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Lowered nutritional quality of prey decrease the growth and biomolecule content of rainbow trout fry

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ABSTRACT

Diet quality is crucial for the development of offspring. Here, we examined how the nutritional quality of prey affects somatic growth and the lipid, carbohydrate, protein, amino acid, and polyunsaturated fatty acid content of rainbow trout (*Oncorhynchus mykiss*) fry using a three-trophic-level experimental setup. Diets differed especially in their content of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), which are physiologically essential polyunsaturated fatty acids for a fish fry. Trout were fed with an artificial diet (fish feed, DHA-rich), marine zooplankton diet (krill/Mysis, DHA-rich), or freshwater zooplankton diet (*Daphnia,* Cladocera, DHA-deficient). The *Daphnia* were grown either on a poor, intermediate, or high-quality algal/microbial diet simulating potential changes in the nutritional prey quality (EPA-content). Trout fed with the fish feed or marine zooplankton entirely replaced their muscle tissue composition with compounds of dietary origin. In contrast, fish tissue renewal was only partial in fish fed any *Daphnia* diet. Furthermore, fish grew five times faster on marine zooplankton than on any of the *Daphnia* diets. This was mainly explained by the higher dietary contents of arachidonic acid (ARA), EPA, and DHA, but also by the higher content of some amino acids in the marine zooplankton than in the *Daphnia* diets. Moreover, fatty acid-specific carbon isotopes revealed that trout fry could not biosynthesize ARA, EPA, or DHA efficiently from their precursors. Our results suggest that changes in the zooplankton and macroinvertebrate communities' structure in freshwater habitats from DHA-rich to DHA-poor species may reduce the somatic growth of fish fry.

1. Introduction

While the nutritional requirements of fish are well-known in aquaculture (e.g., Food and Aquaculture Organization of the United Nations, hereafter FAO), less is known of the quality of natural diets and their influence on fish fry growth and survival. The diet of fish fry consists of different zooplankton taxa, mainly rotifers, cladocerans (*Daphnia*), and copepods, being nutritionally qualitatively divergent due to their feeding preferences and the quality of the available biomolecules (Vesterinen et al., 2021). In both aquatic and terrestrial food webs, linoleic (LIN; 18:2ω6) and alpha-linolenic acid (ALA; 18:3ω3) are essential ω-6 and ω-3 polyunsaturated fatty acids (PUFA), respectively, and serve as precursors for other physiologically active PUFA, such as arachidonic acid (ARA; 20:4ω*6*), eicosapentaenoic acid (EPA; 20:5ω3) and docosahexaenoic acid (DHA; 22:6ω3). EPA and DHA are synthesized by some taxa of phytoplankton in marine and freshwater ecosystems (Galloway and Winder, 2015; Taipale et al., 2013), but these long-chain omega-3 PUFA are only present at trace levels in the terrestrial plants (Hixson et al., 2015). Therefore, the synthesis of EPA and DHA by phytoplankton is important for aquatic organisms and many birds and mammals living at the interface of terrestrial and aquatic ecosystems (Twining et al., 2016).

Eutrophication of aquatic ecosystems modifies phytoplankton community structure by increasing the abundance of cyanobacteria and green algae, resulting in a decrease in the dietary quality of phytoplankton for zooplankton and fish (Jørgensen, 2001; Taipale et al., 2016c). Cyanobacteria and green algae do generally not contain long-chain PUFA but ALA and stearidonic acid (SDA; 18:4ω3) (Los and Mironov, 2015; Taipale et al., 2016a). In lakes and ponds where cyanobacteria and green algae are the major phytoplankton taxa (Dochin

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et al., 2020; Paerl and Huisman, 2008), zooplankton and/or fish need to biosynthesize ARA, EPA, and DHA from their precursors (Murray et al., 2014; Pilecky et al., 2022; Taipale et al., 2022).

The conversion efficiency of EPA and DHA from dietary ALA varies greatly within zooplankton, fish, birds, mammals, and humans (Burdge and Calder, 2005; Koussoroplis et al., 2014; Taipale et al., 2011a). ARA, EPA, or DHA biosynthesis from their precursors is more limited in marine than freshwater fish (Ishikawa et al., 2019; Sargent et al., 1999; Tocher, 2010). Some recent studies on freshwater fish support their ability to biosynthesize EPA and DHA from their precursors, whereas other studies suggest strict regulation of DHA in fish dorsal muscle tissues via dietary DHA (Chaguaceda et al., 2020; Keva et al., 2021; Scharnweber et al., 2021; Taipale et al., 2016b). Early work on dietary lipids for fish noted the importance of ALA and LIN for fish larvae (Watanabe, 1982), but adequate availability of ARA, EPA, and DHA are also crucial for optimal growth and somatic development of fish larvae and fry (Ahlgren et al., 2009; Tocher, 2010; Watanabe, 1993; Wirth et al., 1997). However, the ability for DHA biosynthesis from precursors may also change with fish age (Bell and Sargent, 2003; Buzzi et al., 1996; Taipale et al., 2018; Wirth et al., 1997). Therefore, dietary uptake of ARA, EPA, and DHA is important for fish fry (Bell and Sargent, 2003).

Although carbohydrates are an important energy source for herbivores (or species at lower trophic levels), lipids are usually the most important energy source for carnivorous (or higher trophic level) fish species (Biro et al., 2004; Le Gall and Behmer, 2014; Lee et al., 2006; Skiba-Cassy et al., 2013). The increase of terrestrial organic matter in freshwater ecosystems is predicted to increase the availability of carbohydrates that can support the energy demand for somatic growth and reproduction of herbivorous zooplankton and help them spare essential biomolecules (Taipale et al., 2016c). However, high allochthonous diets decrease the lipid content in zooplankton and consequently in fish fry (Taipale et al., 2018) and can thus also decrease the somatic growth of juvenile fish (Meng et al., 2019) and winter survival for the young fish populations (Hurst and Conover, 1998).

Most fish require relatively high protein content for optimal growth in relation to birds and mammals (Weatherley and Gill, 1983). Dietary proteins include essential amino acids (EAA), enzymes, and proteins required when building new tissues and thus are especially important for the fast growth of fish larvae (Rønnestad et al., 1999; Wilson and Halver, 1986). Fish cannot synthesize EAAs *de novo*, and therefore restricted dietary availability leads to lower growth. However, all phytoplankton taxa can synthesize all essential amino acids, and thus protein quality in marine and freshwater systems is generally high if phytoplankton biomass is sufficiently high (Ahlgren et al., 1992; Brown et al., 1997; Peltomaa et al., 2017). Moreover, the fish fry can compensate for the low amino acid content of prey by high retention of individual amino acids (Taipale et al., 2018). Therefore, it is assumed that any single amino acid does not limit wild fish growth.

Some species of salmonid fish fry grow naturally in lakes (*Salvelinus alpinus*, *Coregonus albula*, *Coregonus lavaretus*, *Thymallus thymallus*), whereas others develop in streams (*Oncorhynchus mykiss*, *Salmo trutta*). In lakes, the diet of salmonid fish fry consists mainly of daphnids and chironomid larvae in streams, which are both deficient in DHA (Ballantyne et al., 2003; Guo et al., 2016; Sánchez-Hernández et al., 2011; Vesterinen et al., 2021). Moreover, pelagic and benthic cyanobacteria blooms are predicted to increase in the future by global change and eutrophication (Paerl and Huisman, 2008; Scott and Marcarelli, 2012) but are also common in fish ponds (Sevrin-Reyssac and Pletikosic, 1990). Microbial pathways dominate carbon and dietary energy flow during cyanobacteria blooming, resulting in reduced EPA and DHA of zooplankton and chironomid larvae (de Kluijver et al., 2012; Taipale et al., 2022). Moreover, increase in temperature has been shown to decrease the EPA content of *Chironomus* larvae (Strandberg et al., 2021). A recent study also showed that the zooplankton community changed from DHA-rich copepods to smaller-sized DHA-deficient cladocerans by increasing the productivity gradients of lakes (Keva et al., 2021). Cladocerans also dominate small ponds and shallow wetlands (Norlin et al., 2006), where fish may need to overcome the low availability of dietary DHA by endogenous biosynthesis. Here, we examined the impact of DHA-rich and DHA-deficient diets on the somatic growth of rainbow trout (*O. mykiss*) fry and their content of macromolecules (lipids, carbohydrates, proteins), essential amino acids, and fatty acids. We tested the hypotheses that; 1) the somatic growth of rainbow trout is determined by the direct dietary availability of DHA, but not EPA, ARA, or amino acid content of natural diets (DHA-rich marine zooplankton vs. DHA-poor cladoceran *Daphnia*); 2) trout growth is equal between dietary marine zooplankton and artificial fish feed, due to the very similar content of lipids and DHA, even though these treatments differ in a given quantity of diet to fish fry, and; 3) trout fry cannot compensate for low ARA, EPA and DHA contents in their prey by biosynthesizing them from precursors, i.e., LIN for ARA, and ALA for EPA and DHA.

2. Methods

2.1. Preparation of bacteria, phytoplankton, and zooplankton

Methylotrophic bacteria (mostly *Hyphomicrobium denitrificans*) were isolated from a fish tank and used to simulate a boreal brown-water lake (Taipale et al., 2011b). Bacteria biomass was cultivated in NMS-medium in 500 mL glass incubation bottles (Wheaton) closed with gas-tight caps and rubber septa. The cultures were incubated at 30 °C and 110 rpm for 72–96 h under atmospheric conditions supplemented with 40–50% (*v/v*) CH_4 (e.g., 150 mL of water, 100 mL of CH_4, and 50 mL of air). Cultures were concentrated by centrifugation (3000 rpm, 10 min, 9 °C; Megafuge 1.0. R, Heraeus, Germany), and pellets were diluted to filter-sterilized artificial *Daphnia* medium, AdaM (Klüttgen et al., 1994). Concentrated samples were stored at −20 °C. The dry weight of the concentrated culture was determined from 5 to 29 mL samples filtered onto pre-dried and pre-weighed GF/C-filters after drying the sample at 70 °C overnight. Since bacteria themselves are an incomplete diet for herbivorous zooplankton, bacterial suspension was combined with green algae when fed to *Daphnia* to represent the poor-quality diet (see below). For the experiment, we selected the fast-growing green alga *Acutodesmus* sp. (from a culture at the University of Basel, Switzerland) to represent an intermediate nutritional quality alga in lakes. Green algae, such as *Acutodesmus* sp., contain high levels of EAA and ALA but lack EPA and DHA (Peltomaa et al., 2017). Therefore, green algae can be considered an intermediate quality diet. We selected *Diatoma tenuis* (CPCC62), *Stephanodiscus hantzschii* (CCAP 1079/4), *Nitzschia* sp. (UTEX FD397), *Cryptomonas erosa* (CPCC 446), *Mallomonas caudata* (CCAP 929/8), *Synura petersenii* (CCAP960/3) and *Peridinium cinctum* (SCCAP K-1721) to represent high-quality diet rich in EAA, ALA and EPA and/or DHA. All microalgae were cultured in MWC medium (Guillard and Lorenzen, 1972) with vitamins from an artificial freshwater medium (AF6, Watanabe et al., 2000) at 18 ± 1 °C in a 14:10 light-dark cycle. Light intensity was 50–70 μmol m^{-1} s^{-1} for all other algae except *Acutodesmus* (90–110 μmol m^{-1} s^{-1}).

2.2. Zooplankton culturing

Daphnia magna, clone DK-35-9, was maintained in the laboratory on *Acutodesmus*. *Daphnia* were cultured in ADaM in glass beakers (1 L) and fed every second day (for 7–14 days) with three separately designated diets consisting of algae or algae and bacteria. Due to the high biomass required for fish feeding, *Daphnia* were cultured in separate batches over a period of two months. *Daphnia* were stored at −20 °C prior to the feeding experiments.

2.3. Fish culturing

Rainbow trout fry originated from a fish farm located in Central Finland and were transported to the laboratory facilities at the

Department of Biological and Environmental Science (University of Jyväskylä). The fish were maintained for 12 weeks prior to the feeding experiment in two 20 L aquaria in borehole water with a flow-through (9.4 L h^{-1}) at 10–12 °C to keep their growth slow and fed with commercial feed (Biomar Inicio Plus, 0.5 mm).

2.4. Experimental design

Our aim was to explore how the change in the nutritional quality of prey impacts the somatic growth of fish fry and its biomolecule content. Our special interest was to compare fish fry growth with DHA-rich diet to DHA-poor diets and when EPA content is also altered. This simulated zooplankton community change from copepod-dominated to Cladocera-dominated zooplankton and green algae and cyanobacteria dominance in lakes (lack of DHA and EPA; diets 35) or change in the macroinvertebrate community from DHA-rich to DHA-poor species and when benthic cyanobacteria have increased. Rainbow trout fry growth and biomolecule content were additionally compared to an artificial diet that represented possibility for the maximal growth.

More specifically, the following five fish diets were used (Table 1; Fig. 1): 1. Artificial diet (Fish feed, Biomar Inicio Plus; 1.0% and 0.5% of ω-3 PUFA and DHA) was used as an optimal diet to achieve maximum growth rate for rainbow trout, 2. Marine zooplankton diet of krill and Mysis (Krill Pacifica and Mysis, Ocean Nutrition; feeding ratio of krill and Mysis: 50% and 50%; Krill/Mysis; 1.5% and 0.6% of ω-3 PUFA and DHA) to simulate DHA-rich diets in lakes and streams, 3. *Daphnia* fed on poor nutritional quality methylotrophic bacteria (grown on methane) and intermediate quality green algae (*Daphnia* 1; bacteria + green algae; 0.5% of ω-3 PUFA of DW), 4. *Daphnia* fed on the intermediate quality diet (*Daphnia* 2; green algae; *Acutodesmus* sp.; 1% of ω-3 PUFA of DW), 5. *Daphnia* fed on a mixture of high quality (*Cryptomonas, Mallomonas, Synura*, *Peridinium*, *Diatoma, Stephanodiscus,* and *Nitzschia*) and intermediate quality algae (*Daphnia* 3: feeding ratio 80% high quality and 20% of green algae; 2% of ω-3 PUFA of DW and 0.2% of EPA of DW). Diets 3–5 represented DHA-deficient diets (cladoceran and most macroinvertebrates in freshwaters). Moreover, *Daphnia*-dominated diets differed in their EPA-content and thus presented poor, intermediate, and high (rich in EPA) nutritional quality. This simulated situation when the dietary availability of EPA for daphnids and macroinvertebrates is limited.

Rainbow trout fry weighed 757 ± 88 mg (FW) at the beginning of the experiment. The mean weight was used to calculate the daily feeding rate for all treatments, which was calculated using the wet weight of the fry and diet. When being fed on zooplankton, a given amount of food was calculated equal to 1.1% daily growth rate of fry, which is similar to growth rates of rainbow trout fry in lakes (Knudson, 2011). For growth conversion efficiency in the zooplankton treatment, we used 15%, which was previously found in juvenile trout in lakes (Jensen et al., 2015). In the fish feed treatment, we simulated maximal growth of fish fry, and the given food amount was adequate to achieve 4.1% daily growth rate when feed efficiency is 1.5 (Dallaire et al., 2007). Therefore, food availability (quantity) was estimated to be high in fish feed treatment and moderate in zooplankton treatments (Table 1).

Four individuals of juvenile trout from initial state before feeding with designated diets (hereafter start) were killed after being euthanized with an overdose of anesthetics (MS-222) for biochemical analyses. During the experiment, fish were kept individually for 20 days in 0.5 L tanks at 13 ± 0.2 °C water temperature at a 12 L:12D (light: dark) cycle. The fish were fed on five different diets, and each diet treatment had four replicate fish. Tanks were oxygenated with a flow-through (9.8 L^{-1}

Table 1
Dietary nutrient requirement of rainbow trout (*Oncorhychus mykis*) fry based on the recommendation of the Food and Agriculture Organization of the United Nations (FAO, 2016) and the biochemical content of diets given in the experiment to fry (% of DW). An artificial diet is a commercial fish feed pellet (Biomar Inicio Plus, Raisioagro, Finland). Marine zooplankton represents DHA-rich copepods, whereas herbivorous cladoceran (*Daphnia*) represent DHA-poor zooplankton. *Daphnia* diets were prepared by feeding Daphnia on the low-quality diet (methane-oxidizing bacteria, MOB; *Daphnia 1*) and supplemented with intermediate quality algae, pure intermediate quality algae (green algae; *Daphnia* 2), or high nutritional quality algae (cryptomonads) supplemented with intermediate quality algae (*Daphnia* 3). The Diet quantity was high in the fish feed treatment and moderate in the other treatments. Specific growth rate (SGR) is the actual growth rate during the 20-day experiment. Letters in the upper case cite statistical differences in the order: d > c > b > a, $p < 0.05$. na = not analyzed.

	Requirement for fry	Artificial diet Fish feed $n = 4$	Marine zooplankton Krill/*Mysis* n = 4	*Daphnia* 1 n = 4	*Daphnia* 2 n = 4	*Daphnia* 3 n = 4
Diet quality		High	High	Low + Intermediate	Intermediate	Intermediate + high
Diet quantity		High	Moderate	Moderate*	Moderate*	Moderate*
Estimated growth (% per day)		4.1	1.1	1.1	1.1	1.1
SGR (% per day)		3.1 ± 0.3^{c}	1.3 ± 0.5^{b}	-0.36 ± 0.79^{a}	0.02 ± 0.23^{a}	0.23 ± 0.17^{a}
Crude protein, % min of DW	45–50	56 ± 0.8^{a}	54 ± 1.5^{a}	51 ± 1.6^{a}	50 ± 0.3^{a}	48 ± 12^{a}
Amino acids, % min of DW						
Arginine	2	na	na	na	na	na
Histidine	0.7	1.3 ± 0.26^{c}	0.91 ± 0.05^{b}	0.13	0.84 ± 0.06^{abc}	0.72 ± 0.15^{a}
Isoleucine	0.8	2.2 ± 0.19^{bc}	2.3 ± 0.15^{c}	2.3	1.9 ± 0.12^{b}	1.7 ± 0.05^{a}
Leucine	1.4	3.6 ± 0.23^{b}	3.5 ± 0.01^{b}	2.7	3.0 ± 0.31^{b}	2.8 ± 0.06^{a}
Lysine	1.8	2.5 ± 0.27^{ab}	3.4 ± 0.01^{c}	1.7	2.5 ± 0.04^{b}	2.2 ± 0.08^{a}
Methionine	1	na	na	na	na	na
Phenylalanine	1.2	2.0 ± 0.31^{a}	2.1 ± 0.12^{a}	1.6	1.7 ± 0.25^{a}	1.7 ± 0.06^{a}
Threonine	0.8	1.8 ± 0.20^{a}	2.1 ± 0.17^{a}	2.1	2.7 ± 0.03^{c}	2.5 ± 0.1^{b}
Tryptophan	0.2	na	na	na	na	na
Valine	1.3	2.7 ± 0.15^{a}	2.6 ± 0.17^{a}	3.3	2.7 ± 0.20^{a}	2.5 ± 0.13^{a}
Lipids, % of DW		13.0 ± 0.4^{b}	9.9 ± 2.5^{b}	10.9 ± 1.2^{a}	15.9 ± 1.1^{c}	12.7 ± 1.1^{ab}
Essential fatty acids, % min of DW						
18:2ω-6 (LIN)		2.4 ± 0.15^{d}	0.21 ± 0.0^{a}	0.16 ± 0.18^{ab}	0.29 ± 0.10^{ab}	0.38 ± 0.02^{bc}
20:4ω-6 (ARA)	0.5	0.05 ± 0.02^{c}	0.17 ± 0.04^{d}	0.02 ± 0.03^{a}	0.01 ± 0.001^{a}	0.08 ± 0.01^{b}
18:3/4ω-3** (ALA + SDA)	1	0.48 ± 0.02^{b}	0.30 ± 0.01^{a}	1.1 ± 1.5^{abc}	2.4 ± 1.1^{cd}	4.1 ± 0.16^{d}
20:5ω-3 (EPA)	1	0.95 ± 0.03^{c}	1.7 ± 0.05^{d}	$<0.01 \pm 0.001^{a}$	$<0.01 \pm 0.001^{a}$	0.31 ± 0.01^{b}
22:6ω-3 (DHA)	0.5	1.2 ± 0.03^{c}	1.6 ± 0.04^{d}	$<0.01 \pm 0.001^{a}$	$<0.01 \pm 0.001^{a}$	$<0.01 \pm 0.001^{b}$
Carbohydrate, % max of DW	12	13.1 ± 2.6^{b}	11.2 ± 4.3^{ab}	12.0 ± 0.5^{a}	7.1 ± 1.4	5.2 ± 1.0

* last five days with high amount.
** based on Watanabe 1982.

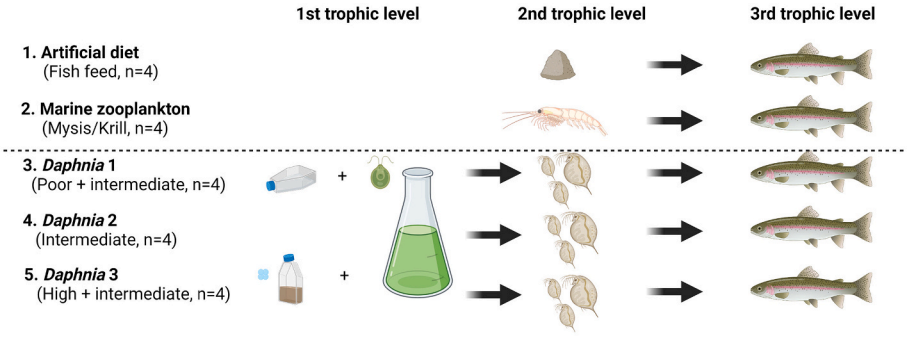

Fig. 1. Experimental setup of the experiment. Rainbow trout fry (~0.7 g) were fed on an artificial diet (fish feed), marine zooplankton (mixture of krill and Mysis), or *Daphnia* diets. *Daphnia* 1 were fed on poor nutritional quality bacteria, and intermediate quality green algae (*Acutodesmus* sp.), *Daphnia* 2 was fed on intermediate quality green algae, and *Daphnia* 3 was fed with a mixture of high nutritional quality algae (cryptophyte, golden algae, diatoms, dinoflagellate) and intermediate quality green algae. Marine zooplankton represents DHA-rich diet where, as *Daphnia* diets represent DHA-deficient diets with altered EPA content. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

h) system. Water flow was stopped twice a day for 15 min (8 a.m. and 5 p.m.) for the feeding. Visual observation during feeding confirmed that all fish fed on the provided food in all treatments.

2.5. Biochemical analysis

Carbohydrate, lipid, and protein content in each trophic level were analyzed using previously described methods (Taipale et al., 2016c). Briefly, total carbohydrate content was analyzed using Dubois's method in which glucose is dehydrated to hydroxymethylfurfural in a hot acidic medium (Dubois, 1956). Carbohydrates were measured with a Shimadzu UV-240 spectrophotometer at 490 nm. Nitrogen content was analyzed using a Carlo-Erba Flash 1112 series Element Analyzer, and protein content was analyzed by multiplying elemental nitrogen percentage with the known nitrogen content of proteins. Here, we used factors of 6.3 for zooplankton and 5.6 for fish (Postel et al., 2000). Lipids were extracted following the Folch protocol (Folch et al., 1957) with chloroform:methanol: water (2:1:0.75) from freeze-dried samples (1–5 mg), and fatty acids were transmethylated with 1% sulfuric acid in methanol and run with a mass spectrometer (GC-MS, Shimadzu Ultra, Kyoto, Japan) using Agilent® DB-23 column (30 m × 0.25 mm × 0.25 μm. Fatty acid methyl esters were identified and quantified as previously published (Taipale et al., 2016a).

Fish (freeze-dried and grounded whole fish) and fish feed were weighed (mean ± SD: 1.47 ± 0.22 mg DW) in glass tubes, and proteins were hydrolyzed with 1 mL of 6 M HCl at 110 °C for 20 h for AAs analysis. Internal standard (30 μg of Norvaline) was added to each sample. We prepared amino acid propyl chloroformates with EZ:faast kit (Phenomenex). Propyl chloroformates were diluted (50–300 μL, depending on expected sample AA content) to drive elution (80 isooctane, 20% chloroform, vol/vol) and run with a GC–MS (GC-2010 Plus and QP-2010 Ultra, Shimadzu) using ZB-AAA column (9.5 m × 0.25 μm × 0.25 mm). The column temperature was raised from the initial temperature, 110 °C, to 320 °C at the rate of 30 °C min^{-1}, after which it was held for 7 min at 320 °C. The injection temperature was 300 °C, and the interface temperature was 290 °C. Total gas flow in the column was 2.4 mL min^{-1} and linear velocity 71.2 cm s^{-1}. AA identification was based EZ:faast library with amino acid-specific ion fingerprints. We used four-point calibration curves derived from the standard mixture (Sigma-Aldrich AAS-18) supplemented with Norvaline to quantify amino acid content in the samples. We used GCMS Solution version 4.42 (Shimadzu) for the identification and calculation of AA content. Sample amino acid contents were recovery corrected. The Mean ± SD of internal standard recovery was 66.2 ± 14.2%. Due to the properties of the EZ:faast kit, we were able to analyze seven EAAs (valine, leucine, isoleucine, threonine, phenylalanine, lysine, and histidine) and six non-EAAs (alanine, glycine, proline, aspartic acid, glutamic acid, and tyrosine).

2.6. Stable isotope analysis

We analyzed the $\delta^{13}C$ values mainly from the second and third trophic levels (Fig. 1). Since we did not sample algae and bacteria, we did not focus on zooplankton-phytoplankton interaction here. For carbon and nitrogen isotopes, we used previously published protocol (Taipale et al., 2014). Briefly, approximately 0.6–1.2 mg of freeze-dried phytoplankton, bacteria, zooplankton, or fish sample was weighed for $\delta^{13}C$ analysis. The $\delta^{13}C$ was measured with an elemental analyzer (Carlo-Erba Flash 1112 series) connected to a continuous flow stable isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage IRMS. Thermo Co., Bremen, Germany) at the University of Jyväskylä.

2.7. Gas chromatography combustion stable isotope ratio mass spectrometry (GC-C-IRMS)

The $\delta^{13}C$ values of fatty acids methyl esters were determined using a GC-C TA III connected to an isotope ratio mass spectrometer (Thermo Finnigan Delta Plus Advantage IRMS. Thermo Co., Bremen, Germany) at the Interuniversity Centre for Aquatic Ecosystems Research WasserCluster Lunz (Lunz am See, Austria). Fatty acids were separated using a 60 m DB-23 column (0.25 mm × 0.15 mm) and then oxidized to carbon dioxide in an oxidation reactor at a temperature of 940 °C with the reduction reactor kept at 630 °C. The temperature program of the GC column started at 60 °C and was kept for 1 min at 60 °C, after which the temperature was raised by 30 °C min^{-1} to 175 °C, and then by 2.6 °C min^{-1} to 245 °C, and held at that temperature for 17 min. The total run time was 48.03 min. The injector temperature was kept at 270 °C. The samples were run against an internal standard, 1,2-Dinonadecanoyl-sn-Glycero-3-Phosphatidylcholine (Larodan, $\delta^{13}C = -28.43‰$), which was used for drift and linear correction. The calculated precision for standard FAME was ±0.4‰, and the accuracy was ±0.3‰. The $\delta^{13}C$ value of individual FAME was manually calculated using individual background values. The $\delta^{13}C$ value of individual FAME was methanol corrected ($\delta^{13}C = -30.9‰$) based on the total carbon number in the chain:

Final $\delta^{13}C$ of value of FA = ((number of C in FAME + $\delta^{13}C$ value of FAME) – ($\delta^{13}C$ of methanol)) / number of C in FA.

2.8. Stable isotope mixing model

The contribution of assimilated diet (fish feed, marine zooplankton, *Daphnia* diets) in fish fry tissues was calculated using $\delta^{13}C$ value measurements of the diet components and IsoError software (version 1.04; (Phillips and Gregg, 2001)). In addition, for the dietary $\delta^{13}C$ value, we used the initial carbon signal from starting point fish to calculate the proportion of dietary origin and initial old carbon in fish. In all cases, we had only two diet sources, and thus, the uncertainty caused by the variability of both sources was accounted for. Replicate results ($n = 4$) for fish diets were used for the calculations. We used this same method for bulk and individual ω-3 and ω-6 PUFA (Table 2).

Table 2
Statistical results for differences in the biochemical content of fish diets and fry using Euclidean distance and univariate PERMANOVA.

Component	Df1	Df2	Pseudo-F	P(MC)
Protein%	4	16	1.2	0.350
Histidine, content	3	14	7.8	**0.006**
Isoleucine, content	3	14	9.4	**0.005**
Leucine, content	3	14	6.0	**0.007**
Lysine, content	3	14	18.1	**0.002**
Phenylalanine, content	3	14	2.9	0.099
Proline, content	3	14	129.8	**0.001**
Theorine, content	3	14	19.4	**0.001**
Valine, content	3	14	0.81	0.496
Lipid, content	5	18	5.3	**0.007**
LIN content	4	17	153.4	**0.001**
ARA, content	4	17	167.7	**0.001**
ALA + SDA, content	4	17	15.9	**0.001**
EPA, content	4	17	26.1	**0.001**
DHA, content	4	17	39.7	**0.001**
Carbohydrate%	5	14	9.0	**0.003**

2.9. Growth rate and statistical testing

Specific Growth Rate (SGR) was calculated from individual fish growth during the experiment as:

$$\text{SGR (\%body weight gain per day)} = ((ln(W_2) - ln(W_1)) \text{ x } 100)/t_2 - t_1,$$

where W_1 and W_2 are the weights (mg wet weight) at the beginning and end of the experiment, t_1 and t_2 denote the duration of the experiment in days (20).

PERMANOVA (main and pairwise test) with Euclidean distance as resemblance matrix was used for univariate analysis (Anderson, 2017). Monte Carlo test was used for *p* values due to the low number of replicates. We used PERMANOVA (Primer 7) analysis and Bray-Curtis similarity to compare the non-essential and essential amino acid composition of prey and fry. Similarity percentages (SIMPER) were used to identify dissimilarities in the amino acid content of essential and non-essential amino acids among treatments. We used linear regression to find out which essential amino acids and fatty acids would best correlate with the fast growth of rainbow trout fry. Bonferroni-adjusted *p*-value (alpha/number of comparisons), 0.05/15 = 0.003, was used to evaluate which biomolecules had the most significant relationship with SGR. Regression analysis was done using IBM SPSS (version 24.0) software.

3. Results

3.1. Lipid, carbohydrate, and protein content of diets

The relative lipid content of fish diets was 12.1 ± 2.3% (mean ± SD) among the artificial diet and all zooplankton diets, however, according to PERMANOVA, the diets differed in their lipid content (Table 1, Fig. 2). The lipid content was lowest in *Daphnia* 1 (poor quality diet), whereas *Daphnia* 2 (intermediate quality diet) had the highest lipid content. The carbohydrate content of the artificial diet, marine zooplankton (Krill/Mysis), and *Daphnia* 1 was similar, whereas *Daphnia* 2 and 3 contained less carbohydrates. However, the statistical difference was not tested for *Daphnia* 2 and 3 due to the lack of replicates. All diets exceeded recommended protein content of the diet (45–50%) and did not differ from each other (Table 1, Fig. 2).

3.2. Essential amino acid and fatty acid content of diets

All diets, excluding *Daphnia* 1 diet, exceeded the nutritional requirements for EAAs (Table 1). *Daphnia* 1 had lower histidine and lysine content as FAO recommendations for trout fry. However, we did not have enough replicates for statistical testing of this diet. Statistical comparison of other diets showed small differences in EAA content

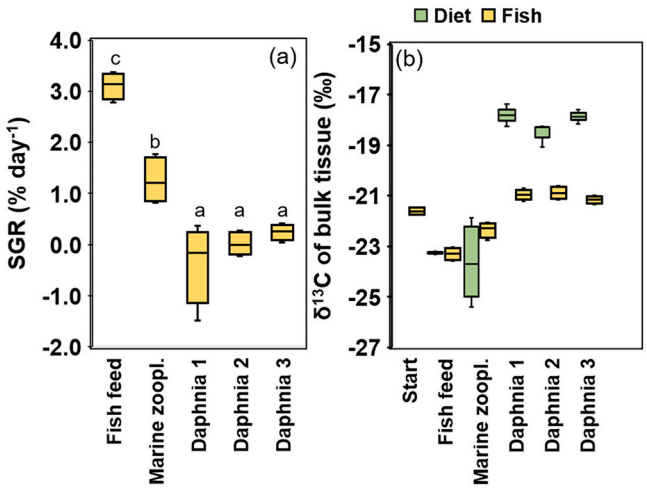

Fig. 2. (a) Specific growth rate (SGR) of rainbow trout fry among feeding treatments (artificial diet (fish feed), marine zooplankton (Krill/Mysis), and different *Daphnia* diets: *Daphnia* 1 = poor quality, *Daphnia* 2 = intermediate quality, *Daphnia* 3 = high quality). Letters cite different statistical groups based on pairwise PERMANOVA ($p < 0.05$). (b) The $d^{13}C$ of bulk isotopes of fish diets and fry after feeding on corresponding diets on 20 days.

(Table 1, 2). Among NEAAs, fish feed had twice as high proline contents as any other diet (4.4 ± 0.1 vs. 2.6 ± 0.1% of DW). Only small differences among AA in fish diets were found (SIMPER dissimilarity analysis: dissimilarity <16%, Supplemental Table 1). Higher dissimilarity was formed by the higher histidine and proline content in the artificial diet than in the zooplankton diets. Additionally, threonine and aspartic acid content was higher in the *Daphnia* diets (1–3) than in the artificial diet, and lysine and aspartic acid in the marine zooplankton than in the fish feed. Comparison between marine zooplankton and *Daphnia* diets revealed higher lysine and leucine content in the marine zooplankton than in the *Daphnia* diets, whereas threonine content was higher in *Daphnia* than in the marine zooplankton (Supplemental Table 1).

3.3. Essential fatty acid content of diets

The LIN content of the artificial diet was nearly 10 times higher than in any of the other diets (Table 1, 2). *Daphnia* diet 3 (good quality) contained slightly more LIN than marine zooplankton. Marine zooplankton contained more ARA than any other diet, and the artificial diet contained slightly more ARA than any *Daphnia* diet. The diets differed in their ALA + SDA, EPA, and DHA content (Table 1, 2). Generally, the ALA + SDA content was higher in the *Daphnia* 2 and 3 diets, and the EPA and DHA content was higher in the marine zooplankton than in any of the other diets. *Daphnia* 3 diet contained more EPA and DHA than *Daphnia* 1 and 2 diets, which contained only traces of EPA and DHA.

3.4. Survival and specific growth rate (SGR) of rainbow trout fry

The survival of fry was 100% in all treatments, and thus we did not find any negative impact of the dietary quality among treatments on fry survival. However, treatments differed in the specific growth rate by their nutritional quality (Table 1, Fig. 2a). SGR of fry was higher with the artificial diet than with any of the other diets (PERMANOVA: Pseudo-$F_{4, 19} = 39.8$, p(MC) = 0.001; Fig. 2a). Furthermore, SGR of fry was higher ($p < 0.05$) with marine zooplankton than with any *Daphnia* diet. Fry had positive SGR only with *Daphnia* 3 diet among all *Daphnia* treatments.

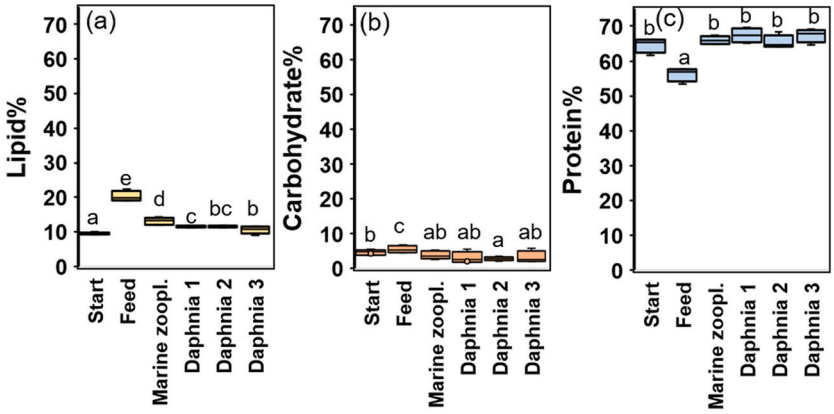

Fig. 3. Lipid (a), carbohydrate (b), and protein (c) percentage of fry at the start of the experiment (start) and after 20 days of the experiment feeding on artificial diet (feed), marine zooplankton (Krill/Mysis) and different *Daphnia* diets (*Daphnia* 1 = poor quality, *Daphnia* 2 = intermediate quality, *Daphnia* 3 = high quality). Letters cite different statistical groups based on pairwise PERMANOVA ($p < 0.05$).

3.5. Diet impact on the nutritional quality of rainbow trout fry

The lipid content of fry was significantly higher in the artificial diet treatment than in any other treatment (Table 1, 2; Fig. 3). Moreover, fish fry in the artificial diet treatment doubled their lipid content in 20 days (lipid gain 10.6 ± 1.5%). Fish fry gained 3.6 ± 1.1% and 1.5 ± 0.9% lipid with marine zooplankton and *Daphnia* 2–3 diets (intermediate-good quality diet) in relation to the starting point, respectively. In contrast, fish fry decreased their lipid content by 1.5 ± 0.7% in poor quality *Daphnia* treatment. Fry increased their carbohydrate content only in the artificial diet treatment from the starting point, and the carbohydrate content of fry was higher in the artificial diet treatment than in any other treatment (Table 1, 3, Fig. 2). The protein content of fry in the marine zooplankton and *Daphnia* treatments did not differ from the initial fish protein content but were higher than in fry with the artificial diet (Fig. 2).

Dissimilarity analysis of the content of individual amino acids of fry revealed only small dissimilarities among treatments (Supplemental Table 2). Generally, individual essential amino acid content was lower in the fry with the artificial diet than in the fry with any other diet (Fig. 4a-g, Table 3). However, statistical difference was found only with leucine, lysine, and phenylalanine. The LIN and ARA content of fry increased with marine zooplankton and the artificial diet from the starting point (Fig. 4h, l). The ARA content of fry was highest with the marine zooplankton diet, whereas the LIN content of fry was highest with the artificial diet. Additionally, the LIN content of fry was lower with *Daphnia* 1 or 2 diets than in the fry at the starting point. The ALA + SDA content of fry was highest in the *Daphnia* 2 and 3 treatments (Fig. 3i, Table 3). Actually, fry with *Daphnia* 2 diet contained 20-times more ALA + SDA than fry at the starting point or with the artificial diet (Fig. 4i). The EPA and DHA content of fry was highest with fish feed and marine zooplankton diets than in the fry with any other diets (Fig. 4 j, k, Table 3). In contrast, the EPA and DHA content of fry with all *Daphnia* diets decreased from the initial starting point. Moreover, the fry's DPA (22:5ω6) content was also higher in the fish feed and marine zooplankton treatment than in any other treatments (Fig. 4m).

Table 3
Statistical results for differences in the biochemical content of trout fry fed with different diets using Euclidean distance and univariate PERMANOVA. Percentage (%) of protein and carbohydrate refers to their contribution to DW, and contents of amino acids and fatty acids refer to the mass fraction (μg of mg DW).

Component	Df1	Df2	Pseudo-F	P(MC)
Protein%	5	23	19.6	**0.001**
Histidine, content	5	23	3.9	**0.011**
Isoleucine, content	5	23	2.2	0.097
Leucine, content	5	23	5.5	**0.003**
Lysine, content	5	23	7.1	**0.001**
Phenylalanine, content	5	23	4.4	**0.009**
Proline, content	5	23	5.3	**0.004**
Theorine, content	5	23	1.1	0.401
Valine, content	5	23	2.0	0.496
Lipid, content	5	18	5.3	0.123
LIN content	5	23	38.9	**0.001**
ARA, content	5	23	31.7	**0.001**
ALA + SDA, content	5	23	43.1	**0.001**
EPA, content	5	23	40.0	**0.001**
DHA, content	5	23	20.5	**0.001**
Carbohydrate%	5	23	2.8	**0.047**

3.6. The $\delta^{13}C$ value of bulk biomass and ω-3 and ω-6 PUFA of diets and fish

The bulk $\delta^{13}C$ value of rainbow trout fry at the start differed from fry after feeding on the artificial diet for 20 days (Table 4), which resulted in equal bulk $\delta^{13}C$ value of fry with their diet (Table 4, 5). Similarly, the bulk $\delta^{13}C$ value of fry coincided with $\delta^{13}C$ values of the marine zooplankton, which consisted of a mixture of krill and *Mysis*, that differed in their carbon isotope values (Table 4, 5, Fig. 2b). When assuming that fry fully changed their carbon in the tissues by feeding on marine zooplankton, the IsoError calculation estimated that 90 ± 6% of their carbon isotope values came from krill and only 10 ± 6% from *Mysis*. The bulk $\delta^{13}C$ value of fry became more ^{13}C-enriched when feeding on *Daphnia* (Table 4, Fig. 2b), however, IsoError calculations estimated that <25% of the carbon signal came from *Daphnia* (Table 5).

The $\delta^{13}C$ values of ω-3 and ω-6 PUFA in fish fry were more depleted in ^{13}C than the bulk biomass in all treatments (Table 4). Similarly, the $\delta^{13}C$ value of LIN and ALA in fry was more depleted than in long-chain ω-3 and ω-6 PUFA in all treatments. Mixing model calculations revealed the high dietary origin of ALA and LIN in fish in all treatments (Table 5). Furthermore, fry fed on fish feed received 100% of their ω-3 and ω-6 PUFA from the diet (Table 5). Similarly, fish feeding on marine zooplankton relied ~100% on dietary ω-3 and ω-6 PUFA, except for ALA. Further quantitative calculation of dietary origin ω-3 and ω-6 PUFA revealed differences among treatments. Fry retained relatively high amounts of ALA from *Daphnia* diets in relation to fry fed on fish feed and marine zooplankton diets (Fig. 5a). Meanwhile, fry fed on fish feed and marine zooplankton retained similar amounts of EPA and DHA, while in fry of the *Daphnia* treatments, the dietary EPA content was much lower than in the fish feed and marine zooplankton treatments (Fig. 5a). Moreover, among all *Daphnia* diets, fry obtained DHA from *Daphnia* 3 diet only (Table 5; Fig. 5a). Fry also assimilated ten times more LIN in the fish feed treatment than in any other treatments. Fry fed on fish feed or marine zooplankton gained similar ARA from their diet, whereas fry

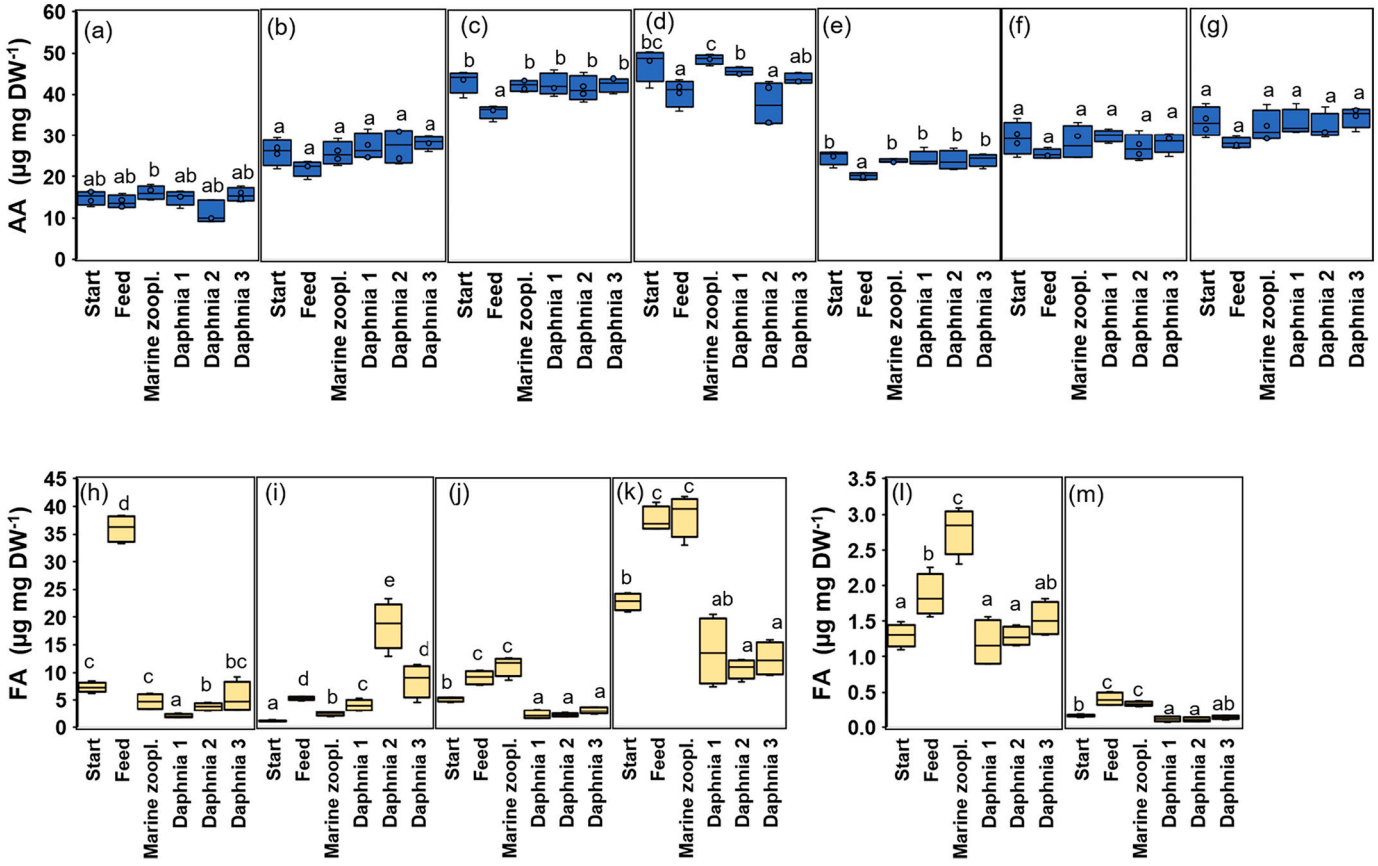

Fig. 4. The content of essential amino acids and fatty acids of fry at the start of the experiment (start) and after 20 days of the experiment feeding on artificial diet (fish feed), marine zooplankton (Krill/Mysis) and different *Daphnia* diets (*Daphnia* 1 = poor quality, *Daphnia* 2 = intermediate quality, *Daphnia* 3 = high quality). The analyzed essential amino acids were histidine (a), isoleucine (b), leucine (c), lysine (d), phenylalanine (e), threonine (f), and valine (g). The measured essential fatty acids were linoleic acid (LIN; h), alpha-linolenic acid + stearidonic acid (ALA + SDA; i), eicosapentaenoic acid (EPA; j), docosahexaenoic acid (DHA; k), arachidonic acid (ARA; l) and docosapentaenoic acid (DPAω6; m). Letters cite different statistical groups based on pairwise PERMANOVA ($p < 0.05$).

Table 4
The $\delta^{13}C$ values (‰; mean ± SD) of bulk samples and of ω-3 and ω-6 PUFA of basal food sources (plankton, see Fig. 1), *Daphnia*, fish feed, and rainbow trout fry.

	$\delta^{13}C$ of	$\delta^{13}C$ of ω3 PUFA			$\delta^{13}C$ of ω6 PUFA	
Treatment	bulk samples	ALA	EPA	DHA	LIN	ARA
Artificial diet						
Fish feed	−23.3 ± 0.1	−32.2 ± 0.1	−27.4 ± 0.2	−24.9 ± 0.1	−30.4 ± 0.2	−24.7 ± 0.8
Fish (start)	−21.6 ± 0.2	−32.9 ± 1.0	−26.6 ± 0.1	−22.4 ± 0.4	−38.3 ± 0.8	−29.2 ± 0.6
Fish (20 d)	−23.3 ± 0.1	−31.6 ± 0.4	−27.4 ± 0.3	−24.8 ± 0.1	−29.9 ± 0.0	−31.5 ± 0.2
Marine zooplankton						
Krill	−22.1 ± 0.3	−33.2 ± 0.1	−27.0 ± 0.2	−27.6 ± 0.4	−30.7 ± 0.4	−24.3 ± 0.3
Mysis	−25.1 ± 0.2	−37.5 ± 0.4	−30.9 ± 0.1	−30.0 ± 0.4	−32.2 ± 0.5	−29.1 ± 0.2
Fish	−22.4 ± 0.3	−34.4 ± 0.8	−28.5 ± 1.6	−28.9 ± 0.2	−30.8 ± 0.3	−26.0 ± 1.2
Daphnia 1						
Daphnia	−17.8 ± 0.6	−23.0 ± 0.2	nd	nd	−21.9 ± 1.1	nd
Fish	−21.0 ± 0.2	−23.4 ± 0.4	−26.2 ± 0.9	−23.0 ± 0.2	−27.1 ± 0.2	−26.3 ± 0.3
Daphnia 2						
Daphnia	−18.5 ± 0.5	−22.6 ± 0.4	nd	nd	−20.9 ± 1.3	nd
Fish	−20.9 ± 0.2	−25.8 ± 0.1	−26.2 ± 0.6	−22.4 ± 0.01	−27.2 ± 0.2	−25.1 ± 0.2
Daphnia 3						
Daphnia	−17.9 ± 0.4	−22.1 ± 1.4	−26.9 ± 0.1	−23.8 ± 0.5	−23.2 ± 0.5	−31.5 ± 0.2
Fish	−21.2 ± 0.1	−23.0 ± 0.4	−26.8 ± 0.1	−23.7 ± 0.3	−26.5 ± 0.6	−31.5 ± 0.2

fed with *Daphnia* 1 and 2 obtained one-quarter of that from their diet. Interestingly, fry feeding on *Daphnia* 3 contained two times more ARA than fish fed on *Daphnia* 1 or 2 diets (Fig. 5b).

3.7. Specific growth rates of fry

Linear regression analysis between SGR of fry and nutritional quality of their diets revealed positive relationships between all three macromolecules, seven essential amino acids, and all ω-3 and ω-6 PUFA, excluding ALA + SDA (Table 6). However, when using Bonferroni-adjusted p-value as significant criteria ($p = 0.003$), linear regressions between SGR and dietary EPA and DHA (average of daily amount) were only statistically significant (Fig. 6). Furthermore, linear regression between SGR and ARA was also marginally significant ($p = 0.006$).

Table 5
The contribution of dietary PUFA (bulk PUFA; ω3 PUFA as ALA, EPA, and DHA; and ω-6 PUFA as LIN and ARA) from the artificial diet (fish feed), marine zooplankton (Krill/Mysis), or *Daphnia* 1–3 diets (*Daphnia* 1 = poor quality, *Daphnia* 2 = intermediate quality, *Daphnia* 3 = high quality) (%; ± standard deviation)) to rainbow trout (*Oncorhynchus mykiss*) fry after 20 days of feeding.

Treatment	Bulk	ALA	EPA	DHA	LIN	ARA
Artificial diet	100 ± 4	100 ± 12	100 ± 21	100 ± 1	100 ± 3	100 ± 17
Marine zooplankton	100 ± 4	59 ± 32	90 ± 33	100 ± 4	100 ± 4	100 ± 26
Daphnia 1	14 ± 4	96 ± 4	14 ± 18*	0 ± 0*	68 ± 1	39 ± 5**
Daphnia 2	23 ± 5	69 ± 1	12 ± 23*	0 ± 0*	64 ± 1	50 ± 2**
Daphnia 3	11 ± 3	92 ± 4	54 ± 23	93 ± 20	78 ± 4	100 ± 8

* calculated from ALA, ** calculated from LIN.

4. Discussion

Trout fry reached five times higher growth with DHA-rich marine zooplankton (krill and *Mysis*) than with any of the *Daphnia* diets. Fish fed on *Daphnia* with higher EPA content grew faster than fry with EPA-deficient *Daphnia* diets, however, this difference was not statistically significant. Nevertheless, our regression analysis revealed a positive correlation between fry growth and diet EPA and DHA content. Since EPA and DHA are physiologically important for fry and juvenile trout and salmon, their absence may prevent fry growth (Ballantyne et al., 2003; Taipale et al., 2018; Wirth et al., 1997). In addition to the EPA and DHA, we found a positive correlation between dietary ARA and the SGR of fry. Therefore, our results support the understanding that EPA, DHA, and ARA are all important for fry growth, as previously found with marine fish (Sargent et al., 1999). This is against our hypothesis 1. However, a deeper understanding of ARA and EPA role in fish fry growth could be achieved by using EPA-rich and ARA-rich supplements.

Low carbon turnover in fish fry with *Daphnia* diets suggests the lower nutritional value of this cladoceran than marine zooplankton or artificial diet. Diet quality impacts the carbon turnover in consumers (Haramis et al., 2001; Mirón et al., 2006; Taipale et al., 2011a) that selectively retain physiologically essential biomolecules in their membranes (Taipale et al., 2021). Moreover, fish feeds containing nutrients highly required for consumers will most likely have much higher digestibility and utilization than the zooplankton that are poor in such nutrients.

Most of the knowledge in fish nutrition comes from artificially supplemented fish pellets and feeds, and there are only a few studies on natural diets. Here, our comparison between marine zooplankton (Krill/Mysis) and artificial diet (fish feed) showed that the DHA content of marine zooplankton exceeded fish feed (per DW). This suggests that the zooplankton diet can contain an adequate amount of DHA for fish growth. However, the given amount of food was higher (until satiation) in fish feed resulting in higher growth, but fish fry can rarely feed until satiation with a high-quality diet in nature. Therefore, the fish growth and nutritional quality should be evaluated by using natural prey and including a mixture of them to gain more understanding of fish nutritional ecology and responses to the decrease in the nutritional quality of their prey. Altogether, our result suggests that a high amount of high-quality diet results in the best growth as previously found with insectivore chickens (Twining et al., 2016). Therefore, we need to reject hypothesis 2. Among all macroinvertebrates in streams, only Crustacea (e.

Table 6
Regression analysis between specific growth rates (SGR) of fry and the nutritional quality of their diet. Bonferroni-adjusted *p*-value (0.003) was used to evaluate the most significant relationship (bolded).

Biomolecule	df 1	df2	F-value	R^2	p-value
EAA					
Histidine	1	4	24.1	0.889	0.016
Isoleucine	1	4	13.5	0.818	0.035
Leucine	1	4	16.6	0.847	0.027
Lysine	1	4	17.9	0.857	0.024
Phenylalanine	1	4	16.2	0.844	0.028
Theorine	1	4	14.4	0.827	0.032
Valine	1	4	12.4	0.805	0.039
ω6 PUFA					
LIN	1	4	13.5	0.820	0.034
ARA	1	4	171.1	0.941	0.006
ω3 PUFA					
ALA + SDA	1	4	0.005	0.020	0.947
EPA	1	4	48.1	0.983	**0.001**
DHA	1	4	75.4	0.962	**0.003**
Macromolecules					
Proteins	1	4	15.1	0.834	0.030
Lipids	1	4	11.5	0.793	0.043
Carbohydrates	1	4	13.3	0.817	0.035

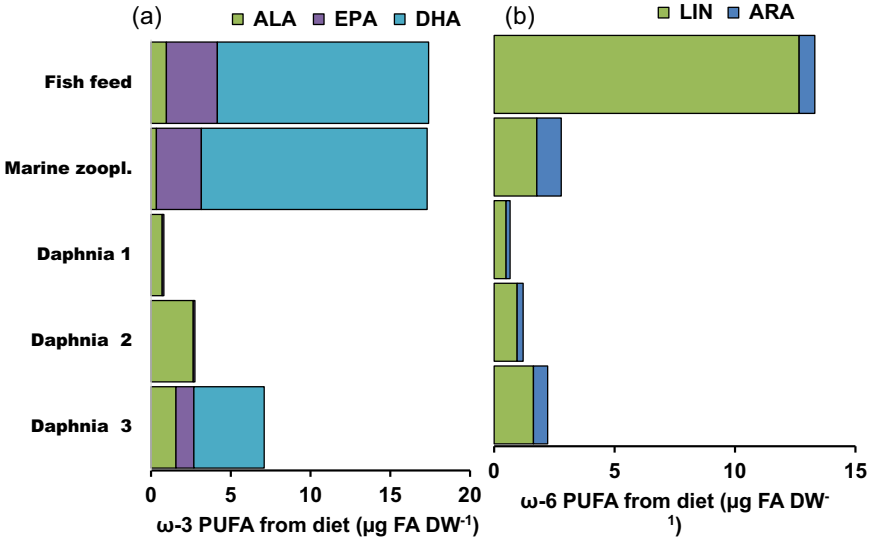

Fig. 5. (a) Calculated amount of alpha-linolenic (ALA), eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and (b) linoleic (LIN) and arachidonic acid (ARA) which originated from the diet. The used diets were: artificial diet (fish feed), marine zooplankton (Krill/Mysis), and different *Daphnia* diets: *Daphnia* 1 = poor diet quality, *Daphnia* 2 = intermediate diet quality, *Daphnia* 3 = high diet quality.

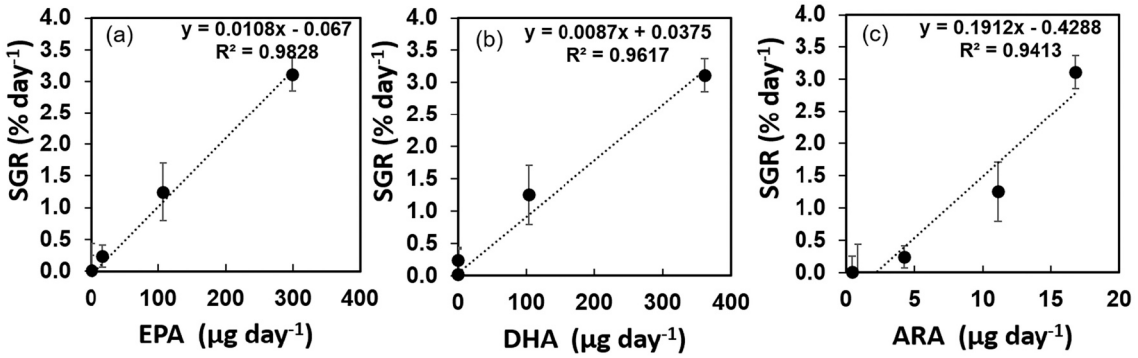

Fig. 6. Linear regression between somatic growth rate (SGR) of fry and (a) EPA, (b) DHA, and (c) ARA in the diet. The p-value for all is <0.05, and Bonferroni adjusted p-value (0.003) was reached with EPA and DHA.

g., *Eulimnogammarus*) and Bivalvia (e.g., *Dreissena*) contain DHA relatively high amounts (Guo et al., 2016; Makhutova et al., 2011). In lakes, copepods are a main source of DHA for planktivorous and juvenile fish and can contain similar amounts of DHA as our marine zooplankton here (Vesterinen et al., 2021). According to our results, it is important that the salmonid fish fry diet contains direct DHA-rich dietary sources as well. In streams, this focuses on the presence of a few taxa of DHA-rich macroinvertebrates (Guo et al., 2016; Makhutova et al., 2011). Thus future studies should determine how different anthropogenic changes influence the nutritional quality of macroinvertebrates in streams, especially the presence of DHA-rich species (Guo et al., 2016). Our result suggests a great impact on the fish fry growth when zooplankton structure shifts from DHA-rich copepods to small-size cladocerans (*Daphnia/Bosmina*) that contain only trace amounts of DHA. A study of Canadian coastal lakes revealed EPA to be highly retained in zooplankton (Kainz et al., 2004), whereas rainbow trout retained more DHA, which suggests that DHA was likely bioconverted from dietary EPA. However, the low EPA content of *Daphnia* used in our experiment represented the extreme situation of intense cyanobacteria blooming and the EPA content of herbivorous cladoceran can be much higher in lakes with higher phytoplankton diversity (Kainz et al., 2004; Keva et al., 2021; Vesterinen et al., 2021).

The somatic growth of fry was lowest with *Daphnia* fed on the poor quality diet, which suggests that diet quality could directly affect fry development, and highlights the importance of high nutritional quality algae for fish growth. Therefore, suppressed nutritional quality of phytoplankton may affect not only the trophic transfer of EPA and DHA in lakes and streams (Guo et al., 2016; Müller-Navarra et al., 2004; Taipale et al., 2016b; Taipale et al., 2019) but also the somatic growth of fish fry. These results emphasize the importance of physiologically required long-chain PUFA (ARA, EPA, and DHA) in the fish fry diet, whereas the high dietary supply of shorter chain ω-6 and ω-3 PUFA (LIN and ALA, respectively) provides less support for somatic growth of fish fry. Moreover, the potential ability of fish fry to bioconvert shorter to longer-chain PUFA does not seem to compensate for the paucity of direct dietary long-chain PUFA supply. Our previous study on trout fry showed high retention efficiency of EAA and thus no dietary impact on the EAA content of trout fry (Taipale et al., 2018). However, in the current study, the fast SGR with fish feed systematically resulted in lower EAA content in the fish fry (µg EAA mg^{-1} DW^{-1}) compared to fry with lower SGR. The fast growth of fry with fish feed did not lower their EPA or DHA content compared to fry fed on marine zooplankton diet. Moreover, the high availability of EPA and DHA in the artificial diet did not increase EPA or DHA content in the fry compared to the moderate availability in the marine zooplankton diet, suggesting strong physiological regulation in their EPA and DHA content in different tissues.

Even though single AA supplementation has been shown to improve fish growth in aquaculture studies (Li and Wu, 2018; Omosowone and Ozorewor, 2019; Xiao et al., 2020), our results showed no difference in EAA or NEAA content among zooplankton (krill/Mysis or *Daphnia*) diets for fry growth. The previous study has shown relatively similar AA content in zooplankton and macroinvertebrates. However, the AA content of herbivorous zooplankton or macroinvertebrates could be reduced by environmental change, e.g., eutrophication, due to the lowered dietary availability of EAA and NEAA (Taipale et al., 2019). Therefore, the EAA and NEAA content of zooplankton, macroinvertebrates, and fish should be more systematically studied from different types of lakes and streams to see if they can efficiently retain different EAA, as shown in the laboratory experiment with three different trophic levels (Taipale et al., 2018).

A decrease in dietary ARA, EPA, and DHA requires consumers to biosynthesize these PUFA from dietary precursors. Biosynthesis of DHA from ALA or ARA from LIN requires several enzymatic conversion steps that are more commonly performed in freshwater than in marine fish (Bell and Sargent, 2003; Buzzi et al., 1996; Sargent et al., 1999). Previous studies have shown that rainbow trout larvae and fry (0.1 g) are unable to biosynthesize DHA from ALA or ARA from LIN (Taipale et al., 2018; Wirth et al., 1997), whereas 2 g and 70 g size trout can biosynthesize DHA and ARA from their precursors (Bell et al., 2003; Buzzi et al., 1996). The conversion was originally studied using ^{14}C-labelled supplementation of LIN and ALA (Bell et al., 2003; Buzzi et al., 1996), but recently ^{13}C-labeling and compound-specific isotopes were successfully used (Taipale et al., 2018). Here we used natural isotope values and compound-specific isotopes, which was possible because of the different isotope values of initial fish and used diets. Our result showed no bioconversion of EPA and DHA from ALA in fry fed on *Daphnia* 1 and 2, whereas fry seemed to successfully bioconvert DHA from EPA when fed with *Daphnia* 3. Therefore, we need to reject hypothesis 3 partially. However, it is not well understood how efficiently fish fry can bioconvert DHA from EPA and could be studied, e.g., using ^{13}C-labelled EPA. With previous studies on fish larvae and juvenile trout (Taipale et al., 2018; Wirth et al., 1997), trout fry must obtain ARA, EPA, and DHA from their diet. Therefore, it is surprising that juvenile rainbow trout and sockeye salmon typically feed mainly on DHA-poor *Daphnia* during their first summer yet still contain a high DHA content (Ballantyne et al., 2003; Beauchamp, 1990). Fry and fingerling trout/salmon can likely catch *Daphnia* more easily than copepods, as found with pike larvae (Drost, 1987). Our results emphasize the dietary importance of the nutritional quality of *the* diet for trout fry. Since *Daphnia* has a very limited ability to biosynthesize EPA from ALA (Taipale et al., 2011a; von Elert, 2002), the *Daphnia* EPA content is determined by the availability of dietary EPA (Galloway et al., 2014; Taipale et al., 2022). Meanwhile, the EPA content of chironomid larvae may also be reduced by eutrophication (Taipale et al., 2022), they also have relatively good ability bioconversion EPA from ALA (Strandberg et al., 2020). Therefore, cyanobacteria blooming may have a different impact on the nutritional quality of fish prey in lakes and streams. Recently, it was shown that three-spined stickleback (*Gasterosteus aculeatus*) was able to express its

fads2 gene (Ishikawa et al., 2019), and it may be thus possible that lack of DHA by intense cyanobacteria blooming favors fish species that can compensate for low dietary EPA and DHA via endogenous biosynthesis of these long-chain PUFA.

5. Conclusion

We compared how DHA-rich and DHA-deficient prey impact the development and biochemical content of rainbow trout (*O. mykiss*) fry by altering the dietary availability of macromolecules (lipids, carbohydrates, proteins) and essential amino acids and fatty acids. The fast somatic growth of trout fry when feeding on a DHA-rich diet compared to DHA-poor diets demonstrates that change in zooplankton or macroinvertebrate communities to DHA-deficient species may reduce the growth of salmonid fish fry. This is especially likely when fish fry cannot efficiently biosynthesize EPA, DHA, or ARA from their precursors and thus cannot overcome suppressed availability of these physiologically essential biomolecules by endogenous biosynthesis.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cbpb.2022.110767.

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