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REVIEW PAPER

PHYTOPLANKTON AS A BASIC NUTRITIONAL SOURCE IN DIETS OF FISH*

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Abstract

This article focuses on the biochemical composition and the role of phytoplankton (primary producer) as a basic nutritional source in diets of fish. In general, an aquatic classical food web comprises phytoplankton, zooplankton and fish (planktivorous and predatory ones) as predominant elements, which in turn define the linear direction of an energy-flow pathway and nutritional transfer. Phytoplankton provides many valuable phytonutrients and biologically active ingredients, especially fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, trace elements, and vitamins, which are directly available for the first larval/juvenile stages of fish or indirectly (through trophic chains) for the more mature forms. Regarding the biochemical composition of phytoplankton, the most nutritional ones are lipids, which affect the growth, health and reproduction of aquatic animals, especially fish species. In general, the freshwater and marine chlorophytes and cryptophytes, and marine diatoms provide more polyunsaturated fatty acids (PUFA>SAFA>MUFA) than other groups, differently to fatty acids supplied by freshwater cyanobacteria (SAFA>MUFA>PUFA). Furthermore, cryptophytes have the highest *n*-3:*n*-6 ratio. A similarly high ratio is recorded in fatty acid composition of marine zooplankton with predominant PUFA. Addition of phytoplankton (e.g. *Isochrysis galbana, Tetraselmis chui* and *Nannochloropsis oculata*) to the process of fish larval rearing shows to improve the digestive functions at the pancreatic and intestinal levels. The most essential fatty acids for all fish are EPA and DHA, and the most popular fish species containing 4.08-11.84 mg g^{-1} of dry weight of EPA and DHA, as the sum, can be given in following order:

Anguila anguila>Rutilus rutilus>Cyprinus carpio>Esox lucius>Blicca bjoerkna>Perca fluviatilis>Abramis brama>Sander lucioperca>Tinca tinca>Carassius carassius.

Keywords: food preference, lipids, fatty acids, proteins, larvae, juvenile fish, aquatic food webs.

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INTRODUCTION

Phytoplankton plays a crucial role in an aquatic ecosystem as the primary producer, i.e. the basic element in a classical food web. Moreover, flagellated algae are also essential for the microbial food web. Phytoplankton assemblages are characterized by their high ability to provide many valuable phytonutrients and biologically active ingredients, e.g. fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, trace elements, vitamins, antioxidants (Dunstan et al. 1992, Guedes, Malcata 2012, Shields, Lupatsch 2012, Kovač et al. 2013). A simplified classical food web in an aquatic ecosystem comprises phytoplankton as staple food for zooplankton, and further zooplankton as food for planktivorous fish, which in turn are food for predatory fish. In the same way, the nutritional value of phytoplankton is transferred to zooplankton, next to fish and finally to humans (with fish consumed). More information, for example on the major carbon-flow direction, is outlined by the microbial food web: consumption of dissolved organic matter and particulate organic matter by heterotrophic protists. Heterotrophic protists play a crucial role in the "trophic-upgrading" concept of food quality developed by Klein Breteler et al. (1999), which can be explained by the lipid composition (Bec et al. 2006). Lipids (especially polyunsaturated fatty acids – PUFA), in turn, are the nutritional factors and essential components in modifying the animal growth, health and even reproduction (Desvilettes, Bec 2009). Therefore, phytoplankton is broadly utilized in aquaculture as nutritional live food for zooplankton (to enrich its nutritional value), bivalve mollusks and larval or even early juvenile stages of some fish species. Thus, the aim of this article was to emphasize the role of primary producers, i.e. phytoplankton, as the basic nutritional source in the diets of fish by reviewing the worldwide literature.

STATE-OF-THE-ART

Biochemical composition of phytoplankton

The biochemical composition of phytoplankton concerning primarily carbohydrates, lipids and proteins (Table 1) is strictly connected with water quality, hence water pollution, e.g. with heavy metals, can significantly affect each component (Abd El-Hady 2014). Close relationships were also recorded with seasonal changes in phytoplankton assemblages. Regarding the exemplary Lake Nasser (Egypt), the highest contents of total proteins (22 mg dm^{-3}), carbohydrates (37 mg dm^{-3}) and total lipids (10 mg dm^{-3}) were noted in winter accompanied by the maximum phytoplankton abundance and chlorophyll *a* concentration (Abd El-Hady et al. 2016). In that period, the zooplankton, especially Copepoda and Cladocera, was also the most abundant (Khalifa et

Table 1

Overview of the biochemical composition of phytoplankton and environmental variables (mean or range concentration)

Environment characteristics			Phytoplankton composition				References
Site	phosphates (mg dm^{-3})	TIN (mg dm^{-3})	chlorophyll a (µg dm^{-3})	carbohydrates (mg dm^{-3})	lipids (mg dm^{-3})	proteins (g dm^{-3})	
Lake Nasser	0.020-0.115	0.596-1.015	46.2	9.5	4.4	3.2	Hussian et al. (2016)
Lake Nasser: Main channel	0.024	0.215	4.3 (1.1-13.3)	2.9 (0.2-6.2)	0.2 (0.1-0.9)	0.04 (0.01-0.1)	Abd El-Karim, Mahmoud (2016)
KTu			7.9	1.7	0.2	0.03	
KDa			4.2	1.3	0.3	0.04	
Lake Nasser:							Abd El-Hady et al. (2016)
KTu	0.027-0.144	0.212-1.080	18.4-302.0	1.7-12.2	1.0-6.3	0.8-3.2	
KW-A	0.014-0.208	0.052-1.746	8.5-255.7	1.0-10.3	0.8-9.6	0.9-8.8	
KKa	0.027-0.185	0.054-0.571	23.3-415.1	1.7-25.4	1.0-2.2	1.0-3.8	
Kko	0.015-0.055	0.201-1.106	10.5-159.4	1.4-36.7	1.1-9.0	0.7-22.3	
Aswan Reservoir	0.063 (0.042-0.099)	0.020-0.181*	1.2-5.2	25.0-87.0	6.0-16.6	2.0-5.6	Abd El-Hady (2014)
River Nile	0.062	0.038*	10.0-20.0	18.0-61.3	5.9-10.3	1.0-6.1	
RN drains	n.a.	n.a.	n.a.	2.0-128.0	2.6-20.0	0.6-7.9	

TIN – total inorganic nitrogen, KTu – Khor Tushka, KDa – Khor Dahmeit, KW-A – Khor Wadi-Abyad, KKa – Khor Kalabsha, KKo – Khor Koroske, RN – River Nile, * only nitrate maxima, n.a. – not analyzed al. 2014). Furthermore, muscles of the analyzed fish *Sarotherdon galielas* L., *Oreochromis niloticus* L. and *Alestes dentex* L. contained the highest protein and lipid contents in winter (Elhaddad, Elshahat 2014).

During the cultivation on the traditional medium and the fish-breeding RAS (Recirculating Aquaculture Systems) wastewater, the accumulation of nutrients in some chlorophytes and cyanobacteria increased together with the active increase of their biomass (Khudyi et al. 2016). The content of total proteins was the highest, up to 64% of the dry weight (in Chlorophyta species), whereas total lipids and carbohydrates constituted up to 16% (in Cyanobacteria species) and 12% (similarly for all species), respectively. Similar mean total lipids content was also recorded in other 10 freshwater and marine microalgae species cultured on taxa-specific medium (Ohse et al. 2015). The content of proteins, carbohydrates, lipids and phosphorus compounds recorded in natural estuarine phytoplankton varied in the ranges of 30.6-50.7%, 14.1-55.8%, 2.1-26.7% and 8.0-21.0% of weight, respectively (reviewed by Ríos et al. 1998). Such variability can be caused by physiological differences among phytoplankton species and their growth phases. An approximately same biochemical composition was demonstrated in other scientific studies on different phytoplankton species (reviewed by Kovač et al. 2013).

Regarding lipids in aquatic ecosystems, phytoplankton can predominantly synthesize PUFA (polyunsaturated fatty acids), which in turn are mainly consumed by zooplankton and benthic invertebrates. The next step in the nutritional lipid transfer is through fish to humans eating fish. The four main groups of phytoplankton, i.e. cryptophytes, diatoms, dinophytes and euglenophytes, can synthesize high amounts of HUFA (highly unsaturated fatty acids – a subset of PUFA), which are transferred and accumulated at progressively higher levels in aquatic organisms (Gladyshev et al. 2009). Thus, an aquatic ecosystem offers the principal dietary sources of *n*-3 HUFA for all aquatic animals and consequently for humans.

Generally, the phytoplankton fatty acids are composed of SAFA (saturated fatty acid), MUFA (monounsaturated fatty acids) and PUFA (including also their derivatives HUFA). According to reviews of Brett et al. (2009), the order of amounts of particular FA components was as follows:

1) PUFA>SAFA>MUFA (freshwater and marine chlorophytes and cryptophytes and marine diatoms);
2) MUFA>PUFA>SAFA (freshwater diatoms);
3) SAFA>MUFA>PUFA (freshwater cyanobacteria).

The mean *n*-3:*n*-6 ratio (of FA) in freshwater phytoplankton ranged from 1.0 (cyanobacteria) to 16.8 (cryptophytes). Whereas ratio from 4.5 (chlorophytes) to 22.4 (cryptophytes) was recorded in marine phytoplankton. Comparing the zooplankton fatty acid composition, the PUFA predominated in both freshwater and marine zooplankton (reviewed by Brett et al. 2009). Zooplankton was characterized by *n*-3:*n*-6 ratio of approximately 3.9 in (freshwater) and 6.7-18.1 (marine).

Live diet composition of fishes

The feeding habits and/or preferences of different fish species vary immensely. However, the composition of diets ingested by the first larval stages is quite similar and, in turn, very important in assessment of the feeding conditions and opportunities to satisfy food requirements in aquatic ecosystems. The composition of a diet is usually determined based on an analysis of the entire gut content (sorting out and identifying mostly or partly digested or even some indigested organisms), and a percentage-based method is used to express the results. Numerous fish larvae are mainly herbivorous, e.g. fish of the families: Sparidae, Clupeidae, Gobiidae and Terapontidae (Ara et al. 2009, 2010, 2011, 2013, Arshad et al. 2013), whose diet consists mainly of phytoplankton (56.3-82.5%), other algae (0.9-11.7%) and plant-like matter (7.3-15.1%) – Table 2. Similarly, roach larvae (the family Cyprinidae) with the body length of 7-8 mm feed totally on phytoplankton, whereas a little larger larvae (12 mm) have a diet with only 16.3% of this food source.

Table 2

Composition of diets (%, mean or range) of selected fish species or family

Fish species/family	Animal		Plants		References
	zooplankton	other	macrophytes	algae	
Tench (adults and fry)	4.0	85.0	10.6	0.4	Prejs (1973)
Common carp (adults and fry)	14.0	70.0	15.7	0.3	Prejs (1973)
Crucian carp (adults and fry)	68.0	26.0	5.6	0.4	Prejs (1973)
Bream (adults and fry)	6.0	87.0	6.7	0.3	Prejs (1973)
Roach (adults and fry)	<1	<1	95	4	Prejs (1973)
larvae (7.0-7.9 mm)	-	-	-	100	Hammer (1985)
larvae (12 mm)	73.9	9.9	-	16.3	Hammer (1985)
larvae (14.6 mm)	98.3	0.7	-	1	Hammer (1985)
larvae (17.6-20.7 mm)	99.5-99.9	0.1-0.5	-	-	Hammer (1985)
group size					
102-130 mm	30-100	0-20	0-20	0-5*	Horppila (1994)
130-159 mm	40-100	0-20	0-22	0-10*	Horppila (1994)
160-189 mm	1-100	0-45	0-45	0-5*	Horppila (1994)
>190 mm	1-90	0-40	0-80	0-20*	Horppila (1994)
Perch larvae (28-72 mm)	80-100	0-20	-	-	Hammer (1985)
Sparidae larvae	9.4	10.2+	7.8**	60.9*** 11.7#	Ara et al. (2009)
Gobiidae larvae	9.8	12.2+	15.1**	9.7 56.3***	Ara et al. (2010)
Clupeidae larvae	3.2	5.6+	7.3**	0.9 82.5***	Ara et al. (2011)
Terapontidae larvae	5.0	6.1+	8.0**	6.7 74.2***	Arshad et al. (2013)

* epiphytes (*Oedogonium*, *Stigeoclonium*), ** plant-like matter, *** phytoplankton, # macroalgae,
\+ including also unidentified items and debris

However, small-size (approximately 8 mm) roach larvae can feed on both phytoplankton and zooplankton (Kirillov 2001). The diet of some roach individuals (>190 mm) consisted in up to 20% from epiphytes (algae *Oedogonium* and *Stigeoclonium*), but they occurred in the littoral zone of a lake (Horppila 1994). On the other hand, many fish larvae can feed on zooplankton (Rust 2003).

The diets of both fry and adult fish primarily consist of fish (predatory fish), benthic fauna and fauna living on plants, zooplankton and macrophytes (non-predatory fish, including seston-feeding, herbivorous, planktofagous and *benthofagous*) (Prejs 1973). Algae usually represent a slight supplement of natural fish's food, usually below 1%, and only sometimes up to 4%, as it was in the roach's diet. However, recent studies on phytoplankton-fish relationships have indicated some significant correlations between dominant adult fish species, e.g. *Coptodon zillii* (previously *Tilapia zillii* (Gervais)) or *Solea* spp., and phytoplankton density (Napiórkowska-Krzebietke et al. 2016). Some results confirmed that the composition of a diet of larval stages with the same or similarly sized body can be differentiated internally and also varied in comparison with early juvenile fish exploiting the same or different food resources (Bogacka-Kapusta, Kapusta 2010, 2014).

Overall, the fish's diet composition depends on the age and the environmental conditions. During the initial stages in larval development, fish prefer phytoplankton, but only for a relatively short time. Next, the morphological features of larvae develop and their ability to find more diversified food resources increase, which can help them in making a dietary shift towards small-size then big-size zooplankton, benthos, macrophytes and/or fish. However, the traces of phytoplankton in an aquatic food web remain as phytonutrients, primarily providing polyunsaturated fatty acids (PUFA), e.g. eicosapentaenoic acid (EPA), arachidonic acid (AA) and docosahexaenoic acid (DHA), usually supplied at a higher-level in consumers. Additionally, environmental conditions significantly influence the metabolic processes, while the quantity and quality of essential micro- and macroelements in food and water are of paramount importance for fish (e.g. Polak-Juszczak, Robak 2015, Terech-Majewska et al. 2016). On the other hand, natural waters are also a source of heavy metal pollution and cyanobacterial toxins, which tend to progressively accumulate along the food chain, thus the highest amounts with respect to each part of body are deposited in fish (e.g. Łuczyńska et al. 2016, Pawlik-Skowrońska et al. 2012).

Live diets versus commercial feed products

The experimental studies on the effect of adding planktonic microalgae, i.e. *Isochrysis galbana* Parke (phylum Haptophyta) to larval rearing of European sea bass *Dicentrarchus labrax* L. proved its significant role, e.g. initiating the hydrolytic functions of cell membranes and improving the digestive functions at the pancreatic and intestinal levels by larvae (Cahu et al.

1998*b*). Nutrient-rich microalgal strains of *I. galbana* and e.g. genera *Tetraselmis, Chlorella, Dunaliella, Haematococcus, Chaetoceros, Skeletonema, Thalossiosira, Navicula*, *Amphora* are currently cultured widely for bivalve, crustacean, rotifer or finfish larvae aquaculture (Shields, Lupatsch 2012). Furthermore, an experiment with larval gilthead seabream *Sparus aurata* L. and the sole, *Solea senegalensis* (Kaup) fed with six different alga treatments (*Tetraselmis chui*, *I. galbana* and *Nannochloropsis oculata*) also demonstrated positive effects (Rocha et al. 2008). The significant improvement of larval growth, feed ingestion and survival was consequently recorded. Furthermore, some experimental studies on live feed enrichment, concerning *Artemia* and copepods, showed a possibility to obtain the sub-optimal lipid profiles and the improvement of the larval rearing success (Battaglene, Cobcroft 2007).

The introduction of other than live food (e.g. fish protein hydrolysate with yeast, soybean proteins with yeast, fish meal) in start-feeding of larval fish proved to be supporting their survival and growth (Cahu et al. 1998*a*). Promising results were also experimentally obtained regarding adult pikeperch, *Sander lucioperca* L. fed with diets of different-level lipids, i.e. no significant difference in the growth rate but the highest slaughter yield and the most advantageous fatty acid profile by the lowest lipid level in feed products (Kowalska et al. 2011).

Recently, the scientists have paid special attention to phospholipid components such as PUFA, which significantly influence the growth, health and reproduction of animals, e.g. fish (Caballero et al. 2002, Boujard et al. 2004, Kowalska et al. 2010, 2011, 2015). The authors concluded that a diet of fish should be well balanced. On the other hand, the quality of fish meat and the consumption value are strictly connected not only with lipid content but also with lipid profiles and quantitative relations. The fatty acid profiles determined in fillets of fish feeding on natural (live) and artificial (granulate enriched with fatty acids) food can be similar when comparing the total content of PUFA, MUFA or even SAFA (Jankowska et al. 2004). The significant differences were recorded, in turn, in total the content of *n*-6 and *n*-3 PUFA and *n*-3 and *n*-6 HUFA, and fish feeding on artificial food absorbed more *n*-3 acids but less *n*-6 acids.

Phytoplankton as well as zooplankton assemblages in natural waters are much differentiated due to seasonal periodicity, environmental conditions and biotic interrelations. Thus, the natural sources of fatty acids are strictly dependent on these aspects. The synthesis of *n*-3 PUFA (omega-3) generally belongs to algae, but some heterotrophic nanoflagelates and protozoans are also able to produce these acids (Desvilettes, Bec 2009). Algae, especially diatoms, are an important source of EPA (20:5*n*-3), cryptophtes and dinoflagellates contain a high content of EPA and DHA (22:6*n*-3) and chlorophytes have much 16C and 18C PUFAs. Essential FA for fish species are primarily EPA and DHA, i.e. components of cell membranes, hormones, pheromones, vitamins and pigments, which in turn positively affect the larval growth and survival (Hafezieh et al. 2009). Their content in freshwater fish can reach up to 13.7 mg g^{-1} of dry weight (EPA) and 11.9 mg g^{-1} of dry weight (DHA), which were reported for *Alosa pseudoharengus* (Wilson) (reviewed by Gladyshev et al. 2009). Whereas in ten popular fish species of Polish waters the summarized EPA and DHA content ranges between 4.08 and 11.84 mg g^{-1} of dry weight, and their amounts were arranged in the descending order:

Anguila anguila>Rutilus rutilus>Cyprinus carpio>Esox lucius>Blicca bjoerkna>Perca fluviatilis>Abramis brama>Sander lucioperca>Tinca tinca>Carassius carassius.

CONCLUSIONS

Based on the above review of the worldwide scientific literature, it can be concluded that phytoplankton (as a primary producer) consititutes the basic nutritional source in diets of fish owing to high amounts of phytonutrients and biologically active ingredients it provides, e.g. fatty acids, amino acids, sterols, organic minerals, enzymes, carotenoids, chlorophyll, trace elements, vitamins. Concerning the valuable phytoplankton fatty acids, the PUFA constitute the largest part of FA in freshwater and marine chlorophytes, freshwater and marine cryptophytes and in marine diatoms (PUFA> SAFA>MUFA), while being the smallest one in freshwater cyanobacteria (SAFA>MUFA>PUFA). This indicates how important the structure of phytoplankton is in the assessment of its nutritional value. The highest average *n*-3:*n*-6 ratio was recorded in freshwater and marine cryptophytes (16.8 and 22.4, respectively), whereas the same ratio reached up to 18.1 in marine zooplankton (calanoid copepod).

Recently, the nutritional quality of phytoplankton has become more important for aquaculture. The larval fish fed with phytoplankton gain many benefits, e.g. significant improvement of growth, feed ingestion, health and survival. By enhancing the nutritional value of all components in an aquatic food web, phytoplankton is a crucial source of oil lipids, primarily omega-3 (*n*-3) and omega-6 (*n*-6) PUFA, sterols and essential amino acids. The transfer of nutritional lipids follows the sequence from phytoplankton-zooplankton -and/or-benthic invertebrates-fish to humans who consume fish.

The review of articles giving scientific insights into the role of the primary producer such as phytoplankton, considered to be the basic nutritional source in diets of fish, can be very useful for successful fish aquaculture management as well as for purposes concerning other aquatic animals. However, proper recognition of phytoplankton (especially species and growth phase) is essential prior to making any prediction about its dietary values in aquatic ecosystems, starting from the base of food webs.

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