REVIEW / SYNTHÈSE

Modelling the role of highly unsaturated fatty acids in planktonic food web processes: a mechanistic approach

Gurbir Perhar, George B. Arhonditsis, and Michael T. Brett

Abstract: Highly unsaturated fatty acids (HUFAs) are a subgroup of fatty acids characterized by chains of 20 or more carbon atoms with multiple double bonds, which potentially limit the growth of zooplankton. Zooplankton require high HUFA concentrations during periods of rapid growth, but co-limitation with nutrients is also likely to occur. Recent modelling results suggest food webs with high quality (nutritional and biochemical) primary producers can attain inverted biomass distributions with efficient energy transfer between trophic levels. In this study, our objective is to highlight the recent advances in studying the role of HUFAs in aquatic food webs. We take a first-principles approach to investigate the chemical nature of HUFAs, and the role they play in zooplankton ecology. To this end, we introduce a novel zooplankton growth sub model that tracks the interplay between nitrogen, phosphorus, and HUFAs in plankton population models. Our aim is to produce a sub model that incorporates the knowledge gained from decades of biochemical research into management-oriented predictive tools.

Key words: polyunsaturated fatty acids, zooplankton, growth limitation, eutrophication, plant–animal interface, mechanistic modelling.

Résumé : Les acides gras fortement non saturés (HGFNS) constituent un groupe d'acides gras caractérisés par des chaines comportant 20 atomes de carbone ou plus avec doubles liaisons, limitant potentiellement la croissance du zooplancton. Le zooplancton nécessite de fortes concentrations en HGFNS pendant la période de croissance rapide, mais une colimitation avec les nutriments pourrait également survenir. De récents résultats de modélisation suggèrent que les chaines alimentaires avec des producteurs primaires de haute qualité (nutritionnelle et biochimique) peuvent connaître des distributions de biomasse inversées avec un transfert efficace d'énergie entre les échelles trophiques. L'objectif des auteurs consiste à mettre en évidence les récents progrès dans l'étude du rôle des HGFNS dans les chaines trophiques aquatiques. Ils partent avec une approche de premier principe pour examiner la nature chimique des HGFNS et le rôle qu'ils jouent dans l'écologie du zooplancton. À cette fin, ils introduisent un nouveau sous modèle de croissance zooplanctonique retraçant les interactions entre l'azote, le phosphore et les HGFNS dans des populations modèles de plancton. Le but consiste à produire des sous modèles incorporant la connaissance acquise au cours de décades de recherches biochimiques dans des outils de prédiction orientés vers l'aménagement.

[Traduit par la Rédaction]

Introduction

In recent years, fatty acids have become a hot topic in both science and the media. Similar to the interest generated by global climate change, research involving fatty acids is receiving more exposure than ever before in a broad number of contexts. Health science researchers continue to publish a wide array of work relating dietary fatty acids to everything from cognitive functioning and nervous system maintenance (Minokoshi et al. 2002), to heart health (Mozaffarian and Rimm 2006), and hormonal imbalances and insulin resistance complications (Yamauchi et al. 2001). The media is also unrelenting in their efforts to sell the public on the benefits of foods and supplements containing essential fatty acids. What is the science behind these carbon structures? Do they possess the potential to address such a broad range of ailments? What makes certain groups of fatty acids essential and others not? In this review, we attempt to fill the gap in understanding fatty acids through a first-principles approach. We begin by building our knowledge from the basics with a solid

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understanding of the core biochemistry, followed by an examination of the basic sources of these molecules, their assimilation in the lower aquatic food web, and finally their importance to human health. A thorough review of the literature will aid us in constructing a nutrient and fatty acid explicit zooplankton growth sub model, accounting for various somatic growth limitations in plankton population models. We conclude this study by presenting preliminary model results from incorporating the sub model into the Lake Washington Eutrophication Model (Arhonditsis and Brett, 2005*a*), and discuss the benefits of integrating such a construct into operational ecosystem management models.

Chemical structure

Some fundamental understanding of fatty acid chemistry is required to appreciate their nature and comprehend their importance. In the most basic of terms, a fatty acid is a long hydrocarbon chain with variant length and degrees of unsaturation that terminates with a carboxylic acid group (see Fig. 1) (Hoffmann-Ostenhof et al. 1978). In biological systems, fatty acids usually contain an even number of carbon atoms, mostly between 14 and 24 (Hoffmann-Ostenhof et al. 1978). The term fatty acid refers to any of the aliphatic monocarboxylic acids that can be created by hydrolysis of naturally occurring fats and oils (Hoffmann-Ostenhof et al. 1978). There are two main types of fatty acids: saturated and unsaturated. The saturated fatty acids (SAFAs) do not contain any double bonds or other functional groups along the hydrocarbon chain; rather, every carbon has the maximum number of possible hydrogens attached to it, giving rise to a linear structure and a relatively high melting point. Unsaturated fatty acids, on the other hand, contain double bonds along the hydrocarbon chain, creating a kinked, irregular structure which can result in a relatively low melting point. In general, double bonds are introduced in fatty acids with 16 or more carbon atoms (Hoffmann-Ostenhof et al. 1978). Double bonds in fatty acids are created by fatty acid desaturases. These enzymes remove two hydrogens, creating carbon-carbon double bonds at specific locations along the fatty acid chain, but are not present in all plants and animals (Vance and Vance 1985). Fatty acids with one double bond are referred to as monounsaturated fatty acids (MUFA) and those with more than one as polyunsaturated fatty acids (PUFA); PUFAs with more than 20 carbons are referred to as HUFAs. With the presence of a double bond, two configurations can occur: the cis-configuration (hydrogens on the same side of the plane), or trans-configuration (hydrogens on opposite sides of the plane). From a purely chemical point of view, a trans fatty acid (TFA) is an unsaturated fatty acid that has one or more double bonds in the trans-configuration (Hoffmann-Ostenhof et al. 1978). TFAs occur naturally in ruminal biohydrogenation, and therefore TFAs are present in dairy products and ruminant meat. Trans fatty acids are also found in industrially synthesized food due to hydrogenation or heat treatment effects on oils and fats (Vance and Vance 1985).

Nomenclature

There are four common conventions for naming fatty acids, three of which specify the number of carbons and the number of double bonds. Common names such as oleic acid do not convey structural information (Hoffmann-Ostenhof et al. 1978). These names are often derived from the source of the fatty acid. For example, oleic acid is derived from oleum (olive oil) and arachidonic acid is found in arachnids (Hoffmann-Ostenhof et al. 1978). The IUPAC convention for naming fatty acids conveys the chain length, and the number and position (relative to the carboxyl end) of all double bonds. In the IUPAC system, the carboxyl carbon is designated as c-1 and double bonds are counted from this end (Hoffmann-Ostenhof et al. 1978). Thus, the systematic name for arachidonic acid is c-5, c-8, c-11, c-14 eicosatetraenoic acid. This name indicates that arachidonic acid has double bonds in the fifth, eighth, eleventh, and fourteenth carbon position and specifies that the double bonds are in the cis-configurations. However, this method is not efficient in recognizing bioconverted compounds, since elongation occurs at the carboxyl end (Davidson and Cantrill 1985). Holman (1964) developed an abbreviation convention, which names the fatty acids based on the chain length and the number of double bonds (this is the convention used in the present study). Unlike the IUPAC method, the carbon at the methyl end (not the carboxyl end) is defined to be c-1 (omega position) and the double bonds are numbered from this methyl end. Each fatty acid belongs to a metabolic family n-x, where x is the position of the first double bond. Utilizing this method, arachidonic acid would be denoted as 20:4 (n-6). This compound contains 20 carbons, four double bonds and the first double bond from the methyl end occurs at the sixth carbon, making this compound a member of the omega-6 fatty acid family.

Fatty acids labeled essential cannot be synthesized *de novo* or be efficiently bioconverted from intermediate forms. As such, somatic requirements must be satisfied entirely through ingestion, making them an essential component of a nutritionally balanced diet. Although the aforementioned desaturases exist in animals, they are usually limited to inserting a double bond into no lower than the n-9 (omega-9) position (Arts et al. 1992), making n-3 and n-6 fatty acids essential. Fish are rich in essential fatty acids (EFAs), but they also lack the desaturases to form n-3 and n-6 EFAs (Arts et al. 1992). The desaturases required to form n-3 and n-6 EFAs exist at the lower trophic levels in aquatic food webs, particularly in phytoplankton.

Impacts on human health

The primary role of HUFAs in mammals is cell signaling (Nakamura and Nara 2004), but n-3 HUFAs may also be important in preventing chronic health conditions, such as Alzheimer's disease, type II diabetes, kidney disease, rheumatoid arthritis, high blood pressure, coronary heart disease, alcoholism, and possibly cancer (Das 2006). Eicosapentaenoic acid (EPA), for example, is a precursor of eicosanoids (signaling hormones), and eicosanoids derived from EPA tend to impede inflammation associated with many chronic diseases (Morris 2011). Alpha-Linolenic acid (ALA), a precursor of EPA, is hypothesized to support the growth and development of infants (Morris 2011). Individuals with ALA deficiency may also experience neurological problems, such as numbness, weakness, and blurry vision (Holman et al. 1982; Trumbo et al. 2002). Docosahexaenoic acid (DHA) is required for the development and maturation of the eyes, constituting up to 80% of total PUFAs in the retina (Innis 2003).

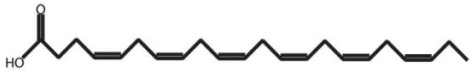
Fig. 1. List of fatty acids in aquatic food webs, their chemical abbreviations, structures, and diagrams. (a) Myristic Acid, SAFA. 14:0, $C_{14}H_{28}O_2$; (b) Palmitoleic Acid, MUFA. 16:1 (n-7), $C_{16}H_{30}O_2$; (c) Arachidonic Acid, PUFA. 20:4 (n-6) ARA, $C_{20}H_{32}O_2$; (d) Linoleic Acid, PUFA. 18:2 (n-6) LA, $C_{18}H_{32}O_2$; (e) Docosahexaenoic Acid, HUFA. 22:6 (n-3) DHA, $C_{22}H_{32}O_2$; (f) Eicosapentaenoic Acid, HUFA. 20:5 (n-3) EPA, $C_{20}H_{30}O_2$; (g) Alpha-Linolenic Acid, PUFA. 18:3 (n-3) ALA, $C_{18}H_{30}O_2$.

(a) Myristic Acid, SAFA

(b) Palmitoleic Acid, MUFA

(c) Arachidonic Acid, PUFA

(d) Linoleic Acid, PUFA



(e) Docosahexaenoic Acid, HUFA

(f) Eicosapentaenoic Acid, HUFA

(g) alpha-Linoleic Acid, PUFA

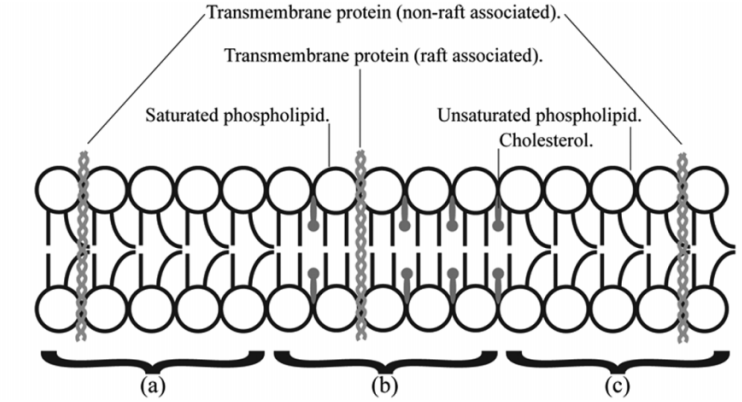
Brain, retina, and sperm are the tissues in the human body with the highest DHA concentrations, and demand for DHA is particularly pressing during the latter stages of pregnancy and early infancy (Arterburn et al. 2006; Morris 2011).

Fatty acids have various fates once assimilated into somatic lipids and can been classified as storage and structural components. Storage lipids, such as wax esters, cholesterols, and triglycerols (TG) are neutral storage reserves, which can be broken down for energy should the need arise (Lee et al. 2006). Cholesterol is a sterol found in animal cells that can account for up to half of the lipid content in a plasma membrane, and thus affects its fluid behavior. Cholesterol molecules typically orient their small hydrophilic hydroxyl group toward the membrane surface, while the remainder is embedded in the lipid bilayer (see Fig. 2, and also Karp 2005). Cholesterol-rich micro-domains tend to float within the more fluid and disordered environment of the bilayer, forming what are referred to as lipid rafts. These floating cholesterol platforms tend to concentrate proteins, whereby membranes are organized into functional compartments (Fig. 2). Lipid rafts provide a favorable local environment for cell-surface receptors to interact with other membrane proteins that transmit signals from the extracellular space to the cell interior (Karp 2005). Structural lipids, such as phospholipids, are polar molecules that form cellular membranes, effectively dividing cells into functional regions (e.g., cell membrane, mitochondrial membrane, nuclear envelope, vacuole membrane). These lipids are arguably the most important in plants, but are generally considered unlikely sources of energy (Lee et al. 2006).

Sources of fatty acids in the human diet range from plant oils from seeds and nuts (Calder 2004), to EFAs found in fatty fish, such as salmon, tuna, herring, and mackerel. These fish species are oil-rich, storing dietary fat as triaglycerides (TGs) in their flesh (Lunn and Buttriss 2008)[1]. ALA is the principle source of n-3 fatty acids in the modern Western diet, but EPA and DHA dietary intake has decreased dramatically in the last century (Burdge and Calder 2005). This decreasing trend is a major health concern, as ALA conversion into long-chain n-3 HUFAs, such as EPA (approximately 8%) and DHA (approximately 4%), is inefficient (Burdge and Calder 2005). In the past, hunter-gatherer societies consumed n-6:n-3 HUFAs in the ratio of roughly 1:4 (De Henauw et al. 2007). The same ratio in the modern western diet is 10–25:1 (Simopoulos 1991)[2]. Prior to the Agricultural Revolution, consumption of wild plants, berries, nuts, and lean meat was substantial. In the last 10 000 years though, a large dependency on cereal grains, which are relatively high in n-6 HUFAs compared to leafy green vegetables has become the norm (Simopoulos 1991). Moreover, there has been an increased usage of vegetable oils instead of fish oils (Simopoulos 2002). The n-6:n-3 consumption balance is important, as

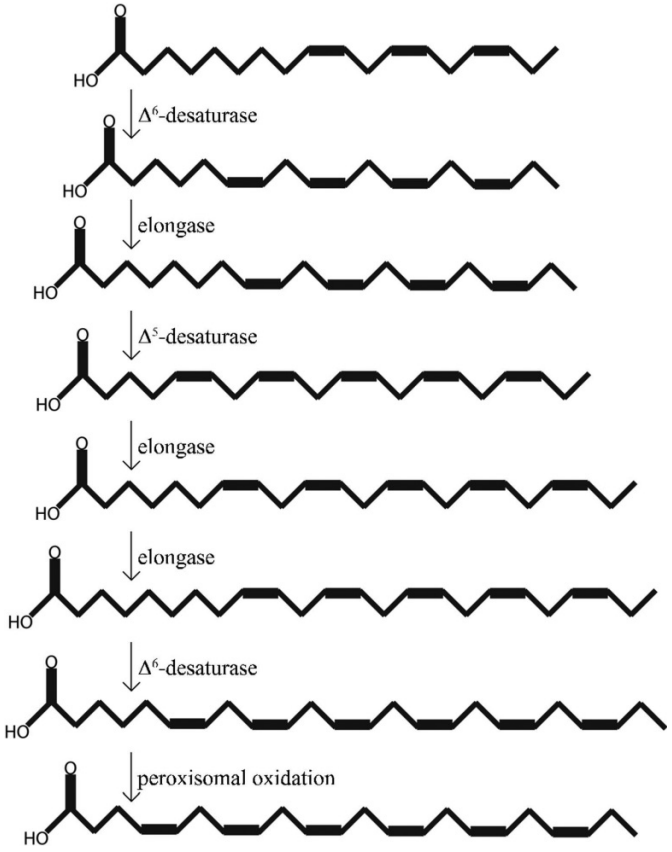
[1]Lean fish can also be favourable for human nutrition; see Ahlgren et al. (1994).
[2]This ratio can conceivably vary, especially in societies heavily dependent on seafood.

Fig. 2. Depiction of phospholipid bilayer, showing regions of lipid raft formation (*b*) and non-raft forming regions (*a* and *c*).



desaturase enzymes responsible for converting LA and ALA to long-chain HUFAs are concentration dependent (Simopoulos 1991; Portolesi et al. 2007). Because there is competition between n-3 and n-6 fatty acids for the same desaturase enzymes, a high consumption of n-6 HUFAs may hinder the bioconversion of ALA to EPA and DHA (Fig. 3).

Fig. 3. Mechanism demonstrating bioconversion of ALA to DHA via desaturation and elongation. Fatty acids as listed from top to bottom: alpha-Linolenic acid (ALA), Stearidonic acid, 20: 4(n-3), Eicosapentaenoic acid (EPA), Docosapentaenoic acid (DPA), 24: 5 (n-3), 24: 6(n-3), Docosahexaenoic acid (DHA). Peroxisomal oxidation occurs when fatty acid chains are too long for mitochondria to handle.



Phytoplankton

Fatty acid synthesis and taxonomic differences

Primary producers and consumers demonstrate greater disparity with regards to their somatic elemental composition relative to other prey–predator relationships in the upper food web (Persson and Vrede 2006). As such, consumers are required to preferentially retain nutrients and other important molecules while recycling excess material. In particular, PUFAs are reported to be higher in zooplankton than seston, whereas MUFAs and SAFAs are higher in seston than in zooplankton (Persson and Vrede 2006). Planktonic primary producers have the ability to synthesize *de novo* n-3 PUFAs, whereby the addition of double bonds to SAFAs via Δ9, Δ12, and Δ15 desaturases leads to ALA formation (Bell and Tocher 2009). High food quality algae and several consumer species use appropriate elongases, enzymes which lengthen fatty acid chains by two carbon atoms on the carboxyl end, to transform ALA to EPA and eventually to DHA (Bell and Tocher 2009). ALA content of seston samples has been reported to be positively related to cyanobacteria abundance, while diatoms, dinoflagellates, and cryptophytes contain higher amounts of longer chained n-3 PUFAs (Brett et al. 2009).

The typical phytoplankton species available to herbivorous zooplankton feeding vary greatly in regards to their fatty acid content (Volkman et al. 1989). Some differences also exist between freshwater and marine phytoplankton, though mostly minor within algal groups (Brett et al. 2009). It has been hypothesized that these differences could stem from different adaptation levels of algae to their environment. Others caution that this pattern may partly be associated with an aquaculture bias, in that most of the marine phytoplankton fatty acid surveys are geared towards identifying taxa with potential value as mariculture food stocks, thereby skewing the marine phytoplankton HUFA values (Brett et al. 2009). Another possible systematic bias underlying the disparity between marine and freshwater studies may be that the former ones have high diatom encounters, whereas freshwater studies are based on higher presence of cyanophytes. Generally, diatoms contain the largest amount of MUFAs (% dry mass), but their PUFA content is relatively low (18 carbons, n-3 and n-6). Diatoms do, however, have relatively high HUFA (>20 carbons) content and n-3:n-6 ratios (Brett et al. 2009). While very few studies are available for marine cyanobacteria,

freshwater studies report high levels of SAFAs, very low levels of n-3 HUFAs (making it very low quality food) as well as very low n-3:n-6 ratios. Both marine and freshwater cryptophytes have low MUFAs, very high PUFAs, moderate to high HUFAs, and a high n-3:n-6 ratio (Brett et al. 2009). Flagellates are shown to contain little EPA, but high proportions of DHA and therefore their use in aquaculture stems from their ease of growth and high DHA content (Brett et al. 2009).

Phytoplankton fatty acid content and seasonal plankton dynamics

A great deal of research has focused on the seasonal variability of the zooplankton fatty acids in conjunction with the contemporaneous changes in phytoplankton community composition and the ambient conditions. Early work by Jeffries (1970) suggested that taxonomy is not the only determining factor of zooplankton fatty acid profiles, and reported a tight causal linkage with the ingested prey. Biogenic seston fatty acid availability follows the phytoplankton succession patterns (Ravet et al. 2010). That is, the interplay between environmental conditions and nutrient loading regimes controls the dominant competitors of the algal community, and subsequently the algal fatty acid content as well as the pertinent fluxes to consumers (Müller-Navarra et al. 2000). In particular, the onset of thermal stratification in the spring along with the high nutrient concentrations promote the rapid growth of PUFA-rich nanoplankton and small diatoms, both of which are heavily grazed by cladocerans (Fraser et al. 1989; Sommer 1986). As grazing exceeds primary production, both populations crash, resulting in the so-called clear-water phase (Sommer 1986; Pinckney et al. 1998). Early to mid-summer phytoplankton abundance and community composition are modulated by silicate depletion rates and (or) soluble phosphorus availability, while cyanobacteria blooms usually occur later in the summer (Sommer 1986). Cyanobacteria are known to be poor food quality as a result of mechanical interference, toxicity, and (or) inadequate biochemical nutritional value for consumers (Müller-Navarra et al. 2000; Perhar and Arhonditsis 2009). Arts et al. (1992) showed the poor food quality of cyanobacteria may stem from their nutritional inadequacy, as consumers exclusively fed cyanobacteria exhibited near-starvation traits. The increasing mixing depth and decreasing day length in the autumn yields the annual minimum of phytoplankton biomass, mainly characterized by chlorophyte dominance (Sommer 1986).

Field samples show a very strong correlation between seston EPA:C and consumer growth, but a moderately weak correlation between seston P:C and consumer growth (Müller-Navarra et al. 2000). Müller-Navarra et al. (2004) explain the effects of total phosphorus loading on zooplankton via fatty acid pathways, whereby increases in total phosphorus loading favors cyanobacteria dominance, which contain little n-3 HUFAs, resulting in limited Daphnia egg production. On the other hand, the food quality of algae that are otherwise nutritionally beneficial to consumers is diminished when facing P-deficient conditions (Ferrão-Filho et al. 2003). As total phosphorus is decreased and algae becomes P stressed, EPA and DHA production appears to cease (Ferrão-Filho et al. 2003). Yet, linoleic and linolenic acids are still produced and can be potentially bioconverted into EPA and DHA, albeit with low efficiency (Farkas and Herodek 1964; Ferrão-Filho et al. 2003). Further, the indirect-limitation hypothesis predicts that P-stressed algae pose problems of ingestion and assimilation for consumers (Van Donk et al. 1997). Namely, algae under poor nutrient conditions undergo morphological changes (e.g., thickening of the cell wall), which can in turn result in reduced digestion by consumers (Van Donk et al. 1997). The findings of Ravet and Brett (2006) lent credibility to the indirect-limitation hypothesis, and noted mutant algal species lacking cell walls (Van Donk et al. 1997) did not pose the same limitation on Daphnia relative to non-mutant strains.

Zooplankton

Historical background

The study of zooplankton fatty acids started with Lovern (1935), who compared the fatty acids composition of the marine calanoid copepod *Calanus finmarchicus*, and three freshwater zooplankton species (*Cyclops strenuous*, *Daphnia galeata*, *Diaptomus gracilis*) vis-à-vis the fatty acids of the fish feeding on them. The resemblance in the corresponding fatty acid profiles led to the conclusion that fish deposit dietary lipids into their tissues with no major modifications. Similar conclusions were drawn by Ackman and Eaton (1966), who compared the fatty acids in krill (*Meganyctiphanes norvegica*) with the fatty acids in the fin whales that consume them. Farkas and Herodek (1964) also noted an increase in zooplankton EPA and DHA concentrations as temperature decreased. In a subsequent study, Farkas (1979) similarly noted that cladocerans tended to accumulate EPA, while copepods accumulated DHA. It was hypothesized that this difference may be associated with their overwintering strategies; namely, cladocerans typically diapause and overwinter as resting eggs, while many copepods remain in an active state. In the same context, Farkas (1979) suggested that copepods may have the ability to bioconvert much of their EPA into DHA as a homeoviscous adaptation to cold weather conditions, whereas daphnids (cladocerans) may have limited capacity to do the same. This cold weather adaptation theory stemmed from the findings of Farkas and Herodek (1964), in which it was reported that the fatty acids in zooplankton always had a lower melting point than the ambient water temperature. One of the most important studies of marine zooplankton fatty acid was published by Lee et al. (1971), who studied the dietary impact on wax esters (neutral storage lipids) in polar and deep living calanoid copepods. They found a relationship between diet and the fatty acid composition of wax esters in copepods, and also reported an increase in fatty acid concentration as food availability increased. It was also shown that structural lipids (phospholipids) were not affected by diet, reflecting the animals' ability to store fatty acids in periods of high food abundance.

Taxonomic differences

Substantial research efforts have been focused on the fatty acid dynamics in copepods (Scott et al. 2002). Marine copepods were shown to be particularly rich in lipids (35%–75% dry mass), primarily in wax esters and secondarily in triglycerides (Lee et al. 2006). The fatty alcohol component of wax esters are synthesized *de novo* from dietary carbohydrates and

proteins, and can therefore be used as fish biomarkers (Dalsgaard et al. 2003). Persson and Vrede (2006) classified zooplankton taxa according to their PUFA and HUFA content. The general trend was consistent with the findings of Farkas (1979) that established the aforementioned seasonal hypothesis, in that cladocerans were found to have high EPA and copepods high DHA concentrations. Farkas and Herodek (1964) also noted that marine environments are generally colder than freshwater, and that marine fish have higher DHA concentrations than freshwater fish, an assertion on par with the cold weather adaptation theory. In an attempt to shed light on the different HUFA accumulation strategies between cladocerans and copepods, Persson and Vrede (2006) argued that may be related to the fact that copepods have more developed and refined nervous systems compared to cladocerans. With intelligent predator avoidance abilities and raptorial prey attack, copepods can respond to stimuli within milliseconds (Lenz et al. 2000). Certain copepod species have chemoreceptors, utilized to taste food and track mates, and thick myelin sheaths covering their axons, thereby achieving quick nerve impulse times. Because of these behavioural differences, the question arising is whether the increased DHA requirement in copepods actually stems from their overwintering strategies or from their nervous system structure (Ravet et al. 2010).

Persson and Vrede (2006) also stressed another important pattern that involves the bioaccumulation of HUFAs up the food chain. Herbivorous zooplankton were found to have higher HUFA content than the grazed seston, while carnivorous zooplankton had higher HUFA concentrations relative to herbivorous animals. Müller-Navarra et al. (2004) attribute the HUFA bioaccumulation up the food chain to the zooplankton feeding patterns and diet. Namely, herbivorous zooplankton feed upon seston (characterized by low fatty acid content), while carnivorous zooplankton selectively feed on rotifers and crustacean zooplankton (both with relatively high fatty acid content).

Impacts of diet on zooplankton fatty acid content

Many laboratory studies have examined the impacts of different diets on zooplankton fatty acid composition, reporting similarities between consumer fatty acid patterns and diet content. Storage lipids are especially sensitive to the diet content (Langdon and Waldock 1981), and can be potentially traced through different trophic levels (Fraser et al. 1989). Dabramo and Sheen (1993) showed that the tissue fatty acid content of the freshwater prawn, *Macrobrachium rosenbergii*, reflected that of its artificially purified diet. SAFA and MUFA concentrations reportedly changed in response to PUFA additions. Further, n-3 PUFA levels decreased unless provided in the diet, while n-6 PUFA remained unchanged (or even increased) possibly due to differential metabolic rates (Dabramo and Sheen 1993). Weers et al. (1997) fed *Daphnia galeata* with combinations of algae and emulsions of varying DHA:EPA values and found that increased DHA:EPA ratios led to an increase of the Daphnia DHA concentration. Yet, there was still up to four times more EPA than DHA, suggesting a retro-conversion mechanism undertaken by Daphnia, whereby ingested DHA was converted into EPA (Taipale et al. 2011). Similarly, Burns et al.'s (2011) experiments with the cladoceran *Ceriodaphnia dubia* showed that this daphnid, even when fed with excess DHA, accumulates very little of this fatty acid. Daphnids also tend to accumulate less SAFAs and more MUFAs than what is available in their diet, possibly due to preferential utilization of SAFAs for catabolic needs, while conserving unsaturated fatty acids (MUFAs and PUFAs) for storage, structure, and bioconversion (Burns et al. 2011).

A different perspective was offered by a handful of field studies that focused on the fatty acid composition of freshwater zooplankton in natural ecosystems. In particular, Persson and Vrede (2006) studied a series of alpine lakes in Sweden, and found zooplankton to be greatly enriched with PUFA and HUFA relative to the seston. Yet, the same study also noted that zooplankton fatty acid composition was nearly independent of the seston fatty acid content, but closely related to the zooplankton taxa. Likewise, Smyntek et al. (2008) reported a strong correlation between zooplankton fatty acid content and their taxonomic affiliation, but virtually no causal connection with seston fatty acid profiles. The conclusions of the two studies were similar to those drawn by Müller-Navarra (2006), who observed a strong dependence in zooplankton fatty acid content when fed with purified algae (consisting of *Scenedesmus obliquus*, *Cryptomonas erosa*, and *Nitzschia palea*), but virtually no relation when fed with natural seston diets. Kainz et al. (2004) studied the accumulation patterns of essential fatty acids across zooplankton size classes in a series of lakes on Vancouver Island, Canada, and concluded that all zooplankton sizes accumulated two to four times the essential fatty acid content of seston. The results are on par with existing evidence that meso-zooplankton (copepods) tends to have higher DHA content, and macro-zooplankton (cladocerans) is characterized by higher EPA levels (Kainz et al. 2004). However, the notion of using animal size as a HUFA preference indicator has been challenged, given that small cladocerans share more in common with large cladocerans than they do with copepods (Kainz et al. 2004; Persson and Vrede 2006).

A careful review of the pertinent literature suggests that the dietary impacts on animal fatty acid content is not always discernible. One plausible explanation that has been proposed highlights the importance of the animal growth strategies. In particular, Brett et al. (2009) argued that this pattern may be due to the fact that fast growing and relatively lean zooplankton species, such as the Daphnia used in the Müller-Navarra (2006) study, require only a short period of time to replace their lipid reserves with new dietary lipids, and thus the signature of the seston fatty acid content is more easily detected. By contrast, marine copepods grow more slowly and build up large lipid reserves over a period of months, and any freshly assimilated fatty acid content would be diluted into their lipid somatic pool (Brett et al. 2009). Consistent with the assertion of Brett et al. (2009), Stübing et al. (2003) studied fatty acid accumulation in larval euphausiids, which have high growth rates and relatively low lipid reserves, and their results showed a clear causal link between fatty acid composition and diet. Persson et al. (2007) offered a somewhat different perspective arguing that freshwater zooplankton in oligotrophic environments may often be limited by the food availability, and therefore allocate less ingested substrate toward replenishing storage lipids. Food quantity limitation may result in individuals not being able to perform basal metabolic

processes and physiological maintenance. Elser and Urabe (1999) predict a critical point, at which food quantity and quality are evenly matched. Increasing quantity beyond this point gives rise to abundant carbon, making food quality the sole limiting factor. Further, Brett et al. (2009) pointed out that zooplankton have the ability to modify somatic fatty acid profiles in response to diet, and that different groups would be expected to have different self-regulatory capacities.

Internal regulation of fatty acids

There is evidence that zooplankton can maintain a quasi-homeostatic response to varying fatty acid availability in their diet. Müller-Navarra (2006) showed daphnids feeding on SAFA-rich cyanobacteria accumulated only half of the available SAFA, but when fed upon MUFA-poor cryptophytes, twice the available MUFAs were accumulated. This study renders support to Jeffries (1970), by placing zooplankton taxonomy ahead of prey characteristics when determining fatty acid profiles and this likelihood brings into focus the internal regulatory mechanisms in consumer species. In this context, Brett et al. (2009) argued that the differences in fatty acid composition and response to diet among different zooplankton orders may be attributed to variable turnover rates. The turnover rate for a particular fatty acid is dependent upon the somatic fatty acid concentration (Jobling 2004). If the initial tissue fatty acid content is low, fatty acid accumulation takes place with little turnover. Once the animal carrying capacity is reached, and accumulation no longer occurs, existing fatty acids can be replaced with new dietary sources (Jobling 2004). Graeve et al. (2005) experimentally tested fatty acid turnover rates, employing ^{13}C labeled diets to determine the time needed for marine copepods to turn over their fatty acid pools. The same study concluded that *Calanus hyperboreus* exchanged nearly all of its lipid pool in 11 days, whereas *Calanus finmarchicus* and *Calanus glacialis* exchanged 22% and 45% of their original pools after 14 days, respectively (Graeve et al. 2005). It is also important to note that lipid turnover rates appear to vary with growth rates, which in turn vary with age, food availability, and water temperature (Graeve et al. 2005).

Reproductive investment

Reproduction requires a large HUFA investment to subitaneous eggs. Müller-Navarra (2006) found substantial variation between cladoceran somatic tissue fatty acids and their subitaneous eggs. *Daphnia* spp. egg fatty acid and n-3 HUFA concentrations were nearly double and triple the concentrations found in maternal somatic tissues, respectively (Müller-Navarra 2006). In a similar study, Wacker and Martin-Creuzburg (2007) found that *Daphnia magna* eggs contained significantly more SAFAs, MUFAs, PUFAs, and HUFAs (both n-3 and n-6) relative to their somatic tissues regardless of their diet. Both studies showed that *Daphnia* invest fatty acids heavily in their eggs, and preferentially enrich them with n-3 PUFAs (Wacker and Martin-Creuzburg 2007). On the other hand, little is known about PUFA requirements in copepods, although their fatty acid variability (for reasons other than their diet) is hypothesized to stem from different developmental stages, differential growth rates and egg production. During egg production, copepods convert storage lipids into phospholipids and transfer them to the gonads, where they become part of the egg yolk (lipovitelin production; Müller-Navarra 2006). HUFAs in resting eggs provide newly hatched juvenile copepods with high growth-yield molecules. Given that cladoceran eggs have high PUFA and HUFA content compared to their somatic tissues, Müller-Navarra (2006) hypothesized that the same may hold true for cladocerans.

Temperature impacts

Nearly five decades after the Farkas and Herodek (1964) study, little has been done to consolidate their findings and gain further insights into temperature impacts on zooplankton fatty acid content. This study was the first to note that actively overwintering zooplankton (e.g., copepods) were able to strongly modify their fatty acid content in response to temperature (i.e., homeoviscous adaptation). Farkas and Herodek (1964) concluded that copepods increased their DHA concentration and decreased their SAFA content when stressed by cold water temperatures. Hazel (1995) noted almost all poikilotherms adapt to cold stress by increasing their PUFA and especially HUFA content. It has been also surmised that when zooplankton have limited ability to modify lipid composition in response to cold weather conditions, then overwintering in a non-active state (e.g., resting eggs) is the only natural selection (Farkas 1979).

More recently, however, Arts et al. (1992) and Schlechtriem et al. (2006) challenged Farkas' notion of DHA importance in cold water adaptation by highlighting the capacity of certain cladocerans to overwinter actively under ice covered lakes. Schlechtriem et al. (2006) showed that Daphnia at 11°C had four times higher EPA levels than in 22°C, but the cold-stressed animals still did not accumulate DHA. Nanton and Castell (1999) reported high concentrations of both EPA and DHA in the tropical marine copepods Amonardia and Tisbe, and thus questioned their importance in the cold weather membrane adaptation hypothesis all together. The latter finding raises the question whether high PUFA content in zooplankton is actually vital for reasons other than membrane fluidity. For example, recent studies have focused on the impact of starvation on zooplankton fatty acid composition (Schlechtriem et al. 2006). Schlechtriem et al. (2006) showed that *Daphnia pulex* starved at 22°C died after 3 days with virtually no change to the fatty acid composition, while those starved at 11° C survived for an additional 3 days. The daphnids studied at the lower temperature had markedly changed their fatty acid profiles, with large decreases in both SAFAs and MUFAs but relatively unchanged PUFAs and HUFAs, which probably suggests preferential catabolism of non-PUFA molecules.

Mechanistic sub model for zooplankton growth

Motivation

As the empirical evidence for the regulatory role of three distinct and not mutually exclusive factors (P limitation, FA limitation, and food quantity) on the strength of the primary producer-grazer coupling has grown, several models have been developed to advance our theoretical understanding of where and when their individual and (or) synergistic effects become important (Mulder and Bowden 2007). Considerable

insights into the potential implications of the ecological stoichiometry have been gained by a series of homeostatic consumer models that explicitly account for the effects of P-deficient food on zooplankton growth rate as well as on consumer-driven P recycling (e.g., Andersen 1997; Anderson et al. 2005). For example, Sterner (1997) modeled the effects of food quantity (C) and quality (P) on the growth of homeostatic heterotrophic consumers, indicating that consumer growth differs between high and low P food concentrations only when food quantity is above a critical level. Hence, two diets might give identical consumer growth rates at low food quantity, but may give different consumer growth at high food quantity. Intriguing results were also presented by Loladze et al. (2001), who modified the Rosenzweig–MacArthur variation of the Lotka–Volterra equations and demonstrated that the chemical heterogeneity in the first two trophic levels can transform the prey and predators into competitors for phosphorus. On the other hand, there is a surprising gap in the literature of predictive frameworks for the FA limitation, i.e., modeling studies that explicitly consider the constraints on zooplankton growth pertinent to the biochemical heterogeneity of the lake seston (Gulati and DeMott 1997; Arhonditsis and Brett 2005*a*, 2005*b*; Zhao et al. 2008). To this end, our objective is to develop a mathematical model that explicitly tracks the interplay between nitrogen, phosphorus, and HUFAs in plankton ecosystem models. The purpose of this modeling construct is to elucidate the mechanisms governing HUFA fate within the animal body, to evaluate the limitations imposed on zooplankton growth, and to subsequently quantify their transport through the food web. It is our hope that the incorporation of the knowledge gained from decades of biochemical research into management-oriented predictive tools will provide a more robust platform to base management decisions off.

Model description

Based on the present review of the fatty acid literature, we propose a modular addition to existing plankton food web models to accommodate the roles of nitrogen, phosphorus, and HUFAs on zooplankton growth. The proposed additions involve the zooplankton differential equation, leaving all other compartments unaltered. Individual-level parameters, such as maximum grazing rate, food selection strategies, and higher predation rate are carried over from the host model. The new additions begin once food enters the feeding apparatus of the grazer and our augmentation aims to track growth-limiting substrates through the zooplankton gut. In short, our proposition is an individual-based model plugged into a plankton population model, thereby offering the opportunity to test animal physiology in conjunction with environmental variability and trophic interactions. We introduce differential equations that describe the dynamics of somatic nitrogen, phosphorus, EPA, and DHA, and thus characterize the limitation of zooplankton growth as a function of the aforementioned resource pools. The following description tracks food particles from the water column through the zooplankter's gut, and illustrates the multiple pathways and fates of the substrates accounted for (see Fig. 4); collected parameter values and ranges from the literature are presented in the Table 1.

The somatic processes taking place within a zooplankter are split into three phases: pre-gut, maintenance, and post maintenance. With the present mathematical depiction of zooplankton physiology, pre-gut processing comprises the ingestion and assimilation of the grazed food. Similar to our earlier work (Arhonditsis and Brett 2005*a*, 2005*b*; Zhao et al. 2008; Perhar and Arhonditsis 2009; Perhar et al. unpublished), we first introduce the variable total food quality concentration in eq. [1]:

$$[1] \qquad \mathrm{FQ_{TOT}} = [\mathrm{FQ_{PHYT}^2}\sqrt{\mathrm{PHYT}} + \mathrm{FQ_{DET}^2}\sqrt{\mathrm{DET_C}}]Z_{\mathrm{PLIM}}$$

Total food quality concentration considers the sum of all food sources (i.e., PHYT and $\mathrm{DET_C}$) weighted by their respective food quality indices (i.e., $\mathrm{FQ_{PHYT}}$ and $\mathrm{FQ_{DET}}$) reflecting the morphological features (i.e., ingestibility, digestibility, and toxicity) of the grazed seston. We have included an additional parameter (Z_{PLIM}; eq. [2] to account for secondary limitation resulting from the imbalance between the P:C ratio of the grazed seston ($\mathrm{GRAZ_P}$; see eq. [5] and the critical minimum phosphorus somatic quota (P_{min}):

$$[2] \qquad \text{if} \quad \mathrm{GRAZ_P} \le P_{\mathrm{min}}, \qquad Z_{\mathrm{PLIM}} = \frac{\mathrm{GRAZ_P}}{P_{\mathrm{min}}}$$
$$\text{if} \quad \mathrm{GRAZ_P} > P_{\mathrm{min}}, \qquad Z_{\mathrm{PLIM}} = 1$$

The inclusion of secondary limitation addresses the indirect-limitation hypothesis that states nutrient-stressed algae demonstrate morphological changes that reduce their digestibility for zooplankton. Total food quality determines the extent to which ingested food is either assimilated or egested (i.e., via sloppy feeding) based on morphological characteristics. Respiration costs are implicitly considered (α_{C1} and α_{C2}) in the calculation of carbon assimilation efficiency (α_{C}; eq. [3]):

$$[3] \qquad \alpha_{\mathrm{C}} = \frac{\alpha_{\mathrm{C1}}\mathrm{FQ_{TOT}}}{\alpha_{\mathrm{C2}} + \mathrm{FQ_{TOT}}}$$

To calculate the carbon assimilation rate (α_{SC}; eq. [4], we first quantify the grazed carbon rate using zooplankton maximum grazing rate (λ), zooplankton grazing preference for phytoplankton (ω_{PHYT}) and detritus (ω_{DET}), available phytoplankton and detritus biomass, and the zooplankton grazing half saturation constant (μ). Multiplying by carbon assimilation efficiency, we calculate the carbon assimilation rate (α_{SC}):

$$[4] \qquad \alpha_{\mathrm{SC}} = \frac{\lambda\alpha_{\mathrm{C}}(\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET_C^2})}{\mu^2 + \omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET_C^2}}$$

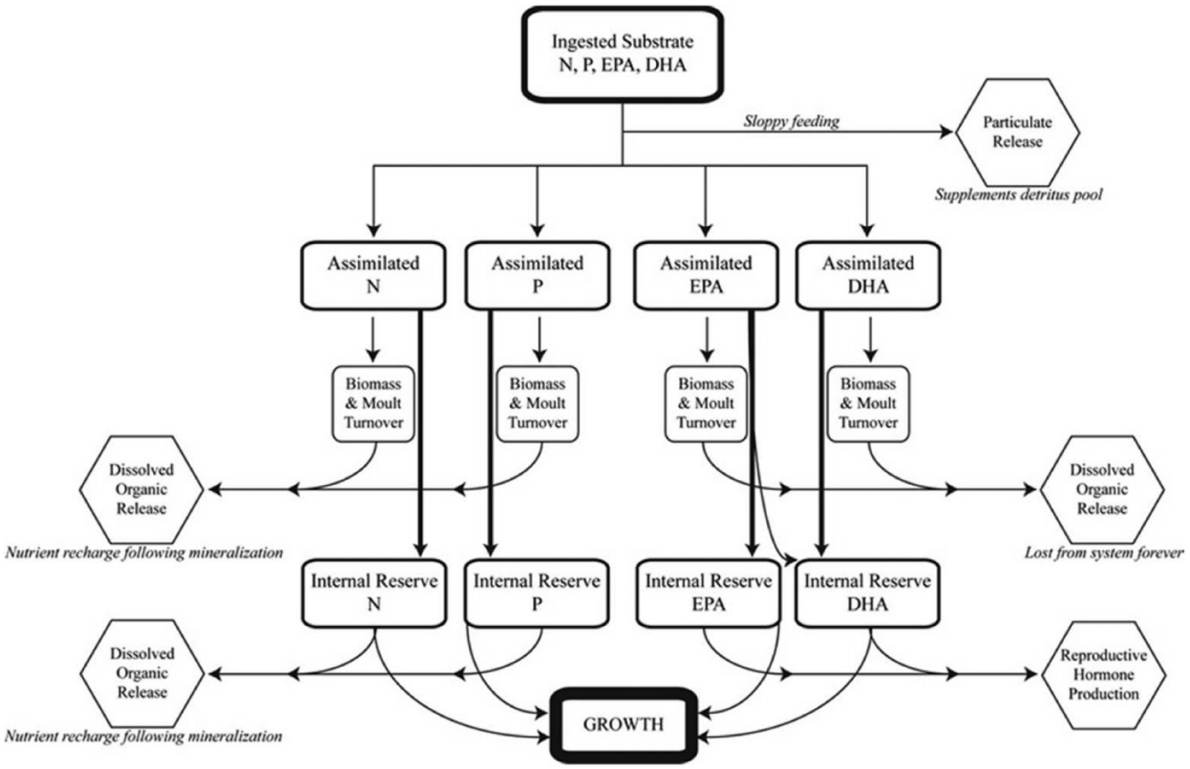
The resource concentrations in algae and detritus (phosphorus: $\mathrm{PC_{PHYT}}$ and $s_{\mathrm{P:C}}$; nitrogen: $\mathrm{NC_{PHYT}}$ and $s_{\mathrm{N:C}}$; EPA: $f_{\mathrm{EPA:C}}$ and $s_{\mathrm{EPA:C}}$; DHA: $f_{\mathrm{DHA:C}}$ and $s_{\mathrm{DHA:C}}$) are weighted by abundance, allowing for the separation of grazed food per unit of biomass into four separate pools, i.e., phosphorus, nitrogen, EPA and DHA per unit biomass; eqs. [5]–[8], respectively:

$$[5] \qquad \mathrm{GRAZ_P} = \frac{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2\mathrm{PC_{PHYT}} + \omega_{\mathrm{DET}}\mathrm{DET_C^2}s_{\mathrm{P:C}}}{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET_C^2}}$$

$$[6] \qquad \mathrm{GRAZ_N} = \frac{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2\mathrm{NC_{PHYT}} + \omega_{\mathrm{DET}}\mathrm{DET_C^2}s_{\mathrm{N:C}}}{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET_C^2}}$$

$$[7] \qquad \mathrm{GRAZ_{EPA}} = \frac{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 f_{\mathrm{EPA:C}} + \omega_{\mathrm{DET}}\mathrm{DET_C^2}s_{\mathrm{EPA:C}}}{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET_C^2}}$$

Fig. 4. Schematic diagram of the sub model that considers the biochemical control of zooplankton growth.



$$[8] \quad \mathrm{GRAZ_{DHA}} = \frac{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 f_{\mathrm{DHA:C}} + \omega_{\mathrm{DET}}\mathrm{DET}_{\mathrm{C}}^2 s_{\mathrm{DHA:C}}}{\omega_{\mathrm{PHYT}}\mathrm{PHYT}^2 + \omega_{\mathrm{DET}}\mathrm{DET}_{\mathrm{C}}^2}$$

Assimilated substrate rate is calculated by multiplying grazed substrate per unit of biomass with the carbon assimilation rate (see eqs. [9]–[12]). The assimilation of a particular substrate depends only on the morphological characteristics and substrate ratios of the grazed seston.

The first physiological need addressed by the substrate pools is maintenance in the form of biomass and moult turnover rates, i.e., τ_{Resource} and τ_{m}, respectively. Parameter estimates for maintenance processes are scarce, but Anderson et al. (2005) provide approximations for nitrogen and phosphorus (see Table 1). We hypothesize that postulated turnover rates and moulting fraction (m) may heavily influence our simulations of organism functionality. For example, an overestimation may deplete internal reserves, halt growth, and decouple grazers from producers. Conversely, an underestimation may not realistically capture the physiological requirements of a grazer, and thus misrepresent the linkage between somatic growth and grazing. Post-maintenance resource pools ($\mathrm{Resource_{PM}}$; eqs. [9]–[12]) reflect assimilated substrates, and substrates removed in somatic maintenance:

$$[9] \quad P_{\mathrm{PM}} = \alpha_{\mathrm{SC}}\mathrm{GRAZ_P} - P_{\mathrm{INT}}(\tau_{\mathrm{P}}(1-m) + m\tau_{\mathrm{m}})$$

$$[10] \quad N_{\mathrm{PM}} = \alpha_{\mathrm{SC}}\mathrm{GRAZ_N} - N_{\mathrm{INT}}(\tau_{\mathrm{N}}(1-m) + m\tau_{\mathrm{m}})$$

$$[11] \quad \mathrm{EPA_{PM}} = (1-\varepsilon) \times [\alpha_{\mathrm{SC}}\mathrm{GRAZ_{EPA}} - \mathrm{EPA_{INT}}(\tau_{\mathrm{EPA}}(1-m) + m\tau_{\mathrm{m}})]$$

$$[12] \quad \mathrm{DHA_{PM}} = \alpha_{\mathrm{SC}}\mathrm{GRAZ_{DHA}} - \mathrm{DHA_{INT}}(\tau_{\mathrm{DHA}}(1-m) + m\tau_{\mathrm{m}}) + \nu\varepsilon[\alpha_{\mathrm{SC}}\mathrm{GRAZ_{EPA}} - \mathrm{EPA_{INT}}(\tau_{\mathrm{EPA}}(1-m) + m\tau_{\mathrm{m}})]$$

Thus far, there is little deviation between nutrient and HUFA processing (with the exception of HUFA elongation). EPA and DHA are subjected to the same ingestion–assimilation–maintenance process as nitrogen and phosphorus. The absence of HUFA maintenance parameters in the literature will need to be addressed with a more conservative approach than nutrient maintenance rates. Unlike Anderson et al. (2005), we are assuming recycled carapace composition to be in the form of POC, as structural and storage lipids are concentrated in internal physiological structures. A fraction of the post-maintenance EPA pool is subject to elongation to DHA, modulated by the EPA fraction allocated to elongation (e_1) and the elongation efficiency (ν); see eqs. [11] and [12]. While retro-conversion of DHA to EPA is also possible (Persson and Vrede 2006), it has not been considered in our framework. Little quantitative data are available for rates of somatic bioconversion, but qualitative information from empirical work (i.e., elongation is very inefficient, copepods are more likely to elongate EPA to DHA) provides a starting point for parameter exploration. Our model also considers the production EPA from C_{18} PUFA to replenish excessive losses due to biomass and moult turnover. If post-maintenance EPA concentration is extremely low, i.e., lower than a critical threshold value (threshold), elongated PUFAs contribute to both EPA and DHA somatic pools. The flux of PUFAs (J_{PUFA}) elon-

Table 1. Zooplankton growth sub model parameter descriptions and literature values; parameters with no literature values not shown.

Parameter	Symbol	Units	Reported Values
Maximum growth rate	π	day^{-1}	0.05-0.30 day^{-1} (Brett and Müller-Navarra 1997), 0.30-0.58 day^{-1} (Brett et al. 2006), 0.15-0.90 day^{-1} (Ferrão-Filho et al. 2003), 0.05-0.55 day^{-1} (Müller-Navarra et al. 2000), 0.15-0.58 day^{-1} (Ravet and Brett 2006), 0.8 day^{-1} (Mulder and Bowden 2007)
Minimum zooplankton somatic phosphorus	P_{min}	mg P (mg C)$^{-1}$	0.004mg/mg (Mulder and Bowden 2007)
Optimal zooplankton somatic phosphorus	P_{opt}	mg P (mg C)$^{-1}$	0.0115molP:molC (Anderson et al. 2005), 0.024mg/mg (Mulder and Bowden 2007)
Minimum zooplankton somatic nitrogen	N_{min}	mg N (mg C)$^{-1}$	
Optimal zooplankton somatic nitrogen	N_{opt}	mg N (mg C)$^{-1}$	0.1695 molN:molC (Anderson et al. 2005)
Minimum zooplankton somatic EPA	EPA_{min}	mg EPA (mg C)$^{-1}$	
Optimal copepod somatic EPA	EPA_{opt}	mg EPA (mg C)$^{-1}$	12.9-23.0%TFA (Farkas and Herodek 1964), 0-1.7%TFA (Graeve et al. 2005), 8mg/gDW (Kainz et al. 2009)
Optimal cladoceran somatic EPA	EPA_{opt}	mg EPA (mg C)$^{-1}$	9.2%TFA (Ballantyne et al. 2003), 7.7–15.8%TFA (Farkas and Herodek 1964), 6.76–6.76 μmol/mgDW (Müller-Navarra 2006), 12.4%TFA (Weers et al. 1997), 11.8%TFA (Persson and Vrede 2007), 8.2 mg/gDW (Kainz et al. 2009)
Minimum zooplankton somatic DHA	DHA_{min}	mg DHA (mg C)$^{-1}$	
Optimal copepod somatic DHA	DHA_{opt}	mg DHA (mg C)$^{-1}$	13.5–35.9%TFA (Farkas and Herodek 1964), 6.3–9.2%TFA (Graeve et al. 2005), 16.1mg/gDW (Kainz et al. 2009)
Optimal cladoceran somatic DHA	DHA_{opt}	mg DHA (mg C)$^{-1}$	1.5%TFA (Ballantyne et al. 2003), 4.9–12.3%TFA (Farkas and Herodek 1964), 0.38–0.61 μmol/mgDW (Müller-Navarra 2006), $0.1_{\%TFA}$ (Weers et al. 1997), 0.9%TFA (Persson and Vrede 2007), 1.4mg/gDW (Kainz et al. 2009)
Seston EPA to carbon ratio	$s_{EPA:C}$	mg EPA (mg C)$^{-1}$	0.71 μmol/mgDW (Müller-Navarra 2006), 1.2-4.5mg/gDW (Kainz et al. 2004), 3.0%TFA (Persson and Vrede 2007), 1.2-2.3mg/gDW (Kainz et al. 2009)
Seston DHA to carbon ratio	$s_{DHA:C}$	mg DHA (mg C)$^{-1}$	0.50 μmol/mgDW (Müller-Navarra 2006), 0.3-2.9mg/gDW (Kainz et al. 2004), 2.1%TFA (Persson and Vrede 2007), 2.7-5.8 mg/gDW (Kainz et al. 2009)
Phytoplankton phosphorus to carbon ratio	$f_{P:C}$	mg P (mg C)$^{-1}$	0.0039 molP:molC (Anderson et al. 2005), 2.5-10mg/mg (Mulder and Bowden 2007)
Phytoplankton nitrogen to carbon ratio	$f_{N:C}$	mg N (mg C)$^{-1}$	0.1042 molN:molC (Anderson et al. 2005)
Diatom EPA to carbon ratio	$f_{EPA:CDIA}$	mg EPA (mg C)$^{-1}$	12.8%TFA (Graeve et al. 2005), 4.6-11.1%TFA (Wacker and Martin-Creuzburg 2007), 0.6-12.5%TFA (Viso and Marty 1993), 16.9%TFA (Ravet et al. 2010)
Diatom DHA to carbon ratio	$f_{DHA:CDIA}$	mg DHA (mg C)$^{-1}$	4.5%TFA (Graeve et al. 2005), 0.1-1.9%TFA (Viso and Marty 1993), 2.5%TFA (Ravet et al. 2010)
Chlorophyte EPA to carbon ratio	$f_{EPA:CCHL}$	mg EPA (mg C)$^{-1}$	0.1%TFA (Brett et al. 2006), 0-0.3 μg/gDW (Ravet and Brett 2006), 0%TFA (Ravet et al. 2010)
Chlorophyte DHA to carbon ratio	$f_{DHA:CCHL}$	mg DHA (mg C)$^{-1}$	0%TFA (Brett et al. 2006), 0-0.1μg/gDW (Ravet and Brett 2006), 0%TFA (Ravet et al. 2010)
Cyanophyte EPA to carbon ratio	$f_{EPA:CCYAN}$	mg EPA (mg C)$^{-1}$	1.5 %TFA (Brett et al. 2006), 1.8-2.2 μg/gDW (Ravet and Brett 2006), 0.7% TFA (Ravet et al. 2010)
Cyanophyte DHA to carbon ratio	$f_{DHA:CCYAN}$	mg DHA (mg C)$^{-1}$	0%TFA (Brett et al. 2006), 0 μg/gDW (Ravet and Brett 2006), 0.6%TFA (Ravet et al. 2010)
Zooplankton biomass phosphorus turnover rate	τ_p	day^{-1}	0.094 day^{-1} (Anderson et al. 2005)
Zooplankton biomass nitrogen turnover rate	τ_n	day^{-1}	0.094 day^{-1}(Anderson et al. 2005)
Zooplankton biomass EPA turnover rate	τ_{epa}	day^{-1}	0.051-0.31 day^{-1} (Shin et al. 2000)
Zooplankton biomass DHA turnover rate	τ_{dha}	day^{-1}	0.051-0.31 day^{-1} (Shin et al. 2000)

Table 1 (*concluded*).

Parameter	Symbol	Units	Reported Values
Zooplankton moult P turnover rate	τ_m	day^{-1}	0.4 day^{-1} (Anderson et al. 2005)
Moult as a fraction of zooplankton biomass	m	dimensionless	0.05 (Anderson et al. 2005)
Maximum zooplankton grazing rate	λ	day^{-1}	0.6 day^{-1} (Perhar and Arhonditsis 2009)
Zooplankton carbon assimilation efficiency	α_{c1}	dimensionless	0.9 (Perhar and Arhonditsis 2009)
Half saturation constant for zooplankton growth efficiency	α_{c2}	$(mg\ C\ L^{-1})^{1/2}$	0.03 $(mg\ C\ L^{-1})^{1/2}$ (Perhar and Arhonditsis 2009)
Phytoplankton food preference	ω_{PHYT}	dimensionless	1.0 (Perhar and Arhonditsis 2009)
Detritus food preference	ω_{DET}	dimensionless	1.0 (Perhar and Arhonditsis 2009)
Diatom food quality	FQ_{DIA}	dimensionless	0.8 (Perhar and Arhonditsis 2009)
Chlorophyte food quality	FQ_{CHL}	dimensionless	0.5 (Perhar and Arhonditsis 2009)
Cyanophyte food quality	FQ_{CYAN}	dimensionless	0.2 (Perhar and Arhonditsis 2009)
Excretion rate	χ	day^{-1}	
Flux of C18 PUFA, precursor to EPA (from diatoms)	J_{PUFA}	mg PUFA (mg C)$^{-1}$ day^{-1}	0.25%DW (Brett and Müller-Navarra 1997), 8.5%TFA (Graeve et al. 2005), 0.8-13.2mg/gDW (Kainz et al. 2004), 2.5-7.2%TFA (Wacker and Martin-Creuzburg 2007), 0.2-5.9%TFA (Viso and Marty 1993), 11.7%TFA (Persson and Vrede 2007), 2.9%TFA (Ravet et al. 2010)
Flux of C18 PUFA, precursor to EPA (from chlorophytes)	J_{PUFA}	mg PUFA (mg C)$^{-1}$ day^{-1}	3%DW (Brett and Müller-Navarra 1997), 0.8-13.2 (Kainz et al. 2004), 2.5-7.2% TFA (Wacker and Martin-Creuzburg 2007), 10.1-26.9 μg/gDW (Ravet and Brett 2006), 11.7%TFA (Persson and Vrede 2007), 25.5%TFA (Ravet et al. 2010)
Flux of C18 PUFA, precursor to EPA (from cyanophytes)	J_{PUFA}	mg PUFA (mg C)$^{-1}$ day^{-1}	0.25%DW (Brett and Müller-Navarra 1997), 0.8-13.2 (Kainz et al. 2004), 2.5-7.2%TFA (Wacker and Martin-Creuzburg 2007), 0.8-3.2 μg/gDW (Ravet and Brett 2006), 11.7%TFA (Persson and Vrede 2007), 7%TFA (Ravet et al. 2010)
Conversion efficiency of ALA to EPA	ρ	mg EPA (mg ALA)$^{-1}$	
Fraction of EPA to DHA conversion via elongation	ε	dimensionless	
Conversion efficiency of EPA to DHA	ν	mg DHA (mg EPA)$^{-1}$	
Hormone production rate	h	day^{-1}	

gated to EPA are controlled by the PUFA-EPA elongation efficiency (ρ; see eqs. [13] and [14]):

$$[13] \quad \text{if} \quad \mathrm{EPA_{PM}} \leq \text{threshold},$$
$$\mathrm{EPA_{PM}} = \alpha_{\mathrm{SC}}\mathrm{GRAZ_{EPA}} - \mathrm{EPA_{INT}}(\tau_{\mathrm{EPA}}(1-m) + m\tau_{\mathrm{m}}) + (1-\varepsilon)J_{\mathrm{PUFA}}\rho$$

$$[14] \quad \mathrm{DHA_{PM}} = \alpha_{\mathrm{SC}}\mathrm{GRAZ_{DHA}} - \mathrm{DHA_{INT}}(\tau_{\mathrm{DHA}}(1-m) + m\tau_{\mathrm{m}}) + \varepsilon J_{\mathrm{PUFA}}\rho\nu$$

In a subsequent study, we investigate model sensitivity and behavior and provide logical rules that determine the sequence of different internal processes and the threshold conditions under which these mechanisms are triggered and (or) switched off (Perhar et al. unpublished).

Finally, there are post-maintenance costs to consider for each resource pool before somatic growth (Growth) and internal resource concentrations are calculated. The nitrogen and phosphorus pools are subjected to a regulated release fraction (χ). Conceptually, these releases represent post-gut excretion in the forms of urine and feces, but can also be thought of as a homeostatic regulation mechanism. Once accounted for, the somatic nutrient concentration differential equations can be calculated as

$$[15] \quad \frac{\mathrm{d}P_{\mathrm{INT}}}{\mathrm{d}t} = P_{\mathrm{PM}} - \mathrm{Growth}P_{\mathrm{INT}} - \chi_{\mathrm{P}}P_{\mathrm{INT}}$$

$$[16] \quad \frac{\mathrm{d}N_{\mathrm{INT}}}{\mathrm{d}t} = N_{\mathrm{PM}} - \mathrm{Growth}N_{\mathrm{INT}} - \chi_{\mathrm{N}}N_{\mathrm{INT}}$$

Pre-gut regulation released substrate into the water column in particulate form (via sloppy feeding), but maintenance by-products and post-maintenance release can be fractionated into particulate and dissolved forms, and broken down into different dissolved nutrient forms (e.g., nitrate, ammonium, dissolved organic nitrogen), depending on the specification of the host plankton population model. Empirical work has shown cladocerans release phosphorus even under severe phosphorus limiting conditions (DeMott et al. 1998), but this may reflect maintenance turnover rather than post-growth regulation/release. Fractions of post- maintenance EPA and DHA pools are subjected to hormone production (h_{EPA} and h_{DHA}). These HUFA subtractions are analogous to reproductive investments (via hormone production and HUFA amassing in eggs); once accounted for, the somatic HUFA concentration differential equations can be specified as follows:

$$[17] \quad \frac{\mathrm{dEPA_{INT}}}{\mathrm{d}t} = \mathrm{EPA_{PM}} - \mathrm{Growth}\mathrm{EPA_{INT}} - h_{\mathrm{EPA}}\mathrm{EPA_{INT}}$$

$$[18] \quad \frac{\mathrm{dDHA_{INT}}}{\mathrm{d}t} = \mathrm{DHA_{PM}} - \mathrm{Growth}\mathrm{DHA_{INT}} - h_{\mathrm{DHA}}\mathrm{DHA_{INT}}$$

We do not explicitly account for reproduction, but have qualitative data to parameterize our proxy parameter (h; Smyntek et al. 2008). Once all somatic processes are accounted for, resource saturation quotients ($g_{\mathrm{LIM_{RESOURCE}}}$) are calculated:

$$[19] \quad g_{\mathrm{LIM_P}} = \frac{P_{\mathrm{INT}} - P_{\mathrm{min}}}{P_{\mathrm{opt}} - P_{\mathrm{min}}}$$

$$[20] \quad g_{\mathrm{LIM_N}} = \frac{N_{\mathrm{INT}} - N_{\mathrm{min}}}{N_{\mathrm{opt}} - N_{\mathrm{min}}}$$

$$[21] \quad g_{\mathrm{LIM_{EPA}}} = \frac{\mathrm{EPA_{INT}} - \mathrm{EPA_{min}}}{\mathrm{EPA_{opt}} - \mathrm{EPA_{min}}}$$

$$[22] \quad g_{\mathrm{LIM_{DHA}}} = \frac{\mathrm{DHA_{INT}} - \mathrm{DHA_{min}}}{\mathrm{DHA_{opt}} - \mathrm{DHA_{min}}}$$

The purpose of the saturation quotients is to evaluate the degree to which somatic quotas are being met. To define the saturation quotients, the differences between internal and minimum quotas are used as dividends, and the divisors are the differences between optimal and minimum quotas. These saturation quotients (ranging from 0 when a resource pool is depleted, to 1 when a resource pool is saturated) are the drivers of zooplankton somatic growth, and may be considered in a number of ways. One possible approach resembles Liebig's Law of the Minimum, postulating that the grazer growth is limited by the resource in shortest supply, with no regard for whether it stems from a mineral or HUFA deficiency. The second approach separately considers the effects of mineral and HUFA limitation. That is, the product of the lowest mineral limitation (i.e., nitrogen or phosphorus), the lowest HUFA limitation (i.e., EPA or DHA) and maximum growth rate (π) yield zooplankton actual growth rate. The third approach considers all four saturation quotients in a multiplicative form; zooplankton actual growth rate would be the product of maximum growth rate, nitrogen, phosphorus, EPA, and DHA saturation. The three strategies outlined put increasing pressure on growth limitation. Consider for example that each resource pool is 75% saturated. Under the first strategy, zooplankton growth rate is 75% of the maximum growth rate; with the second this falls to 56% of the maximum growth rate ($0.75^2 = 0.56$); while the final approach falls the growth rate to 32% of the maximum growth rate ($0.75^4 = 0.32$). As such, the second or third approach may excessively limit zooplankton somatic growth. It is important to note, however, that when growth is limited by an individual resource, the limitation does not imply resource starvation, but that it is the resource least saturated in the grazer's body and thus determines the growth rate.

$$[23] \quad \mathrm{Growth} = \pi \min[g_{\mathrm{LIM_P}}, g_{\mathrm{LIM_N}}, g_{\mathrm{LIM_{EPA}}}, g_{\mathrm{LIM_{DHA}}}]$$

The challenge with the explicit consideration of HUFAs and minerals in a population model is the inevitable fusion of homeostatic processes at the individual level with population-scale dynamics. Anderson et al. (2005) noted this difficulty, stating,

> "introducing stoichiometric constraints into theoretical and empirical studies of population dynamics will require a far more comprehensive and explicit integration of population and ecosystem perspectives than has previously been achieved."

Integrating a homeostatic individual-based sub model with a plankton population model yields an expensive construct, based on the number of parameters considered. While many of the processes are lacking data in the literature, future sensitivity analyses and calibration exercises are likely to delineate plausible regions and may facilitate their realistic characterization (Perhar et al. unpublished). In this regard, the proliferation of a physiologically explicit approach to modeling plankton communities may not only make management decisions via water quality models more robust, but should also provide a starting point for empirical work to validate/challenge the mathematical outputs and hypotheses generated.

Application

The dynamics and sensitivity of the zooplankton growth model proposed in the current study are tested rigorously in Perhar et. al., (unpublished). The primary objective, however, remains the adoption into a management-oriented model replicating real world patterns for a given study site. The eutrophication model for Lake Washington designed by Arhonditsis and Brett (2005*a*) is seasonally forced and considers three phytoplankton (diatoms, greens, cyanobacteria) and two zooplankton (cladocerans and copepods) functional groups, and multiple nutrient cycles. For illustrative purposes, we present here preliminary results from the integration of our proposed resource-explicit growth submodel with the eutrophication model (henceforth referred to as the host model). We replaced the host model's appraisal of food quality with the dynamics outlined in eq. [1], and modified the zooplankton growth terms with eq. [23]. The only other change made to the host model was to assign HUFA concentrations to the food sources (i.e., algae and detritus). We conducted a calibration of the new parameters (i.e., sub model parameters and food HUFA concentrations) to match empirical data of the zooplankton nutrient and fatty acid content in Lake Washington (Ravet et al. 2010), while holding all host model parameters constant as in the original application (Arhonditsis and Brett 2005*a*, 2005*b*). We present seasonal algal biomass, zooplankton biomass, and cladoceran growth limitation patterns across three loading scenarios.

Time series plots from running the calibrated sub model with the host model, utilizing the default loading forcing that approximates the mesotrophic conditions currently prevailing in Lake Washington, show cladocerans experience both nutrient and biochemical limitation (Fig. 5). EPA limitation is apparent from mid-summer to late winter, while seston EPA availability in the spring bloom is abundant. Somatic growth limitation for each resource is quantified between 0 (fully limited) and 1 (somatic requirements met). Thus, under the growth calculation employed in our sub model, cladoceran growth is driven by available phosphorus in the spring bloom, as it is the most limiting resource considered (see Fig. 5*c*). Copepod growth patterns (not shown) exhibit a similar trend, but the signature of DHA limitation was more distinct. Severely reducing the phosphorus loading into the system drastically alters the phytoplankton community composition (Fig. 6*a*), which in turn affects zooplankton growth. By reducing phosphorus loading by 65%, we approximate oligotrophic conditions in which cyanobacteria are gradually phased out, the relative abundance of diatoms increases, and total chlorophyll-a is approximately half of the mesotrophic condition. Zooplankton are limited by food availability, and their biomass drops in response to the structural changes of the phytoplankton assemblage (Fig. 6*b*). Phosphorus limitation

Fig. 5. Seasonal succession plankton patterns and limiting factors of zooplankton growth in a typical mesotrophic environment.

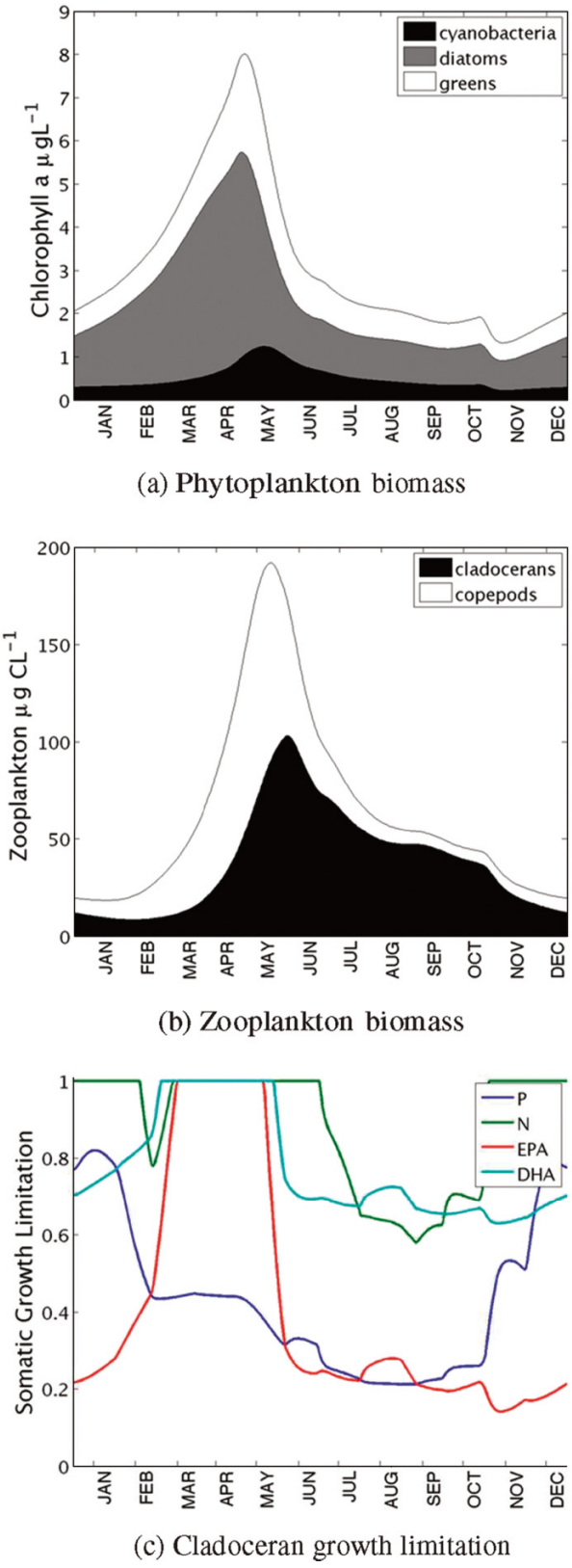


Fig. 6. Seasonal succession plankton patterns and limiting factors of zooplankton growth in a typical oligotrophic environment.

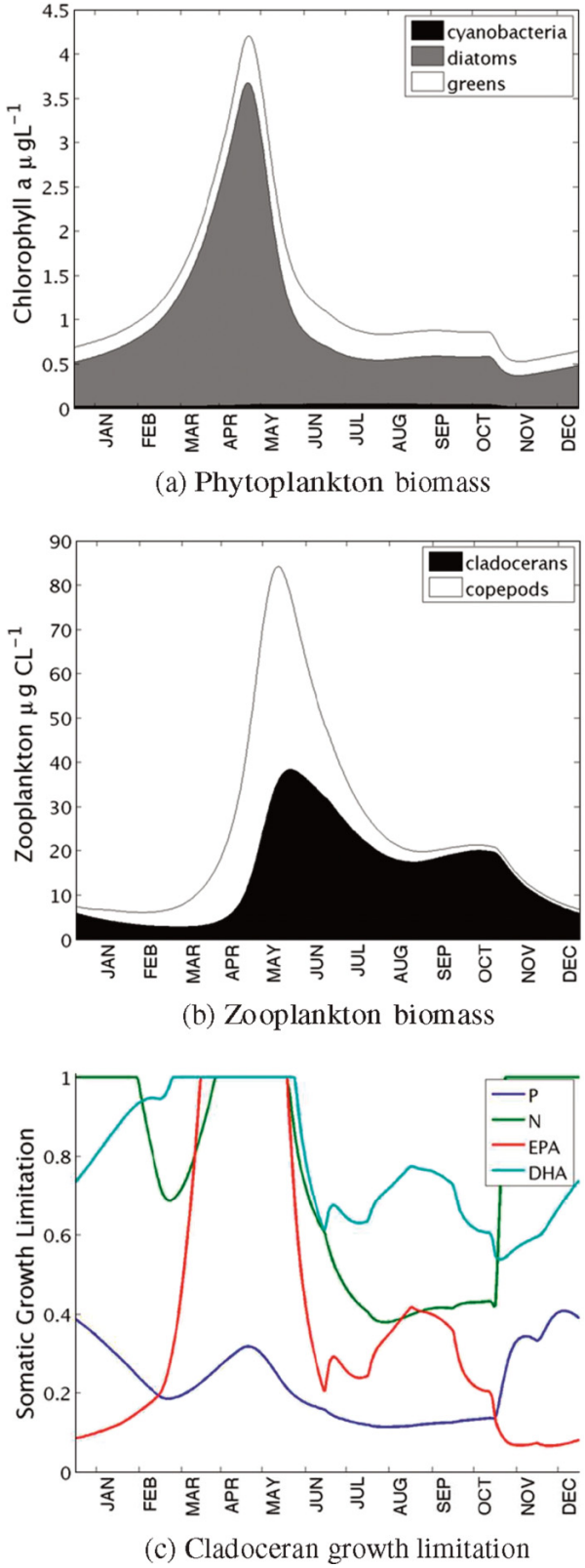
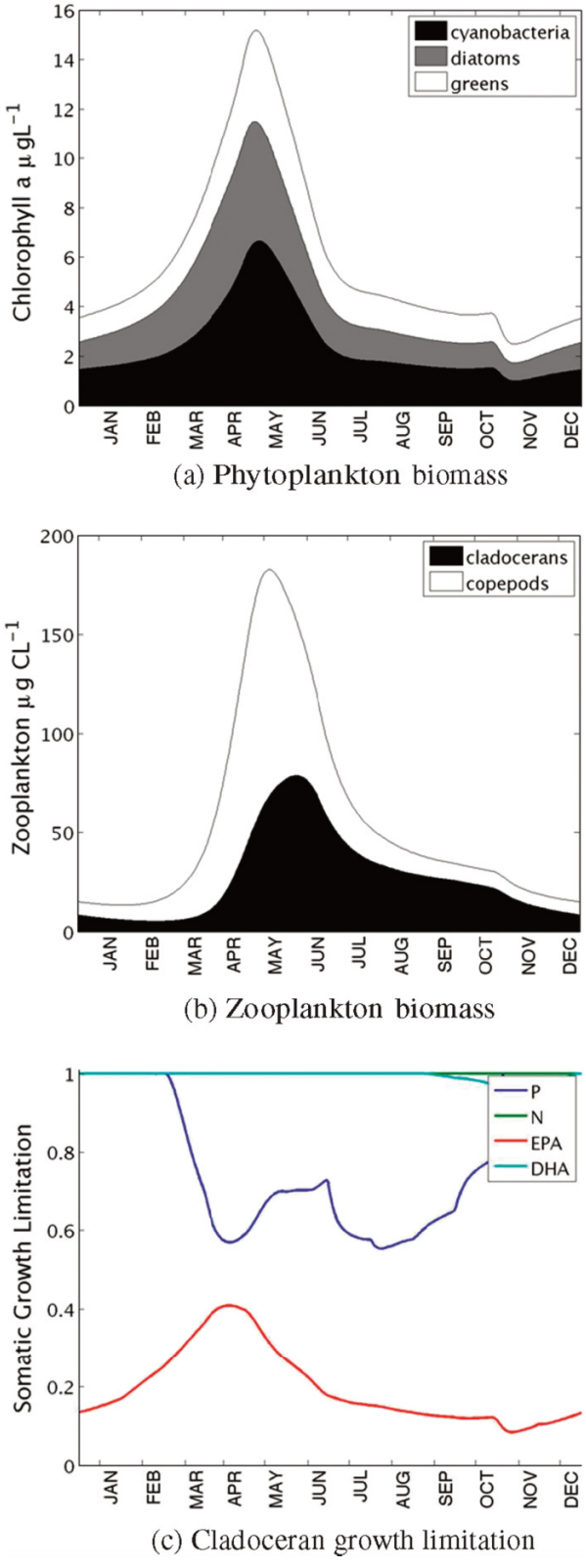


Fig. 7. Seasonal succession plankton patterns and limiting factors of zooplankton growth in a typical eutrotrophic environment.



on cladoceran growth is more pronounced throughout the year, with the exception of winter when EPA limitation is apparent (Fig. 6*c*). When phosphorus loading is increased by 75% over the loading of the mesotrophic conditions, there is a substantial increase in chlorophyll-a (Fig. 7*a*). Over 40% of the phytoplankton community is comprised of cyanobacteria, and cladoceran somatic growth is limited entirely by EPA (Fig. 7*c*). The lack of increase of zooplankton biomass

(Fig. 7*b*) yields a scenario with considerable producer accumulation.

It is important to clarify our stance on how zooplankton growth should be modelled. Our preliminary results illustrate that the sole consideration of nutrient or HUFA limitation strategies to modelling zooplankton growth may not capture the full range of dynamics experienced. Thus, the incorporation of both nutrient and HUFA limitation in our zooplankton growth sub model holds potential for a more accurate portrayal of the dynamics of planktonic food webs.

Conclusions and future perspectives

Stemming from their structure and functional role in ecosystem dynamics, HUFA availability may have far reaching consequences. Aquatic primary producers are a primary source of the world's EPA and DHA (Connor 2000). While somatic bioconversions are possible, the consumer inability to synthesize HUFAs *de novo* often results in the unaltered incorporation of dietary HUFAs into somatic tissues, thereby permitting the use of HUFAs as trophic biomarkers and tracers (Brett et al. 2006). The practice of using fatty acids as trophic markers (FATM) is based on observations that primary producers' fatty acid signatures are recognizable in consumers due to conservative transfer and assimilation (Dalsgaard et al. 2003). Gut content analysis is also utilized to analyze energetic pathways, but severe drawbacks are the tight temporal link and the digestibility of ingested food. Namely, gut content analysis will only reveal what has been eaten recently, whereas the conservative handling of fatty acids can yield dietary information over longer periods of time. Secondly, gut content analysis may overemphasize hard to digest food items, as they are retained for a longer period of time, whereas easily digestible material will be quickly processed. The ideal trophic marker is one which has an easily identified origin, is conservative and non-harmful to organisms, not selectively processed during ingestion/assimilation, and metabolically stable (Dalsgaard et al. 2003). HUFAs are a good choice for trophic markers, but are not ideal. While fatty acids alone cannot be used as toxicity indicators, fatty acid signatures may be used to deduce primary producer species presence, including toxic species (Fahl and Kattner 1993; Viso and Marty 1993; Napolitano 1999; Volkman et al. 1989).

A large portion of the fatty acid literature is dedicated to studying food web pathways via EFAs, while fewer studies consider the importance of fatty acids at the level of the producer and consumer and their interactions, and considerably more focus on EFAs in fish. Despite the tremendous interest in fatty acids, they have rarely been examined in planktonic food web modeling studies. We have proposed a HUFA-explicit sub model designed to be appended to existing plankton population models, thereby considering not only stoichiometric and morphological constraints at the plant-animal interface, but biochemical -namely HUFA- limitations as well. It is important to clarify our stance on limitations at the plant-animal interface: HUFAs alone may not control energy flow across trophic levels; the same can be said of tracking only stoichiometric constraints. Instead, a combined synergistic approach considering multiple limitation factors should be more informative. The literature shows multiple studies discussing the competitive advantages and overall fitness of individuals based on the handling of internal nutrient reserves in variable environments (e.g., Sommer 1985, Sommer 1989 and Grover 1991 in the context of phytoplankton; Brett et al. 2009 in the context of zooplankton). For example, a zooplankter with a large internal phosphorus reserve has a greater chance of survival in periods of starvation, and can potentially meet more effectively the maintenance, growth, and reproductive needs. Explicit accounting of HUFAs may be helpful in further investigating this outcome, as individual quotas (i.e., phosphorus vs. nitrogen vs. EPA vs. DHA) are tightly linked to life history patterns and physiological functioning. Individuals saturated with phosphorus may still exhibit poor ecological fitness, due to depleted HUFA pools. Our HUFA sub model was designed to address issues of fitness, trophic transfer efficiency, and physiological response to various perturbations at the plant-animal interface.

Models are useful tools for investigating environmentally-driven ecology (Neuheimer et al. 2009), but ignoring aspects of life history, individual physiology and phenology may limit their usefulness. The literature provides sufficient information to delineate realistic regions for some parameters, but there are still other parameters that are difficult to measure in controlled environments. In a follow up study, we carried out a thorough sensitivity analysis narrow the plausible regions for the poorly constrained parameters, while still producing ecologically sound results at the population level (Perhar et al. unpublished). The incorporation of our explicit zooplankton growth model into a large scale management-type model is also presented in a subsequent study (Perhar et al. unpublished). In this exercise, we present our sub model calibration, model response to various loading scenarios and a series of statistical analyses highlighting the bulk of information gained by incorporating a resource-explicit zooplankton growth term. We think that this structural augmentation -once properly calibrated against observed data- holds the potential to improve our capacity to reproduce plankton dynamics, and thus yield a more robust platform upon which to make management decisions.

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