± 0.4	11.5 ± 0.8
iso 15:1	n.d	n.d	0.2 ± 0.0	0.2 ± 0.1	4.6 ± 0.2	5.5 ± 0.7
iso 16:0	n.d	n.d	n.d	n.d	0.3 ± 0.3	0.4 ± 0.3
iso 17:0	n.d	n.d	n.d	n.d	0.2 ± 0.2	0.2 ± 0.2
Σ Branched	1.5 ± 0.0	1.8 ± 0.2	2.2 ± 0.0	1.8 ± 0.5	20.7 ± 1.5	24.5 ± 0.6
16:1(n-5)	n.d	n.d	n.d	n.d	1.0 ± 0.1	1.2 ± 0.2
16:1(n-7)	18.7 ± 0.3	22.8 ± 2.9	18.1 ± 0.2	14.6 ± 4.1	10.3 ± 0.3	12.2 ± 0.6
16:1(n-9)	0.3 ± 0.2	0.3 ± 0.3	0.4 ± 0.0	0.3 ± 0.1	2.3 ± 0.2	2.8 ± 0.4
18:1(n-7)	1.2 ± 0.2	1.5 ± 0.3	2.6 ± 0.1	2.1 ± 0.6	26.3 ± 0.7	31.2 ± 2.6
18:1(n-9)	6.3 ± 0.6	7.7 ± 1.5	4.9 ± 0.2	4.0 ± 1.1	5.4 ± 0.4	6.4 ± 1.0
22:1(n-9)	n.d	n.d	n.d	n.d	n.d	n.d
24:1(n-9)	n.d	n.d	n.d	n.d	n.d	n.d
Σ MUFA	26.5 ± 1.2	32.3 ± 4.8	26.0 ± 0.4	20.9 ± 5.8	45.3 ± 1.0	53.8 ± 4.7
16:2(n-4)	n.d	n.d	n.d	n.d	n.d	n.d
16:2(n-7)	n.d	n.d	n.d	n.d	n.d	n.d
16:3(n-4)	n.d	n.d	n.d	n.d	n.d	n.d
16:4(n-1)	n.d	n.d	n.d	n.d	n.d	n.d
18:2(n-6)	14.6 ± 0.2	17.7 ± 2.1	8.4 ± 0.1	6.7 ± 1.8	2.4 ± 0.1	2.9 ± 0.3
18:3(n-3)	24.1 ± 1.2	29.1 ± 2.2	31.1 ± 0.4	25.0 ± 6.9	3.5 ± 0.3	4.2 ± 0.4
18:3(n-6)	n.d	n.d	0.3 ± 0.0	0.2 ± 0.1	0.1 ± 0.2	0.1 ± 0.2
18:4(n-1)	n.d	n.d	n.d	n.d	n.d	n.d
18:4(n-3)	n.d	n.d	2.8 ± 0.1	2.2 ± 0.6	3.9 ± 0.2	4.7 ± 0.5
18:4(n-4)	n.d	n.d	n.d	n.d	n.d	n.d
20:2(n-9)	n.d	n.d	0.3 ± 0.0	0.3 ± 0.1	n.d	n.d
20:3(n-3)	0.2 ± 0.1	0.2 ± 0.2	0.5 ± 0.0	0.4 ± 0.1	n.d	n.d
20:4(n-3)	n.d	n.d	n.d	n.d	n.d	n.d
20:4(n-6)	n.d	n.d	n.d	n.d	n.d	n.d
20:5(n-3)	n.d	n.d	n.d	n.d	n.d	n.d
22:6(n-3)	n.d	n.d	n.d	n.d	n.d	n.d
Σ (n-3)	24.2 ± 1.1	29.3 ± 2.4	34.3 ± 0.4	27.6 ± 7.7	7.5 ± 0.5	8.9 ± 0.9
Σ (n-6)	14.6 ± 0.2	17.7 ± 2.1	8.6 ± 0.1	6.9 ± 1.9	2.5 ± 0.2	3.0 ± 0.3
Σ PUFA	38.8 ± 1.0	47.1 ± 4.3	43.3 ± 0.5	34.8 ± 9.6	10.0 ± 0.6	11.9 ± 1.2
Σ Unidentified	1.8 ± 0.1	2.2 ± 0.1	2.9 ± 0.0	2.3 ± 0.6	6.8 ± 0.9	8.1 ± 0.5

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