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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

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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 7.5and 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).

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 on-going 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, 2005; 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 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 2000 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 -80ºC 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 –80ºC 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 x 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.

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 (15000 x g, 10 min, 4ºC), 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 (15000 x 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 serum albumin (BSA, Sigma A-7638) in 0.5 M NaOH as standard and 0.5M 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 (106ºC, 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 x 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 (µmoles/mg DW of analysed material) were converted to the equivalent protein content and expressed both in molar terms of the various amino acids (µmoles/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 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 -80ºC 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 4ºC. Separation was performed using tandem installed Chromspher 5 mm C18 columns (100 mm x 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,

388 which separates the vitamin D isomers. Vitamin D_3 was quantified by using vitamin
389 D_2 as internal standard and vice versa. Vitamin D_2 was not detected at all in the
390 samples. The method and instrumentation are described in detail in Horvli and Lie
391 (1994) and CEN (1999a).
392
393 Vitamin A was subjected to normal phase HPLC with UV detection at 325 nm and
394 quantified by external standards according to method and instrumentation described in
395 Moren et al. (2004a). This method gives a large peak with similar retention time as all
396 trans retinol in samples from Artemia. However, later work has shown, by the use of a
397 diode array detector, which produces UV spectra of the peaks, that this compound is
398 not vitamin A (Moren et al., 2005). The tocopherols (vitamin E isomers) were also
399 analysed by normal phase HPLC, detected by fluorescence at 295 nm excitation and
400 330 nm emission and quantified using external standards (CEN 1999b). Given relative
401 to wet weight of the sample, the detection and quantification limits of the analytical
402 methods are 6 and 20 ng/g for vitamin D, 8 and 28 ng/g for vitamin A, 11 and 38 ng/g
403 for α-tocopherol, and 8 and 28 ng/g for the other tocopherols, respectively.
404
405 The samples for ascorbic acid (vitamin C) were homogenised and extracted in meta-
406 phosphoric acid with dithiothreitol, which reduces de-hydro ascorbic acid to ascorbic
407 acid. Compounds in the extract were separated by reverse phase HPLC, and ascorbic
408 acid was detected by amperiometrically at 0.6 V and quantified using external
409 standards (Mæland and Waagbø, 1998). The B vitamins, thiamine and riboflavin, were
410 analysed by semi-automated microbiological methods which are detailed in Mæland et
411 al. (2000). Detection and quantification limits of the methods relative to wet weight of
412 the sample are 0.35 and 1.1 µg/g for vitamin C, 1.3 and 4.3 µg/g for riboflavin, and
413 0.02 and 0.2 µg/g for thiamine, respectively.
414
415 *2.4. Statistical analysis*
416
417 Differences in biochemical indices were tested by Students t-test after checking for
418 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 7-9°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, 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 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 Nitzschia 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 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

542 should also be noted that the algae-derived neutral glycoglycerolipids (galactocides) in
543 combination with neutral glycosphingolipids (cerebrocides) or sulfoglycoglycerolipids
544 (sulfolipids) were more or less absent in rotifers and Artemia (Table 2: MGDG+CB
545 and DGDG+SL). Significant differences in copepod neutral lipids between the two
546 years were only detected for MGDG+CB.
547
548 Amounts of polar lipids in the copepods averaged 6.2 and 6.3% of copepod DW for
549 2000 and 2001, respectively, with a relatively stable fraction averaging 57.1% (2000)
550 and 58.2% (2001) of TL (Table 2, Fig. 4). Polar lipid content in rotifers was more
551 similar to copepods, constituting 6.1% of DW but corresponding only to 39.8% of TL.
552 Copepod nauplii and the zooplankton sample had somewhat lower content of polar
553 lipids (5.4 and 5.1% of DW, equivalent to 63.1 and 36.0% of TL, respectively). In
554 contrast, polar lipids in Artemia were lower and between 3.9 and 5.0% of DW (15.4-
555 20.5% of TL). The major phospholipids in copepods and copepod nauplii were PC and
556 PE, each having average levels between 1.5 and 2.0% of DW and 17.6-20.5% of TL
557 (Table 2, Fig. 4). Significant difference in copepod phospholipid class composition
558 between the two years was only found for PS. PC and PE also dominated
559 phospholipids in the zooplankton and the rotifer samples (1.5-2.1% of DW and 10.6-
560 13.7% of TL), as well as in the Artemia samples (1.2-1.9% of DW and 4.8-7.6% of
561 TL).
562
563 In the copepods, PUFA dominated the TL fatty acid composition, accounting for 63.3
564 and 64.2% of TL in 2000 and 2001, respectively (Table 2). Variation in PUFA was
565 low between the samples within each year. Although not significantly different from
566 the copepods, PUFA fraction in copepod nauplii was even higher (69.4% of TL), on
567 the expense of MUFA. Zooplankton was more similar to rotifers and Artemia, with
568 PUFA levels ranging between 43.6 and 48.5% of TL. Compared to copepods, these
569 reduced levels of PUFA were balanced by increased fractions of MUFA (20.1-34.8%
570 of TL).
571

572 Among the single fatty acids, DHA was abundant in the copepod samples, averaging
573 34.4 and 32.9% of TL for 2000 and 2001, respectively (Table 2, Fig. 5). The copepod
574 nauplii averaged 40.5% DHA, which was significantly higher than for the copepod
575 samples in 2001. These high levels contrasted the DHA fraction of 17.3% found in the
576 zooplankton sample. In the intensively produced live feed, DHA was between 10.6
577 and 20.0%, with highest level in the 3-day on-grown Artemia. In the copepods,
578 averages of EPA were between 16.2 and 17.4% of TL, including copepod nauplii and
579 zooplankton. However, in rotifers and Artemia EPA was lower, ranging between 7.1
580 and 9.2%, respectively. Another abundant fatty acid was palmitic acid (16:0), which
581 was between 13.7 to 19.7% of TL in all groups (Table 2). Among other important fatty
582 acids, ARA was very low in the copepod and copepod nauplii samples and even below
583 detection limit in many samples. This contrasted that of zooplankton, rotifers, and
584 Artemia where ARA was more abundant, ranging between 1.6 and 3.2% of TL.
585 Significant differences in fatty acids composition between the copepod samples from
586 2000 and 2001 were mainly found among the fatty acids with 18 carbon atoms (C18),
587 along with myristic acid (14:0). Similarly, significant lower fractions among C18 fatty
588 acids were also found for copepod nauplii when compared with the copepod samples
589 from the same year (Table 2).
590
591 The average DHA/EPA ratio was 2.1 and 2.2 for copepods in 2000 and 2001,
592 respectively (Table 2, Fig. 5). Copepod nauplii had somewhat higher DHA/EPA ratio,
593 but not significantly different from the 2001 copepods. The zooplankton had the
594 lowest DHA/EPA ratio (1.1), while intensively reared live feed varied between 1.4 and
595 2.2, the latter belonging to 3-day on-grown Artemia. The EPA/ARA ratio was in
596 general very high in copepods and copepod nauplii (on average between 23.2 and
597 27.7), and also relatively high in the zooplankton sample (10.3). This contrasted the
598 EPA/ARA ratios in rotifers (3.7) and Artemia (2.9-4.0). A similar pattern was seen for
599 the (n-3)/(n-6) ratio, which was highest in copepods and lowest in the rotifers (Table 2,
600 Fig. 5).
601
602 *3.5. Protein and protein-bound amino acids*

603

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

621

622 The concentration of protein-bound amino acids (PAA_c) was lowest in rotifers (2.3
623 µmoles/mg DW), being almost half of that in copepods in 2000 (4.1 µmoles/mg DW)
624 (Table 3). All concentration-specific PAA and IAA indices applied on the copepod
625 samples were significantly different between 2000 and 2001, but with low variation
626 within each of the years (Table 3, Fig. 6). Considering all prey types sampled, the
627 concentration-specific IAA fraction of PAA (IAA_c/PAA_c) was between 40.4 and
628 43.7%. Similarly, the IAA_c/DAA_c ratio of the hydrolysed protein averaged 0.68 and
629 0.70 in the copepod samples from 2000 and 2001, respectively (Table 3), while for the
630 rotifers and Artemia it was higher (between 0.75 and 0.78). In contrast, the
631 IAA_c/DAA_c ratio in the zooplankton sample was 0.71, and more in accordance with
632 the copepods.

633

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, 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/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 (Fig. 6, 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 of 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

696 DW in mid-October, while in 2001 the levels continued to rise and peaked in mid-
697 November at 1422 µg/g DW. In the zooplankton sample, astaxanthin was about 25%
698 of the average copepod pigment content in the corresponding year, while the rotifers
699 similarly contained 3.8% of the copepod astaxanthin content. In all cases, only free
700 astaxanthin was found, and no esters were observed. All Artemia samples were devoid
701 of astaxanthin, but contained canthaxanthin in the same ranges as copepod astaxanthin
702 (Table 5). Further, β-carotene was not detected in any of the samples.
703
704 Of the lipid-soluble vitamins, vitamin D_3 was either not detected in the copepod
705 samples or found in trace amounts (three of the samples). On average, it was therefore
706 considered below the detection limits of the method (Table 5). The zooplankton
707 sample was also free of vitamin D_3, while levels in rotifers and Artemia were 0.9 and
708 0.7-1.8 µg/g DW, respectively. Further, vitamin A was found in low levels or beyond
709 quantification limits in the copepods. In many samples, vitamin A was even below
710 detection limit, particularly in 2001 (Table 5). Zooplankton and rotifers were also low
711 in Vitamin A (0.2 µg/g DW), and in Artemia realistic values for vitamin A were not
712 possible to quantify due to analytical problems (see section 2.3.4.). Vitamin E was
713 abundant in all samples (Fig. 8) and was dominated by the isomer, E_α (Table 5),
714 constituting between 90 and 100% of total vitamin E. No other isomers were detected
715 in the zooplankton sample, while the remaining vitamin E in the copepods was E_γ and
716 E_β, the latter only observed in 2000. Both rotifers and Artemia showed low levels of
717 vitamin E_γ, and in addition Artemia displayed low but consistent levels of vitamin E_δ,
718 not found in the other feed types.
719
720 In the water-soluble vitamins, copepods showed high but variable levels of vitamin C
721 (Table 5, Fig. 8). Vitamin C in zooplankton, rotifers, and Artemia was within the range
722 of one standard deviation of the average values observed in the copepods. In copepods,
723 levels of thiamine was consistent and well above the quantification limit of the
724 method, with some variation between the years at different seasons (Fig. 8). Thiamine
725 was also abundant in zooplankton, rotifers and Artemia (Table 5). In contrast,

726 riboflavin values varied around quantification limit of the method in copepods (Fig. 8),
727 zooplankton and rotifers, while Artemia had slightly higher levels (Table 5).
728
729
730 **4. Discussion**
731
732 The biochemical composition of the copepods from Svartatjern was generally
733 characterised by substantial amounts of polar lipids, high levels of n-3 PUFA
734 (particularly DHA and EPA), protein with a diverse amino acid contribution in the
735 PAA profile (both for IAA and DAA, FAA dominated by few amino acids (gly, tau in
736 DAA and arg in IAA), high levels of astaxanthin, and considerable amounts of vitamin
737 C and vitamin E. In addition, compounds like β-carotene and vitamin D_3 were
738 virtually absent in the copepods, while vitamin A and riboflavin were in the range of
739 trace limit concentrations. Further, the biochemical composition showed surprisingly
740 high stability between years or seasons within a year, despite large changes in copepod
741 species composition and environmental conditions (e.g. photoperiod, temperature and
742 salinity). However, the zooplankton sample from the Hyltro lagoon contrasts that of
743 Svartatjern copepods in containing more lipids with less PUFA and DHA. In addition,
744 the zooplankton had less protein, somewhat different FAA profile with more
745 dispensable FAA, along with lower astaxanthin and vitamin C content. These
746 discrepancies may most likely be explained by differences in phytoplankton
747 communities and densities, but also by a different composition of crustacean taxa,
748 since decapod larvae contributed to 32.4% of enumerated plankton in the zooplankton
749 sample. This may also clarify occurrence of slightly heavier individuals with higher
750 fraction of dry matter and ash in the zooplankton, probably because decapod zoeae are
751 more heavily armoured with carapace spines than copepods.
752
753 An important question is to what extent Svartatjern represents natural ecosystems, and
754 how this pond-like system may affect the biochemical composition of copepods? The
755 Svartatjern pond system is managed by a specific protocol that implies fertilisation to
756 boost primary production, mixing to prevent stratification, and emptying and refilling

757 according to renewal of copepod plankton from resting eggs (Naas et al., 1991; Næss,
758 1991). In this sense, copepods from Svartatjern may be regarded as "reared" copepods,
759 although reared on a diverse and natural assemblage of phytoplankton in a large
760 outdoor ecosystem. However, regarding dry matter, ash content, total lipids, and FAA
761 content the Svartatjern copepods were close to or within the mode values for other
762 copepods (reviewed by Båmstedt, 1986), but lower in protein content which on the
763 other hand was in accordance with data reported by Mæland et al. (2000). Protein
764 content may depend on the analytical method, and at present back calculation based on
765 PAA is regarded to be the most precise method for other larval prey (Hamre et al.,
766 2007). Analyses of lipid class composition in copepods are mostly from high-latitude
767 oceanic calanoids (e.g. Calanus sp), which normally are rich in wax esters used as
768 energy source during overwintering and reproduction (Lee et al., 1971; Sargent and
769 Falk-Petersen, 1988; Fraser et al., 1989). The copepod species included in the present
770 investigation are neritic calanoid species that do not overwinter as adults in the pond
771 system. Instead, they use resting eggs as a reproductive mode to ensure survival from
772 one generation to another during unfavourable conditions, e.g. during the seasonable
773 disruption of the production cycles (Næss, 1996). Storage of wax esters may therefore
774 not be required to the same extent as in the larger Calanus sp. The Svartatjern
775 copepods rather resembled naupliar and early copepodid stages of Calanus sp, which
776 are rich in structural phospholipids and contain TAG as main storage lipid (Sargent
777 and Henderson, 1986; Sargent and Falk-Petersen, 1988). In this respect, it should be
778 noted that nauplii and the young copepodid stages of Calanus sp are the primary prey
779 for larvae of many fish species.
780
781 Lipid content and composition in copepods have been found to be relatively diverse,
782 and to vary with developmental stage, species, feed preference, latitude, season, and
783 life cycle strategy (Båmstedt, 1986; Sargent and Falk-Petersen, 1988; Fraser et al.,
784 1989; Norrbin et al., 1990; Støttrup, 2003). The Svartatjern copepod lipid composition
785 may therefore be regarded as within the natural variation among copepods. Supporting
786 this is also the high levels of certain fatty acids like 16:0, EPA, and DHA, which are in
787 concordance with several other studies on neritic calanoid copepod species (Evjemo

and Olsen, 1997; Evejemo 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 a 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

819 fatty acid requirements of marine fish larvae have pointed out the importance of
820 phospholipids, DHA, EPA, ARA, and the ratios of such PUFA for optimal lipid
821 digestion, normal larval development, larval survival and growth, and stress tolerance
822 (Olsen et al., 1991; Coutteau, 1997; Kanazawa, 1997; Sargent et al., 1999; Shields et
823 al., 1999; Izquierdo et al., 2001; Bell et al., 2003; Cahu et al., 2003; Hadas et al., 2003;
824 Støttrup, 2003). Compared to rotifers and Artemia, the Svartatjern copepods were
825 loaded with EPA and DHA. DHA was particularly abundant in the copepod nauplii,
826 indicating the importance of this fatty acid in the nutrition of young fish larvae whose
827 initial exogenous feed would be such prey. The high EPA/ARA ratio in the copepods
828 should be noted, as successful pigmentation during metamorphosis in flatfish larvae
829 may be dependent on this (Hamre et al., 2007). Considering the fraction of
830 phospholipids relative to total lipid, copepods were rich in phospholipids (57-63%)
831 compared to rotifers (40%) and particularly to Artemia (15-20%). However, taking
832 into account phospholipids per mg live prey biomass, differences were lesser (Table
833 2), probably due to the higher lipid content of the intensive prey types from
834 enrichment. Most enrichment oils for rotifers and Artemia are usually TAG, and
835 enhancing the phospholipid content of the prey by enrichment has turned out to be
836 difficult (Rainuzzo et al., 1997; Harel et al., 1999). This is expressed as accumulation
837 of TAG with increasing lipid levels, with the potential for imbalances in both lipid
838 class and PUFA composition. Dietary phospholipids may enhance larval ingestion
839 (Koven et al., 1998), and phospholipids seem to be necessary for optimal lipid
840 transport and synthesis in the larval digestive system, as well as a number of cell
841 membrane and signalling functions (Bell et al., 2003; Cahu et al., 2003). Also the
842 relative abundance of different phospholipid classes may be of importance for larval
843 growth and development (Geurden et al., 1998). In the present data, both rotifers and
844 Artemia displayed many similarities with copepods when the relative composition of
845 the phospholipid profile was compared, indicating that structural lipids in the marine
846 food web are to some extent conservative. Quantitative deviations from the copepod
847 phospholipids were however evident, particularly in Artemia. More focus on
848 phospholipid enrichment of live feed and phospholipid supplement in formulated feed

849 is therefore necessary, with the goal to reach balanced levels of lipid classes and
850 PUFA as observed in copepods.
851
852 The gut system of young fish larvae has initially high assimilation capability of FAA
853 and low protein digestibility, with a gradual maturation of the proteolytic capacity
854 throughout ontogenesis (Cahu and Zambonino Infante, 2001; Rønnestad and
855 Conceição, 2005, Kvåle et al., 2007). FAA may serve as both energy substrate and
856 sustain protein synthesis in marine fish larvae (Rønnestad et al., 1999b; Wright and
857 Fyhn, 2001; Rønnestad et al., 2003). The Svartatjern copepods were rich in FAA, and
858 the FAA concentration relative to DW was found to correlate with salinity. This
859 correlation may be explained by the need for copepods to use FAA in osmoregulation
860 (Båmstedt, 1986; Fyhn et al., 1993). Fish larvae may be very efficient in retaining and
861 absorbing FAA from the gut lumen, in particularly IAA (Conceição et al., 2002).
862 However, larval growth potential is in most cases very high, and daily weight gain
863 may exceed 20% even in coldwater species (van der Meeren et al., 1994; Finn et al.,
864 2003). The observed FAA levels alone in larval live prey cannot sustain the amino
865 acid requirements surged by the protein deposition rate necessary to maintain such
866 high growth rates, and protein digestion must play a significant role in total amino acid
867 availability, absorption, and subsequent protein synthesis. Concordantly, recent studies
868 have shown that young marine fish larvae also are able to utilize peptide chains in
869 protein hydrolysates (Zambonino Infante et al., 1997; Cahu et al., 1999; Hamre et al.,
870 2001), and that amino acids supplied in the diet in this form may reduce larval spinal
871 malformations (Cahu et al., 2003). Peptide digestion may be aided by high activity of
872 peptidases in young fish larvae (Cahu and Zambonino Infante, 2001). Although young
873 fish larvae have limited proteolytic capacity, access to peptide chains and amino acids
874 from dietary protein may be facilitated by autolysis of the ingested prey (Fyhn et al.,
875 1993; Kolkovski, 2001). In this respect, Luizi et al. (1999) noted that copepods were
876 much more readily digested in Atlantic halibut larvae than Artemia. Furthermore, in
877 vitro digestibility studies with pancreatic enzymes chosen to mimic the conditions in
878 the larval intestine, show that water-soluble protein is more digestible than insoluble
879 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 (Carvallo 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; Aragão 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 (Aragão et al., 2004a). In absence of more detailed knowledge about specific amino

910 acid requirements, copepods might therefore be regarded as a baseline recipe for
911 protein, PAA, and FAA contents and profiles in feed for marine fish larvae.
912
913 Requirements for dietary micronutrients like pigments, vitamins, minerals, and trace
914 elements are little investigated in marine fish, and such studies are particularly scarce
915 for larval and early juvenile stages. Regarding minerals and trace elements, only iodine
916 was analysed from the Svartatjern copepods, as presented elsewhere (Moren et al.,
917 2006). Compared to adult fish, the high growth rates and rapid organogenesis may
918 account for elevated micronutrient requirements and turnover during early
919 developmental in fish (Lie et al., 1997), and recommendations suggested for adult fish
920 (e.g. in NRC, 1993) may therefore not be valid for younger life stages (Mæland et al.,
921 2000). Levels of micronutrients found in copepods that sustain growth and normal
922 development, may be better indices for requirements in larval and juvenile marine fish,
923 and the present study is an attempt to provide such baseline data.
924
925 Regarding pigments, the consistent high levels of astaxanthin in the copepods suggest
926 that this compound should receive more attention in larval fish nutrition. Together
927 with canthaxanthin commonly found in Artemia, astaxanthin, lutein, and β-carotene
928 belong to the carotenoid family that may serve as precursors for vitamin A in fish
929 (Bendich and Olson, 1989; Christiansen and Torrissen, 1996; Moren et al., 2005;
930 Palace and Werner, 2006). Since β-carotene was not detected in the copepods,
931 astaxanthin and possibly also lutein may be important provitamin A compounds in
932 such plankton (Rønnestad et al., 1998). Astaxanthin have also demonstrated profound
933 antioxidant properties, particularly as a coantioxidant working synergistically with
934 vitamin E in suppressing lipid peroxidation (Bell et al., 2000). Antioxidant action on
935 active oxygen radicals in marine organisms has also been suggested for a number of
936 other carotenoids, including canthaxanthin (Shimidzu et al., 1996), and carotenoids
937 enhanced survival and reduced lipid peroxidation in Japanese flounder larvae
938 (Okimasu et al., 1992). Carotenoids may therefore assist the enzymatic antioxidant
939 system in fish, which is already functional during early larval stages (Peters and
940 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).

971 Another vitamin not found in the copepods was cholecalcipherol (vitamin D_3). This
972 was unexpected, as this vitamin may play important roles in calcium and phosphorous
973 metabolism and affect bone formation and remodelling in vertebrates. Vitamin D_3 is
974 the main storage form in the liver of marine teleosts, and may be converted to 25-
975 hydroxyl vitamin D_3 isomers in various fish tissues (Takeuchi et al., 1991; Graff et al.,
976 1999; Holick, 2003; Lall and Lewis-McCrea, 2007). However, data on effects of
977 dietary vitamin D_3 in larval fish are very scarce. In a recent study of young juvenile
978 Japanese flounder, hypermelanosis on the blind side and vertebral deformities have
979 been reported when dietary levels exceed 5 µg/g vitamin D_3 or 0.5 µg/g 1,25$(OH)_2$
980 vitamin D_3 (Haga et al., 2004). Copepods may contain vitamin D_3 levels below the
981 analytical detection and quantification limits, or they may contain precursors as the 7-
982 dehydrocholesterol (7-DCH), which is the provitamin responsible for vitamin D_3
983 production in the skin of terrestrial vertebrates under UV light irradiation. In this
984 respect, several studies agree on that copepods displays either lack of vitamin D_2 and
985 D_3 while 7-DCH is detected in reasonable amounts (Geiger, 1958; Takeuchi et al.,
986 1991; Kenny et al., 2004). Approximately 1.4% of the Svartatjern copepod DW was
987 cholesterol and sterol esters, but 7-DCH was not specifically analysed for. In adult
988 fish, both photo-conversion in the skin and enzymatic dark-transfer of 7-DCH in the
989 liver to vitamin D_3 has been reported (Holick, 2003; Blondin et al., 1967), but also
990 disputed (Takeuchi et al., 1991). No data have been presented on this matter for fish
991 larvae, and this calls for further exploration. If fish is able to convert this provitamin to
992 vitamin D_3 it may account for 7-DCH as a potential important vitamin D source in
993 most stages of planktivorous fish, and explain the paradox of vitamin D_3 enrichment at
994 this trophic level in the marine food web. Photo-conversion implies that such fish has
995 to reside close to daylight at the surface, which e.g. fish larvae or pelagic schooling
996 fish often do. It also means that indoor rearing of larval fish in absence of UV-light
997 might require dietary vitamin D_3, which is actually supplied in rotifers and Artemia
998 due to use of fish oils in the enrichment emulsions (Table 5). Since 7-DCH occurs
999 naturally in fish liver (Takeuchi et al., 1987), enzymatic dark-conversion is an
1000 intriguing aspect that also needs further investigation. However, with the enormous
1001 potential of prey ingestion in larval fish, bioaccumulation from ingested zooplankton

1002 containing traces of vitamin D_3 may not be ruled out as a sufficient source. Analogue
1003 to retinoic acid, vitamin D isomers may be involved in regulation of gene transcription
1004 in a ligand-dependent manner through their interaction with specific DNA sequences
1005 (Crisp et al., 1998; Hamre et al., 2007), and should therefore be added to the larval diet
1006 with care as long as larval storage capabilities and metabolic pathways are unknown.
1007
1008 The high fraction of phospholipids and PUFA in the copepods may require substantial
1009 protection against oxidation by free radicals. The main function of vitamin E is to
1010 reduce peroxyl radicals in membrane lipids and prevent the chain reaction leading to
1011 lipid peroxidation, and vitamin E is therefore crucial for normal development of
1012 tissues, including bone and cartilage (Lall and Lewis-McCrea, 2007). Vitamin E may
1013 also inhibit the oxidations induced by the electronically excited singlet oxygen, and
1014 have a number of other effects as reviewed by Kamal-Eldin and Appelqvist (1996) and
1015 Azzi and Stocker (2000). Due to the lipid protective activity, it is not surprising that
1016 the copepods were rich in vitamin E and other synergists like carotenoids and vitamin
1017 C, the latter being important in regenerating the antioxidative properties of vitamin E
1018 by converting the oxidised form (α-tocopheroxyl) to α-tocopherol (Hamre et al., 1997)
1019 which is the most abundant and bioactive form of the vitamin E isomers (Kamal-Eldin
1020 and Appelqvist, 1996, Hamre et al., 1998). Rapid growth and formation of cell
1021 membranes in larval fish count for high PUFA requirements, with the risk of high
1022 oxidative stress. Dietary vitamin E in larval fish should therefore relate to PUFA
1023 intake (Martínez-Álvarez et al., 2005), and will as a free radical scavenger support the
1024 antioxidation enzyme systems encountered in fish larvae (Mourente et al., 1999b;
1025 Tocher et al., 2002). Due to the high metabolic turnover in larval fish, the specific
1026 vitamin E requirements suggested by NRC (1993) for older stages may not be
1027 appropriate, and higher levels have been suggested (Lie et al., 1997). However,
1028 restoration of vitamin E by other micronutrients implies that body contents of
1029 regenerative compounds and their dietary intake, together with restoration rates need
1030 to be accounted for in study of larval vitamin E deficiency. The fact that other
1031 micronutrients also effectively contribute as antioxidants makes assessment of specific
1032 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 cofactor 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

1064 levels in other copepods (Mæland et al., 2000). No data on thiamine deficiency or
1065 requirements of larval marine fish have to our knowledge been reported. Thiamine
1066 combines with pyrophosphate in a coenzyme used for oxidative decarboxylation of α-
1067 keto acids and transketolase reaction in the pentose shunt, and therefore relate closely
1068 to energy production (NRC, 1993; Woodward, 1994). In fish, deficiency in thiamine
1069 has been associated with the M74 and Cayuga syndromes in salmonids, leading to high
1070 mortality during early life stages in wild fish (Fisher et al., 1996; Åkerman et al., 1998;
1071 Pickova et al., 1998; Ketola et al., 2000). In thiamine deficient farmed fish,
1072 malfunctioning of the nervous system, including loss of equilibrium accompanied by
1073 whirling, melanotic appearance, inability to feed, progressive weakness, and paralysis
1074 were described by Woodbury (1943). In other vertebrates, thiamine deprivation causes
1075 pan-necrosis affecting the nuclei of the brain stem and diencephalons (Dreyfus and
1076 Victor, 1961). The use of Svartatjern copepods for successful rearing of cold-water
1077 species accounts for satisfactory thiamine levels in these copepods, which is above the
1078 levels suggested for adult fish by NRC (1993), but research is needed to verify larval
1079 requirements.
1080
1081 The observed riboflavin levels in the copepods exceeded the recommended minimum
1082 requirements for fish, including for juveniles that do not seem to have elevated needs
1083 for riboflavin compared to older fish (NRC, 1993; Serrini et al., 1996; Bjørnstad et al.,
1084 2002; Deng and Wilson, 2003). However, most of these data are collected from studies
1085 of freshwater or anadromous fish species, and no investigations on riboflavin
1086 requirements of marine fish larvae have been published. Through its involvement in
1087 two coenzymes, riboflavin functions as electron mediator in oxidation-reduction
1088 reactions involved in metabolism of keto-acids, fatty acids, and amino acids in the
1089 mitochondrial electron system (NRC, 1993). Symptoms of riboflavin deficiency may
1090 be species-specific, and include elevated mortality, reduced weight gain, rapid
1091 opercular movements, aneroxia, lethargy, dark or light body colour, severe fin erosion,
1092 cataracts, photophobia, reduced hepatic D-amino acid oxidase activity, and
1093 haemorrhages (Woodward, 1984; NRC, 1993; Serrini et al., 1996; Deng and Wilson,
1094 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.

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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1607 Figure legends:

1608 Figure 1. Densities of major phytoplankton groups and protozoans in the Svartatjern
1609 pond during collection of copepods in 2000 and 2001. The grey areas indicate when
1610 the pond was drained and refilled during the summer season. Note that the ordinate is
1611 logarithmic. Values indicated by lines below the abscissa equal zero.
1612
1613 Figure 2. Relative abundance (percent of numbers) of the copepods Eurytemora
1614 affinis, Centropagus hamatus, and Acartia grani, the cladoceran Podon sp., and
1615 miscellaneous zooplankton (including other copepods) in the samples collected from
1616 the Svartatjern pond in 2000 and 2001.
1617
1618 Figure 3. Dry weight of individual copepods, dry matter (% of wet weight), and ash
1619 content (% of dry weight) from the 2000 and 2001 samples collected in the Svartatjern
1620 pond. Note that the ordinate is broken in the lower panel.
1621
1622 Figure 4. Total lipid content relative to dry weight (DW) and relative abundance of
1623 major lipid classes in the copepod samples from the Svartatjern pond in 2000 and
1624 2001. See table 2 for explanation of abbreviations.
1625
1626 Figure 5. Fatty acid ratios and major fatty acids extracted from total lipids of copepods
1627 samples from the Svartatjern pond in 2000 and 2001. See table 2 for explanation of
1628 abbreviations.
1629
1630 Figure 6. Protein fraction relative to dry weight (DW) and calculated from protein-
1631 bound amino acids (PAA), fraction of indispensable PAA, and relative abundance of
1632 amino acids with a major contribution to PAA in the copepod samples from the
1633 Svartatjern pond in 2000 and 2001. See table 3 for explanation of abbreviations. Note
1634 that the right ordinate is broken in the upper panel.
1635
1636 Figure 7. Fraction of free amino acids (FAA) relative to dry weight (DW), fraction of
1637 indispensable FAA, and relative abundance of amino acids with a major contribution

1638 to FAA in the copepod samples from the Svartatjern pond in 2000 and 2001. See table
1639 4 for explanation of abbreviations.
1640
1641 Figure 8. Content of pigments and vitamins relative to dry weight (DW) in the 2000
1642 and 2001 copepod samples from the Svartatjern pond. Dotted line in lower panel
1643 indicates quantification limit for riboflavin at the present analytical method.

1644 Table 1

1645 Hydrographical data from the Svartatjern pond during collection of copepods.

1646

	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

1647 [a] Not measured in 2000

1648 Table 2
1649 Individual size (dry weight: DW), dry matter content (% of wet weight: WW), ash
1650 content, and lipid components from copepods, copepod nauplii, zooplankton
1651 (copepods and decapod zoeae), rotifers, and Artemia (1-day or 3-day after hatching).
1652 Data are given as mean ± SD when number of samples >1. Values below detection
1653 limits of the analytical method are denoted n.d.
1654

		Svartatjern			Hyltro	Intensive live feed			
	Abbr.	Copepods	Copepods	Cop. nauplii	Zoopl.	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[A]± 2.5	8.1± 2.7	0.63[B]± 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[B]± 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[B]± 3.6	15.5	11.6	14.6	18.4	18.7
Monogalactosides+Cerebrocides	MGDG+CB	1.6[A]± 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[A]± 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[A]± 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^{B}± 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^{B}± 0.5	3.1	4.9	5.5	6.3	5.4
Linoleic acid	18:2(n-6)	1.5^{A}± 0.5	2.3± 0.7	1.5^{B}± 0.5	2.2	15.3	6.6	5.8	4.2
α-Linolenic acid	18:3(n-3)	1.9^{A}± 1.0	2.4± 1.1	1.5^{B}± 0.9	1.4	1.2	12.2	16.2	10.2
Stearidonic acid	18:4(n-3)	2.3^{A}± 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^{B}± 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^{A}± 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^{A}± 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^{A}± 4.5	49.1± 6.8	56.8^{B}± 6.8	33.6	19.4	18.4	19.0	29.2
Fatty acid ratios	(n-3)/(n-6)	11.3^{A}± 2.7	9.8± 2.5	12.5^{B}± 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

[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.

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

[B] Significant difference between copepod nauplii and copepods from 2001.

1660 Table 3
1661 Content of protein (P) and protein-bound amino acids (PAA) in copepods, zooplankton
1662 (copepods and decapod zoeae), rotifers, and Artemia (1-day or 3-day after hatching).
1663 Values are relative to dry weight (DW) and are given as mean ± SD when number of
1664 samples >1. Values below detection limits of the analytical method are denoted n.d.
1665 The subscripts "w" and "c" indicate data given as weight and concentration,
1666 respectively.
1667

		Svartatjern		Hyltro	Intensive live feed			
	Abbr.	Copepods	Copepods	Zoopl.	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[A]± 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[A]± 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[A]± 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 (µmoles/mg DW)	PAA_c	4.1[A]± 0.4	3.8± 0.4	2.8	2.3	2.5	2.7	3.4
Indispensable amino acids (µmoles/mg DW)	IAA_c	1.7[A]± 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[A]± 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[A]± 0.04	0.70± 0.03	0.71	0.78	0.76	0.75	0.75
Indispensable amino acids (nmoles/mg DW)								
Leucine	LEU	349.0[A]± 38.5	320.5± 33.8	246.4	230.1	225.6	295.2	237.0
Valine	VAL	291.8[A]± 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[A]± 26.3	187.3± 20.1	146.5	143.3	137.6	186.8	148.7
Arginine	ARG	121.7[A]± 27.6	161.7± 14.1	126.4	83.7	115.7	149.4	108.8
Phenylalanine	PHE	154.4[A]± 18.7	143.4± 15.7	112.1	114.9	105.7	138.8	111.8
Threonine	THR	128.7[A]± 13.1	120.0± 14.1	95.1	70.7	86.3	114.6	89.1
Methionine	MET	122.3[A]± 13.4	77.7± 38.1	69.4	47.6	56.8	40.5	63.2
Histidine	HIS	53.7[A]± 26.2	63.7± 10.1	10.5	6.9	43.2	60.1	45.9
Tryptophan	TRP	44.6[A]± 84.1	0.7± 2.7	n.d.	4.4	2.3	7.0	3.5
Dispensable amino acids (nmoles/mg DW)								
Glutamic acid+Glutamine	GLU+GLN	577.8[A]± 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[A]± 54.8	392.3± 38.2	284.7	189.0	230.2	306.9	252.4
Glycine	GLY	441.2[A]± 94.7	352.1± 49.7	286.1	181.0	224.7	321.8	245.8

Serine	SER	204.9 [A] ± 22.3	190.7 ± 19.2	152.0	136.8	136.7	186.3	143.7
Proline	PRO	252.0 [A] ± 59.9	186.8 ± 24.8	164.4	134.4	157.4	217.4	200.8
Tyrosine	TYR	122.2 [A] ± 36.1	154.5 ± 16.9	40.5	19.9	88.7	109.5	89.5

[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).

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

Table 4

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

		Svartatjern		Hyltro	Intensive live feed			
	Abbr.	Copepods	Copepods	Zoopl.	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[A] ± 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[A] ± 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[A] ± 0.06	0.40 ± 0.07	0.29	0.53	0.15	0.20	0.23
FAA concentration (nmoles/mg DW)	FAA_c	471.7[A] ± 89.8	580.1 ± 95.1	766.6	124.5	277.6	254.0	219.0
Indispensable amino acids (nmoles/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[A] ± 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[A] ± 0.03	0.24 ± 0.03	0.18	0.44	0.11	0.17	0.19
Indispensable amiono acids (nmoles/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[A] ± 1.9	4.6 ± 1.5	7.5	4.0	1.9	5.0	4.6
Histidine	HIS	5.1[A] ± 1.7	9.3 ± 5.7	21.3	4.9	2.9	6.2	3.2
Leucine	LEU	3.6[A] ± 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[A] ± 1.1	1.9 ± 0.8	3.2	3.0	1.1	1.8	2.0
Phenylalanine	PHE	2.0[A] ± 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 amiono acids (nmoles/mg DW)								
Glycine	GLY	126.5[A] ± 37.1	231.4[d] ± 58.2	235.3[d]	8.8	5.4	8.2	9.7
Taurine	TAU	84.3[A] ± 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[A] ± 7.1	24.5 ± 7.0	45.0	14.6	27.0	35.2	31.2

Proline	PRO	24.3[A]± 19.7	38.3± 38.9	125.9	3.9	50.7	34.6	25.0
Aspartic acid+Phosphoserine	ASP+PHS	17.9[A]± 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[A]± 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[A]± 1.1	2.5± 0.7	3.9	10.6	7.3	5.8	3.5
Asparagine	ASN	2.7[A]± 0.8	2.0± 0.6	2.9	8.7	6.8	10.0	6.2

1684 [a] Institute of Marine Research: rotifers grown on Rotimac and Isochrysis galbana algae.

1685 [b] Institute of Marine Research: Artemia enriched with DC-DHA Selco.

1686 [c] Austevoll Marin Yngel AS: Artemia fed DC-DHA Selco and Algamac 2000.

1687 [d] In the 2001 samples, high glycine content caused masking of threonine (next eluated top in the
1688 chromatogram).

1689 [A] Significant difference between copepods from 2000 and 2001.

Table 5

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

		Svartatjern		Hyltro	Intensive live feed			
	Abbr.	Copepods	Copepods	Zoopl.	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]
Cholecalcipherol	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[A]± 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

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

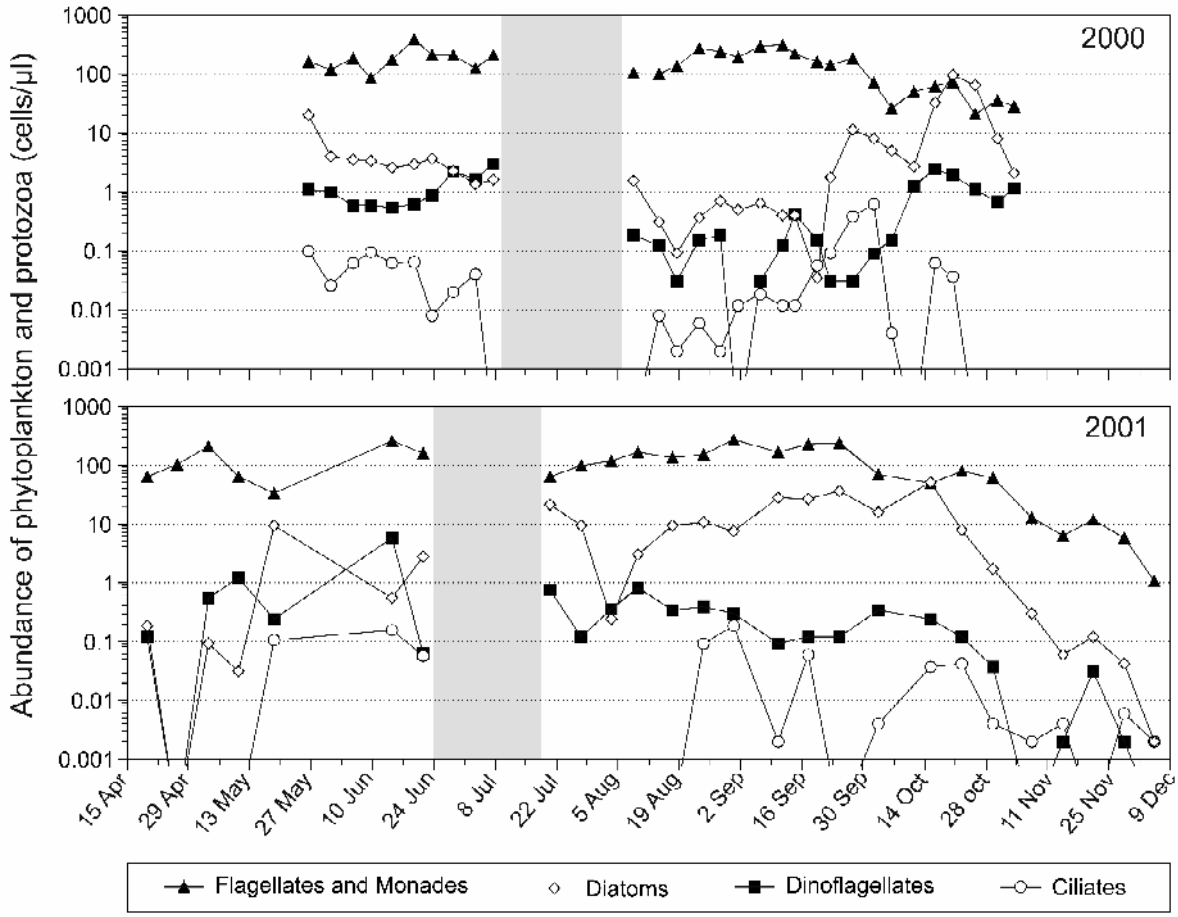
[b] Institute of Marine Research: Artemia enriched with DC-DHA Selco.

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

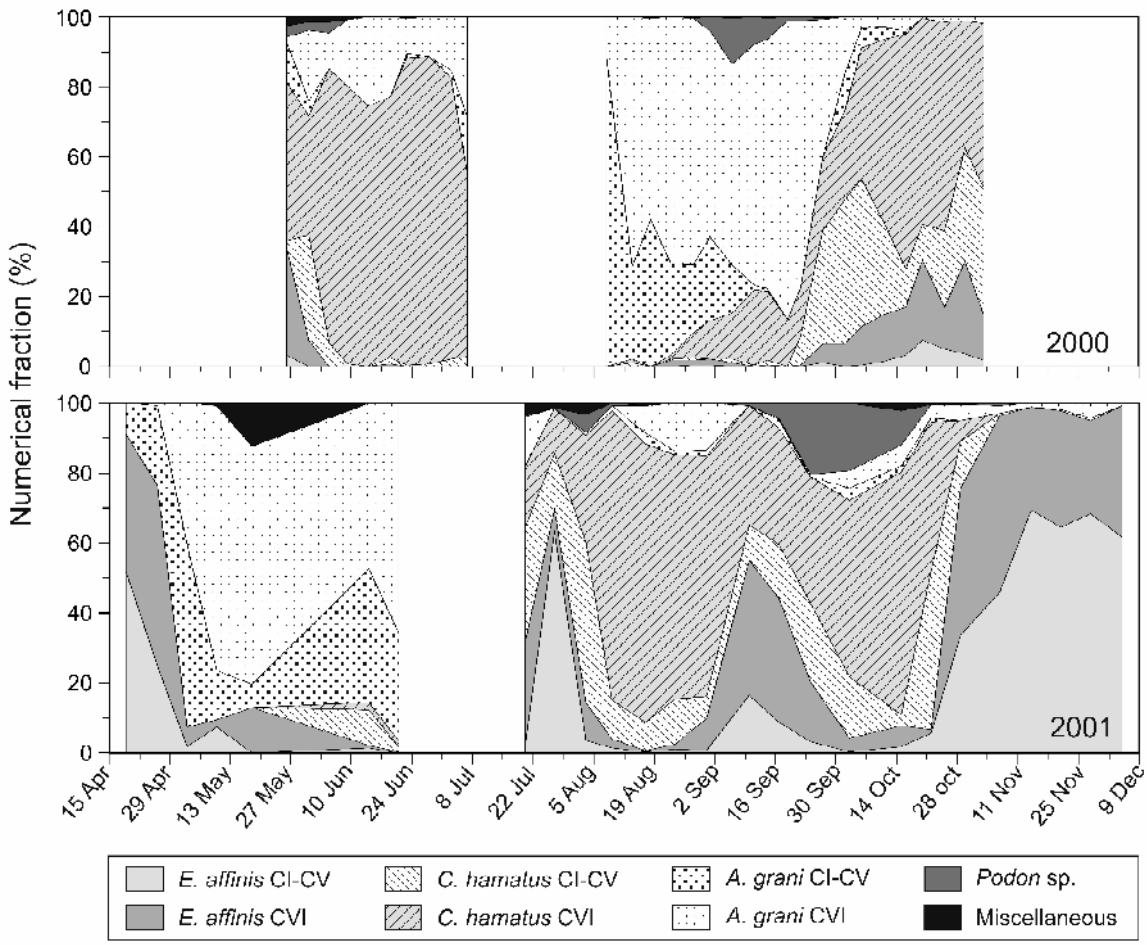
[d] Interactions in the analytical method caused too high retinol readings for Artemia, see section 2.3.4.

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

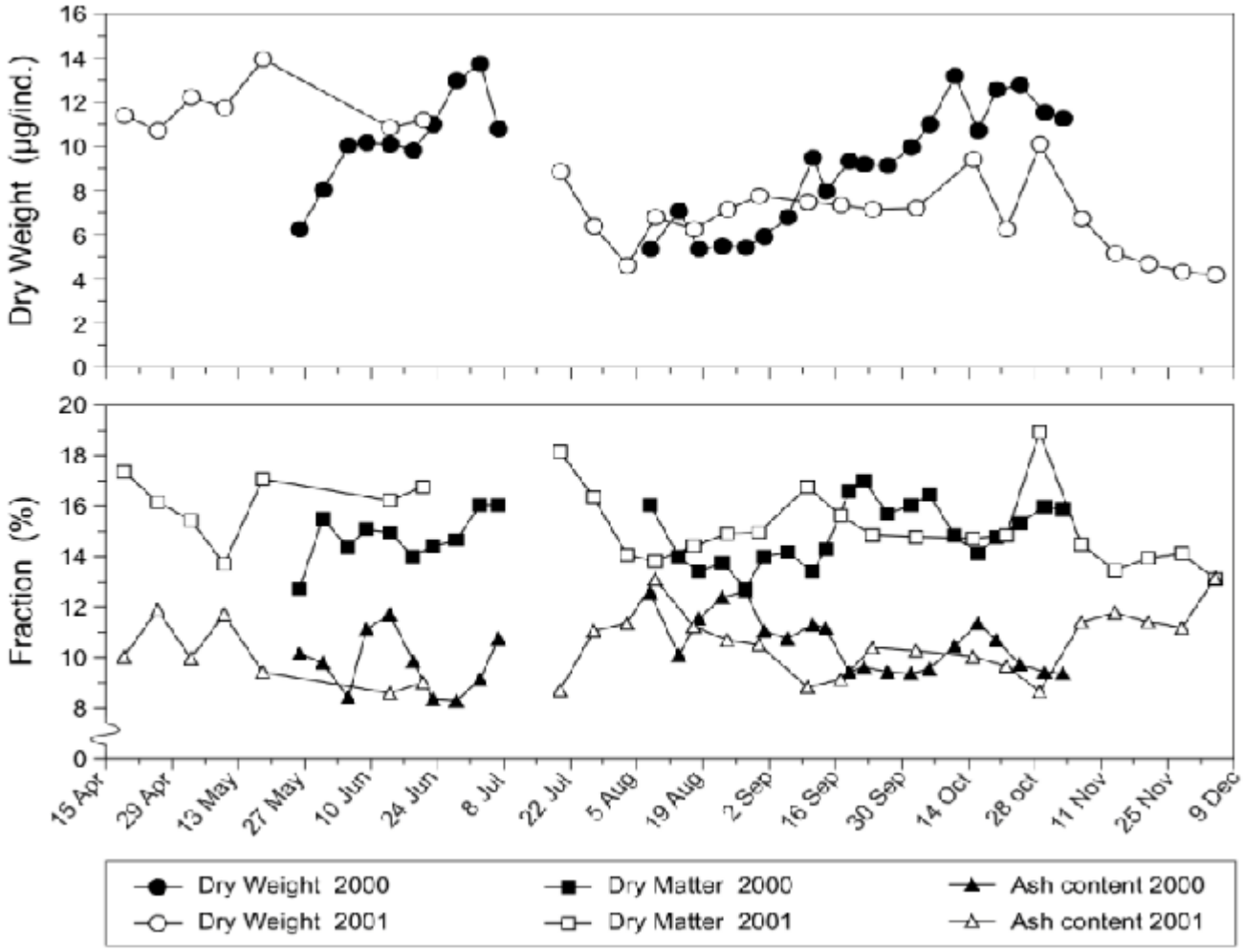
1702



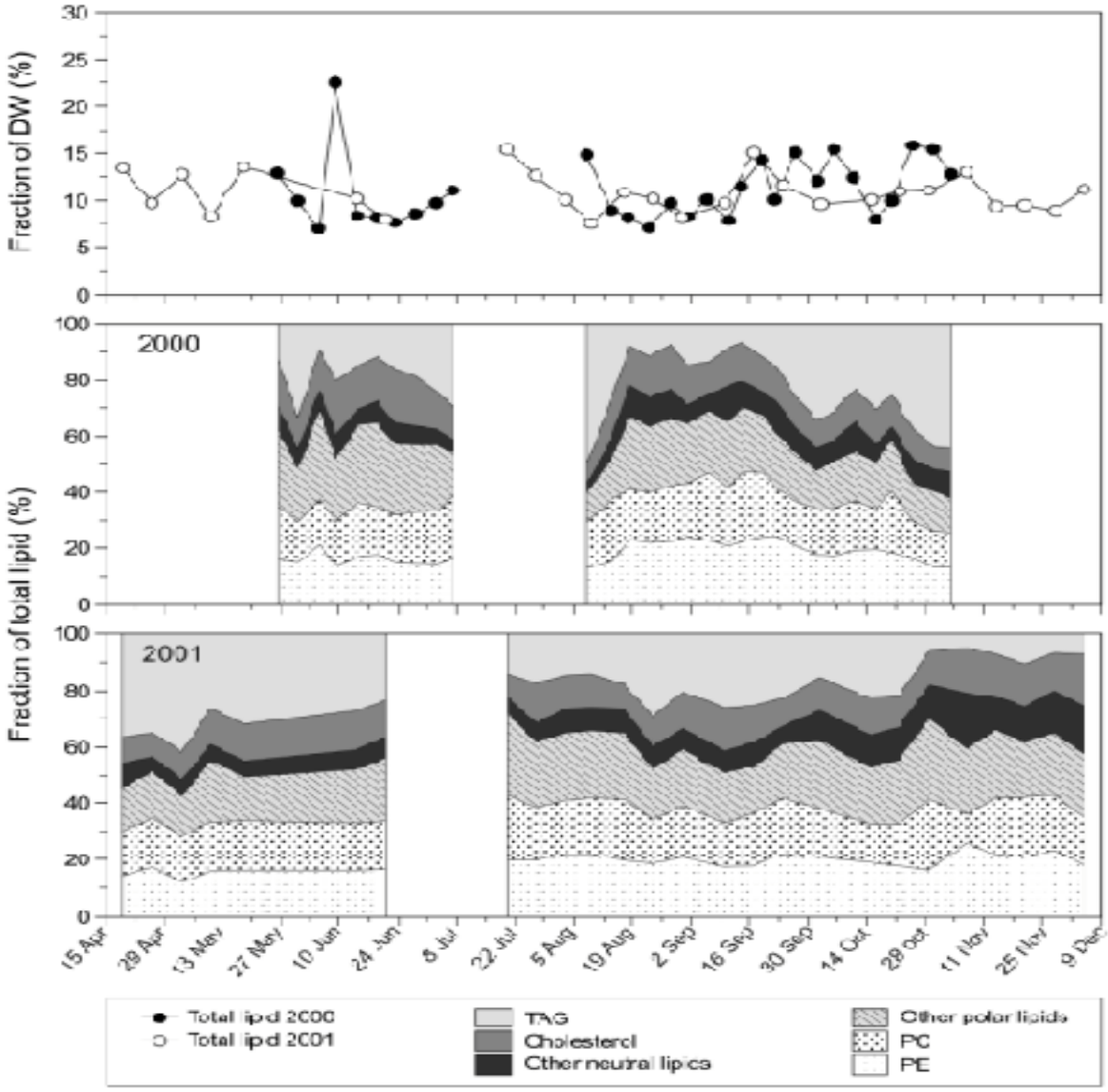
1703 Figure 1



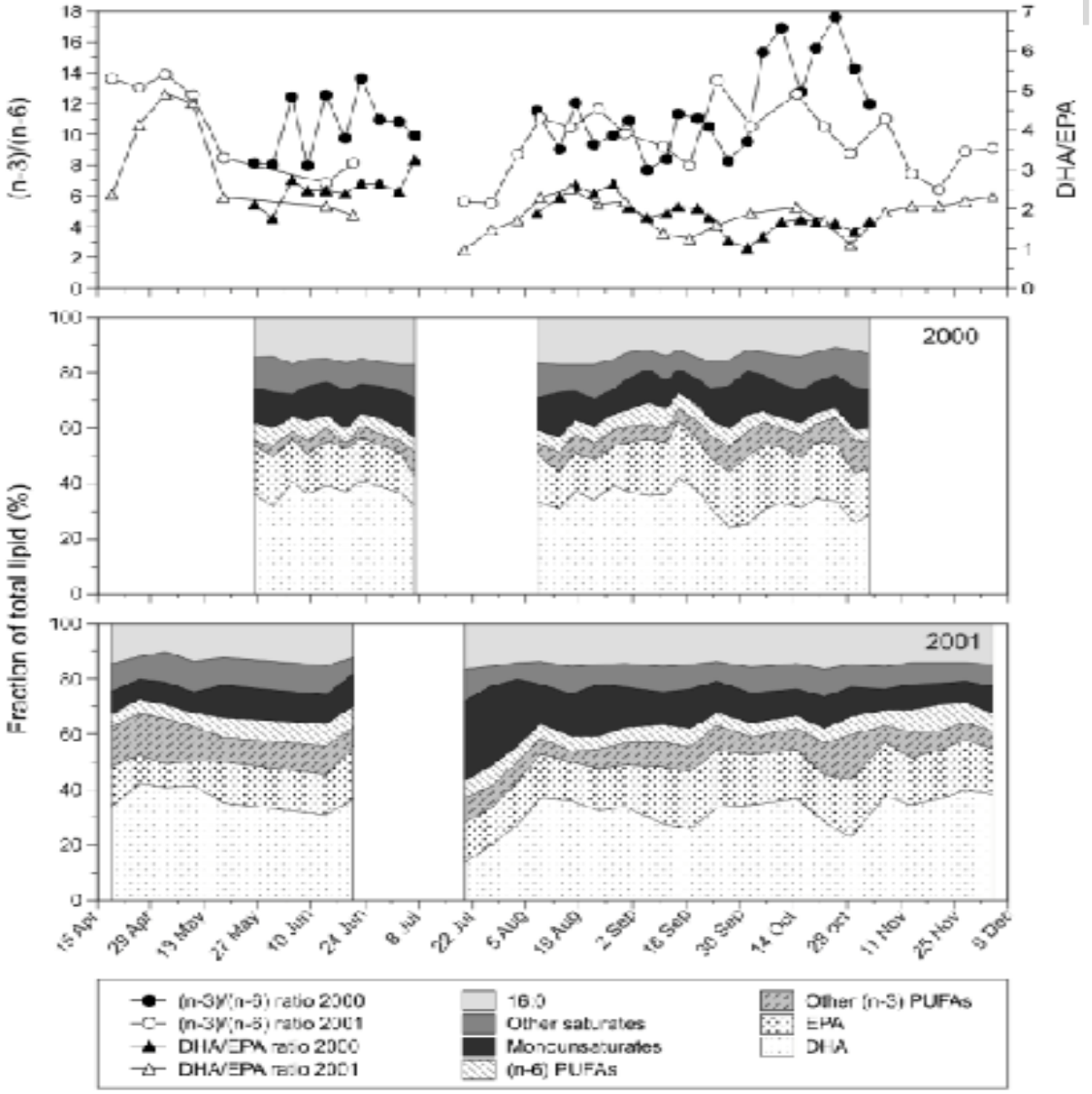
1704 Figure 2



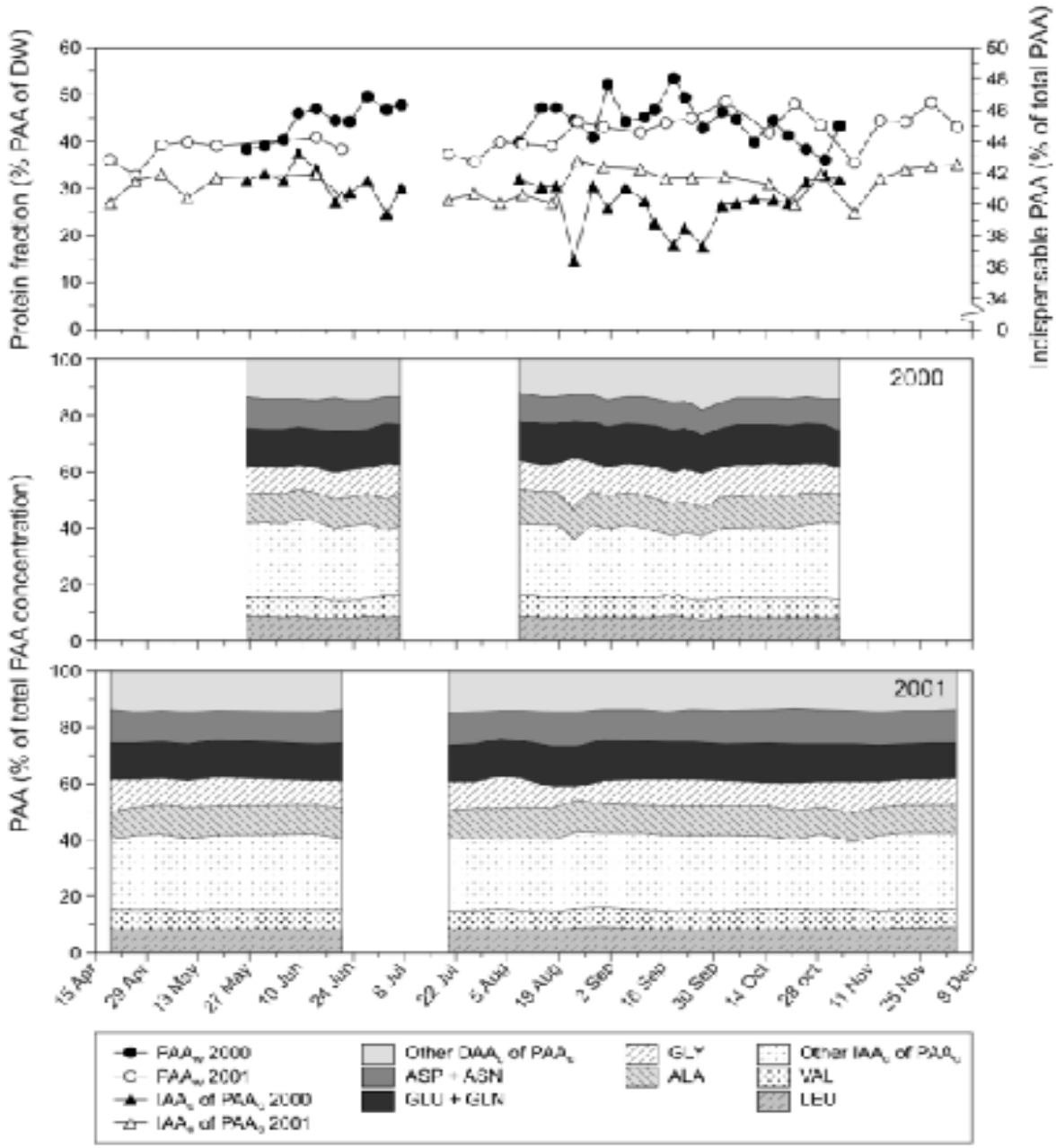
1705 Figure 3



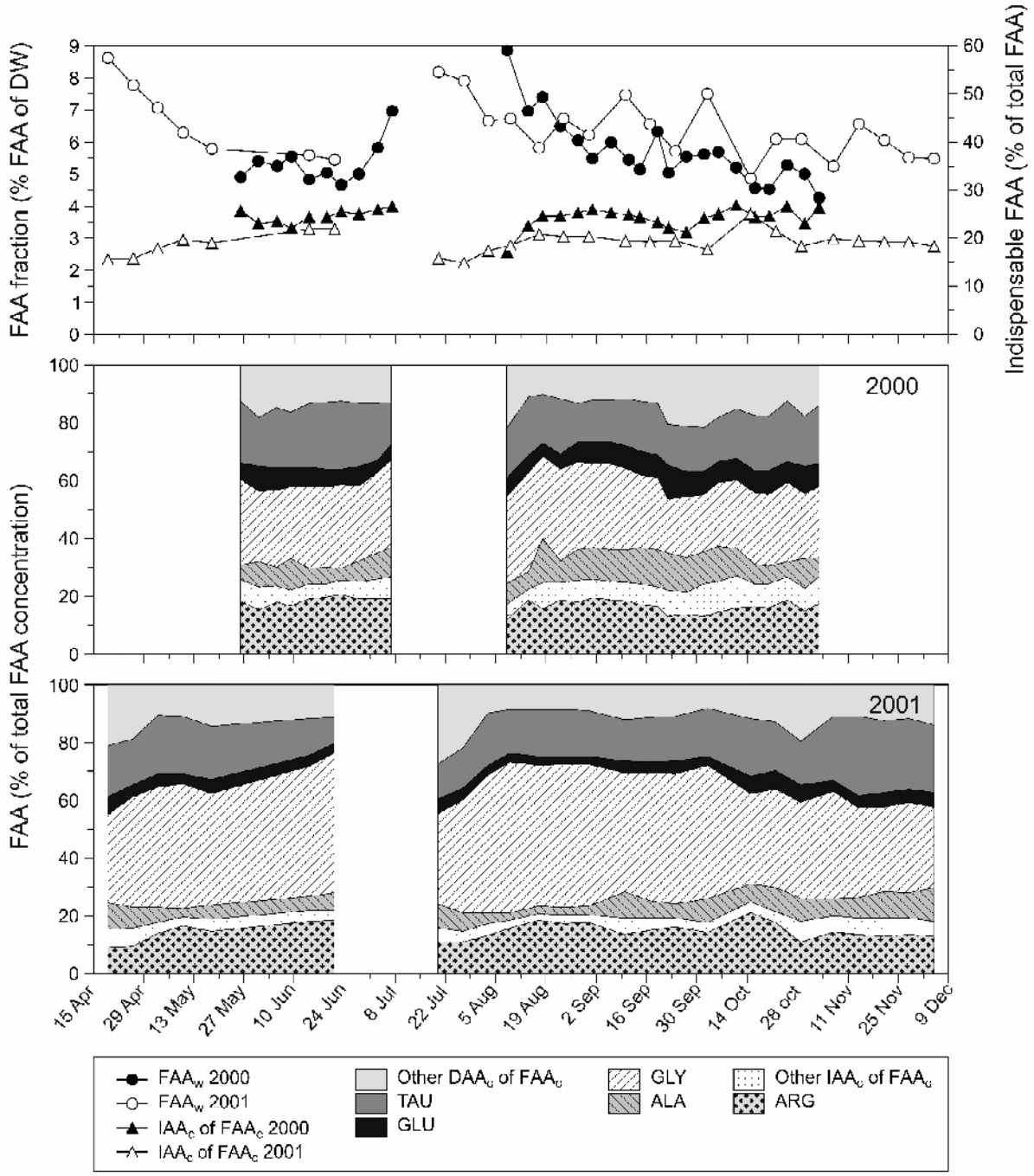
1706 Figure 4



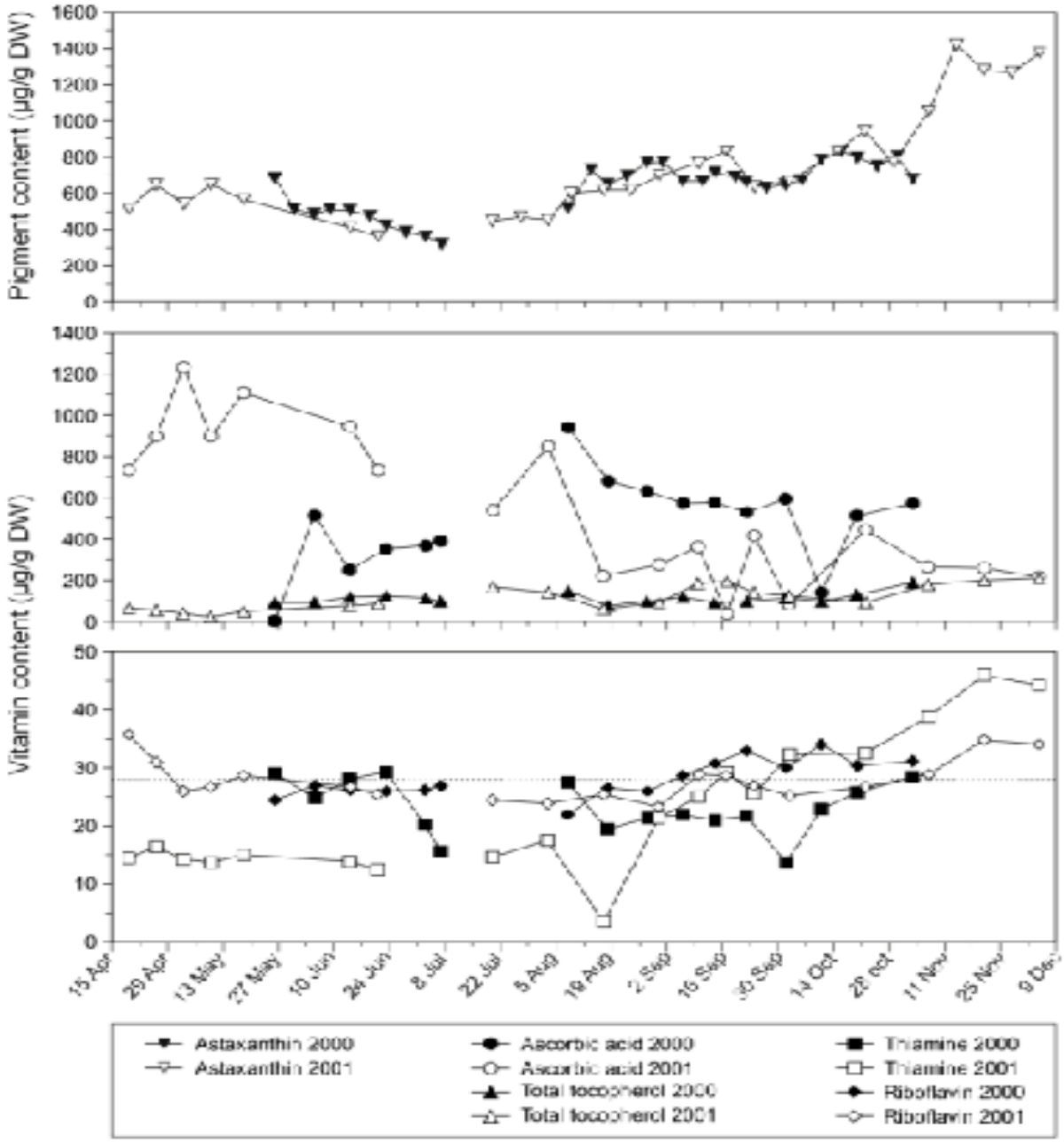
1707 Figure 5



1708 Figure 6



1709 Figure 7



1710 Figure 8