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Biochemical composition of copepods for evaluation of feed quality in production of juvenile marine fish

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Abstract

To increase current knowledge on the nutritional value of natural prey organisms, the biochemical components of mainly three copepods (*Acartia grani*, *Centropages hamatus*, and *Eurytemora affinis*) from a marine pond system were analysed once a week from spring until late fall, over two years. The analysed components were total lipid, lipid class composition, total lipid fatty acid composition, free amino acids, total protein, protein-bound amino acids, pigment (astaxanthin and ß-carotene), and vitamins (A, thiamine, riboflavin, C, D_3, and E). Copepod dry weight (DW), dry matter (% of wet weight), and ash content (% of DW) were also determined. The data are unique due to the homogenous content of copepods in the samples and the long time span of sampling. The copepods were characterised by moderate levels of lipids (6.9–22.5% of DW), with polar lipids accounting for 37.9 to70.2% of the total lipid. The most abundant fatty acids in total lipid (as % of total lipid) were 16:0 (palmitic acid, 10.8–17.1%), 20:5n-3 (EPA, 8.3–24.6%), and 22:6n-3 (DHA, 13.9–42.3%). The amount of 20:4n-6 (ARA) was generally low (0–2.6%), giving an EPA/ARA range between 7.5 and 49.5. The DHA/EPA ratio was between 1.0 and 4.9. Free amino acids (FAA) constituted between 4.3 and 8.9% of copepod DW, and varied with salinity. Glycine, taurine, and arginine dominated FAA, and the fraction of indispensable amino acids varied between 15.5 and 26.8%. Protein, as back-calculated from the protein-bound amino acids (PAA), amounted to 32.7–53.6% of copepod DW, and contained a stable fraction of indispensable amino acids (37.3–43.2% of PAA). Glutamine/glutamic acid, asparagine/aspartic acid, leucine, alanine, and glycine were the most abundant PAA. Astaxanthin was abundant in the copepods (413–1422 μg/g DW), while ß-carotene was not found. High but variable concentrations of vitamin C (38–1232 μg/g DW) and vitamin E (23–209 μg/g DW) were found, while vitamin A and D_3 occurred in trace amounts or were not detected. Detectable levels were found for both thiamine (3.5–46.0 μg/g DW) and riboflavin (23.2–35.7 μg/g DW). The data may generate an important base for improvement of live feed enrichment emulsions or formulated feeds used during larval and early juvenile stages in marine fish culture.

Keywords: Lipid class composition; Fatty acids; PUFA; DHA; EPA; TAG; Phospholipid; Protein content; Free amino acids; Pigments; Astaxanthin; Vitamin A; Ascorbic acid; Vitamin D; Vitamin E; Thiamine; Riboflavin; Larval nutrition; Essential nutrients

1. Introduction

High survival and growth, normal pigmentation, and low frequencies of skeletal deformities are characteristics of marine fish reared on natural assemblages of marine zooplankton that mainly consists of copepods (Næss et al., 1995; van der Meeren and Naas, 1997; Støttrup et al., 1998; Shields et al., 1999; Finn et al., 2002; Hamre et al., 2002). This has been particularly evident for Atlantic halibut (*Hippoglossus hippoglossus*) and Atlantic cod (*Gadus morhua*). In the latter case, lagoon or mesocosm rearing is still superior to intensive fry production with rotifers and *Artemia* as feed. Using copepods as feed compared to intensive rearing of cod larvae on rotifers has indicated a significant nutritional influence on juvenile quality and growth (Imsland et al., 2006). The superiority of copepods for larviculture of marine fish has recently increased the interest for controlled culture of copepods (Støttrup, 2003; Lee et al., 2005).

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A number of beneficial effects have been linked to copepod nutrient composition in relation to early larval nutrition. In particular, emphasis has been put on lipid composition, and the content and ratio of the polyunsaturated fatty acids (PUFA) docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), and arachidonic acid (ARA) (Scott and Middelton, 1979; Seikai, 1985; Kanazawa, 1993; Reitan et al., 1994; Reitan et al., 1997; Nanton and Castell, 1998; Venizelos and Benetti, 1999; Bell et al., 2003). The composition of lipid classes and distribution of certain fatty acids between neutral and polar lipids has also gained some attention in lipid nutrition of fish (Olsen et al., 1991; Coutteau et al., 1997; Geurden et al., 1998; McEvoy et al., 1998; Sargent et al., 1999).

Further, Nakamura et al. (1986) concluded that insufficient skin pigmentation (melanin) was a result of rhodopsin deficiency, and hence deficiency in the rhodopsin precursors DHA and retinol (vitamin A). In this respect, deficiencies in compounds like carotenoids, thiamine, riboflavin, and cholecalciferol (vitamin D_3) may be considered. Nutrients with antioxidative properties, comprising astaxanthin, ascorbic acid (vitamin C), and tocopherol (vitamin E), may also be of importance. For example, vitamin C appears to enhance the ability of fish larvae to resist stress and infections (Merchie et al., 1997).

As marine fish larvae have a high growth potential, they have high dietary requirements for protein and essential amino acids. In addition, fish larvae use of amino acids for energy (Rønnestad et al., 1999b; Wright and Fyhn, 2001), which will further increase the demand for dietary amino acids and protein. Consequently, some essential amino acids have been suggested as limiting for larval growth (Conceição et al., 1997; Aragao et al., 2004b). Thus, increased knowledge on the variation in both content and composition of free amino acids and protein in the natural diet will be essential in current understanding on the importance of these factors in larval development and survival.

Data on biochemical composition of copepods are fragmentary, both with respect to what parameters investigated, and how they vary between copepod species and seasons. Most previous work has concentrated on lipid and fatty acid compositions (Gatten et al., 1983; Watanabe et al., 1983; Witt et al., 1984; Sargent and Henderson, 1986; Fraser and Sargent, 1989; Klungsøyr et al., 1989; Olsen et al., 1991; van der Meeren et al., 1993; Norsker and Støttrup, 1994; Evjemo and Olsen, 1997; Evjemo et al., 2003; Morehead et al., 2005). But there are also some data on amino acids and protein (Fyhn et al., 1993, 1995; Helland et al., 2003a,b,c), pigments (Rønnestad et al., 1998), and vitamins (Mæland et al., 2000). There are however, to our knowledge, no studies describing the seasonal variation in both macro- and micronutrients in natural prey organisms of fish larvae. The present work includes copepods sampled weekly from a marine pond system over two years from spring to late autumn, and is an attempt to establish more comprehensive database on a number of biochemical components in copepods that are nutritionally important for fish larvae. The work includes analyses of dry matter, ash content, lipids, fatty acids, protein content, protein-bound amino acids, free amino acids, pigments, and vitamins. Such data will be valuable in the ongoing research to improve enrichment emulsions and nutritional quality of live feed used in marine fish culture, as well as for development of formulated starter or early weaning diets for marine fish larvae.

2. Materials and methods

2.1. Copepod production and collection system

Copepods were collected during 2000 and 2001 from the marine pond system "Svartatjern" (Naas et al., 1991; van der Meeren, 2003), which is situated near Institute of Marine Research (IMR), Austevoll Research Station at 60°N on the west coast of Norway. Svartatjern is a 20,000 m^3 seawater pond, with largest depth of 3.5 m, and in which all the water can be pumped out and replaced over 3–4 weeks period. A management protocol has been established since the system was started in 1984, which includes draining and refilling the pond twice a year (in early February and early July). Seawater was pumped from 35 m depth in the open fjord outside the pond, and filtered through a UNIK-900 wheel filter (Unik Filtersystem AS, Os, Norway) with 80 µm mesh size (Støttrup, 2003; van der Meeren and Naas, 1997). From March to mid-October, the pond was fertilised weekly or daily depending on weather with agricultural NPK 21-4-10 fertiliser (no trace elements were listed: Yara Norge AS, Oslo, Norway). Fertilisation was always stopped when secci-disk readings became less than 1.5 m. This would ensure a net primary production in the whole water column. The pond was also gently mixed with a propeller placed at 2 m depth. This prevented stratification and formation of oxygen depletion in the bottom layer. This production cycle gives relatively pure populations of mainly calanoid copepods, which are the dominant plankton of Norwegian coastal lagoon systems (Næss, 1996). During winter and pond draining, the copepods survive in the sediments as resting or dormant eggs (Næss, 1991).

In addition to filtering the incoming water, the UNIK-900 wheel filter was also used for copepod collection from Svartatjern (van der Meeren, 2003). The collection and concentration system was placed inside a small building on a raft in the middle of Svartatjern, and consisted of a slow-impeller-pump (1250 rpm) with up to 1000 l/min capacity, the filter, and six collection and settling tanks. The pump was submerged to 2 m depth and lifted pond water into the first compartment of the wheel filter. A rotating fibreglass wheel equipped with 800 µm plankton net sorted out objects too big for being copepods (e.g. hydromedusas), and the water entered the second compartment which was limited by a second wheel with 250 µm plankton net. The copepods were trapped on this latter wheel filter, flushed off into a funnel, and drained down into a set of six 250 l round fibreglass tanks with conical bottoms. When these tanks were filled to the outlet, outputs from the filter bypassed these collection tanks, enabling sedimentation of dead plankton and other organic debris. A timer controlled the wheel filter and pump so collection and sedimentation could take place automatically during night and early morning. In this manner, the remaining live zooplankton could immediately be concentrated in the morning by slowly flushing the tank content through an 80 µm conical plankton net submerged in the pond water. In the tanks, an inner tube with openings 15 cm above the cone prevented settled material from entering the drained water. Further, air and oxygen were supplied at the bottom of the submerged net to prevent the collected copepods from settling in the net cone. From experience, settling would induce heavy mortality among the copepods.

In addition to collection of copepods, 60 ml water samples were taken at 2 m depth and preserved in 0.6 ml of a glutaraldehyde–Lugol solution (Rousseau et al., 1990) for determination and enumeration of algal species and groups in the pond. Hydrographical data (Table 1) were monitored twice a week with WTW portable meters (WTW LF 330 with Tetra Con 325 probe for salinity and temperature, and WTW Oxi 330 with CellOx 325 electrode for oxygen; WTW GmbH, Weilheim, Germany). Water samples for pH measurements and nutrient analyses were collected once a week and analysed for nitrate (including nitrite), orthophosphate, and silicate, using standard procedures (Koroleff, 1983). A Radiometer PHM 210 (London Scientific Ltd, London Ontario, Canada) was used for pH readings, and nutrients were quantified on a Shimadzu UV-160 UV–visible Recording Spectrophotometer (Shimadzu Corp., Kyoto, Japan). Copepods, nutrient and algal samples, and hydrography were always collected between at 09:00 and 10:00 h.

In 2001, a single sample of zooplankton was also collected from the Hyltro lagoon in Austevoll, another coastal marine lagoon system previously used for

Table 1
Hydrographical data from the Svartatjern pond during collection of copepods

	2000			2001		
	Mean±SD	Min.	Max.	Mean±SD	Min.	Max.
Temperature (°C)	14.2±2.9	8.5	19.1	14.1±3.7	7.1	19.3
Salinity (‰)	25.2±2.9	21.1	31.2	26.3±3.2	19.9	31.4
Oxygen (% saturation)	105±19	77	145	98±43	15	160
pH[a]				8.2±0.5	7.4	9.1
Secci depth (m)	1.4±0.4	1.0	2.2	1.7±0.6	1.0	3.3
Nitrate (μM)	1.8±1.2	0.0	4.5	4.5±5.0	0.5	15.3
Phosphate (μM)	0.6±0.3	0.2	1.1	0.7±0.3	0.2	1.3
Silicate[a] (μM)				3.4±4.9	0.3	19.0

[a] Not measured in 2000.

copepod production and juvenile marine fish rearing (Øiestad et al., 1985). However, low copepod biomass prevented further collection from this system. Therefore, no hydrography, nutrients, or phytoplankton samples were collected from the Hyltro lagoon. Moreover, to be able to directly compare the copepod samples with intensive-produced live feed for marine fish larvae, one sample of the rotifer *Brachionus plicatilis* and three samples of *Artemia franciscana* (Great Salt Lake strain) were included during the 2000 season. The rotifers were reared at IMR with *Isochrysis galbana* and Rotimac (Bio-Marine Aquafauna Inc., Hawthorne, CA, USA) as feed. Two of the *Artemia* samples were 1-day-old metanauplii obtained from IMR and from the commercial cod and halibut fry producer Austevoll Marin Yngel AS (AMY), respectively. Both these *Artemia* groups were enriched with DC-DHA Selco (INVE Aquaculture, Dendermonde, Belgium). The third sample was 3-day-old *Artemia* from AMY, which also used Algamac 2000 (Bio-Marine Aquafauna Inc.) as feed in addition to the DC-DHA Selco for this on-grown *Artemia* group. To compare biochemical components of copepod nauplii (sieved through 150 μm and retained on 80 μm plankton nets) and the older stages of copepods in the 250–800 μm fraction, three samples of nauplii from Svartatjern were included during the 2001 season. The collected nauplii biomasses were insufficient for other analyses than lipids, dry weight, and content of dry matter and ash.

In the following, samples from the Svartatjern pond are referred to as copepods and nauplii, the sample from the Hyltro lagoon as zooplankton, and the samples of the intensive-produced live feed as rotifers and *Artemia*.

2.2. Sample preparation

The collected copepods were transported live for 10 min in a black 12-l-bucket to the sample preparation laboratory. Here, the copepods were placed in a mixing column of 6 l volume and 9.5 cm diameter (van der Meeren, 2003), with densities between 400 and 900 copepods/ml. To ensure proper mixing and sufficient oxygen supply, air and oxygen were mixed and bubbled gently from the tip of the cone at the bottom of the column. With this arrangement, copepods could easily be kept alive for more than 4 h, which was sufficient to prepare the samples for biochemical analyses. The bubbling also led to a homogenous distribution of copepods in the column, as shown from a biomass of 2.6 g±0.12 (mean wet weight±SD) among 10 subsequent samples of equal volume collected through a silicon tube placed 15 cm above the cone bottom. Further, the relationship between sample size in ml (V) and sample wet weight in grams (WW) showed high correlation among 5 replicate samples of unequal volume in the range of 50 to 500 ml ($V=258.98\,WW-26.379$, $R^2=0.9989$). Similarly, the relationship between actual counts of copepods from these samples (N) and V also showed high correlation ($N=138.46\,V+753.26$, $R^2=0.9942$). In this way, uniformity of collected biomass among repeated samples from the column was demonstrated.

Aliquots of copepods were sampled from the column for the following biochemical analyses: lipid classes and total lipid fatty acids, pigments, protein and free amino acids, lipid-soluble vitamins, and water-soluble vitamins. In addition, one aliquot was collected to determine individual copepod wet weight, followed by another aliquot for determination of dry matter and ash content. Between 0.5 and 2.7 g copepod wet weight were sampled for each analysis. Finally, an aliquot of 50 ml was preserved with 0.9 ml Lugol solution for identification of copepod species and stages, as well as other zooplankton species. Copepod samples were also made available for iodine analyses (published in Moren et al., 2006).

Wet weight was determined in all unpreserved samples by weak vacuum filtration at 680 mm Hg (van der Meeren, 2003). The unit was equipped with 52 mm diameter filter disks of 60 μm mesh size plankton net (Sefar Nitex 03-60/35, Sefar Holding Inc., Freibach, Switzerland). To remove salt, the samples were flushed 2–3 times with 10‰ salt water made from distilled water and 0.2 μm filtered 35‰ seawater. Salinity lower than 10‰ was observed to burst the copepod exoskeleton, with subsequent loss of biomass. The resulting semi-dry "cake" of copepods was further divided into sub-samples by a spatula and transferred to pre-weighed Nunc cryotubes with an externally treaded lid. The cryotubes were then quickly weighed to nearest 0.1 mg on a Mettler AE200 (Mettler-Toledo Inc., Columbus, OH, USA). Lipid samples were then immediately frozen in liquid nitrogen, while the samples for the other biochemical components were quickly placed in an −80 °C freezer. By this procedure, a short time (3–5 min) was ensured from sample collection to placement in freezer.

The sample for determination of individual copepod WW was first filtered and weighed as described above, then 75 to 100 ml of 10‰ salt water was added along with a few drops of Lugol solution to improve contrast, and finally ten well-mixed aliquots of 0.2–0.5 ml were collected from the sample and counted to determine the total number of copepods. A Leica MS5 stereo Microscope with options for both light and dark field (Leica Microsystems GmbH, Wetzlar, Germany) was used for counting. Variation among the 10 counts was low, with an average coefficient of variation of 11%.

After freezing, the sample for determination of dry matter content was dried in a Heto FD8 freeze-drier (Heto-Holten AS, Allerød, Denmark). A freeze-drying period of 72 h was necessary to reach stable weight. To ensure reliable dry weight (DW) measurements over a range of different sample sizes, the sample DW in g was regressed on the corresponding WW for 9 replicate samples between 0.5 and 5.0 g wet weight. This sample series showed high linear correlation ($DW=0.140\,WW+0.004$, $R^2=0.999$). Amount of dry matter (% of WW) was calculated, and ash content (% of DW) was determined by combusting at 550 °C for 24 h in pre-weighed porcelain crucibles.

The frozen samples for analysis of total protein, protein-bound amino acids, and free amino acids were also freeze-dried and weighed for determination of DW before being shipped in dry condition to the laboratory for analysis. All other samples were packed on dry ice and kept frozen when shipped to the analytical laboratories within 3 h. Preparation of the zooplankton, rotifer, and *Artemia* samples was in all respects similar to the copepod samples.

After the sample preparations were completed, copepod viability of the remaining biomass was checked by a light–dark test. A sample of copepods was placed on a Petri dish with seawater, and partly covered by aluminium foil. The cover was then moved to the other half of the disk. In both cases, almost 100% of the copepods gathered under the shadowed area within a short time. This was consistent throughout the sampling seasons, showing no mortality during sample collection. In addition, the samples were inspected under the Leica stereo microscope for damages on the copepod antennae and tail, and for content of organic debris (van der Meeren, 2003).

2.3. Analytical methods

2.3.1. Lipids and fatty acids

Frozen samples were homogenized in solvent using an Ultra Turrax (IKA Werke GmbH, Staufen, Germany) and total lipid extracted according to the method of Folch et al. (1957). After evacuation of the solvent under nitrogen, water was evacuated under vacuum over dry sodium hydroxide, and total lipid quantified gravimetrically. The lipid was then stored in chloroform:methanol (2:1) under nitrogen at −80 °C until used for further analysis. Lipid class composition was assessed using the HPTLC double development method of Olsen and Henderson (1989). For fatty acid analysis of total lipid, portions of the samples were subjected to the sulphuric acid catalysed transesterification method of Christie (1982), extracted into hexane, and stored at −80 °C until analysed. Quantitative analysis of fatty acid methyl esters were carried out by gas liquid chromatography using a HP 5890 gas chromatograph (Hewlett Packard Labs Inc., Palo Alto, CA, USA) equipped with a J&N Scientific Inc DB-23 fused silica column (30 m×0.25 mm i.d.) as described by Olsen et al. (2004). Abbreviations for lipid classes and fatty acids used in the text are given in Table 2.

Table 2
Individual size (dry weight: DW), dry matter content (% of wet weight: WW), ash content, and lipid components from copepods, copepod nauplii, zooplankton (copepods and decapod zoeae), rotifers, and *Artemia* (1-day or 3-day after hatching)

	Abbreviations	Svartatjern			Hyltro	Intensive live feed			
		Copepods	Copepods	Copepod nauplii	Zooplankton	Rotifers	*Artemia*		
		2000	2001	2001	2001	IMR [a]	1-day [b]	1-day [c]	3-day [c]
Individual size, dry matter, and ash									
Number of samples	*N*	30	26	3	1	1	1	1	1
Dry weight (μg/individual)	DW	9.4 [d] ± 2.5	8.1 ± 2.7	0.63 [e] ± 0.7	9.9	0.61	2.1	2.1	2.5
Dry matter (% of WW)	DM	14.9 ± 1.1	15.3 ± 1.5	15.2 ± 1.9	17.7	13.2	10.2	10.8	8.9
Ash content (% of DW)	ASH	10.3 ± 1.2	10.5 ± 1.3	9.9 ± 0.5	15.3	9.6	10.4	9.6	9.5
Total lipid (μg/mg DW)	TL	111 ± 35	108 ± 21	86 [e] ± 12	143	154	254	243	249
Neutral lipids (μg/mg DW)	NL	49.4 ± 23.4	45.4 ± 13.3	32.6 ± 13.5	91.5	92.5	215.0	193.4	206.0
Sterol esters + Wax esters	SE + WE	1.5 ± 1.5	1.1 ± 1.2	1.3 ± 0.5	22.6	11.6	n.d.	1.3	n.d.
Triacylglycerol	TAG	26.3 ± 19.5	22.1 ± 13.1	14.0 ± 13.3	42.1	60.6	195.9	167.9	178.4
Free fatty acids	FFA	3.3 ± 2.1	3.3 ± 1.6	3.9 ± 2.1	6.6	6.9	4.4	5.8	8.9
Cholesterol	C	14.5 ± 6.3	13.3 ± 3.5	9.5 [e] ± 3.6	15.5	11.6	14.6	18.4	18.7
Monogalactosides + Cerebrocides	MGDG + CB	1.6 [d] ± 1.0	2.7 ± 2.6	2.1 ± 1.3	1.3	n.d.	n.d.	n.d.	n.d.
Digalactosides + Sulfolipids	DGDG + SL	2.3 ± 1.1	2.9 ± 1.5	1.8 ± 0.8	3.4	1.9	n.d.	n.d.	n.d.
Polar lipids (μg/mg DW)	PL	61.9 ± 16.8	62.6 ± 14.4	53.7 ± 2.7	51.5	61.1	39.2	49.9	43.3
Phosphatidylethanolamine	PE	19.9 ± 5.8	20.4 ± 4.8	17.3 ± 2.1	15.2	21.0	12.3	16.8	14.3
Cardiolipin	CL	5.7 ± 2.0	5.8 ± 1.6	5.2 ± 0.3	2.5	3.1	2.2	3.0	2.5
Phosphatidylglycerol	PG	2.2 ± 2.3	1.5 ± 1.0	0.8 ± 0.2	1.4	1.5	0.5	0.8	0.5
Phosphatidylinositol	PI	3.9 ± 1.7	4.2 ± 1.4	5.1 ± 2.1	5.6	10.6	3.8	5.1	4.2
Phosphatidylserine	PS	5.5 [d] ± 1.5	6.6 ± 2.0	6.4 ± 0.7	4.8	5.3	3.1	4.1	3.2
Phosphatidylcholine	PC	20.0 ± 6.0	19.4 ± 5.3	15.0 ± 1.3	19.0	18.5	16.4	18.6	17.4
Lysophosphatidylcholine + Sphingomyelin	LPC + SM	4.7 ± 1.6	4.8 ± 1.6	3.9 ± 0.2	2.9	1.1	0.9	1.4	1.1
Fatty acids *(% of total lipid)*									
Myristic acid	14:0	3.4 [d] ± 1.7	1.7 ± 1.1	1.3 ± 0.8	3.8	6.7	1.7	1.5	2.4
Palmitic acid	16:0	14.5 ± 1.9	14.4 ± 1.4	13.7 ± 2.5	14.1	19.7	14.9	14.4	15.8
Palmitoleic acid	16:1(n-7)	3.4 ± 1.8	4.4 ± 4.7	1.8 ± 1.4	7.6	9.2	4.8	1.0	3.0
Stearic acid	18:0	3.5 ± 1.0	3.7 ± 0.7	3.9 ± 1.0	4.1	3.9	5.0	5.0	5.4
Oleic acid	18:1(n-9)	2.3 ± 1.1	2.6 ± 1.4	1.3 [e] ± 0.7	7.3	7.8	23.3	22.8	17.8
Vaccenic (Asclepic) acid	18:1(n-7)	2.7 ± 0.6	2.9 ± 0.7	2.0 [e] ± 0.5	3.1	4.9	5.5	6.3	5.4
Linoleic acid	18:2(n-6)	1.5 [d] ± 0.5	2.3 ± 0.7	1.5 [e] ± 0.5	2.2	15.3	6.6	5.8	4.2
α-Linolenic acid	18:3(n-3)	1.9 [d] ± 1.0	2.4 ± 1.1	1.5 [e] ± 0.9	1.4	1.2	12.2	16.2	10.2
Stearidonic acid	18:4(n-3)	2.3 [d] ± 1.4	4.1 ± 2.9	4.5 ± 5.7	5.2	2.0	2.8	3.2	1.7
Arachidonic acid (ARA)	20:4(n-6)	0.8 ± 0.5	0.9 ± 0.7	0.6 ± 0.3	1.6	1.9	2.0	2.0	3.2
Eicosapentaenoic acid (EPA)	20:5(n-3)	17.4 ± 3.1	16.2 ± 3.4	16.3 ± 6.4	16.4	7.1	7.8	7.8	9.2
Docosahexaenoic acid (DHA)	22:6(n-3)	34.4 ± 4.6	32.9 ± 6.8	40.5 [e] ± 2.4	17.3	12.4	10.6	11.1	20.0
Other saturated fatty acids		3.1 ± 1.1	3.3 ± 1.2	3.9 ± 2.7	9.7	n.d.	n.d.	n.d.	n.d.
Other monounsaturated fatty acids		3.7 [d] ± 1.0	2.8 ± 0.9	2.7 ± 1.2	2.0	4.2	1.2	1.5	1.7
Other polyunsaturated fatty acids		5.1 ± 1.2	5.4 ± 1.5	4.6 ± 1.0	4.2	3.8	1.6	1.4	n.d.
Total amounts of fatty acid groups *(%)*									
Saturated fatty acids	SFA	24.6 [d] ± 2.9	23.1 ± 2.2	22.7 ± 2.9	31.7	30.3	21.6	20.9	23.7
Monounsaturated fatty acids	MUFA	12.1 ± 2.1	12.7 ± 6.1	7.8 ± 3.2	20.1	26.1	34.8	31.6	27.8
Polyunsaturated fatty acids	PUFA	63.3 ± 3.7	64.2 ± 6.8	69.4 ± 5.8	48.3	43.7	43.6	47.5	48.5
Highly unsaturated (n-3) fatty acids	DHA + EPA	51.8 [d] ± 4.5	49.1 ± 6.8	56.8 [e] ± 6.8	33.6	19.4	18.4	19.0	29.2
Fatty acid ratios	(n-3)/(n-6)	11.3 [d] ± 2.7	9.8 ± 2.5	12.5 [e] ± 3.0	7.0	1.5	3.9	4.2	5.5
	DHA/EPA	2.1 ± 0.5	2.2 ± 1.0	2.8 ± 1.3	1.1	1.7	1.4	1.4	2.2
	EPA/ARA	24.7 ± 9.2	23.2 ± 10.1	27.7 ± 4.0	10.3	3.7	4.0	4.0	2.9

Data are given as mean ± SD when number of samples > 1. Values below detection limits of the analytical method are denoted n.d.
[a] Institute of Marine Research: rotifers grown on Rotimac and *Isochrysis galbana* algae.
[b] Institute of Marine Research: *Artemia* enriched with DC-DHA Selco.
[c] Austevoll Marin Yngel AS: *Artemia* fed DC-DHA Selco and Algamac 2000.
[d] Significant difference between copepods from 2000 and 2001.
[e] Significant difference between copepod nauplii and copepods from 2001.

2.3.2. Protein and amino acids

Sub-samples (15–25 mg) of the freeze-dried samples were extracted in Eppendorf tubes in 1 ml 6% tri-chloro-acetic acid (TCA) under rotation (Heto Rota-Mix) for 24 h at 4 °C. After centrifugation (15,000 × g, 10 min, 4 °C), the supernatant was used for free amino acid (FAA) analysis after appropriate dilution in borate buffer (100 mM, pH 10.4). The precipitate was washed once in 6% TCA, re-centrifuged, and transferred to a 10 ml tube and dissolved in 4 ml of 1 M NaOH by rotation for 48 h at room temperature for analysis of total protein and protein-bound amino acids (PAA). After centrifugation (15,000 × g, 10 min, 20 °C), the supernatant was collected and appropriately diluted to 0.5 M NaOH with distilled water, and used for determination of total protein by the method of Lowry et al. (1951), using the micro-modification of Rutter (1967) with bovine

Table 3
Content of protein (P) and protein-bound amino acids (PAA) in copepods, zooplankton (copepods and decapod zoeae), rotifers, and *Artemia* (1-day or 3-day after hatching)

	Abbreviations	Svartatjern		Hyltro	Intensive live feed			
		Copepods	Copepods	Zooplankton	Rotifers	*Artemia*		
		2000	2001	2001	IMR [a]	1-day [b]	1-day [c]	3-day [c]
Number of samples	*N*	30	26	1	1	1	1	1
Protein [d] (μg/mg DW)	P	382.6 [f] ± 25.5	565.4 ± 40.0	366.3	243.4	287.9	309.2	326.2
PAA in weight [e] (μg/mg DW)	PAA_w	443.6 [f] ± 41.6	412.6 ± 41.0	302.5	247.7	277.5	293.8	367.6
Indispensable amino acids (μg/mg DW)	IAA_w	201.3 [f] ± 16.8	189.6 ± 20.8	141.8	120.0	133.3	140.3	175.2
Indispensable amino acids (%)	IAA_w/PAA_w	45.4 ± 1.5	45.9 ± 0.9	46.9	48.4	48.0	47.8	47.7
Indispensable to dispensable ratio	IAA_w/DAA_w	0.83 ± 0.05	0.85 ± 0.03	0.88	0.94	0.92	0.91	0.91
PAA concentration (μmol/mg DW)	PAA_c	4.1 [f] ± 0.4	3.8 ± 0.4	2.8	2.3	2.5	2.7	3.4
Indispensable amino acids (μmol/mg DW)	IAA_c	1.7 [f] ± 0.1	1.6 ± 0.2	1.2	1.0	1.1	1.2	1.4
Indispensable amino acids (%)	IAA_c/PAA_c	40.4 [f] ± 1.5	41.3 ± 0.9	41.5	43.7	43.3	42.8	42.7
Indispensable to dispensable ratio	IAA_c/DAA_c	0.68 [f] ± 0.04	0.70 ± 0.03	0.71	0.78	0.76	0.75	0.75
Indispensable amino acids *(nmol/mg DW)*								
Leucine	LEU	349.0 [f] ± 38.5	320.5 ± 33.8	246.4	230.1	225.6	295.2	237.0
Valine	VAL	291.8 [f] ± 36.4	253.0 ± 24.9	200.7	160.1	175.1	233.6	183.9
Lysine	LYS	241.3 ± 43.0	231.1 ± 34.7	163.8	136.6	149.0	222.6	166.7
Isoleucine	ILE	209.6 [f] ± 26.3	187.3 ± 20.1	146.5	143.3	137.6	186.8	148.7
Arginine	ARG	121.7 [f] ± 27.6	161.7 ± 14.1	126.4	83.7	115.7	149.4	108.8
Phenylalanine	PHE	154.4 [f] ± 18.7	143.4 ± 15.7	112.1	114.9	105.7	138.8	111.8
Threonine	THR	128.7 [f] ± 13.1	120.0 ± 14.1	95.1	70.7	86.3	114.6	89.1
Methionine	MET	122.3 [f] ± 13.4	77.7 ± 38.1	69.4	47.6	56.8	40.5	63.2
Histidine	HIS	53.7 [f] ± 26.2	63.7 ± 10.1	10.5	6.9	43.2	60.1	45.9
Tryptophan	TRP	44.6 [f] ± 84.1	0.7 ± 2.7	n.d.	4.4	2.3	7.0	3.5
Dispensable amino acids *(nmol/mg DW)*								
Glutamic acid + Glutamine	GLU + GLN	577.8 [f] ± 66.4	505.2 ± 52.7	384.7	325.0	325.4	427.4	328.2
Aspartic acid + Asparagine	ASP + ASN	411.1 ± 43.4	432.3 ± 54.0	335.8	293.0	271.9	363.5	282.4
Alanine	ALA	463.4 [f] ± 54.8	392.3 ± 38.2	284.7	189.0	230.2	306.9	252.4
Glycine	GLY	441.2 [f] ± 94.7	352.1 ± 49.7	286.1	181.0	224.7	321.8	245.8
Serine	SER	204.9 [f] ± 22.3	190.7 ± 19.2	152.0	136.8	136.7	186.3	143.7
Proline	PRO	252.0 [f] ± 59.9	186.8 ± 24.8	164.4	134.4	157.4	217.4	200.8
Tyrosine	TYR	122.2 [f] ± 36.1	154.5 ± 16.9	40.5	19.9	88.7	109.5	89.5

Values are relative to dry weight (DW) and are given as mean ± SD when number of samples > 1. Values below detection limits of the analytical method are denoted n.d. The subscripts "w" and "c" indicate data given as weight and concentration, respectively.

[a] Institute of Marine Research: rotifers grown on Rotimac and *Isochrysis galbana* algae.

[b] Institute of Marine Research: *Artemia* enriched with DC-DHA Selco.

[c] Austevoll Marin Yngel AS: *Artemia* fed DC-DHA Selco and Algamac 2000.

[d] Protein determined with the Bovine serum albumin method of Lowry et al. (1951) and Rutter (1967).

[e] PAA in weight are calculated as protein (i.e. from the amino acid mole weight subtracted by the mole weight of a water molecule, which resembles the PAA before hydrolysis).

[f] Significant difference between copepods from 2000 and 2001.

serum albumin (BSA, Sigma A-7638) in 0.5 M NaOH as standard and 0.5 M NaOH as blank. The colour was allowed to develop in darkness for 30 min and, after an additional mixing, the sample absorbance was read on a Perkin Elmer Biolambda spectrophotometer (PerkinElmer Inc., Waltham, MA, USA) at 750 nm. Preliminary tests showed no increase in the protein or FAA contents of the freeze-dried copepod, *Artemia,* or rotifer material by Potter–Elvehjem glass–glass homogenisation, so direct extraction of the freeze-dried material in TCA or NaOH was routinely used in this study.

An aliquot (200 μl) of the NaOH supernatant was added concentrated HCl to reach final concentration of 6 M HCl to allow acid protein hydrolysis (106 °C, 24 h) in N_2-flushed stoppered glass vials. Samples of 6 M HCl were included in the hydrolysis as blank controls. The hydrolysed samples were neutralised by addition of equal volume of 6 M NaOH and appropriately diluted in the borate buffer before analysis. All reagents used in the analyses were prepared from glass-distilled, ion-exchanged (Millipore Milli-Q) water with a resistance of 18 MΩ.

Amino acid analysis was performed by reversed-phase chromatography using a Gilson HPLC (Gilson Medical Electronics Inc., Middleton, WI, USA) with fluorometric detection (OPA and FMOC reagents) and connected to an ASTED (Automated Sequential Trace Enrichment of Dialysates) sample robot and a 3 × 150 mm, 3 μm particle size Inertsil ODS-3 column from Varian (Varian Inc., Palo Alto, CA, USA). The analytical reproducibility based on repetitive analyses of standards was <1% for all amino acids except proline (4%). The applied HPLC procedure did not separate phosphoserine and aspartic acid. In the analysis of FAA of the 2001 samples, the glycine peak dominated the following threonine peak so it could not be resolved or quantified. Protein-bound tryptophan is difficult to quantify after acid hydrolysis since it is partly destroyed by the treatment. Gilson Unipoint 715 Software, version 2.10 was used for peak analysis and sample integration.

The PAA values (μmol/mg DW of analysed material) were converted to the equivalent protein content and expressed both in molar terms of the various amino acids (μmol/mg DW), and in weight-specific terms as an equivalent to protein content (μg/mg DW). Abbreviations for the amino acids used in the text are the lower case equivalents to abbreviations used in Tables 3 and 4. The terminology of dispensable (DAA) and indispensable (IAA) amino acids are used according to Harper (1983) and the following 10 amino acids are termed

Table 4
Free amino acids (FAA) in copepods, zooplankton (copepods and decapod zoeae), rotifers, and *Artemia* (1-day or 3-day after hatching)

	Abbreviations	Svartatjern		Hyltro	Intensive live feed			
		Copepods	Copepods	Zooplankton	Rotifers	*Artemia*		
		2000	2001	2001	IMR [a]	1-day [b]	1-day [c]	3-day [c]
Number of samples	*N*	30	26	1	1	1	1	1
FAA in weight (μg/mg DW)	FAA_w	56.1 [e] ± 9.7	64.7 ± 9.8	86.0	16.6	33.7	32.1	27.5
Indispensable amino acids (μg/mg DW)	IAA_w	18.4 ± 3.0	18.2 ± 1.8	19.3	5.8	4.4	5.5	5.1
Indispensable amino acids (%)	IAA_w/FAA_w	32.9 [e] ± 2.7	28.5 ± 3.3	22.4	34.7	12.9	17.0	18.7
Indispensable to dispensable ratio	IAA_w/DAA_w	0.49 [e] ± 0.06	0.40 ± 0.07	0.29	0.53	0.15	0.20	0.23
FAA concentration (nmol/mg DW)	FAA_c	471.7 [e] ± 89.8	580.1 ± 95.1	766.6	124.5	277.6	254.0	219.0
Indispensable amino acids (nmol/mg DW)	IAA_c	113.6 ± 18.2	109.0 ± 10.7	119.2	38.0	27.7	36.4	34.3
Indispensable amino acids (%)	IAA_c/FAA_c	24.3 [e] ± 2.0	19.1 ± 2.2	15.5	30.6	10.0	14.3	15.6
Indispensable to dispensable ratio	IAA_c/DAA_c	0.32 [e] ± 0.03	0.24 ± 0.03	0.18	0.44	0.11	0.17	0.19
Indispensable amino acids *(nmol/mg DW)*								
Arginine	ARG	79.6 ± 15.8	83.1 ± 13.7	68.3	13.6	13.7	12.3	13.4
Threonine	THR	10.2 ± 2.5	– [d]	– [d]	3.0	0.9	2.9	3.1
Valine	VAL	5.8 [e] ± 1.9	4.6 ± 1.5	7.5	4.0	1.9	5.0	4.6
Histidine	HIS	5.1 [e] ± 1.7	9.3 ± 5.7	21.3	4.9	2.9	6.2	3.2
Leucine	LEU	3.6 [e] ± 1.4	3.0 ± 1.0	5.0	2.0	1.9	2.9	2.9
Lysine	LYS	3.3 ± 1.1	3.9 ± 1.8	6.7	5.2	4.4	3.1	3.4
Isoleucine	ILE	2.5 [e] ± 1.1	1.9 ± 0.8	3.2	3.0	1.1	1.8	2.0
Phenylalanine	PHE	2.0 [e] ± 0.9	1.5 ± 0.5	2.6	1.6	0.8	1.1	1.0
Methionine	MET	1.3 ± 0.5	1.4 ± 0.9	4.4	0.6	0.1	0.9	0.5
Tryptophan	TRP	0.3 ± 0.2	0.3 ± 0.1	0.3	0.2	n.d.	0.2	0.1
Dispensable amino acids *(nmol/mg DW)*								
Glycine	GLY	126.5 [e] ± 37.1	231.4 [d] ± 58.2	235.3 [d]	8.8	5.4	8.2	9.7
Taurine	TAU	84.3 [e] ± 16.7	101.1 ± 23.3	136.0	2.9	65.5	57.8	58.2
Alanine	ALA	43.5 ± 18.1	36.4 ± 16.0	68.0	8.9	65.7	34.2	28.0
Glutamic acid	GLU	33.5 [e] ± 7.1	24.5 ± 7.0	45.0	14.6	27.0	35.2	31.2
Proline	PRO	24.3 [e] ± 19.7	38.3 ± 38.9	125.9	3.9	50.7	34.6	25.0
Aspartic acid + Phosphoserine	ASP + PHS	17.9 [e] ± 3.1	13.7 ± 3.7	9.3	4.6	6.2	6.9	5.3
Glutamine	GLN	10.3 ± 1.7	10.4 ± 3.0	7.6	6.2	11.7	17.0	9.4
Serine	SER	8.6 [e] ± 2.3	7.0 ± 2.0	9.3	16.4	2.1	5.0	6.3
Gamma-amino butyric acid	GABA	3.6 ± 1.5	3.8 ± 1.1	4.4	0.8	1.5	2.9	1.9
Tyrosine	TYR	3.1 [e] ± 1.1	2.5 ± 0.7	3.9	10.6	7.3	5.8	3.5
Asparagine	ASN	2.7 [e] ± 0.8	2.0 ± 0.6	2.9	8.7	6.8	10.0	6.2

Values are relative to dry weight (DW) and are given as mean ± SD when number of samples > 1. Values below detection limits of the analytical method are denoted n.d. The subscripts "w" and "c" indicate data given as weight and concentration, respectively.

[a] Institute of Marine Research: rotifers grown on Rotimac and *Isochrysis galbana* algae.

[b] Institute of Marine Research: *Artemia* enriched with DC-DHA Selco.

[c] Austevoll Marin Yngel AS: *Artemia* fed DC-DHA Selco and Algamac 2000.

[d] In the 2001 samples, high glycine content caused masking of threonine (next eluated top in the chromatogram).

[e] Significant difference between copepods from 2000 and 2001.

IAA for fishes according to Wilson (1985): arg, his, ile, leu, lys, met, phe, thr, trp, and val. The inclusion of arg and tyr among the IAA in this study of the natural feed organisms of fish larvae is in agreement with results on embryonic and neonatal vertebrate nutrition which document their strong dependency on amino acids (e.g. Rønnestad et al., 2003; Wu et al., 2004; Dabrowski et al., 2005; Urschel et al., 2006, 2007).

2.3.3. Pigments

The frozen samples were added acetone and homogenized on ice using an Ultra Turrax homogenizer. Moisture was removed by means of Na_2SO_4 and samples stored at −80 °C until analysed. Astaxanthin and β-carotene were quantified using a HP automated sample injector (G1329A ALS), a G1315A DAD diode array detector and G1316A ColComp column temperature controller, maintained at a constant temperature of 4 °C. Separation was performed using tandem installed Chromspher 5 mm C18 columns (100 mm × 3 mm i.d.) with a guard column of C18 material (Chromsep guard column SS) preceding the main column. The mobile phase was acetonitrile:dichlormethane:methanol:propionic acid:water (61:20:7.6:5.7:5.7), which was filtered before use. Vitamin C (263 mg/l) was added to the mobile phase as an antioxidant. The flow rate was isocratic at 1 ml/min. Both column and auto injector temperatures were maintained at 1 °C. Peaks were detected at 476 nm for astaxanthin and β-carotene, and subsequently quantified with reference to authentic standards. Each sample was analysed in triplicates. Data were stored and processed using HP Chemstation software.

2.3.4. Vitamins

All analyses of vitamins were performed on thawed samples and related to wet sample weight. After analysis, data were converted relative to DW by dividing with the dry matter fraction obtained from separate samples as described above in Section 2.2. Whenever vitamin concentration was between the detection and quantification limits, it was denoted as trace amounts. However, to reduce error and variation, particularly at low vitamin concentrations, the trace values were included in the calculations of average vitamin levels.

Samples for analyses of the lipid-soluble vitamins were homogenised and weighed into screw-capped glass tubes, saponified, and extracted with hexane. Vitamin D was up-concentrated by passage over a preparative normal phase HPLC column, where the isomeres D_2 and D_3 eluted as one peak, which was

collected. The collected fraction was then subjected to analytical reverse phase HPLC with UV detection at 275 nm, which separates the vitamin D isomers. Vitamin D_3 was quantified by using vitamin D_2 as internal standard and vice versa. Vitamin D_2 was not detected at all in the samples. The method and instrumentation are described in detail in Horvli and Lie (1994) and CEN (1999a).

Vitamin A was subjected to normal phase HPLC with UV detection at 325 nm and quantified by external standards according to method and instrumentation described in Moren et al. (2004a). This method gives a large peak with similar retention time as all trans retinol in samples from *Artemia*. However, later work has shown, by the use of a diode array detector, which produces UV spectra of the peaks, that this compound is not vitamin A (Moren et al., 2005). The tocopherols (vitamin E isomers) were also analysed by normal phase HPLC, detected by fluorescence at 295 nm excitation and 330 nm emission and quantified using external standards (CEN, 1999b). Given relative to wet weight of the sample, the detection and quantification limits of the analytical methods are 6 and 20 ng/g for vitamin D, 8 and 28 ng/g for vitamin A, 11 and 38 ng/g for α-tocopherol, and 8 and 28 ng/g for the other tocopherols, respectively.

The samples for ascorbic acid (vitamin C) were homogenised and extracted in meta-phosphoric acid with dithiothreitol, which reduces de-hydro ascorbic acid to ascorbic acid. Compounds in the extract were separated by reverse phase HPLC, and ascorbic acid was detected by amperiometrically at 0.6 V and quantified using external standards (Mæland and Waagbø, 1998). The B vitamins, thiamine and riboflavin, were analysed by semi-automated microbiological methods which are detailed in Mæland et al. (2000). Detection and quantification limits of the methods relative to wet weight of the sample are 0.35 and 1.1 µg/g for vitamin C, 1.3 and 4.3 µg/g for riboflavin, and 0.02 and 0.2 µg/g for thiamine, respectively.

2.4. Statistical analysis

Differences in biochemical indices were tested by Students *t*-test after checking for normal distribution by Kolmogorov–Smirnov tests for normality (goodness of fit, Lilliefors *P*-values). Student *t*-tests were carried out for copepods between the two years, and between copepods and copepod nauplii in 2001. Whenever the biochemical indices were percentages, arcsine transformation was carried out before statistical testing as suggested by Sokal and Rohlf (1995). Differences among means were considered statistically significant at $P<0.05$.

3. Results

3.1. Hydrography and phytoplankton

Temperature in Svartatjern during sample collection (Table 1) typically started between 7 and 9 °C in the spring, rising in May to around 15–16 °C with a peak of 18–19 °C before emptying the pond in mid-summer. After refilling in late July, temperature was in the range of 17–18 °C until early September, and dropped gradually to 7–6 °C at early December. Salinity started in the range of 30–31‰ every time the pond was filled, but dropped slowly over time due to precipitation run-off. At salinities below 24‰, new salt water was pumped into the system. Average salinity was 25.2 and 26.3‰ for 2000 and 2001, respectively (Table 1). Oxygen saturation fluctuated with algal production, being highest during periods of net primary production at good light conditions (March to October). During intensive primary production in May and June, water became supersaturated with oxygen (up to 160% saturation) and with corresponding high pH level up to 9.1 (Table 1). Average Secci disc readings were 1.4 and 1.7 m in 2000 and 2001, respectively. Algal nutrients (Table 1) were low during the seasons of net primary production, but increased quickly from mid-October when light intensity and photoperiod declined.

Many of the phytoplankton species present in the pond were small (3–5 µm) single-celled specimens that were not possible to identify. This confined between 81.9 and 99.9% of monads and flagellates, which overall was the most abundant phytoplankton group (Fig. 1), with densities in the range of 21 to 378 cells/µl (2000), and 1 to 269 cells/µl (2001). Both years, cell densities of monads and flagellates fell

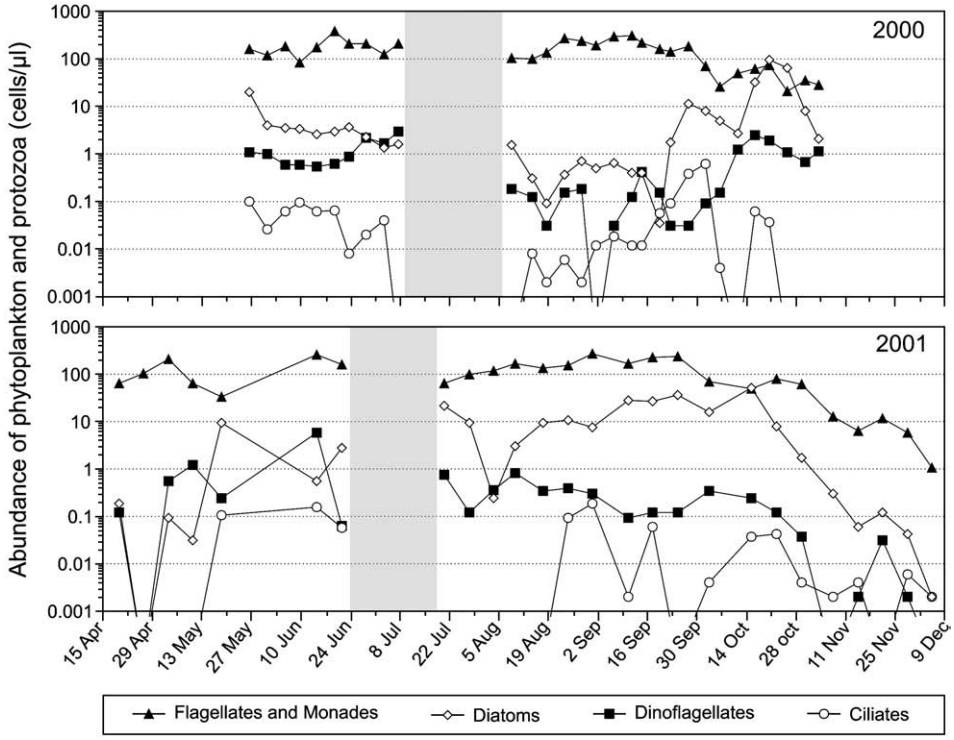

Fig. 1. Densities of major phytoplankton groups and protozoans in the Svartatjern pond during collection of copepods in 2000 and 2001. The grey areas indicate when the pond was drained and refilled during the summer season. Note that the ordinate is logarithmic. Values indicated by lines below the abscissa equal zero.

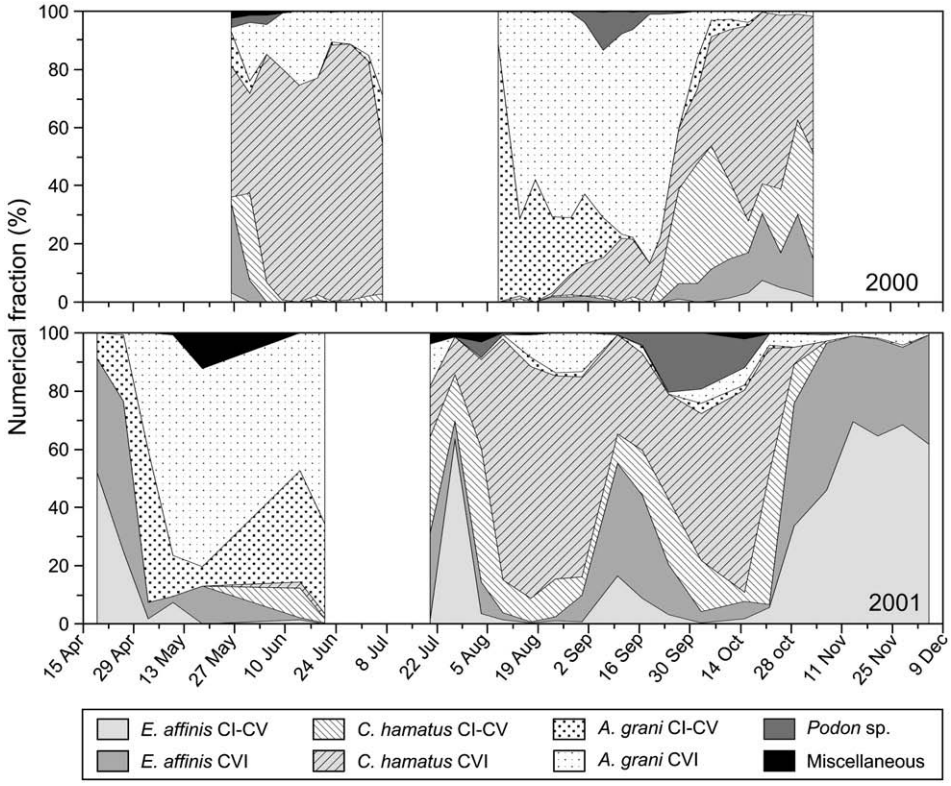

Fig. 2. Relative abundance (percent of numbers) of the copepods *Eurytemora affinis*, *Centropagus hamatus*, and *Acartia grani*, the cladoceran *Podon* sp., and miscellaneous zooplankton (including other copepods) in the samples collected from the Svartatjern pond in 2000 and 2001.

below 30 cells/µl at end of October. Similarly, all other phytoplankton groups also quickly declined in late autumn (Fig. 1). Considering abundances above 5 cells/µl, *Rhizosolenia fragilissima* was initially the most abundant diatom (Bacillariophyceae) with 19 cells/µl during late May of the 2000 season. This was followed by the green algae (Chlorophyceae) *Gloeocystis* sp. (5 cells/µl) and *Oocystis* sp. (11 cells/µl) in last half of June, with late September appearance of the diatoms *Skeletonema costatum* (10 cells/µl) and a small *Chaetoceros* sp. (93 cells/µl) in October.

In 2001, the green alga *Nephrocytium* sp. (11 cells/µl) was abundant in April and first half of May, followed by *Gloeocystis* sp. (22 cells/µl) and *Oocystis* sp. (16 cells/µl) that lasted until end of August. *R. fragilissima* peaked at 9 cells/µl in late May, but was abundant until late July. Among the diatoms, a small *Thalassiosira* sp. bloomed to 12 cells/µl in late July and lasted to mid-October, while N*itzschia closterium* went up to a maximum of 43 cells/µl during it's blooming period in September and October. Other algae just exceeding 5 cells/µl in 2001 were *Katodinium* sp. (Dinophyceae) in mid-June and *Emiliania huxleyi* (Haptophyceae) in late July. Ciliates were often dominated by *Strombidium* sp, and reached high levels of more than 100 cells/ml several times during late spring and autumn both years (Fig. 1).

3.2. Copepod species and stages

Three species of copepods dominated the samples from Svartatjern: *Eurytemora affinis*, *Centropages hamatus*, and *Acartia grani* (Fig. 2). These copepods typically occurred in single or paired dominance, and a substantial fraction of all three species together was therefore rarely observed and only during short transitions. In 2001, the common succession pattern previously observed in Svartatjern from spring to autumn (*Eurytemora–Centropages–Acartia–Centropages–Eurytemora*) was shifted, as *A. grani* had its main season before the pond was emptied at mid-summer, and therefore overlapped with *E. affinis* in May. In this sense, the seasonal succession pattern diverged the two years of copepod collection. Other copepod species constituted maxima of 2.4% (2000) and 3.4% (2001) of the total zooplankton items in the samples (Fig. 2). Of non-copepod zooplankton species in Svartatjern, the cladoceran *Podon* sp. occurred only during short periods and contributed up to 13.1% (2000) and 20.5% (2001) of single samples (Fig. 2). *Podon* sp. was most abundant during September both years. The other brief contributor to the non-copepod zooplankton was young medusa stages of *Sarsia* sp, with 11.3% of the plankton numbers and only found in the 18-May-sample of 2001.

The nauplii sample from April 2001 contained both copepod nauplii (55%) and first copepodid stages (45%). In this sample, 32% was *A. grani*, while *C. hamatus* and *E. affinis* constituted the rest. Copepodids were not found in the other two nauplii samples from late July and mid-September 2001, in which *A. grani* comprised 65 and 39%, respectively. In the Hyltro lagoon sample, *E. affinis* constituted 43.3% of enumerated zooplankton, while other observed zooplankton species or groups were the copepod *Paracalanus parvus* (2.7%), copepod nauplii (16.7%), decapod zoeae (32.4%), and *Sarsia* sp. medusae (4.8%).

3.3. Zooplankton size, dry matter and ash content

Individual copepod DW (Fig. 3, Table 2) was in the ranges of 5.3–13.7 µg (2000) and 4.2–13.9 µg (2001). In 2000, DW increased with time and reached maximum values in late June, and another maximum in October. In contrast, the 2001 copepods were biggest in late May, and smallest in November. The DW of individual zooplankton from the Hyltro lagoon was 9.9 µg. Copepod nauplii (Table 2) had low DW in two of the samples (0.18 and 0.25 µg per nauplius at end of July and

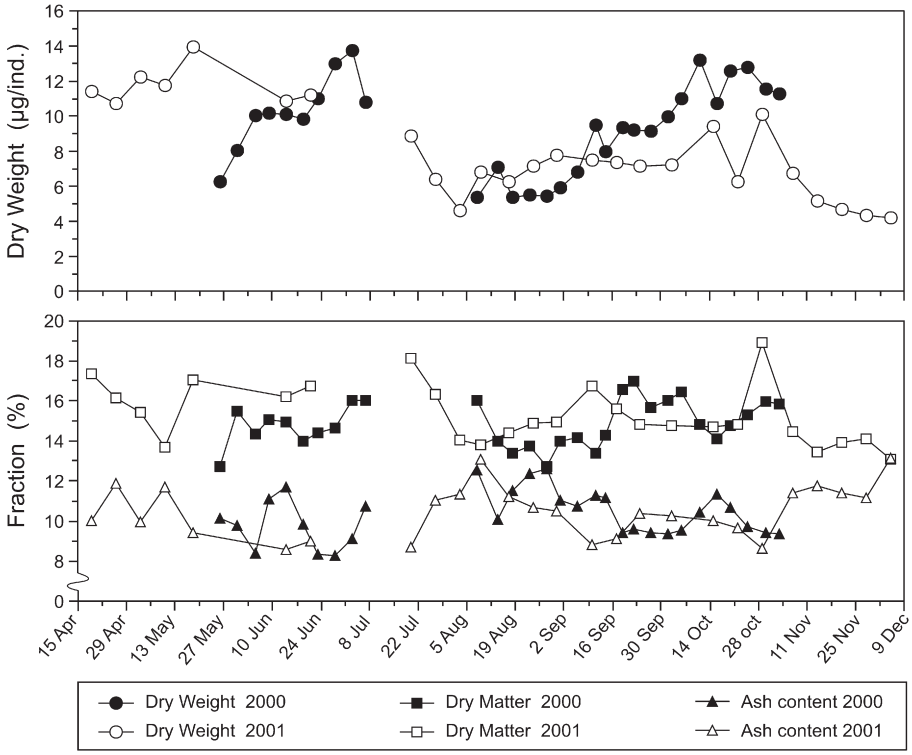

Fig. 3. Dry weight of individual copepods, dry matter (% of wet weight), and ash content (% of dry weight) from the 2000 and 2001 samples collected in the Svartatjern pond. Note that the ordinate is broken in the lower panel.

mid-September, respectively), while DW was 1.46 µg per nauplius in the late-April sample, reflecting a higher content of young copepodid stages observed in this latter sample. The rotifers weighed 0.61 µg per individual (Table 2), while 1-day-old *Artemia* was 2.12 and 2.14 µg and 3-day-old *Artemia* was 2.48 µg.

Dry matter content in the copepods was quite stable and averaged 14.9 and 15.3% of WW for 2000 and 2001, respectively (Fig. 3, Table 2). Dry matter content of the nauplii was quite similar to the copepods (Table 2). In contrast, the zooplankton contained more dry matter (17.7%). Rotifers contained less dry matter (13.2%) than copepods, and *Artemia* even less (8.9–10.8%).

Average ash content was quite constant both years, and within 9.5 and 10.5% of DW for the copepods, nauplii, rotifers, and *Artemia* (Table 2, Fig. 3). This contrasted the zooplankton sample, which contained 14.3% ash.

3.4. Lipids and fatty acids

The total lipid content (TL) in the copepods was relatively low and stable, with the exception of one sample that was 220 µg/mg DW, corresponding to 22% of DW (Table 2, Fig. 4). Average copepod TL in 2000 and 2001 was close (108 and 111 µg/mg DW), while TL of the copepod nauplii (86 µg/mg DW) was significantly lower than in the copepods. The zooplankton had higher TL than that found in copepods and was more similar to the rotifers (Table 2), while *Artemia* was loaded up with a lipid content of approximately 25% of DW.

Regarding lipid class composition, the main components of the copepod neutral lipids were TAG and cholesterol. TAG averaged 2.6 and 2.2% of copepod DW in 2000 and 2001, respectively, which corresponded to 21.9 and 20.2% of TL for the two years (Table 2, Fig. 4). Similarly, mean cholesterol levels were 1.5 and 1.3% of copepod DW, equivalent to 13.2 and 12.4% of TL in 2000 and 2001, respectively. Copepod nauplii had lower fractions of TAG and cholesterol than the average values of the copepods samples, but only statistically significant for cholesterol. TAG showed a large variation among both copepod and nauplii samples. The zooplankton displayed almost twice the amount of TAG (4.2% of DW and 29.4% of TL) compared to copepods, and rotifers had even more TAG (6.1% of DW and 39.4% of TL). In *Artemia*, TAG constituted as much as 16.8–19.6% of DW (69.0–77.1% of TL). It should also be noted that the algae-derived neutral glycoglycerolipids (galactocides) in combination with neutral glycosphingolipids (cerebrocides) or sulfoglycoglycerolipids (sulfolipids) were more or less absent in rotifers and *Artemia* (Table 2: MGDG+CB and DGDG+SL). Significant differences in copepod neutral lipids between the two years were only detected for MGDG+CB.

Amounts of polar lipids in the copepods averaged 6.2 and 6.3% of copepod DW for 2000 and 2001, respectively, with a relatively stable fraction averaging 57.1% (2000) and 58.2% (2001) of TL (Table 2, Fig. 4). Polar lipid content in rotifers was more similar to copepods, constituting 6.1% of DW but corresponding only to 39.8% of TL. Copepod nauplii and the zooplankton sample had somewhat lower content of polar lipids (5.4 and 5.1% of DW, equivalent to 63.1 and 36.0% of TL, respectively). In contrast, polar lipids in *Artemia* were lower and between 3.9 and 5.0% of DW (15.4–20.5% of TL). The major phospholipids in copepods and copepod nauplii were PC and PE, each having average levels between 1.5 and 2.0% of DW and 17.6–20.5% of TL (Table 2, Fig. 4). Significant difference in copepod phospholipid class composition between the two years was only found for PS. PC and PE also dominated phospholipids in the zooplankton and the rotifer samples (1.5–2.1% of DW and 10.6–13.7% of TL), as well as in the *Artemia* samples (1.2–1.9% of DW and 4.8–7.6% of TL).

In the copepods, PUFA dominated the TL fatty acid composition, accounting for 63.3 and 64.2% of TL in 2000 and 2001, respectively (Table 2). Variation in PUFA was low between the samples within each

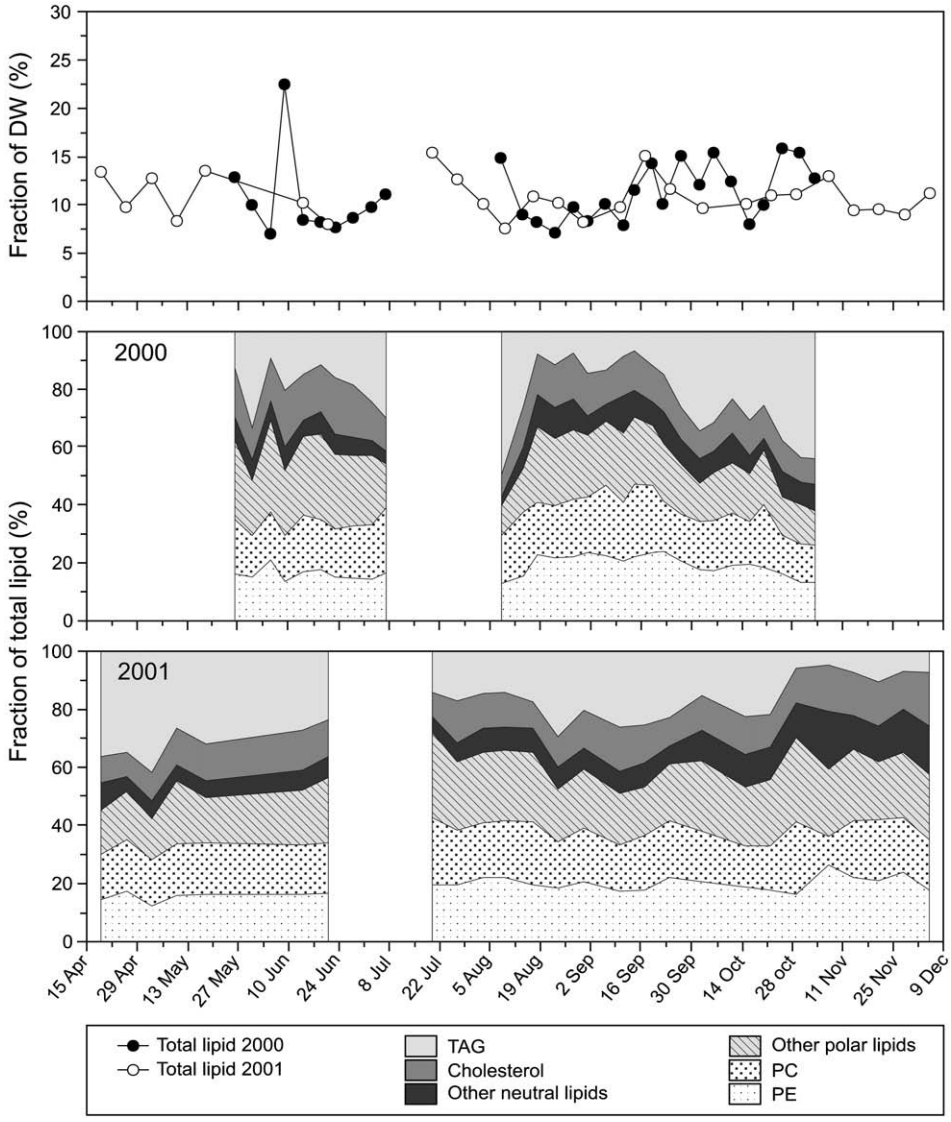

Fig. 4. Total lipid content relative to dry weight (DW) and relative abundance of major lipid classes in the copepod samples from the Svartatjern pond in 2000 and 2001. See Table 2 for explanation of abbreviations.

year. Although not significantly different from the copepods, PUFA fraction in copepod nauplii was even higher (69.4% of TL), on the expense of MUFA. Zooplankton was more similar to rotifers and *Artemia*, with PUFA levels ranging between 43.6 and 48.5% of TL. Compared to copepods, these reduced levels of PUFA were balanced by increased fractions of MUFA (20.1–34.8% of TL).

Among the single fatty acids, DHA was abundant in the copepod samples, averaging 34.4 and 32.9% of TL for 2000 and 2001, respectively (Table 2, Fig. 5). The copepod nauplii averaged 40.5% DHA, which was significantly higher than for the copepod samples in 2001. These high levels contrasted the DHA fraction of 17.3% found in the zooplankton sample. In the intensively produced live feed, DHA was between 10.6 and 20.0%, with highest level in the 3-day on-grown *Artemia*. In the copepods, averages of EPA were between 16.2 and 17.4% of TL, including copepod nauplii and zooplankton. However, in rotifers and *Artemia* EPA was lower, ranging between 7.1 and 9.2%, respectively. Another abundant fatty acid was palmitic acid (16:0), which was between 13.7 to 19.7% of TL in all groups (Table 2). Among other important fatty acids, ARA was very low in the copepod and copepod nauplii samples and even below detection limit in many samples. This contrasted that of zooplankton, rotifers, and *Artemia* where ARA was more abundant, ranging between 1.6 and 3.2% of TL. Significant differences in fatty acids composition between the copepod samples from 2000 and 2001 were mainly found among the fatty acids with 18 carbon atoms (C18), along with myristic acid (14:0). Similarly, significant lower fractions among C18 fatty acids were also found for copepod nauplii when compared with the copepod samples from the same year (Table 2).

The average DHA/EPA ratio was 2.1 and 2.2 for copepods in 2000 and 2001, respectively (Table 2, Fig. 5). Copepod nauplii had somewhat higher DHA/EPA ratio, but not significantly different from the 2001 copepods. The zooplankton had the lowest DHA/EPA ratio (1.1), while intensively reared live feed varied between 1.4 and 2.2, the latter belonging to 3-day on-grown *Artemia*. The EPA/ARA ratio was in general very high in copepods and copepod nauplii (on average between 23.2 and 27.7), and also relatively high in the zooplankton sample (10.3). This contrasted the EPA/ARA ratios in rotifers (3.7) and *Artemia* (2.9–4.0). A similar pattern was seen for the (n-3)/(n-6) ratio,

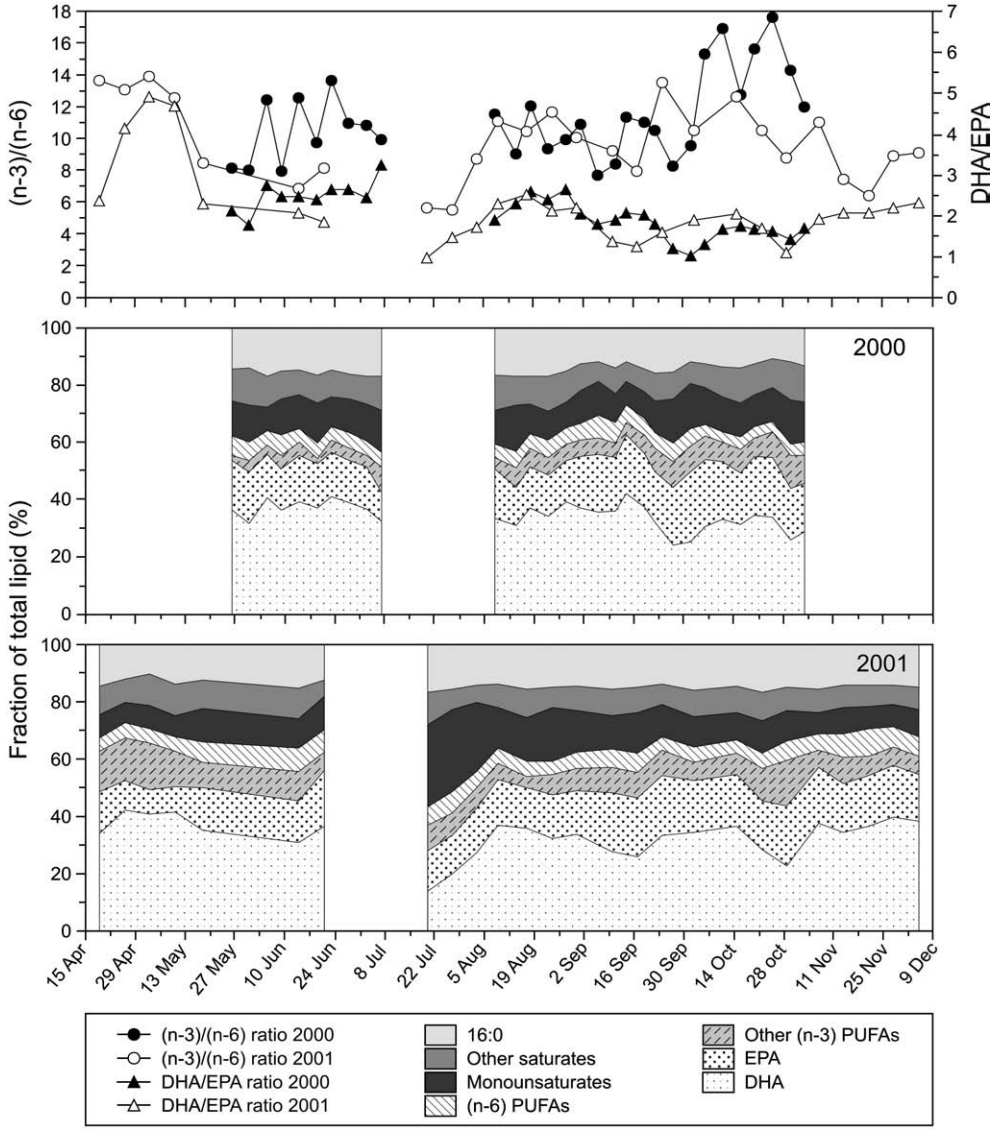

Fig. 5. Fatty acid ratios and major fatty acids extracted from total lipids of copepods samples from the Svartatjern pond in 2000 and 2001. See Table 2 for explanation of abbreviations.

which was highest in copepods and lowest in the rotifers (Table 2, Fig. 5).

3.5. Protein and protein-bound amino acids

The protein content determined by the Lowry method using BSA as reference standard averaged 38.3 and 56.5% of copepod DW for 2000 and 2001, respectively (given as µg/mg DW in Table 3). This difference was significant, but did not correspond to a similar magnitude in the protein calculated from weight-specific protein-bound amino acids (PAA_w). Although still significantly different, the average PAA_w values in copepods from the two years were more similar, and corresponded to 44.4 and 41.3% of copepod DW in 2000 and 2001, respectively. Variation in PAA_w over time was low (Table 3, Fig. 6) as indicated by a coefficient of variation close to 10%. No significant correlations were observed between protein determined by the Lowry method and protein calculated as PAA_w for any of the two years with copepod samples. Some discrepancy also occurred between the two methods of protein content determination in the zooplankton sample (36.6 vs. 30.3% for the Lowry vs. PAA_w method), while protein contents determined by the two methods were more in agreement for rotifers and *Artemia* samples (Table 3). Rotifers were lowest in PAA_w-calculated protein content (24.8% of DW), followed by 1-day-old and 3-day-old *Artemia* (27.8 to 36.8% of DW). The reasons for the discrepancies in protein determination between the Lowry and the PAA_w methods for zooplankton and copepods were not clarified.

The concentration of protein-bound amino acids (PAA_c) was lowest in rotifers (2.3 µmol/mg DW), being almost half of that in copepods in 2000 (4.1 µmol/mg DW) (Table 3). All concentration-specific PAA and IAA indices applied on the copepod samples were significantly different between 2000 and 2001, but with low variation within each of the years (Table 3, Fig. 6). Considering all prey types sampled, the concentration-specific IAA fraction of PAA (IAA_c/PAA_c) was between 40.4 and 43.7%. Similarly, the IAA_c/DAA_c ratio of the hydrolysed protein averaged 0.68 and 0.70 in the copepod samples from 2000 and 2001, respectively (Table 3), while for the rotifers and *Artemia* it was higher (between 0.75 and 0.78). In contrast, the IAA_c/DAA_c ratio in the zooplankton sample was 0.71, and more in accordance with the copepods.

In the PAA_c, leu, val, lys, and ile were the most dominant IAA in all samples, followed by arg, phe, and thr (Table 3). Among DAA, glu + gln,

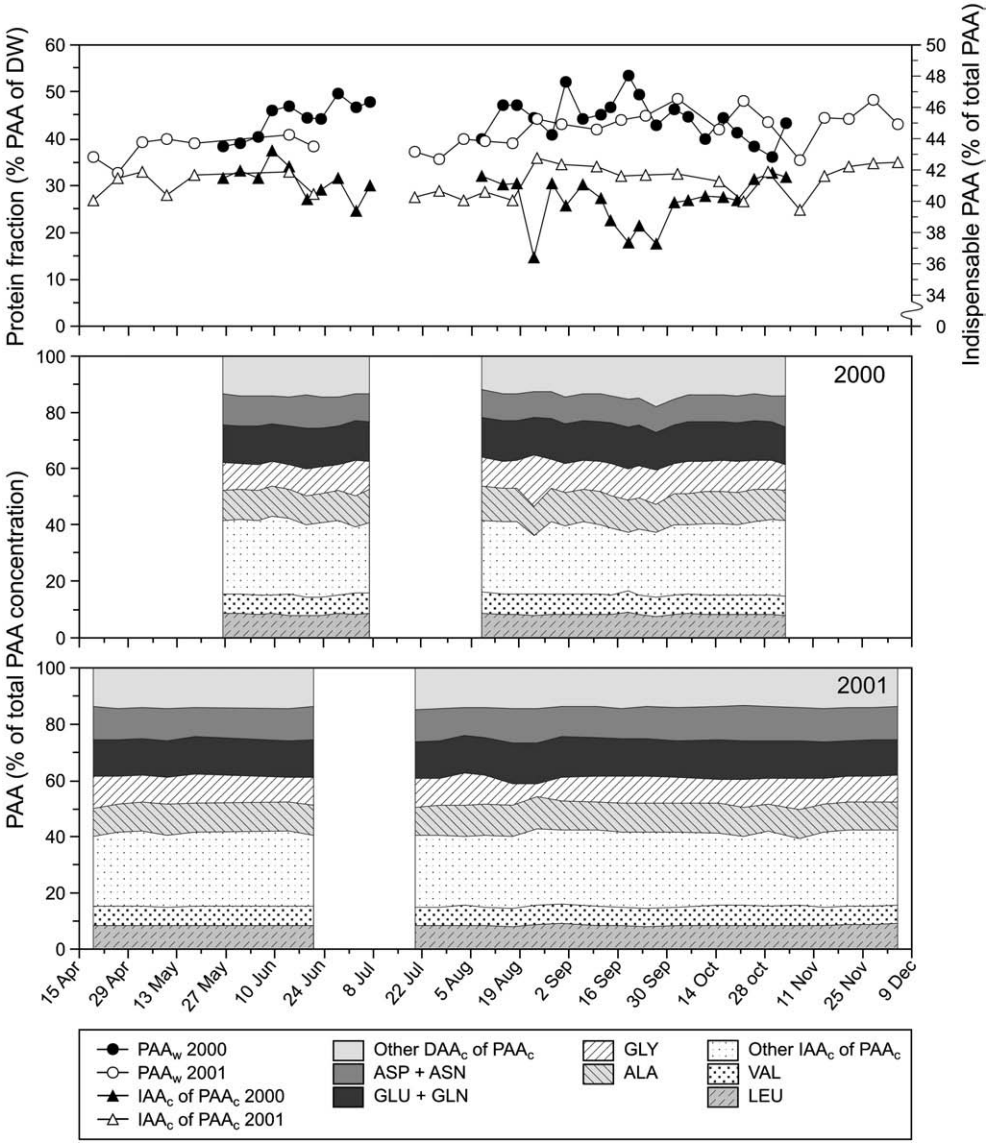

Fig. 6. Protein fraction relative to dry weight (DW) and calculated from protein-bound amino acids (PAA), fraction of indispensable PAA, and relative abundance of amino acids with a major contribution to PAA in the copepod samples from the Svartatjern pond in 2000 and 2001. See Table 3 for explanation of abbreviations. Note that the right ordinate is broken in the upper panel.

asp+asn, ala, and gly were the most abundant amino acids. Concentrations of all amino acids, except lys and asp+asn, were significantly different between the copepod samples of the two years (Table 3). In absolute values, amino acid concentrations were generally lower in the zooplankton, rotifers, and *Artemia*, compared to the copepods (Table 3). However, regarding the amino acid profiles expressed as percentage of the hydrolysed copepod protein, they were similar the two years of sampling (Fig. 6), with no significant differences found for major IAA as thr, leu, lys, and ile. Also the zooplankton, rotifers, and *Artemia* PAA profiles showed similarities with the copepods. The observed differences can be attributed to very low variation in fractions of single amino acids in the hydrolysed protein (Fig. 6), typically displaying coefficients of variation between 3 and 15%.

3.6. Free amino acids

The weight-specific content of free amino acids (FAA_w) in the copepod samples from Svartatjern varied between 4.3 and 8.9% of copepod DW, averaging 5.6 and 6.5% for 2000 and 2001, respectively (given as μg/mg DW in Table 4). The average FAA_w content of the copepods was significantly different between the two years. In the zooplankton sample, FAA_w was in the upper range of the levels observed in the copepods and composed 8.6% of the zooplankton DW, while in the intensive reared live feed FAA_w was considerably lower than in copepods and corresponded to 1.7% in rotifers and 2.6 to 3.4% in *Artemia*.

Concentration of free amino acids (FAA_c) was lowest in rotifers and *Artemia*, higher in copepods, and highest in the zooplankton (Table 4). The absolute levels of indispensable free amino acid concentration (IAA_c) in copepods were not significantly different between 2000 and 2001. However, significant differences among copepods occurred between the two years when other concentration-specific IAA indices like IAA_c/FAA_c and IAA_c/DAA_c ratios were considered, and among concentrations of most individual FAA (Table 4). Only the rotifers had a higher IAA_c/FAA_c fraction (30.6%) than the copepods (19.1–24.3%), with *Artemia* and zooplankton displaying the lowest $IAA_c/$

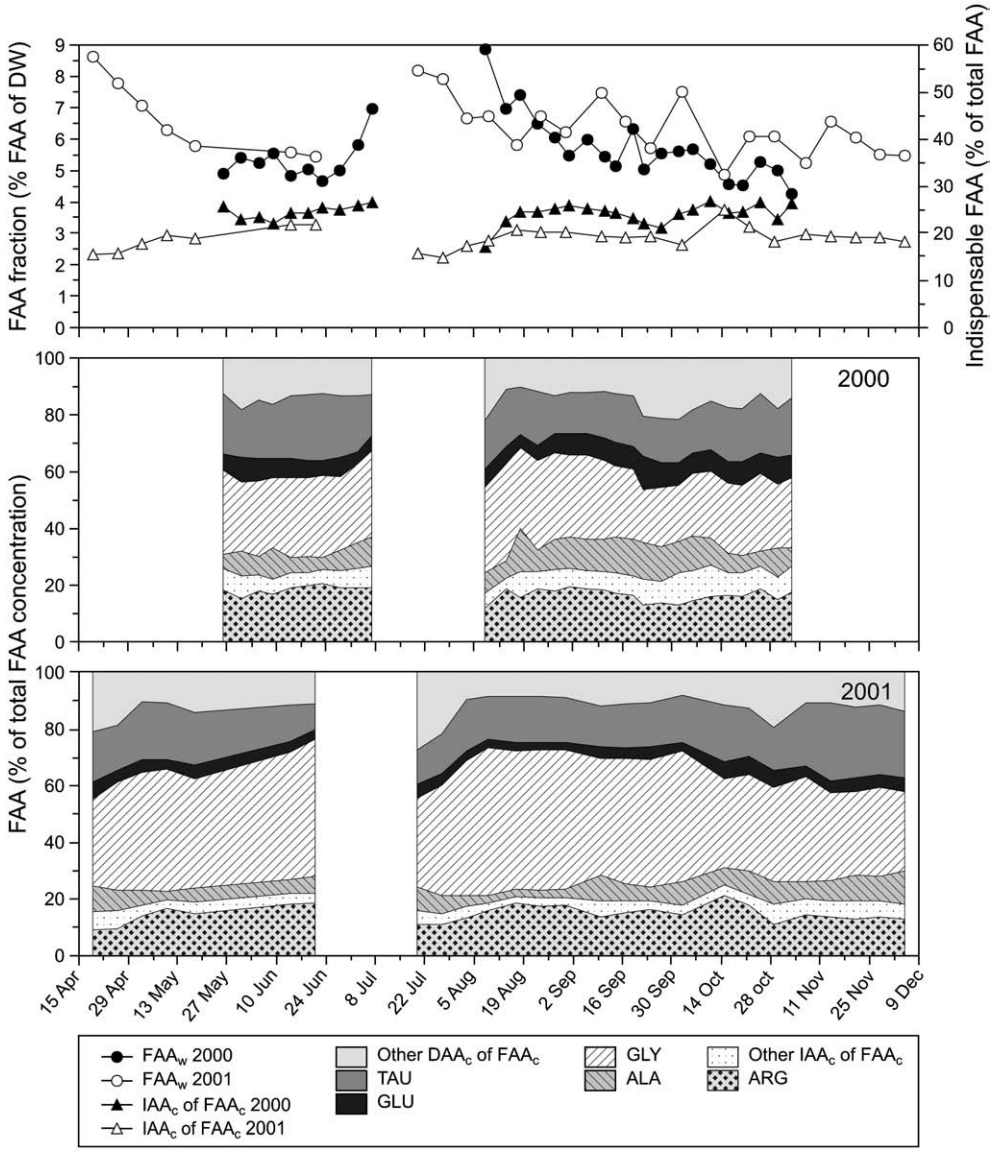

Fig. 7. Fraction of free amino acids (FAA) relative to dry weight (DW), fraction of indispensable FAA, and relative abundance of amino acids with a major contribution to FAA in the copepod samples from the Svartatjern pond in 2000 and 2001. See Table 4 for explanation of abbreviations.

FAA_c fractions (10.0–15.6%). A similar pattern was demonstrated for the IAA_c/DAA_c ratio. Variation in all IAA_c indices was low among the copepod samples each year (Table 4, Fig. 7).

Assuming similar levels of thr in 2001 as in 2000, the averaged copepod FAA_c profiles expressed as percentage (relative abundance) were dominated in decreasing order by gly, tau, arg, and ala (26.9–9.0%, totalling 70.6% of FAA_c in 2000, and 39.0–6.1%, totalling 76.9% of FAA_c in 2001). In the zooplankton sample, the four most abundant amino acids were in decreasing order gly, tau, pro, and arg (30.3–8.8%, totalling 72.8% of FAA_c), with also ala being abundant (8.8%). In rotifers, the FAA_c profile was more diverse, and the four most abundant amino acids included ser, glu, arg, and tyr (13.2–8.5%, adding up to 44.3% of FAA_c). The four most abundant FAA_c in the *Artemia* samples were all DAA and comprised tau, ala, pro, and glu (averaged to 24.3–12.6% which summed up to 68.0% of total FAA_c). Relative abundance of single amino acids in the FAA_c profiles throughout the sampling season was more variable compared to the PAA_c profiles (Figs. 6 and 7).

Considering all copepod samples of both years, total FAA concentration correlated significantly with salinity ($R^2=0.379$, $P<0.0001$), where increased salinity elevated the total FAA_c level. Among individual amino acids of the FAA_c pool, significant positive correlation with salinity was found for gly ($R^2=0.466$, $P<0.0001$), pro ($R^2=0.174$, $P=0.0013$), and arg ($R^2=0.131$, $P=0.0061$), while asn had a weak but significant negative correlation ($R^2=0.122$, $P=0.0083$).

3.7. Pigments and vitamins

Astaxanthin was abundant in the copepods, and the levels were relatively similar between 2000 and 2001 (Table 5, Fig. 8). The copepod astaxanthin content was lowest during the two weeks after mid-summer, with minimums of 321 and 362 μg/g DW in 2000 and 2001, respectively. In 2000, the copepod astaxanthin level reached 832 μg/g DW in mid-October, while in 2001 the levels continued to rise and peaked in mid-November at 1422 μg/g DW. In the zooplankton sample, astaxanthin was about 25% of the average copepod pigment

Table 5
Pigments and vitamins in copepods, zooplankton (copepods and decapod zoeae), rotifers, and *Artemia* (1-day or 3-day after hatching)

	Abbreviations	Svartatjern		Hyltro	Intensive live feed			
		Copepods	Copepods	Zooplanktn	Rotifers	*Artemia*		
		2000	2001	2001	IMR [a]	1-day [b]	1-day [c]	3-day [c]
Pigments *(μg/g DW)*								
Number of samples	*N*	30	26	1	1	1	1	1
Astaxanthin		626.9±139.1	747.7±296.8	197.9	24.0	n.d.	n.d.	n.d.
Canthaxanthin		n.d.	n.d.	n.d.	n.d.	752.4	744.7	654.0
β-Carotene		n.d.	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
Lipid-soluble vitamins *(μg/g DW)*								
Number of samples	*N*	16	19	1	1	1	1	1
Retinol	Vitamin A	tr.	n.d.	0.2	0.2	– [d]	– [d]	– [d]
Cholecalciferol	Vitamin D_3	n.d.	n.d.	n.d.	0.9	0.7	1.8	1.0
Total Tocopherol	Vitamin E_{tot}	112.0±28.1	114.0±61.3	114.0	513.1	571.8	340.2	465.3
α-Tocopherol	Vitamin E_α	108.0±28.5	113.5±61.1	114.0	509.0	562.0	327.8	424.3
β-Tocopherol	Vitamin E_β	0.5±0.2	n.d.	n.d.	n.d.	n.d.	n.d.	n.d.
γ-Tocopherol	Vitamin E_γ	3.5 [e] ±2.3	0.4±1.4	n.d.	4.1	7.4	9.4	32.9
δ-Tocopherol	Vitamin E_δ	n.d.	n.d.	n.d.	n.d.	2.4	3.0	8.1
Water-soluble vitamins *(μg/g DW)*								
Number of samples	*N*	16	19	1	1	1	1	1
Thiamine	Vitamin B_1	23.1±4.7	22.7±11.7	9.2	48.6	18.2	13.0	20.9
Riboflavin	Vitamin B_2	tr.	28.0±3.6	28.9	30.7	53.1	52.1	51.9
Ascorbic acid	Vitamin C	476.6±224.6	552.9±360.2	271.1	220.1	530.6	361.3	372.6

Values are relative to dry weight (DW) and are given as mean±SD when number of samples >1. Values below detection limits (n.d.) and trace amounts (tr.) between detection and quantification limits of the analytical method are indicated.
[a] Institute of Marine Research: rotifers grown on Rotimac and *Isochrysis galbana* algae.
[b] Institute of Marine Research: *Artemia* enriched with DC-DHA Selco.
[c] Austevoll Marin Yngel AS: *Artemia* fed DC-DHA Selco and Algamac 2000.
[d] Interactions in the analytical method caused too high retinol readings for *Artemia*, see Section 2.3.4.
[e] Significant difference between copepods from 2000 and 2001.

content in the corresponding year, while the rotifers similarly contained 3.8% of the copepod astaxanthin content. In all cases, only free astaxanthin was found, and no esters were observed. All *Artemia* samples were devoid of astaxanthin, but contained canthaxanthin in the same ranges as copepod astaxanthin (Table 5). Further, β-carotene was not detected in any of the samples.

Of the lipid-soluble vitamins, vitamin D_3 was either not detected in the copepod samples or found in trace amounts (three of the samples). On average, it was therefore considered below the detection limits of the method (Table 5). The zooplankton sample was also free of vitamin D_3, while levels in rotifers and *Artemia* were 0.9 and 0.7–1.8 μg/g DW, respectively. Further, vitamin A was found in low levels or beyond quantification limits in the copepods. In many samples, vitamin A was even below detection limit, particularly in 2001 (Table 5). Zooplankton and rotifers were also low in Vitamin A (0.2 μg/g DW), and in *Artemia* realistic values for vitamin A were not possible to quantify due to analytical problems (see Section 2.3.4). Vitamin E was abundant in all samples (Fig. 8) and was dominated by the isomer, E_α (Table 5), constituting between 90 and 100% of total vitamin E. No other isomers were detected in the zooplankton sample, while the remaining vitamin E in the copepods was E_γ and E_β, the latter only observed in 2000. Both rotifers and *Artemia* showed low levels of vitamin E_γ, and in addition *Artemia* displayed low but consistent levels of vitamin E_δ, not found in the other feed types.

In the water-soluble vitamins, copepods showed high but variable levels of vitamin C (Table 5, Fig. 8). Vitamin C in zooplankton, rotifers, and *Artemia* was within the range of one standard deviation of the average values observed in the copepods. In copepods, levels of thiamine was consistent and well above the quantification limit of the method, with some variation between the years at different seasons (Fig. 8). Thiamine was also abundant in zooplankton, rotifers and *Artemia* (Table 5). In contrast, riboflavin values varied around quantification limit of the method in copepods (Fig. 8), zooplankton and rotifers, while *Artemia* had slightly higher levels (Table 5).

4. Discussion

The biochemical composition of the copepods from Svartatjern was generally characterised by substantial amounts of polar lipids, high levels of n-3 PUFA (particularly DHA and EPA), protein with a diverse amino acid contribution in the PAA profile (both for IAA and DAA), FAA dominated by few amino acids (gly, tau in DAA and arg in IAA), high levels of astaxanthin, and considerable amounts of vitamin C and vitamin E. In addition, compounds like β-carotene and vitamin D_3 were virtually absent in the copepods, while vitamin A and riboflavin were in the range of trace limit concentrations. Further, the biochemical composition showed surprisingly high stability between years or seasons within a year, despite large changes in copepod species composition and environmental conditions (e.g. photoperiod, temperature and salinity). However, the zooplankton sample from the Hyltro lagoon contrasts that of Svartatjern copepods in containing more lipids with less PUFA and DHA. In addition, the zooplankton had less protein, somewhat different FAA profile

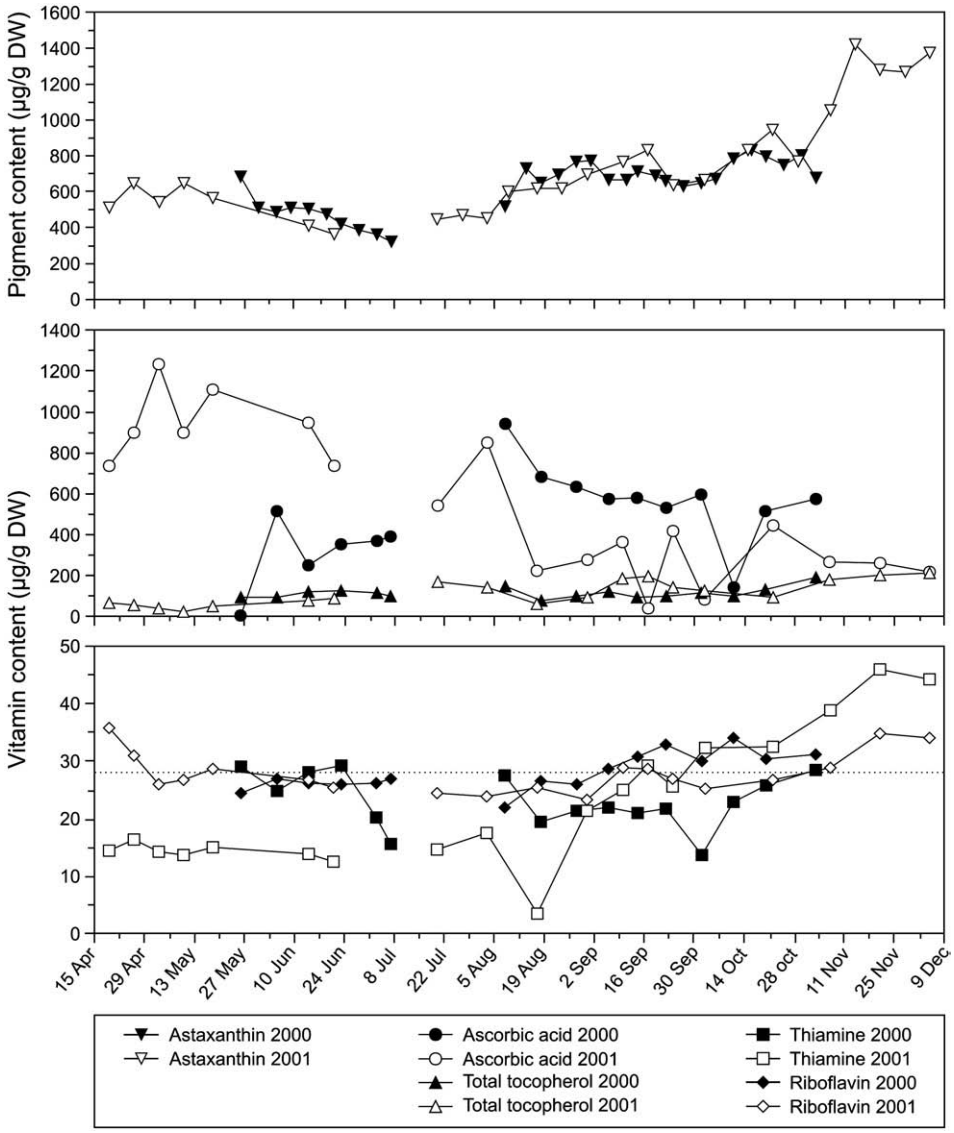

Fig. 8. Content of pigments and vitamins relative to dry weight (DW) in the 2000 and 2001 copepod samples from the Svartatjern pond. Dotted line in lower panel indicates quantification limit for riboflavin at the present analytical method.

with more dispensable FAA, along with lower astaxanthin and vitamin C content. These discrepancies may most likely be explained by differences in phytoplankton communities and densities, but also by a different composition of crustacean taxa, since decapod larvae contributed to 32.4% of enumerated plankton in the zooplankton sample. This may also clarify occurrence of slightly heavier individuals with higher fraction of dry matter and ash in the zooplankton, probably because decapod zoeae are more heavily armoured with carapace spines than copepods.

An important question is to what extent Svartatjern represents natural ecosystems, and how this pond-like system may affect the biochemical composition of copepods? The Svartatjern pond system is managed by a specific protocol that implies fertilisation to boost primary production, mixing to prevent stratification, and emptying and refilling according to renewal of copepod plankton from resting eggs (Naas et al., 1991; Næss, 1991). In this sense, copepods from Svartatjern may be regarded as "reared" copepods, although reared on a diverse and natural assemblage of phytoplankton in a large outdoor ecosystem. However, regarding dry matter, ash content, total lipids, and FAA content the Svartatjern copepods were close to or within the mode values for other copepods (reviewed by Båmstedt, 1986), but lower in protein content which on the other hand was in accordance with data reported by Mæland et al. (2000). Protein content may depend on the analytical method, and at present back calculation based on PAA is regarded to be the most precise method for other larval prey (Hamre et al., 2007). Analyses of lipid class composition in copepods are mostly from high-latitude oceanic calanoids (e.g. *Calanus* sp.), which normally are rich in wax esters used as energy source during overwintering and reproduction (Lee et al., 1971; Sargent and Falk-Petersen, 1988; Fraser et al., 1989). The copepod species included in the present investigation are neritic calanoid species that do not overwinter as adults in the pond system. Instead, they use resting eggs as a reproductive mode to ensure survival from one generation to another during unfavourable conditions, e.g. during the seasonable

disruption of the production cycles (Næss, 1996). Storage of wax esters may therefore not be required to the same extent as in the larger *Calanus* sp. The Svartatjern copepods rather resembled naupliar and early copepodid stages of *Calanus* sp, which are rich in structural phospholipids and contain TAG as main storage lipid (Sargent and Henderson, 1986; Sargent and Falk-Petersen, 1988). In this respect, it should be noted that nauplii and the young copepodid stages of *Calanus* sp. are the primary prey for larvae of many fish species.

Lipid content and composition in copepods have been found to be relatively diverse, and to vary with developmental stage, species, feed preference, latitude, season, and life cycle strategy (Båmstedt, 1986; Sargent and Falk-Petersen, 1988; Fraser et al., 1989; Norrbin et al., 1990; Støttrup, 2003). The Svartatjern copepod lipid composition may therefore be regarded as within the natural variation among copepods. Supporting this is also the high levels of certain fatty acids like 16:0, EPA, and DHA, which are in concordance with several other studies on neritic calanoid copepod species (Evjemo and Olsen, 1997; Evjemo et al., 2003; Sørensen et al., 2007). Further, FAA in the Svartatjern copepods was dominated by gly, tau, arg, ala, and pro, in a similar order and magnitude as in other calanoid copepods (Båmstedt, 1986; Helland et al., 2003a,c). Astaxanthin, thiamine, riboflavin, vitamin C, and vitamin E were within the ranges previously reported for copepods (Fisher et al., 1964; Hapette and Poulet, 1990; Rønnestad et al., 1999a; Mæland et al., 2000). It may therefore be concluded that in most biochemical indices, the Svartatjern copepods fell well within the variation observed for copepods collected elsewhere. Thus, despite the manipulations imposed for enhancement of primary production in the Svartatjern pond system, the copepods preserved their similarities with wild copepods. Similar preservation of nutritional composition has been reported from other zooplankton production systems (Mischke et al., 2003). This indicates that the diverse phytoplankton and protozoan communities in Svartatjern were conserved, preventing extreme lipid and fatty acid profiles which can appear when one or two sub-optimal algal species are used in intensive copepod culture systems (McKinnon et al., 2003). Copepods from Svartatjern have been used in several larval finfish studies, and have shown to support very high growth and survival rates, and good juvenile quality (van der Meeren et al., 1993, 1994; Næss et al., 1995; Conceição et al., 1997; McEvoy et al., 1998; van der Meeren and Lønøy, 1998; Finn et al., 2002; Hamre et al., 2002; van der Meeren and Moksness, 2003). Consequently, these copepods should represent a nutritionally adequate feed for many larval fish species, and the data on biochemical composition may therefore serve as a base for nutritional improvements of enrichment media used in culture of intensive-produced live feed for marine fish larvae, as well as for nutritional optimisation of early weaning formulated diets.

Inadequate nutritional composition of intensive-produced live prey has been considered an important bottleneck in the production of high-quality juvenile marine fish, and a substantial effort has been put into development of adequate live feed enrichments (Støttrup, 2003; Marcus, 2005). Comparison between copepods, rotifers, and *Artemia* data of the present study suggests a considerable potential for improvement of enrichment emulsions. Recent advances in knowledge about lipid and fatty acid requirements of marine fish larvae have pointed out the importance of phospholipids, DHA, EPA, ARA, and the ratios of such PUFA for optimal lipid digestion, normal larval development, larval survival and growth, and stress tolerance (Olsen et al., 1991; Coutteau et al., 1997; Kanazawa, 1997; Sargent et al., 1999; Shields et al., 1999; Izquierdo et al., 2001; Bell et al., 2003; Cahu et al., 2003; Hadas et al., 2003; Støttrup, 2003). Compared to rotifers and *Artemia*, the Svartatjern copepods were loaded with EPA and DHA. DHA was particularly abundant in the copepod nauplii, indicating the importance of this fatty acid in the nutrition of young fish larvae whose initial exogenous feed would be such prey. The high EPA/ARA ratio in the copepods should be noted, as successful pigmentation during metamorphosis in flatfish larvae may be dependent on this (Hamre et al., 2007). Considering the fraction of phospholipids relative to total lipid, copepods were rich in phospholipids (57–63%) compared to rotifers (40%) and particularly to *Artemia* (15–20%). However, taking into account phospholipids per mg live prey biomass, differences were lesser (Table 2), probably due to the higher lipid content of the intensive prey types from enrichment. Most enrichment oils for rotifers and *Artemia* are usually TAG, and enhancing the phospholipid content of the prey by enrichment has turned out to be difficult (Rainuzzo et al., 1997; Harel et al., 1999). This is expressed as accumulation of TAG with increasing lipid levels, with the potential for imbalances in both lipid class and PUFA composition. Dietary phospholipids may enhance larval ingestion (Koven et al., 1998), and phospholipids seem to be necessary for optimal lipid transport and synthesis in the larval digestive system, as well as a number of cell membrane and signalling functions (Bell et al., 2003; Cahu et al., 2003). Also the relative abundance of different phospholipid classes may be of importance for larval growth and development (Geurden et al., 1998). In the present data, both rotifers and *Artemia* displayed many similarities with copepods when the relative composition of the phospholipid profile was compared, indicating that structural lipids in the marine food web are to some extent conservative. Quantitative deviations from the copepod phospholipids were however evident, particularly in *Artemia*. More focus on phospholipid enrichment of live feed and phospholipid supplement in formulated feed is therefore necessary, with the goal to reach balanced levels of lipid classes and PUFA as observed in copepods.

The gut system of young fish larvae has initially high assimilation capability of FAA and low protein digestibility, with a gradual maturation of the proteolytic capacity throughout ontogenesis (Cahu and Zambonino Infante, 2001; Rønnestad and Conceicao, 2005, Kvåle et al., 2007). FAA may serve as both energy substrate and sustain protein synthesis in marine fish larvae (Rønnestad et al., 1999b; Wright and Fyhn, 2001; Rønnestad et al., 2003). The Svartatjern copepods were rich in FAA, and the FAA concentration relative to DW was found to correlate with salinity. This correlation may be explained by the need for copepods to use FAA in osmoregulation (Båmstedt, 1986; Fyhn et al., 1993). Fish larvae may be very efficient in retaining and absorbing FAA from the gut lumen, in particularly IAA (Conceição et al., 2002). However, larval growth potential is

in most cases very high, and daily weight gain may exceed 20% even in coldwater species (van der Meeren and Næss, 1993; van der Meeren et al., 1994; Finn et al., 2002). The observed FAA levels alone in larval live prey cannot sustain the amino acid requirements surged by the protein deposition rate necessary to maintain such high growth rates, and protein digestion must play a significant role in total amino acid availability, absorption, and subsequent protein synthesis. Concordantly, recent studies have shown that young marine fish larvae also are able to utilize peptide chains in protein hydrolysates (Zambonino Infante et al., 1997; Cahu et al., 1999; Hamre et al., 2001), and that amino acids supplied in the diet in this form may reduce larval spinal malformations (Cahu et al., 2003). Peptide digestion may be aided by high activity of peptidases in young fish larvae (Cahu and Zambonino Infante, 2001). Although young fish larvae have limited proteolytic capacity, access to peptide chains and amino acids from dietary protein may be facilitated by autolysis of the ingested prey (Fyhn et al., 1993; Kolkovski, 2001). In this respect, Luizi et al. (1999) noted that copepods were much more readily digested in Atlantic halibut larvae than *Artemia*. Furthermore, in vitro digestibility studies with pancreatic enzymes chosen to mimic the conditions in the larval intestine, show that water-soluble protein is more digestible than insoluble protein (Tonheim et al., 2007). Both in intensive live feed and in copepods there are a large fraction (approximately 50%) of water-soluble protein which has been suggested to be highly bioavailable (Carvalhoa et al., 2003; Srivastava et al., 2006; Kvåle et al., 2007; Tonheim et al., 2007).

Due to the high growth rate of fish larvae the demand for dietary amino acids for protein accretion is especially high, and the supply of all amino acids, IAA as well as DAA, may become critical for sustaining optimal growth. Thus, in juvenile rainbow trout (*Oncorhynchus mykiss*) addition of crystalline DAAs (gln, gly, glu) to an otherwise complete diet significantly increased growth rate and feed efficiency (Schuhmacher et al., 1995). Such experiments have not been performed with marine fish larvae although the suggestions have been made (Wright and Fyhn, 2001). Total amino acid requirements may be related to larval growth rate which again may be affected by a number of physical and biological factors (e.g. temperature, species, larval size and age, and diet characteristics). In salmonids, deficiency in a single amino acid (trp) during the first 4 weeks of exogenous feeding induced scoliosis (Akiyama et al., 1986). Other amino acids (thr, leu, arg, met, lys, and his) have been suggested as limiting when rotifers or *Artemia* is used as feed for marine fish larvae (Conceição et al., 1997, 2003; Aragao et al., 2004b). Deficiencies in these amino acids are mostly inferred from imbalances between larval fish and prey profiles. However, these amino acids were abundant in the Svartajern copepods, either in PAA, FAA, or both. With some exceptions in rotifers, relative composition of amino acid profiles in protein seems to be conserved between different plankton taxa. Since a substantial part of dietary amino acids are in the form of PAA, amino acid deficiency may rather be a matter of protein content in the feed, and how much of this protein that is digestible and thereby available to absorption in the larval gut. In this respect, protein content in rotifers and *Artemia* was lower compared to copepods. Further, dissimilarities in FAA profiles of copepods, rotifers and *Artemia* were more pronounced, and FAA content was highest in copepods. Use of live algae versus commercial enrichment products has induced considerable variation in total amino acid profiles of rotifers and *Artemia* (Aragao et al., 2004a). In absence of more detailed knowledge about specific amino acid requirements, copepods might therefore be regarded as a baseline recipe for protein, PAA, and FAA contents and profiles in feed for marine fish larvae.

Requirements for dietary micronutrients like pigments, vitamins, minerals, and trace elements are little investigated in marine fish, and such studies are particularly scarce for larval and early juvenile stages. Regarding minerals and trace elements, only iodine was analysed from the Svartatjern copepods, as presented elsewhere (Moren et al., 2006). Compared to adult fish, the high growth rates and rapid organogenesis may account for elevated micronutrient requirements and turnover during early developmental in fish (Lie et al., 1997), and recommendations suggested for adult fish (e.g. in NRC, 1993) may therefore not be valid for younger life stages (Mæland et al., 2000). Levels of micronutrients found in copepods that sustain growth and normal development, may be better indices for requirements in larval and juvenile marine fish, and the present study is an attempt to provide such baseline data.

Regarding pigments, the consistent high levels of astaxanthin in the copepods suggest that this compound should receive more attention in larval fish nutrition. Together with canthaxanthin commonly found in *Artemia*, astaxanthin, lutein, and β-carotene belong to the carotenoid family that may serve as precursors for vitamin A in fish (Bendich and Olson, 1989; Christiansen and Torrissen, 1996; Moren et al., 2005; Palace and Werner, 2006). Since β-carotene was not detected in the copepods, astaxanthin and possibly also lutein may be important provitamin A compounds in such plankton (Rønnestad et al., 1998). Astaxanthin have also demonstrated profound antioxidant properties, particularly as a coantioxidant working synergistically with vitamin E in suppressing lipid peroxidation (Bell et al., 2000). Antioxidant action on active oxygen radicals in marine organisms has also been suggested for a number of other carotenoids, including canthaxanthin (Shimidzu et al., 1996), and carotenoids enhanced survival and reduced lipid peroxidation in Japanese flounder larvae (Okimasu et al., 1992). Carotenoids may therefore assist the enzymatic antioxidant system in fish, which is already functional during early larval stages (Peters and Livingstone, 1996; Mourente et al., 1999a; Martínez-Álvarez et al., 2005). Data on biological activities of pigments in fish are scarce, but effects of astaxanthin on skin and muscle coloration are well documented (Torrissen et al., 1989; Chatzifotis et al., 2005). Low intake of astaxanthin may reduce growth in salmonids (Christiansen and Torrissen, 1996), and maternal deficiency may significantly reduce transfer of astaxanthin to the fish eggs and possibly erode survival in the larval stages (Pickova et al., 1998).

Use of dietary carotenoids may be a safe way to provide vitamin A in larval fish, as dietary surplus of vitamin A or its derivates (retinoids) may have detrimental effects on normal bone development (Dedi et al., 1995; Cahu et al., 2003). Retinol and other retinoids seem to be very low or absent in copepods,

and the hidden source of vitamin A in larval fish is probably carotenoids, which are enzymatically cleaved to form retinoids in fish (Moren et al., 2005). In this way, carotenoids may be converted to vitamin A, depending on the retinoid and protein status of the animal (Bendich and Olson, 1989). Similarly to retinoids, β-carotene also seems to be very low or absent in copepods, which may explain why fish, compared to land vertebrates, display less specificity for this carotenoid as a vitamin A source (Palace and Werner, 2006). However, conversion of β-carotene to retinols at a higher rate than with other carotenoids has been demonstrated in juveniles of Atlantic halibut (Moren et al., 2004a), although quantification of this conversion remains to be determined for larval fish. Under the assumption that larval halibut has a vitamin A requirement in the same range as juvenile halibut, astaxanthin levels in copepods or canthaxanthin in *Artemia* could cover the need for this vitamin (Moren et al., 2004a,b). Alternatively, covering vitamin A requirements for larval fish in terms of dietary retinoids needs more attention, since certain retinoids may inflict disruptive actions on fish physiology, development, growth, and survival (Woodward, 1994; Dedi et al., 1995; Furuita et al., 2001; Haga et al., 2002; Moren et al., 2004a; Palace and Werner, 2006), including teratogenic effects on bone development at the level of gene expression (Cahu et al., 2003; Hamre et al., 2007; Lall and Lewis-McCrea, 2007).

Another vitamin not found in the copepods was cholecalciferol (vitamin D_3). This was unexpected, as this vitamin may play important roles in calcium and phosphorous metabolism and affect bone formation and remodelling in vertebrates. Vitamin D_3 is the main storage form in the liver of marine teleosts, and may be converted to 25-hydroxyl vitamin D_3 isomers in various fish tissues (Takeuchi et al., 1991; Graff et al., 1999; Holick, 2003; Lall and Lewis-McCrea, 2007). However, data on effects of dietary vitamin D_3 in larval fish are very scarce. In a recent study of young juvenile Japanese flounder, hypermelanosis on the blind side and vertebral deformities have been reported when dietary levels exceed 5 μg/g vitamin D_3 or 0.5 μg/g 1,25$(OH)_2$ vitamin D_3 (Haga et al., 2004). Copepods may contain vitamin D_3 levels below the analytical detection and quantification limits, or they may contain precursors as the 7-dehydrocholesterol (7-DCH), which is the provitamin responsible for vitamin D_3 production in the skin of terrestrial vertebrates under UV-light irradiation. In this respect, several studies agree on that copepods display either lack of vitamin D_2 and D_3 while 7-DCH is detected in reasonable amounts (Geiger, 1958; Takeuchi et al., 1991; Kenny et al., 2004). Approximately 1.4% of the Svartatjern copepod DW was cholesterol and sterol esters, but 7-DCH was not specifically analysed for. In adult fish, both photo-conversion in the skin and enzymatic dark-transfer of 7-DCH in the liver to vitamin D_3 has been reported (Holick, 2003; Blondin et al., 1967), but also disputed (Takeuchi et al., 1991). No data have been presented on this matter for fish larvae, and this calls for further exploration. If fish is able to convert this provitamin to vitamin D_3 it may account for 7-DCH as a potential important vitamin D source in most stages of planktivorous fish, and explain the paradox of vitamin D_3 enrichment at this trophic level in the marine food web. Photo-conversion implies that such fish has to reside close to daylight at the surface, which e.g. fish larvae or pelagic schooling fish often do. It also means that indoor rearing of larval fish in absence of UV-light might require dietary vitamin D_3, which is actually supplied in rotifers and *Artemia* due to use of fish oils in the enrichment emulsions (Table 5). Since 7-DCH occurs naturally in fish liver (Takeuchi et al., 1987), enzymatic dark-conversion is an intriguing aspect that also needs further investigation. However, with the enormous potential of prey ingestion in larval fish, bioaccumulation from ingested zooplankton containing traces of vitamin D_3 may not be ruled out as a sufficient source. Analogue to retinoic acid, vitamin D isomers may be involved in regulation of gene transcription in a ligand-dependent manner through their interaction with specific DNA sequences (Crisp et al., 1998; Hamre et al., 2007), and should therefore be added to the larval diet with care as long as larval storage capabilities and metabolic pathways are unknown.

The high fraction of phospholipids and PUFA in the copepods may require substantial protection against oxidation by free radicals. The main function of vitamin E is to reduce peroxyl radicals in membrane lipids and prevent the chain reaction leading to lipid peroxidation, and vitamin E is therefore crucial for normal development of tissues, including bone and cartilage (Lall and Lewis-McCrea, 2007). Vitamin E may also inhibit the oxidations induced by the electronically excited singlet oxygen, and have a number of other effects as reviewed by Kamal-Eldin and Appelqvist (1996) and Azzi and Stocker (2000). Due to the lipid protective activity, it is not surprising that the copepods were rich in vitamin E and other synergists like carotenoids and vitamin C, the latter being important in regenerating the antioxidative properties of vitamin E by converting the oxidised form (α-tocopheroxyl) to α-tocopherol (Hamre et al., 1997) which is the most abundant and bioactive form of the vitamin E isomers (Kamal-Eldin and Appelqvist, 1996, Hamre et al., 1998). Rapid growth and formation of cell membranes in larval fish count for high PUFA requirements, with the risk of high oxidative stress. Dietary vitamin E in larval fish should therefore relate to PUFA intake (Martínez-Álvarez et al., 2005), and will as a free radical scavenger support the antioxidation enzyme systems encountered in fish larvae (Mourente et al., 1999b; Tocher et al., 2002). Due to the high metabolic turnover in larval fish, the specific vitamin E requirements suggested by NRC (1993) for older stages may not be appropriate, and higher levels have been suggested (Lie et al., 1997). However, restoration of vitamin E by other micronutrients implies that body contents of regenerative compounds and their dietary intake, together with restoration rates need to be accounted for in study of larval vitamin E deficiency. The fact that other micronutrients also effectively contribute as antioxidants makes assessment of specific larval α-tocopherol requirements even more challenging. Vitamin E levels in copepods were low compared to rotifers and *Artemia*, but high levels of other synergistic compounds like astaxanthin and vitamin C are suggesting that the copepods might provide sufficient antioxidant potential for fish larvae. The high levels of vitamin E provided through enrichment emulsions may therefore not be necessary, but more research should be carried out to determine requirements and metabolic pathways of tocopherols in larval fish.

The vitamin C in the copepods was high but variable. Copepod vitamin C content originates from dietary phytoplankton since biosynthesis of ascorbic acid does not occur in copepods (Hapette and Poulet, 1990). The omnivorous nature of many copepod species may explain some of the variation in copepod vitamin C levels, induced by variations in algal vitamin C content, copepod gracing, and food selection. Most fish cannot synthesise vitamin C, which is a strong reducing agent that can be restored enzymatically, and that acts as a co-factor in production of procollagen, a precursor of collagen (NRC, 1993). Vitamin C is therefore important for development of connective tissue, wound repair, and formation of bone matrix. Vitamin C may also enhance immune function (Woodward, 1994), and deficiencies may affect hepatocyte cellular compartmentation (Merchie et al., 1997). Vitamin C requirements in larval and early juvenile fish have been indicated in the range of 20 and 500 μg/g DW, while in some species enhanced growth, increased stress tolerance, and reduced incidence of opercular deformities occurred at levels up to 1750 μg/g DW (Merchie et al., 1997; Gapasin et al., 1998). Opercular abnormalities are distortion of gill filament cartilages resulting from de-calcification, and are characteristic of scorbutic fish (Cahu et al., 2003). The above-mentioned differences in dietary requirements between species or stage of development may be explained by metabolic activity (Merchie et al., 1997). The Svartatjern copepods should therefore have no problem in supporting dietary needs of vitamin C for both temperate and cold-water larval and juvenile marine fish species.

The copepod thiamine content resembled rotifer levels reached after more than 10 h of enrichment on the algae *Isochrysis galbana* (Lie et al., 1997), and corresponded to levels in other copepods (Mæland et al., 2000). No data on thiamine deficiency or requirements of larval marine fish have to our knowledge been reported. Thiamine combines with pyrophosphate in a coenzyme used for oxidative decarboxylation of α-keto-acids and transketolase reaction in the pentose shunt, and therefore relate closely to energy production (NRC, 1993; Woodward, 1994). In fish, deficiency in thiamine has been associated with the M74 and Cayuga syndromes in salmonids, leading to high mortality during early life stages in wild fish (Fisher et al., 1996; Åkerman et al., 1998; Pickova et al., 1998; Ketola et al., 2000). In thiamine deficient farmed fish, malfunctioning of the nervous system, including loss of equilibrium accompanied by whirling, melanotic appearance, inability to feed, progressive weakness, and paralysis were described by Woodbury (1943). In other vertebrates, thiamine deprivation causes pan-necrosis affecting the nuclei of the brain stem and diencephalons (Dreyfus and Victor, 1961). The use of Svartatjern copepods for successful rearing of cold-water species accounts for satisfactory thiamine levels in these copepods, which is above the levels suggested for adult fish by NRC (1993), but research is needed to verify larval requirements.

The observed riboflavin levels in the copepods exceeded the recommended minimum requirements for fish, including for juveniles that do not seem to have elevated needs for riboflavin compared to older fish (NRC, 1993; Serrini et al., 1996; Brønstad et al., 2002; Deng and Wilson, 2003). However, most of these data are collected from studies of freshwater or anadromous fish species, and no investigations on riboflavin requirements of marine fish larvae have been published. Through its involvement in two coenzymes, riboflavin functions as electron mediator in oxidation–reduction reactions involved in metabolism of keto-acids, fatty acids, and amino acids in the mitochondrial electron system (NRC, 1993). Symptoms of riboflavin deficiency may be species-specific, and include elevated mortality, reduced weight gain, rapid opercular movements, anaeroxia, lethargy, dark or light body colour, severe fin erosion, cataracts, photophobia, reduced hepatic D-amino acid oxidase activity, and haemorrhages (Woodward, 1984; NRC, 1993; Serrini et al., 1996; Deng and Wilson, 2003). The riboflavin levels in the Svartatjern copepods were lower than in the rotifers and *Artemia*, but slightly above the levels presented for the copepod *Temora longicornis* by Mæland et al. (2000). Since no riboflavin-related deficiency symptoms have been observed when feeding the copepods to larval coldwater fish, use of rotifers and *Artemia* should therefore assumingly cover the requirements. But controlled experiments to verify riboflavin requirements in marine fish larvae are still lacking.

5. Conclusions

From present knowledge about nutritional requirements of marine fish larvae, small neritic calanoid copepods display a macronutrient composition that seems to satisfy the demands of the larvae. In particular, this comprises medium protein and high FAA contents with balanced amino acid profiles, medium to low lipid content, high fractions of phospholipids, DHA, and EPA, with optimal ratios regarding DHA/EPA and EPA/ARA. The low content of wax esters resembles nauplii and young copepodid stages of *Calanus* sp., which are a major component of the larval feed in many marine ecosystems. Among the micronutrients, copepods are rich in pigment, and particularly astaxanthin, which may be an important source of retinoids for larval fish since β-carotene and vitamin A are scarce in copepods. Absence of vitamin D_3 in the copepods may indicate dietary precursors as source of cholecalcipherol in larval fish, but data on potential precursors are lacking. In contrast, copepods are rich in vitamin E and ascorbic acid, which together with astaxanthin are pointing to high antioxidative capacity needed to protect against peroxidation of membrane lipids. Vitamin C was most abundant, making the copepods particularly suitable for fish larvae with a high growth potential. The copepod content of thiamine and riboflavin may be sufficient to sustain larval development in marine fish, but data on larval requirements are absent in the literature. High metabolism linked to the rapid growth rates often displayed by young marine fish larvae may account for elevated micronutrient needs beyond what are suggested for older fish. Determination of optimal larval requirements are lacking for many of the micronutrients, and such data should be collected since insufficient dietary supply of some micronutrients already has demonstrated impairment of normal larval development. Copepods have successfully been applied as feed for marine fish larvae, also in intensive rearing systems. Since copepods are the principal prey of marine fish larvae, this suggests specific larval adaptations to universal traits of copepod

biochemical composition. Thus, evolution of the larval digestive and metabolic systems may have set limits to tolerance of nutritional variability in the larval prey, limits that were surpassed when *Artemia* and rotifers were introduced in intensive production of marine fish juveniles. Alteration of nutritional composition of rotifers, *Artemia*, and formulated feed should therefore be made in the direction of copepods, and the present data provide a comprehensive outline of this direction.

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References

Åkerman, G., Tjärnlund, U., Noaksson, E., Balk, L., 1998. Studies with oxythiamine to mimic reproduction disorders among fish early life stages. Mar. Environ. Res. 46, 493–497.

Akiyama, T., Murai, T., Nose, T., 1986. Oral administration of serotonin against spinal deformity of chum salmon fry induced tryptophan deficiency. Bull. Jpn. Soc. Sci. Fish. 52, 1249–1254.

Aragao, C., Conceicao, L.E.C., Dinis, M.T., Fyhn, H.J., 2004a. Amino acid pools of rotifers and *Artemia* under different conditions: nutritional implications for fish larvae. Aquaculture 234, 429–445.

Aragao, C., Conceicao, L.E.C., Fyhn, H.J., Dinis, M.T., 2004b. Estimated amino acid requirements during early ontogeny in fish with different life styles: Gilthead seabream (*Sparus aurata*) and Senegalese sole (*Solea senegalensis*). Aquaculture 242, 589–605.

Azzi, A., Stocker, A., 2000. Vitamin E: non-antioxidant roles. Prog. Lipid Res. 39, 231–255.

Båmstedt, U., 1986. Chemical composition and energy content. In: Corner, E.D.S., O'Hara, S.C.M. (Eds.), The Biological Chemistry of Marine Copepods. Clarendon Press, Oxford, pp. 1–58.

Bell, J.G., McEvoy, J., Tocher, D.R., Sargent, J.R., 2000. Depletion of α-tocopherol and astaxanthin in Atlantic salmon (*Salmo salar*) affects autoxidative defense and fatty acid metabolism. J. Nutr. 130, 1800–1808.

Bell, J.G., McEvoy, L.A., Estevez, A., Shields, R.J., Sargent, J.R., 2003. Optimising lipid nutrition in first-feeding flatfish larvae. Aquaculture 227, 211–220.

Bendich, A., Olson, J.A., 1989. Biological actions of carotenoids. FASEB J. 3, 1927–1932.

Blondin, G.A., Kulkarni, B.D., Nes, W.R., 1967. A study of the origin of vitamin-D from 7-dehydrocholesterol in fish. Comp. Biochem. Physiol. 20, 379–390.

Brønstad, I., Bjerkås, I., Waagbø, R., 2002. The need for riboflavin supplementation in high and low energy diets for Atlantic salmon *Salmo salar* L. parr. Aquac. Nutr. 8, 209–220.

Cahu, C., Zambonino Infante, J., 2001. Substitution of live food by formulated diets in marine fish larvae. Aquaculture 200, 161–180.

Cahu, C.L., Zambonino Infante, J.L., Quazuguel, P., Le Gall, M.M., 1999. Protein hydrolysate vs. fish meal in compound diets for 10-day old sea bass *Dicentrarchus labrax* larvae. Aquaculture 171, 109–119.

Cahu, C., Zambonino Infante, J., Takeuchi, T., 2003. Nutritional components affecting skeletal development in fish larvae. Aquaculture 227, 245–258.

Carvalhoa, A.P., Oliva-Telesa, A., Bergot, P., 2003. A preliminary study on the molecular weight profile of soluble protein nitrogen in live food organisms for fish larvae. Aquaculture 225, 445–449.

CEN (Comitè Europèen de Normalisation), prEN 12821, 1999a. Foodstuffs — Determination of vitamin D by high performance liquid chromatography — Measurement of cholecalciferol (D_3) and ergocalciferol (D_2).

CEN (Comitè Europèen de Normalisation), prEN 12822, 1999b. Foodstuffs — Determination of vitamin E by high performance liquid chromatography — Measurement of α-, β-, γ- and δ-tocopherols.

Chatzifotis, S., Pavlidis, M., Jimeno, C.D., Vardanis, G., Sterioti, A., Divanach, P., 2005. The effect of different carotenoid sources on skin coloration of cultured red porgy (*Pagrus pagrus*). Aquac. Res. 36, 1517–1525.

Christiansen, R., Torrissen, O.J., 1996. Growth and survival of Atlantic salmon, *Salmo salar* L. fed different dietary levels of astaxanthin. Juv. Aquac. Nutr. 2, 55–62.

Christie, W.W., 1982. The preparation of derivatives of lipids, In: Christie, W.W. (Ed.), Lipid analysis. Isolation, Separation, Identification and Structural Analysis of Lipids, 2nd edition. Pergamon Press, Oxford, UK, pp. 51–62.

Conceição, L.E.C., Rønnestad, I., Tonheim, S.K., 2002. Metabolic budgets for lysine and glutamate in unfed herring (*Clupea harengus*) larvae. Aquaculture 206, 305–312.

Conceição, L.E.C., Grasdalen, H., Rønnestad, I., 2003. Amino acid requirements of fish larvae and post-larvae: new tools and recent findings. Aquaculture 227, 221–232.

Conceição, L., van der Meeren, T., Verreth, J.A.J., Evjen, M.S., Houlihan, D.F., Fyhn, H.J., 1997. Amino acid metabolism and protein turnover in larval turbot (*Scophthalmus maximus*) fed natural zooplankton or *Artemia*. Mar. Biol. 129, 255–265.

Coutteau, P., Geurden, I., Camara, M.R., Bergot, P., Sorgeloos, P., 1997. Review on the dietary effects of phospholipids in fish and crustacean larviculture. Aquaculture 155, 149–164.

Crisp, T.M., Clegg, E.D., Cooper, R.L., Wood, W.P., Anderson, D.G., Baetcke, K.P., Hoffmann, J.L., Morrow, M.S., Rodier, D.J., Schaeffer, J.E., Touart, L.W., Zeeman, M.G., Patel, Y.M., 1998. Environmental endocrine disruption: an effects assessment and analysis. Environ. Health Perspect. 106 (Suppl. 1), 11–56.

Dabrowski, K., Terjesen, B.F., Zhang, Y., Phang, J.M., Lee, K.-J., 2005. A concept of dietary dipeptides: a step to resolve the problem of amino acid availability in the early life of vertebrates. J. Exp. Biol. 208, 2885–2894.

Dedi, J., Takeuchi, T., Seikai, T., Watanabe, T., 1995. Hypervitaminosis and safe levels of vitamin A for larval flounder (*Paralichthys olivaceus*) fed *Artemia* nauplii. Aquaculture 133, 135–146.

Deng, D.-F., Wilson, R.P., 2003. Dietary riboflavin requirement of juvenile sunshine bass (*Morone chrysops* ♀ × *Morone saxatilis* ♂. Aquaculture 218, 695–701.

Dreyfus, P.M., Victor, M., 1961. Effects of thiamine deficiency on the central nervous system. Am. J. Clin. Nutr. 9, 414–425.

Evjemo, J.O., Olsen, Y., 1997. Lipid and fatty acid content in cultivated live feed organisms compared to marine copepods. Hydrobiologia 358, 159–162.

Evjemo, J.O., Reitan, K.I., Olsen, Y., 2003. Copepods as live food organisms in the larval rearing of halibut larvae (*Hippoglossus hippoglossus* L.) with special emphasis on the nutritional value. Aquaculture 227, 191–210.

Finn, R.N., Rønnestad, I., van der Meeren, T., Fyhn, H.J., 2002. Fuel and metabolic scaling during the early life stages of Atlantic cod *Gadus morhua*. Mar. Ecol., Prog. Ser. 243, 217–234.

Fisher, J.P., Fitzsimons, J.D., Combs, G.F., Spitsbergen, J.M., 1996. Naturally occurring thiamine deficiency causing reproductive failure in Finger Lakes Atlantic salmon and Great Lakes lake trout. Trans. Am. Fish. Soc. 125, 167–178.

Fisher, L.R., Kon, S.K., Thompsom, S.Y., 1964. Vitamin A and carotenoids in certain invertebrates. VII. Crustacea: Copepoda. J. Mar. Biol. Assoc. U.K. 44, 685–692.

Folch, J., Lees, M., Sloane-Stanley, G.H., 1957. A simple method for the isolation and purification of total lipids from animal tissues. J. Biol. Chem. 226, 497–509.

Fraser, A.J., Sargent, J.R., 1989. Formation and transfer of fatty acids in an enclosed marine food chain comprising phytoplankton, zooplankton and herring (*Clupea harengus* L.) larvae. Mar. Chem. 27, 1–18.

Fraser, A.J., Sargent, J.R., Gamble, J.C., 1989. Lipid class and fatty acid composition of *Calanus finmarchicus* (Gunnerus), *Pseudocalanus* sp. and *Temora longicornis* Muller from a nutrient-enriched seawater enclosure. J. Exp. Mar. Biol. Ecol. 130, 81–92.

Furuita, H., Tanaka, H., Yamamoto, T., Shiraishi, M., Takeuchi, T., 2001. Effects of high dose of vitamin A on reproduction and egg quality of Japanese flounder *Paralichthys olivaceus*. Fish. Sci. 67, 606–613.

Fyhn, H.J., Finn, R.N., Helland, S., Rønnestad, I., Lømsland, E.R., 1993. Nutritional value of phyto- and zooplankton as live food for marine fish larvae. In: Reinertsen, H., Dahle, L.A., Jørgensen, K., Tvinnereim, K. (Eds.), Fish farming technology. Balkema, Rotterdam, pp. 121–126.

Fyhn, H.J., Rønnestad, I., Berg, L., 1995. Variation in protein and free amino acid content of marine copepods during the spring bloom. Spec. Publ. Eur. Aquaculture Soc. 24, 321–324.

Gapasin, R.S.J., Bombeo, R., Lavens, P., Sorgeloos, P., Nelis, H., 1998. Enrichment of live food with essential fatty acids and vitamin C: effects on milkfish (*Chanos chanos*) larval performance. Aquaculture 162, 269–286.

Gatten, R.R., Sargent, J.R., Gamble, J.C., 1983. Diet-induced changes in fatty acid composition of herring larvae reared in enclosed ecosystem. J. Mar. Biol. Assoc. U.K. 63, 575–584.

Geiger, E., 1958. Problems connected with the possible use of plankton for human nutrition. Am. J. Clin. Nutr. 6, 394–400.

Geurden, I., Bergot, P., Schwarz, L., Sorgeloos, P., 1998. Relationship between dietary phospholipid classes and neutral lipid absorption in newly weaned turbot, *Scophthalmus maximus*. Fish Physiol. Biochem. 19, 217–228.

Graff, I.E., Lie, Ø., Aksnes, L., 1999. *In vitro* hydroxylation of vitamin D_3 and 25-hydroxy vitamin D_3 in tissues of Atlantic salmon *Salmo salar*, Atlantic mackerel *Scomber scombrus*, Atlantic halibut *Hippoglossus hippoglossus* and Atlantic cod *Gadus morhua*. Aquac. Nutr. 5, 23–32.

Hadas, E., Koven, W., Sklan, D., Tandler, A., 2003. The effect of dietary phosphatidylcholine on the assimilation and distribution of ingested free oleic acid (18:1n-9) in gilthead seabream (*Sparus aurata*) larvae. Aquaculture 217, 577–588.

Haga, Y., Suzuki, T., Takeuchi, T., 2002. Retinoic acid isomers produce malformations in postembryonic development of the Japanese flounder, *Paralichthys olivaceus*. Zool. Sci. 19, 1105–1112.

Haga, Y., Takeuchi, T., Murayama, Y., Ohta, K., Fukunaga, T., 2004. Vitamin D_3 compounds induce hypermelanosis on the blind side and vertebral deformity in juvenile Japanese flounder *Paralichthys olivaceus*. Fish. Sci. 70, 59–67.

Hamre, K., Waagbø, R., Berge, R.K., Lie, Ø., 1997. Vitamins C and E interact in juvenile Atlantic salmon (*Salmo salar,* L.). Free Radic. Biol. Med. 22, 137–149.

Hamre, K., Berge, R.K., Lie, Ø., 1998. Turnover of α-, γ-, and δ-tocopherol and distribution in subcellular and lipoprotein fractions indicate presence of an hepatic tocopherol binding protein in Atlantic salmon (*Salmo salar* L.). Fish Physiol. Biochem. 18, 71–83.

Hamre, K., Næss, T., Espe, M., Holm, J.C., Lie, Ø., 2001. A formulated diet for Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae. Aquac. Nutr. 7, 123–132.

Hamre, K., Opstad, I., Espe, M., Solbakken, J., Hemre, G.-I., Pittman, K., 2002. Nutrient composition and metamorphosis success of Atlantic halibut (*Hippoglossus hippoglossus*, L.) larvae fed natural zooplankton or *Artemia*. Aquac. Nutr. 8, 139–148.

Hamre, K., Holen, E., Moren, M., 2007. Pigmentation and eye migration in Atlantic halibut (*Hippoglossus hippoglossus* L.) larvae: new findings and hypotheses. Aquac. Nutr. 13, 65–80.

Hapette, A.M., Poulet, S.A., 1990. Variation of vitamin C in some common species of marine plankton. Mar. Ecol., Prog. Ser. 64, 69–79.

Harel, M., Ozkizilcik, S., Lund, E., Behrens, P., Place, A.R., 1999. Enhanced absorption of docosahexaenoic acid (DHA, 22:6n-3) in *Artemia* nauplii using a dietary combination of DHA-rich phospholipids and DHA-sodium salts. Comp. Biochem. Physiol., B 124, 169–176.

Harper, A.E., 1983. Dispensable and indispensable amino acid relationships. In: Blackburn, G.L., Grant, J.P., Young, V.R. (Eds.), Amino Acids — Metabolism and Medical Applications. John Wright, PSG Inc., Boston, pp. 105–121.

Helland, S., Nejstgaard, J.C., Fyhn, H.J., Egge, J.K., Båmstedt, U., 2003a. Effects of starvation, season, and diet on the free amino acid and protein content of *Calanus finmarchicus* females. Mar. Biol. 143, 297–306.

Helland, S., Nejstgaard, J.C., Humlen, R., Fyhn, H.J., Båmstedt, U., 2003b. Effects of season and maternal food on *Calanus finmarchicus* reproduction, with emphasis on free amino acids. Mar. Biol. 142, 1141–1151.

Helland, S., Terjesen, B.F., Berg, L., 2003c. Free amino acid and protein content in the planktonic copepod *Temora longicornis* compared to *Artemia franciscana*. Aquaculture 215, 213–228.

Holick, M.F., 2003. Vitamin D: a millenium perspective. J. Cell. Biochem. 88, 296–307.

Horvli, O., Lie, Ø., 1994. Determination of vitamin D_3 in fish meals by HPLC. Fisk. Dir. Skr. Ser. Ernæring 6, 163–175.

Imsland, A.K., Foss, A., Koedijk, R., Folkvord, A., Stefansson, S.O., Jonassen, T.M., 2006. Short- and long-term differences in growth, feed conversion efficiency and deformities in juvenile Atlantic cod (*Gadus morhua*) startfed on rotifers or zooplankton. Aquac. Res. 37, 1015–1027.

Izquierdo, M.S., Tandler, A., Salhi, M., Kolkovski, S., 2001. Influence of dietary polar lipids' quantity and quality on ingestion and assimilation of labelled fatty acids by larval gilthead seabream. Aquac. Nutr. 7, 153–160.

Kamal-Eldin, A., Appelqvist, L.-Å., 1996. The chemistry and antioxidant properties of tocopherols and tocotrienols. Lipids 31, 671–701.

Kanazawa, A., 1993. Nutritional mechanisms involved in the occurrence of abnormal pigmentation in hatchery-reared flatfish. J. World Aquac. Soc. 24, 162–166.

Kanazawa, A., 1997. Effects of docosahexaenoic acid and phospholipids on stress tolerance of fish. Aquaculture 155, 129–134.

Kenny, D.E., O'Hara, T.M., Chen, T.C., Lu, Z., Tian, X., Holick, M.F., 2004. Vitamin D content in Alaskan arctic zooplankton, fishes, and marine mammals. Zoo Biol. 23, 33–43.

Ketola, H.G., Bowser, P.R., Wooster, G.A., Wedge, L.R., Hurst, S.S., 2000. Effects of thiamine on reproduction of Atlantic salmon and a new hypothesis for their extirpation in Lake Ontario. Trans. Am. Fish. Soc. 129, 607–612.

Klungsøyr, J., Tilseth, S., Wilhelmsen, S., Falk-Petersen, S., Sargent, J.R., 1989. Fatty acid composition as an indicator of food intake in cod larvae *Gadus morhua* from Lofoten, Northern Norway. Mar. Biol. 102, 183–188.

Kolkovski, S., 2001. Digestive enzymes in fish larvae and juveniles—implications and applications to formulated diets. Aquaculture 200, 181–201.

Koroleff, F., 1983. Determination of nutrients. In: Grasshoff, K., Ehrhardt, M., Kremling, K. (Eds.), Methods of Seawater Analysis. Verlag Chemie, Weinheim, pp. 125–187.

Koven, W.M., Parra, G., Kolkovski, S., Tandler, A., 1998. The effect of dietary phosphatidylcholine and its constituent fatty acids on microdiet ingestion and fatty acid absorption rate in gilthead sea bream, *Sparus auratus*, larvae. Aquac. Nutr. 4, 39–45.

Kvåle, A., Nordgreen, A., Tonheim, S.K., Hamre, K., 2007. The problem of meeting dietary protein requirements in intensive aquaculture of marine fish larvae, with emphasis on Atlantic halibut (*Hippoglossus hippoglossus* L.). Aquac. Nutr. 13, 170–185.

Lall, S.P., Lewis-McCrea, L.M., 2007. Role of nutrients in skeletal metabolism and pathology in fish — An overview. Aquaculture 267, 3–19.

Lee, C.-S., O'Bryen, P.J., Marcus, N.H., 2005. Copepods in Aquaculture. Blackwell Publishing, Oxford. 269 pp.

Lee, R.F., Nevenzel, J.G., Paffenhöfer, G.-A., 1971. Importance of wax esters and other lipids in the marine food chain: phytoplankton and copepods. Mar. Biol. 9, 99–108.

Lie, Ø., Haaland, H., Hemre, G.-I., Maage, A., Lied, E., Rosenlund, G., Sandnes, K., Olsen, Y., 1997. Nutritional composition of rotifers following a change in diet from yeast and emulsified oil to microalgae. Aquac. Int. 5, 427–438.

Lowry, O.H., Rosebrough, N.J., Farr, A.L., Randall, R.J., 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265–275.

Luizi, F.S., Gara, B., Shields, R.J., Bromage, N.R., 1999. Further description of the development of the digestive organs in Atlantic halibut (*Hippoglossus hippoglossus*) larvae, with notes on differential absorption of copepod and *Artemia* prey. Aquaculture 176, 101–116.

Mæland, A., Waagbø, R., 1998. Examination of the qualitative ability of some cold water marine teleosts to synthesise ascorbic acid. Comp. Biochem. Physiol., A 121, 249–255.

Mæland, A., Rønnestad, I., Fyhn, H.J., Berg, L., Waagbø, R., 2000. Water-soluble vitamins in natural plankton (copepods) during two consecutive

spring blooms compared to vitamins in *Artemia franciscana* nauplii and metanauplii. Mar. Biol. 136, 765–772.
Marcus, N.H., 2005. Calanoid copepods, resting eggs, and aquaculture. In: Lee, C.-S., O'Bryen, P.J., Marcus, N.H. (Eds.), Copepods in Aquaculture. Blackwell Publishing, Oxford, pp. 3–9.
Martínez-Álvarez, R.M., Morales, A.E., Sanz, A., 2005. Antioxidant defences in fish: biotic and abiotic factors. Rev. Fish Biol. Fish. 15, 75–88.
McEvoy, L.A., Næss, T., Bell, J.G., Lie, Ø., 1998. Lipid and fatty acid composition of normal and malpigmented Atlantic halibut (*Hippoglossus hippoglossus*) fed enriched *Artemia*: a comparison with fry fed wild copepods. Aquaculture 163, 235–248.
McKinnon, A.D., Duggan, S., Nichols, P.D., Rimmer, M.A., Semmens, G., Robino, B., 2003. The potential of tropical paracalanid copepods as live feeds in aquaculture. Aquaculture 223, 89–106.
Merchie, G., Lavens, P., Sorgeloos, P., 1997. Optimization of dietary vitamin C in fish and crustacean larvae: a review. Aquaculture 155, 165–181.
Mischke, C.C., Li, M.H., Zimba, P.V., 2003. Pond fertilization does not affect nutritional value of zooplankton in channel catfish nursery ponds. N. Am. J. Aquac. 65, 248–254.
Morehead, D.T., Battaglene, S.C., Metillo, E.B., Brandsen, M.P., Dunstan, G.A., 2005. Calanoid copepods, resting eggs, and aquaculture. In: Lee, C.-S., O'Bryen, P.J., Marcus, N.H. (Eds.), Copepods in Aquaculture. Blackwell Publishing, Oxford, pp. 195–207.
Moren, M., Næss, T., Hamre, K., 2004a. Conversion of β-carotene, canthaxanthin and astaxanthin to vitamin A in Atlantic halibut (*Hippoglossus hippoglossus* L.) juveniles. Fish Physiol. Biochem. 27, 71–80.
Moren, M., Opstad, I., Hamre, K., 2004b. A comparison of retinol, retinal and retinyl ester concentrations in larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) fed *Artemia* or zooplankton. Aquac. Nutr. 10, 253–259.
Moren, M., Gundersen, T.E., Hamre, K., 2005. Quantitative and qualitative analysis of retinoids in *Artemia* and copepods by HPLC and diode array detection. Aquaculture 246, 359–365.
Moren, M., Opstad, I., van der Meeren, T., Hamre, K., 2006. Iodine enrichment of *Artemia* and enhanced levels of iodine in Atlantic halibut larvae (*Hippoglossus hippoglossus* L.) fed the enriched *Artemia*. Aquac. Nutr. 12, 97–102.
Mourente, G., Tocher, D.R., Diaz, E., Grau, A., Pastor, E., 1999a. Relationships between antioxidants, antioxidant enzyme activities and lipid peroxidation products during early development in *Dentex dentex* eggs and larvae. Aquaculture 179, 309–324.
Mourente, G., Tocher, D.R., Diaz-Salvago, E., Grau, A., Pastor, E., 1999b. Study of the n-3 highly unsaturated fatty acids requirement and antioxidant status of *Dentex dentex* larvae at the *Artemia* feeding stage. Aquaculture 179, 291–307.
Naas, K.E., van der Meeren, T., Aksnes, D.L., 1991. Plankton succession and responses to manipulations in a marine basin for larval rearing. Mar. Ecol., Prog. Ser. 74, 161–173.
Næss, T., 1991. Marine calanoid resting egg in Norway: abundance and distribution of two copepod species in the sediment of an enclosed marine basin. Mar. Biol. 110, 261–266.
Næss, T., 1996. Benthic resting eggs of calanoid copepods in Norwegian enclosures used in mariculture: abundance, species composition and hatching. Hydrobiologia 320, 161–168.
Næss, T., Germain-Henry, M., Naas, K.E., 1995. First feeding of Atlantic halibut (*Hippoglossus hippoglossus*) using different combinations of *Artemia* or wild zooplankton. Aquaculture 130, 235–250.
Nanton, D.A., Castell, J.D., 1998. The effects of dietary fatty acids on the fatty acid composition of the harpacticoid copepod, *Tisbe* sp., for use as a live food for marine fish larvae. Aquaculture 163, 251–261.
Nakamura, K., Iida, H., Nakano, H., 1986. Riboflavin in the skin of albinic flatfish *Liopsetta obscura*. Bull. Jpn. Soc. Sci. Fish. Nissuishi 52 (12), 2207.
Norrbin, M.F., Olsen, R.E., Tande, K.S., 1990. Seasonal-variation in lipid class and fatty-acid composition of 2 small copepods in Balsfjorden, northern Norway. Mar. Biol. 105, 205–211.
Norsker, N.-H., Støttrup, J.G., 1994. The importance of dietary HUFAs for fecundity and HUFA content in the harpacticoid, *Tisbe holothuriae* Humes. Aquaculture 125, 155–166.
NRC (National Research Council), 1993. Nutrient Requirements of Fish. National Academy Press, Washington DC. 115 pp.
Øiestad, V., Kvenseth, P.G., Folkvord, A., 1985. Mass production of Atlantic cod juveniles *Gadus morhua* in a Norwegian saltwater pond. Trans. Am. Fish. Soc. 114, 590–595.
Okimasu, E., Matsumoto, M., Yoshida, Y., Amemura, A., 1992. The effect of pigments of Rhodobacter capsulatus on free radicals and application of the bacterium as feed to fish larvae. Nippon Suisan Gakkaishi 58, 1487–1491.
Olsen, R.E., Henderson, R.J., 1989. The rapid analysis of neutral and polar marine lipids using double-development HPTLC and scanning densitometry. J. Exp. Mar. Biol. Ecol. 129, 189–197.
Olsen, R.E., Henderson, R.J., Pedersen, T., 1991. The influence of dietary lipid classes on the fatty acid composition of small cod *Gadus morhua* L. juveniles reared in an enclosure in northern Norway. J. Exp. Mar. Biol. Ecol. 148, 59–76.
Olsen, R.E., Dragnes, B.T., Myklebust, R., Ringø, E., 2004. Effect of soybean oil and soybean lecithin on intestinal lipid composition and lipid droplet accumulation of rainbow trout, *Oncorhynchus mykiss* Walbaum. Fish Physiol. Biochem. 29, 181–192.
Palace, V.P., Werner, J., 2006. Vitamins A and E in the maternal diet influence egg quality and early life stage development in fish: a review. In: Olivar, M.P., Govoni, J.J. (Eds.), Recent Advances in the Study of Fish Eggs and Larvae. Sci. Mar., vol. 70S2, pp. 41–57.
Peters, L.D., Livingstone, D.R., 1996. Antioxidant enzyme activities in embryologic and early larval stages of turbot. J. Fish Biol. 49, 986–997.
Pickova, J., Kiessling, A., Pettersson, A., Dutta, P.C., 1998. Comparison of fatty acid composition and astaxanthin content in healthy and by M74 affected salmon eggs from three Swedish river stocks. Comp. Biochem. Physiol., B 120, 265–271.
Rainuzzo, J.R., Reitan, K.I., Olsen, Y., 1997. The significance of lipids at early stages of marine fish: a review. Aquaculture 155, 103–115.
Reitan, K.I., Rainuzzo, J.R., Olsen, Y., 1994. Influence of lipid composition of live feed on growth, survival and pigmentation of turbot larvae. Aquac. Int. 2, 33–48.
Reitan, K.I., Rainuzzo, J.R., Øie, G., Olsen, Y., 1997. A review of the nutritional effects of algae in marine fish larvae. Aquaculture 155, 207–221.
Rønnestad, I., Conceicao, L.E.C., 2005. Aspects of protein and amino acid digestion and utilisation by marine fish larvae. In: Starck, J.M., Wang, T. (Eds.), Physiological and Ecological Adaptations to Feeding in Vertebrates. Science Publishers, Enfield, New Hampshire, pp. 389–416.
Rønnestad, I., Helland, S., Lie, Ø., 1998. Feeding *Artemia* to larvae of Atlantic halibut (*Hippoglossus hippoglossus* L.) results in lower larval vitamin A content compared with feeding copepods. Aquaculture 165, 159–164.
Rønnestad, I., Hamre, K., Lie, Ø., Waagbø, R., 1999a. Ascorbic acid and α-tocopherol levels in larvae of Atlantic halibut before and after exogenous feeding. J. Fish Biol. 55, 720–731.
Rønnestad, I., Thorsen, A., Finn, R.N., 1999b. Fish larval nutrition: a review of recent advances in the roles of amino acids. Aquaculture 177, 201–216.
Rousseau, V., Mathot, S., Lancelot, C., 1990. Calculating carbonbiomass of *Phaeocystis* sp. from microscopic observations. Mar. Biol. 107, 305–314.
Rutter, W.J., 1967. Protein determinations in embryos. In: Wilt, F.H., Wessels, N.K. (Eds.), Methods in Developmental Biology. Academic Press, London, pp. 671–684.
Sargent, J., Bell, G., McEvoy, L., Tocher, D., Estevez, A., 1999. Recent developments in the essential fatty acid nutrition of fish. Aquaculture 177, 191–199.
Sargent, J.R., Falk-Petersen, S., 1988. The lipid biochemistry of calanoid copepods. Hydrobiologia 167/168, 101–114.
Sargent, J.R., Henderson, R.J., 1986. Lipids. In: Corner, E.D.S., O'Hara, S.C.M. (Eds.), The Biological Chemistry of Marine Copepods. Oxford University Press, Oxford, pp. 59–108.
Rønnestad, I., Tonheim, S.K., Fyhn, H.J., Rojas-Garcia, C.R., Kamisaka, Y., Koven, W., Finn, R.N., Terjesen, B.F., Barr, Y., Conceicao, L.E.C., 2003. The supply of amino acids during early feeding stages of marine fish larvae: a review of recent findings. Aquaculture 227, 147–164.
Schuhmacher, A., Münch, M., Gropp, J.M., 1995. Non-essential amino acid sources in crystalline amino acid diets for trout (*Oncorhynchus mykiss*). J. Appl. Ichthyol. 11, 317–321.
Scott, A.P., Middelton, C., 1979. Unicellular algae as a food for turbot (*Scophthalmus maximus* L.) larvae — the importance if dietary long-chain polyunsaturated fatty acids. Aquaculture 18, 227–240.

Seikai, T., 1985. Reduction in occurrence frequency of albinism in juvenile flounder *Paralichthys olivaceus* hatchery-reared on wild zooplankton. Nippon Suisan Gakkaishi 51, 1261–1267.

Serrini, G., Zhang, Z., Wilson, R.P., 1996. Dietary riboflavin requirement of fingerling channel catfish (*Ictalurus punctatus*). Aquaculture 139, 285–290.

Shields, R.J., Bell, J.G., Luizi, F.S., Gara, B., Bromage, N.R., Sargent, J.R., 1999. Natural copepods are superior to enriched *Artemia* nauplii as feed for halibut larvae (*Hippoglossus hippoglossus*) in terms of survival, pigmentation and retinal morphology: relation to dietary essential fatty acids. J. Nutr. 129, 1186–1194.

Shimidzu, N., Goto, M., Miki, W., 1996. Carotenoids as singlet oxygen quenchers in marine organisms. Fish. Sci. 62, 134–137.

Sokal, R.R., Rohlf, F.J., 1995. Biometry, 3rd edn. W.H. Freeman and Company, NewYork. 885 pp.

Sørensen, T.F., Drillet, G., Engell-Sørensen, K., Hansen, B.W., Ramløv, H., 2007. Production and biochemical composition of eggs from neritic calanoid copepods reared in large outdoor tanks (Limfjord, Denmark). Aquaculture 236, 84–96.

Srivastava, A., Hamre, K., Stoss, J., Chakrabarti, R., Tonheim, S.K., 2006. Protein content and amino acid composition of the live feed rotifer (*Brachionus plicatilis*): with emphasis on the water soluble fraction. Aquaculture 254, 534–543.

Støttrup, J.G., 2003. Production and nutrition value of copepods. In: Støttrup, J.G., McEvoy, L.A. (Eds.), Live Feeds in Marine Aquaculture. Blackwell Publishing, Oxford, pp. 145–205.

Støttrup, J.G., Shields, R., Gillespie, M., Gara, M.B., Sargent, J.R., Bell, J.G., Henderson, R.J., Tocher, D.R., Sutherland, R., Næss, T., Mangor-Jensen, A., Naas, K., van der Meeren, T., Harboe, T., Sanchez, F.J., Sorgeloos, P., Dhert, P., Fitzgerald, R., 1998. The production and use of copepods in larval rearing of halibut, turbot and cod. Bull. Aquac. Assoc. Can. 4, 41–45.

Takeuchi, A., Okano, T., Torii, M., Hatanaka, Y., Kobayashi, T., 1987. Comparative studies on the contents of vitamin D_3, 25-hydroxy vitamin D_3 and 7-dehydrocholesterol in fish liver. Comp. Biochem. Physiol., B 88, 569–573.

Takeuchi, A., Okano, T., Tanda, M., Kobayashi, T., 1991. Possible origin of extremely high contents vitamin D_3 in some kinds of fish liver. Comp. Biochem. Physiol. 100A, 483–487.

Tocher, D.R., Mourente, G., van der Eecken, A., Evjemo, J.O., Diaz, E., Bell, J.G., Geurden, I., Lavens, P., Olsen, Y., 2002. Effects of dietary vitamin E on antioxidant defence mechanisms of juvenile turbot (*Scophthalmus maximus* L.), halibut (*Hippoglossus hippoglossus* L.) and sea bream (*Sparus aurata* L.). Aquac. Nutr. 8, 195–207.

Tonheim, S.K., Nordgreen, A., Høgøy, I., Hamre, K., Rønnestad, I., 2007. In vitro digestibility of water-soluble and water-insoluble protein fractions of some common fish larval feeds and feed ingredients. Aquaculture 262, 426–435.

Torrissen, O.J., Hardy, R.W., Shearer, K.D., 1989. Pigmentation of salmonids — carotenoid deposition and metabolism. Rev. Aquat. Sci. 1, 209–225.

Urschel, K.L., Shoveller, A.K., Uwiera, R.R.E., Pencharz, P.B., Ball, R.O., 2006. Citrulline is an effective arginine precursor in enterally fed neonatal piglets. J. Nutr. 136, 1806–1813.

Urschel, K.L., Evans, A.R., Wilkinson, C.W., Pencharz, P.B., Ball, R.O., 2007. Parenterally fed neonatal piglets have a low rate of endogenous arginine synthesis from circulating proline. J. Nutr. 137, 601–606.

van der Meeren, T., Næss, T., 1993. How does cod (*Gadus morhua* L.) cope with variability in the feeding conditions during early larval stages? Mar. Biol. 116, 637–647.

van der Meeren, T., Naas, K.E., 1997. Development of rearing techniques using large enclosed ecosystems in the mass production of marine fish fry. Rev. Fish. Sci. 5, 367–390.

van der Meeren, T., Lønøy, T., 1998. Use of mesocosms in larval rearing of saithe [*Pollachius virens* (L.)], goldsinny [*Ctenolabrus rupestris* (L.)], and corkwing [*Crenilabrus melops* (L.)]. Aquac. Eng. 17, 253–260.

van der Meeren, T., 2003. Kartlegging av biokjemisk innhold i copepoder som basis for kvalitetsvurdering av fôr i oppdrett av marin fiskeyngel. Fisken Havet 5–2003, 39 pp. http://www.imr.no/dokumenter/fisken_og_havet/nr5_2003.pdf (*In Norwegian with English abstract, figure, and table legends*).

van der Meeren, T., Moksness, E., 2003. Growth and mortality patterns evaluated from otolith microstructure in Atlantic cod *Gadus morhua* larvae reared on different feeding regimes in mesocosms. Mar. Ecol., Prog. Ser. 264, 95–107.

van der Meeren, T., Klungsøyr, J., Wilhelmsen, S., Kvenseth, P.G., 1993. Fatty acid composition of unfed cod larvae *Gadus morhua* L. and cod larvae feeding on natural plankton in large enclosures. J. World Aquac. Soc. 24, 167–185.

van der Meeren, T., Jørstad, K.E., Solemdal, P., Kjesbu, O.S., 1994. Growth and survival of cod larvae (*Gadus morhua* L.): comparative enclosure studies of Northeast Arctic cod and coastal cod from western Norway. ICES Mar. Sci. Symp. 198, 633–645.

Venizelos, A., Benetti, D.D., 1999. Pigmentation abnormalities in flatfish. Aquaculture 176, 181–188.

Watanabe, T., Kitajima, C., Fujita, S., 1983. Nutritional values of live organisms used in Japan for mass propagation of fish: a review. Aquaculture 34, 115–143.

Wilson, R.P., 1985. Amino acid and protein requirements of fish. In: Cowey, C.B., mackie, A.M., Bell, J.G. (Eds.), Nutrition and Feeding in Fish. Academic Press, London, pp. 1–16.

Witt, U., Quantz, G., Kuhlmann, D., Kattner, G., 1984. Survival and growth of turbot larvae *Scophthalmus maximus* L. reared on different food organisms with special regard to long-chain polyunsaturated fatty acids. Aquac. Eng. 3, 177–190.

Woodbury, L.A., 1943. Vitamin B_1 deficiency in hatchery reared rainbow trout. Trans. Am. Fish. Soc. 72, 30–34.

Woodward, B., 1984. Symptoms of severe riboflavin deficiency without ocular opacity in rainbow trout (*Salmo gairdneri*). Aquaculture 37, 275–281.

Woodward, B., 1994. Dietary vitamin requirements of cultured young fish, with emphasis on quantitative estimates for salmonids. Aquaculture 124, 133–168.

Wright, P.A., Fyhn, H.J., 2001. Ontogeny of nitrogen metabolism and excretion. In: Wright, P.A., Anderson, P.M. (Eds.), Nitrogen Excretion. Fish Physiology Series, vol. 20. Academic Press, San Diego, pp. 149–201.

Wu, G., Knabe, D.A., Kim, S.W., 2004. Arginine nutrition in neonatal pigs. J. Nutr. 134, 2783S–2790S.

Zambonino Infante, J.L., Cahu, C.L., Péres, A., 1997. Partial substitution of di- and tripeptides for native proteins in sea bass diet improves *Dicentrarchus labrax* larval development. J. Nutr. 127, 608–614.