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Variable fatty acid composition of the pelagic appendicularian *Oikopleura dioica* in response to dietary quality and quantity

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ABSTRACT: In marine zooplankton communities, appendicularians are second in abundance to copepods, and there is evidence that they are an important dietary component for larvae of several fish species. As various algal species upon which the appendicularian *Oikopleura dioica* grazes differ significantly in their fatty acid compositions, we have examined how this filter feeder translates diets that differ in quality or quantity into its own fatty acid composition. By feeding *O. dioica* diets containing either a single diatom or single flagellate species, we demonstrated that the fatty acid composition of *O. dioica* reflects dietary quality. Quantitative differences in the amount of fatty acids in the diet also directly led to quantitative differences in fatty acid content of the animal, consistent with differences in reproductive output reported earlier. Under the same dietary regimes, males differed from females in fatty acid composition. Among these differences, females had higher amounts of eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA), important for larval development. Analysis of fatty acid composition of fecal pellets revealed selective assimilation of some fatty acids by the animals, including EPA and DHA. Overall, consistent with data on other zooplanktonic filter feeders, the fatty acid composition of *O. dioica* reflected dietary composition, indicating that the nutritional quality of primary production would be transferred to predators of this component of zooplankton without substantial intermediary modifications.

KEY WORDS: Life history · Zooplankton · Filter feeding · Larval development · Flagellate · Diatom

INTRODUCTION

Appendicularians generate a complex, extracellular, gelatinous, filter feeding house composed of mucopolysaccharides (Spada et al. 2001, Thompson et al. 2001). This filter feeding structure enables appendicularians to feed on algae, as well as particulate organic carbon (POC) down to 0.2 µm (Flood et al. 1992, Fernández et al. 2004). This makes them an important component in marine ecosystems, where they provide a shortcut in the food web by directly transferring energy from very small particles (e.g. submicron colloids, prochlorophytes, cyanobacteria, bacterioplankton, nanoflagellates) to much larger predators such as larval and adult fish (Deibel 1998).

Among appendicularians, *Oikopleura dioica* is found in all major oceans (Fenaux et al. 1998). The generation time, which is extremely short for a complex metazoan (7 d at 15°C), combined with high fecundity, yields a relationship between animal size and maximal intrinsic rate of natural increase (r_{max}) that considerably exceeds values recorded for other poikilothermic metazoans (Troedsson et al. 2002). Appendicularians are, thus, able to exploit favorable environments quickly, resulting in patchy distributions and densities attaining 53 000 ind. m^{-3} (Uye & Ichino 1995). An increased understanding of the general life history parameters of *O. dioica* has been gained in recent years, where filter feeding, development and growth rates have been the main focus. However, data on the

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composition and nutritional value of appendicularians are very limited. Deibel et al. (1992) studied the major lipid classes of *O. vanhoffeni* at different ontogenetic stages under both pre- and post-bloom conditions, and demonstrated a change in the total fatty acid composition before and after the spring bloom. They suggested that there is little evidence of energy storage in *O. vanhoffeni*, with the predominant strategy seeming to be an allocation towards rapid somatic and reproductive growth. *O. dioica* has a very short generation time, with unusually high population increases (Troedsson et al. 2002). Energy will be allocated into reproductive tissues in an opportunistic way and storage for long-term survival is limited. However, a strategy of fixed somatic growth with no apparent storage could lead to high death rates, even over short durations of food depletion. Storage, particularly in the form of fat droplets, would therefore enhance survival as well as reducing the risk of food depletion as reproductive allocation begins.

In view of their appropriate size range for ingestion by fish larvae, and capacity for rapid blooms, appendicularians are also likely to be an important factor for successful recruitment in some fish populations, and this has been documented in several fish species (Shelbourne 1962, Gadomski & Boelert 1984, Mousseau et al. 1998, Ticina et al. 2000, Watanabe et al. 2002, Hasegawa et al. 2003). Studies of marine fishes have shown that fish larvae are sensitive to low levels of some n-3 polyunsaturated fatty acids (PUFAs), particularly eicosapentaenoic acid (EPA; 20:5n3) and docosahexaenoic acid (DHA; 22:6n3) (Støttrup & Attramadal 1992, Koven et al. 1993, Kraul et al. 1993, Lochmann & Gatlin 1993, Watanabe 1993, Whyte et al. 1994). In some fish, the level of DHA has a significant effect on survival as well as on growth rates (Watanabe 1993). Marine animals have limited abilities to synthesize long-chain PUFA from short-chain PUFA (Dunstan et al. 1996). This means that they must obtain these fatty acids from the diet (essential fatty acids). As appendicularians are thought to be an important food source for a number of fish species, a significant question is how the fatty acid composition of appendicularians changes with different composition and quantity of food. To investigate how *Oikopleura dioica* assimilates and modifies fatty acids when experiencing diets differing qualitatively in fatty acid content, animals were fed either a strict diet of *Isochrysis* sp., which is high in DHA and low in EPA content, or *Chaetoceros calcitrans*, which, in contrast, is high in EPA and low in DHA (Napolitano et al. 1990). Subsequently, we examined the storage and assimilation of fatty acids under abundant and limited food regimes, which were identical in fatty acid composition but differed in total fatty acid content (Troedsson et al. 2002).

MATERIALS AND METHODS

Culture of algae. Fresh inoculates were made from static cultures of *Isochrysis* sp. (Prymnesiophyta, CCAP 927/14; diameter = 4.5 µm, carbon = 10.6 ± 0.3 pg cell^{-1}, fatty acid = 1.3 ± 0.4 pg cell^{-1}) and *Chaetoceros calcitrans* (Bacillariophyta, CCAP 1010/11; diameter = 3 µm, length = 4.5 µm, carbon = 4.6 ± 0.3 pg cell^{-1}, fatty acid = 1.0 ± 0.4 pg cell^{-1}). Nutrients and silica (for *C. calcitrans*) were added, and they were grown in 2 l plastic bags with constant light and air bubbles for agitation. The same strain was used throughout the experiments. To avoid fluctuations in fatty acid composition of the algae, qualitative fatty acid analysis was carried out at regular intervals after inoculation. Experiments were then conducted using algae in the exponential growth phase (2 to 6 × 10^6 cells ml^{-1} for *Isochrysis* sp.; 4 to 8 × 10^6 cells ml^{-1} for *C. calcitrans*) during which they had stable lipid composition. These algae were selected for experimentation because they have been well characterised in the culture of *Oikopleura dioica* (Gorsky 1980, Troedsson et al. 2002), were similar in total fatty acid content, but differed significantly in fatty acid composition (Tables A & B of Appendix 1, see www.int-res.com/journals/suppl/troedsson_appendix.pdf), and they are representative of the progression from diatom to flagellate blooms in natural ecosystems.

Collection and culture of animals. *Oikopleura dioica* was collected in the coastal area of Bergen, Norway, using a plankton net with a large volume codend, and cultured in wet laboratory facilities. Animals were cultured in 6 l plastic beakers (Cambro) using a plastic paddle (25 cm deep × 7 cm wide) rotating at 15 rpm to keep the animals and algae suspended in the water column. Seawater from a depth of 4 to 8 m was filtered through 3 Hytrex II Cartridge Filters (20, 10 and 1 µm, respectively). The filtered seawater was then passed through a Merck charcoal filter with a 0.25 to 1 mm gradient and exposed to an Aqua-Care UV-light (254 nm, 10 W) to sterilize the seawater. Cultures were run at 15°C, illuminated by 36W/20 cool white fluorescent light tubes. To establish a generation of animals, 40 to 45 mature females and 30 to 35 mature males were placed in 4 l of seawater. The animals were monitored and forced to release their gametes into the water by gentle aspiration in a Pasteur pipette at full maturity. This was done to synchronize the population. After fertilization, embryos were transferred, using 1 l beakers, into 2 fresh 6 l beakers with the designated food regimes. After 24 h, the content of each beaker was diluted into 3 new 6 l beakers, and after an additional 24 h, the contents of each of these beakers was in turn diluted into two 6 l beakers, attaining a total of 12 beakers. These serial dilutions allowed addition of

fresh seawater at 24 h intervals and promoted consistently better survival than 1, more extensive, initial dilution. After each dilution, the assigned food regime was added. Each successive 24 h after this, the animals were transferred with a 10 ml Sterilin pipette with a cut tip, the diameter of which exceeded the maximum width of the house, to a fresh 6 l beaker under the same experimental conditions.

Culture of animals for fatty acid analysis. Four experimental conditions were set up to investigate the fatty acid composition in *Oikopleura dioica*, given qualitatively and quantitatively different food regimes.

Different quality of algae: A generation of animals was split in 2 after hatching and 1 population was given a standard food regime of only *Isochrysis* sp., while the other received a standard food regime of only *Chaetoceros calcitrans* (Table 1). Animals from both food regimes were sampled (10 replicates) each 24 h after fertilization, and the fatty acid contents were analyzed. Fecal pellets from both food regimes were sampled (5 replicates) each 24 h starting 4 d after fertilization, and the fatty acid content analyzed. The number of animals and fecal pellets in each replicate varied with age (Table 2).

Different quantity of algae: A generation of animals was split in 2 after hatching and 1 population was given a standard food regime of both algae, while the other was given a limited food regime of both algae (Table 1). Animals and fecal pellets from both food regimes were sampled as in the above experiment.

Fatty acid analyses. Sampled animals were prodded to escape their house and left in fresh sterile-filtered sea water (0.2 µm) for 15 min. The animals were then inspected with a stereo microscope to confirm absence of fecal pellets in the stomach. Animals were transferred with a micropipette to a 1 ml thick-walled glass vial with a conical bottom and a Teflon-lined screw cap. The number of animals for each replicate varied with the age of the animals (Table 2). A 1-step extraction-methylation procedure was used (Ulberth & Henninger 1995). Residual water was evaporated under a stream of nitrogen gas. An internal standard (0.12 µg, 19:0, NuChek Prep.) dissolved in chloroform was added to each sample and the solvent was evaporated under nitrogen. To methanolyze the fatty acids, 50 µl of 2 M dry methanolic-HCl was added, and the vial was capped and incubated at 90°C for 2 h. The methanolic-HCl was evaporated with nitrogen gas and the remaining methyl esters were dissolved in 40 µl HPLC-grade hexane. Polar components were extracted with 20 µl of H_2O. The samples were vortexed and placed at –20°C until the polar portion was frozen. The non-polar hexane layer was transferred with a Hamilton pipette into a conical bottom crimp-top GC microvial. Nitrogen gas was quickly purged over the samples to eliminate any oxygen in order to avoid oxidation of fatty acids, and the vial was capped. The methyl esters were then analyzed by gas chromatography (Hewlett Packard HP 5890 II with a HP 7673 A automatic injection system) as previously described (Grahl-Nielsen et al. 2003). Full results containing all fatty acids at all time points are given in Tables A to F of Appendix 1. Tables 3 to 7 reported in the text present average values for phases 2, 3 and 4 of the life cycle (Troedsson et al. 2002) of a subset of fatty acids. Phase 2 was defined from the expansion of the first house to when the reproductive organ started to grow. Phase 3 was defined from the start of reproductive organ growth until the beginning of spawning. Phase 4 was defined as mature animals with gametes nearing the time of release. Table 8 shows a subset of fatty acids in fecal pellets at 3 time points.

Table 2. Number of animals and fecal pellets per replicate fatty acid analysis

Age (d)	Animals	[a]Fecal pellets
1	50	–
2	40	–
3	35	–
4	8	100
5	2	80
6 (mature)	1	30

[a]Fecal pellets were not sampled at early stages because of their small size

Table 1. Food regimes for quantitative and qualitative protocols

Protocol	Food regime	Algae[a]	0–96 h (cells ml^{-1})	96 h–maturity (cells ml^{-1})
Qualitative	*Isochrysis*	*Isochrysis*	4000	8000
	Chaetoceros	*Chaetoceros*	4000	8000
Quantitative	Standard	*Isochrysis*	2000	4000
		Chaetoceros	2000	4000
	Limited	*Isochrysis*	333	666
		Chaetoceros	333	666

[a]The algal strains used were *Isochrysis* sp. and *Chaetoceros calcitrans*

Net lipid accumulation efficiency. Net lipid accumulation efficiency was calculated using the quantity of fatty acids cleared from the given experimental environment and allocated to body mass. The total fatty acid quantity per cell of *Isochrysis* sp. or *Chaetoceros calcitrans* was analyzed using 19:0 (NuChek Prep.) as an internal standard. The number of cells cleared from the water column per time unit was cor-

related to the food concentration according to Selander & Tiselius (2003). We used the same somatic size of animals as Selander & Tiselius to avoid size effects (Broms & Tiselius 2003). The instantaneous net lipid accumulation efficiency ($NLAE_i$) was calculated as the percentage of lipids assimilated and used for fatty acid increase:

$$NLAE_i = [G_{animals}/(IR_{FA} - P_{FA})] \times 100 \quad (1)$$

where $G_{animals}$ (ng FA ind.$^{-1}$ h^{-1}) is the fatty acid increase in animals over 1 h, IR_{FA} (ng FA ind.$^{-1}$ h^{-1}) is the amount of fatty acids cleared from the water column in 1 h, and P_{FA} (ng FA ind^{-1} h^{-1}) is the amount of fatty acid that is excreted in fecal pellets in 1 h. P_{FA} is dependent on the number of pellets excreted per hour and we, therefore, used the gut passage time (GPT) estimated by López-Urrutia & Acuña (1999):

$$GPT = 51.67(FC^{-0.245}) \times e^{-0.0376T} \quad (2)$$

where FC represents the concentration of available food (µg C l^{-1}) and T represents temperature (°C).

Statistical analysis. Chromatographic data were treated by multivariate statistics, using principal component analysis in the SIRIUS 6.5 package (Kvalheim & Karstang 1987). Data were normalized by expressing the methyl esters as a percentage of the total amount in the sample. The percentages as well as the total quantity of fatty acids in the tables are given to 2 insignificant digits. The data were logarithmically transformed to avoid dominance of highly abundant methyl esters. The samples were placed in an n-dimensional space (n = the number of methyl esters in the sample). Two new coordinates (principal components or PCs) were generated through the center of gravity, explaining the largest and the second largest variation in the samples. Two-dimensional plots (PCs) were made from this analysis as a representation of the separation between samples.

Quantification of total fatty acids was calculated using 19:0 as an internal standard. Linear regression analysis of the population was performed with Statistix8. The data were log-transformed and a normality test (Shapiro-Wilks) and test of co-linearity were run to ensure requirements were fulfilled to perform a linear regression analysis. To investigate differences in a particular fatty acid quantity between 2 groups at a fixed time point, a *t*-test was performed after verification of homogeneity in the variance.

RESULTS AND DISCUSSION

The major fatty acids in *Oikopleura dioica* were myristic acid (14:0), palmitic acid (16:0), palmitoleic acid (16:1n7), stearic acid (18:0), stearidonic acid (18:4n3), eicosapentaenoic acid (20:5n3 [EPA]) and docosahexaenoic acid (22:6n3 [DHA]), with 18:4n3, EPA and DHA seeming to be increasingly important as the reproductive organs started to grow (Phase 3; Troedsson et al. 2002) (Tables 3 to 6). Indeed, these fatty acids accumulated in mature animals (Table 7). Selective assimilation of some important fatty acids in *O. dioica* is apparent by looking at fecal pellet composition. The major fatty acids remaining in the fecal pellet were 14:0, 16:0, 16:1n7 and 18:0, while 18:4n3, EPA and DHA were preferentially absorbed by the animals (Table 8). Synthesis of 18:4n3 via α-linolenic acid, catalyzed by δ-6-desaturase, is a starting point for further

Table 3. *Isochrysis* sp. and *Oikopleura dioica*. Fatty acid compositions of *Isochrysis* sp. and *O. dioica* fed a diet containing only *Isochrysis* sp. Mean fatty acid compositions in the 3 growth phases of *O. dioica*. The full data set with samples every 24 h is given in Table A of Appendix 1 (www.int-res.com/journals/suppl/troedsson_appendix.pdf). The major fatty acids are given as the percentage of total composition with standard deviation. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	*Isochrysis* sp.	*Oikopleura dioica* Phase 2	Phase 3	Phase 4
14:0	12.8 ± 5.1	5.6 ± 0.9	10.1 ± 1.6	9.1 ± 0.7
14:1n5	0.3 ± 0.1[b]	0.7 ± 0.4	0.6 ± 0.2	0.4 ± 0.2
16:0	8.2 ± 1.5	17.5 ± 3.0	15.6 ± 1.9	17.3 ± 3.4
16:1n7[a]	2.1 ± 1.0[b]	5.8 ± 1.7	10.5 ± 1.5	13.7 ± 1.0
16:2n6[a]	0.4 ± 0.2[b]	1.6 ± 0.4	0.6 ± 0.3	0.3 ± 0.1
17:0	0.1 ± 0.0[b]	0.6 ± 0.2	0.6 ± 0.1	0.7 ± 0.1
18:0	1.0 ± 0.3[b]	7.9 ± 1.8	2.4 ± 1.0	2.8 ± 1.2
18:1n9[a]	5.9 ± 1.7[b]	2.0 ± 0.9	0.9 ± 0.8	0.3 ± 0.1
18:1n7[a]	0.9 ± 0.1[b]	5.2 ± 2.1	2.2 ± 0.7	2.4 ± 0.5
18:2n6[a]	4.3 ± 2.6[b]	1.4 ± 0.6	1.8 ± 0.3	1.8 ± 0.3
18:2n4[a]	0.2 ± 0.2[b]	2.0 ± 0.7	0.7 ± 0.5	0.4 ± 0.2
18:3n3[a]	3.3 ± 1.8[b]	1.6 ± 0.8	3.0 ± 0.6	2.7 ± 0.7
18:4n3[a]	9.3 ± 4.9[b]	1.6 ± 0.6	10.4 ± 2.3	9.1 ± 3.4
18:5n1	0.6 ± 0.2[b]	2.8 ± 1.0	1.1 ± 0.9	0.5 ± 0.4
20:1n9	0.4 ± 0.2[b]	2.0 ± 0.6	0.6 ± 0.2	0.4 ± 0.2
20:2n6	0.2 ± 0.2[b]	0.1 ± 0.1	0.1 ± 0.0	0.2 ± 0.1
20:4n6	0.2 ± 0.1	0.2 ± 0.1	0.6 ± 0.2	0.7 ± 0.2
20:3n3	0.2 ± 0.2[b]	0.4 ± 0.2	0.3 ± 0.2	0.2 ± 0.1
20:5n3[a]	2.5 ± 1.0[b]	5.4 ± 1.4	8.6 ± 0.9	8.2 ± 2.1
22:0	0.2 ± 0.0[b]	0.6 ± 0.2	0.2 ± 0.1	0.3 ± 0.2
21:5n3[a]	0.1 ± 0.1[b]	1.2 ± 0.4	0.5 ± 0.3	0.4 ± 0.2
22:5n6[a]	1.2 ± 0.3[b]	0.3 ± 0.3	1.5 ± 0.4	1.0 ± 0.4
22:5n3[a]	3.5 ± 1.6	1.8 ± 0.7	0.6 ± 0.4	0.3 ± 0.2
24:0[a]	0.2 ± 0.0	0.6 ± 0.2	0.3 ± 0.1	0.2 ± 0.1
22:6n3[a]	6.9 ± 4.3[b]	1.7 ± 0.8	7.7 ± 1.7	6.7 ± 2.7
Groups				
SFA	22.9 ± 5.4	35.4 ± 5.4	30.5 ± 2.8	31.6 ± 3.7
MUFA	11.0 ± 2.0	17.1 ± 3.4	15.5 ± 1.8	17.5 ± 1.2
PUFA	32.9 ± 7.4	22.2 ± 4.4	37.4 ± 4.7	32.4 ± 4.9
n-3	26.0 ± 7.0	13.7 ± 3.4	31.1 ± 4.7	27.6 ± 4.9
n-6	6.3 ± 2.6	3.6 ± 0.8	4.6 ± 0.6	4.0 ± 0.4

[a] Fatty acids showing a significant difference (regression analysis: $p < 0.05$) between animals fed *Isochrysis* sp. compared to *Chaetoceros calcitrans* throughout the life cycle

[b] Fatty acids that differ significantly (*t*-test: $p < 0.05$) from the respective values for *Chaetoceros calcitrans* (Table 4)

Table 4. *Chaetoceros calcitrans* and *Oikopleura dioica*. Fatty acid compositions of *C. calcitrans* and *O. dioica* fed a diet containing only *C. calcitrans*. Mean fatty acid compositions in the 3 growth phases of *O. dioica*. The full data set with samples every 24 h is given in Table B of Appendix 1. The major fatty acids are given as the percentage of total composition with standard deviation. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	*Chaetoceros calcitrans*	*Oikopleura dioica* Phase 2	*Oikopleura dioica* Phase 3	*Oikopleura dioica* Phase 4
14:0	9.2 ± 6.7	4.8 ± 1.5	9.2 ± 2.9	6.8 ± 1.5
14:1n5	0.2 ± 0.1[b]	0.6 ± 0.3	0.7 ± 0.1	0.6 ± 0.2
16:0	6.5 ± 4.9[b]	16.9 ± 3.7	15.4 ± 2.7	15.5 ± 2.2
16:1n7[a]	8.5 ± 5.5[b]	5.3 ± 1.6	14.5 ± 4.1	15.2 ± 3.2
16:2n6[a]	1.7 ± 1.4[b]	1.7 ± 0.8	0.5 ± 0.5	0.4 ± 0.3
17:0	0.3 ± 0.3[b]	0.6 ± 0.2	0.5 ± 0.1	0.7 ± 0.2
18:0	1.9 ± 1.4[b]	8.4 ± 1.7	2.6 ± 1.5	2.6 ± 0.9
18:1n9[a]	0.3 ± 0.4[b]	2.5 ± 1.7	1.3 ± 1.7	1.1 ± 0.9
18:1n7[a]	0.3 ± 0.1[b]	5.2 ± 2.4	1.6 ± 1.0	1.6 ± 0.5
18:2n6[a]	0.3 ± 0.2[b]	1.3 ± 0.4	1.0 ± 0.2	1.2 ± 0.2
18:2n4[a]	0.4 ± 0.1[b]	2.3 ± 1.3	0.6 ± 0.7	1.0 ± 1.0
18:3n3[a]	0.1 ± 0.1[b]	1.6 ± 0.8	0.7 ± 0.2	1.1 ± 0.2
18:4n3[a]	0.2 ± 0.1[b]	1.3 ± 0.7	2.5 ± 0.8	4.6 ± 1.2
18:5n1	1.3 ± 0.5[b]	3.2 ± 2.0	0.9 ± 0.9	1.3 ± 1.2
20:1n9	0.1 ± 0.0[b]	2.2 ± 1.0	0.5 ± 0.2	0.4 ± 0.1
20:2n6	0.5 ± 0.3[b]	0.1 ± 0.1	0.2 ± 0.1	0.2 ± 0.1
20:4n6	0.3 ± 0.2	0.2 ± 0.1	0.9 ± 0.3	0.5 ± 0.1
20:3n3	0.5 ± 0.3[b]	0.4 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
20:5n3[a]	6.5 ± 3.1[b]	5.9 ± 1.2	18.3 ± 5.3	9.9 ± 2.3
22:0	1.1 ± 0.5[b]	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1
21:5n3[a]	–[b]	1.1 ± 0.6	0.5 ± 0.4	0.7 ± 0.7
22:5n6[a]	0.2 ± 0.2[b]	0.1 ± 0.2	0.2 ± 0.1	0.1 ± 0.1
22:5n3[a]	6.6 ± 4.8	1.6 ± 0.9	0.6 ± 0.5	0.5 ± 0.4
24:0[a]	0.5 ± 0.3	0.6 ± 0.3	0.2 ± 0.1	0.2 ± 0.1
22:6n3[a]	0.3 ± 0.1[b]	1.6 ± 0.9	4.0 ± 0.9	4.5 ± 1.5
Groups				
SFA	20.2 ± 8.4	35.4 ± 5.4	30.5 ± 2.8	27.5 ± 2.8
MUFA	11.8 ± 5.8	17.3 ± 3.3	19.3 ± 2.0	19.1 ± 2.3
PUFA	19.0 ± 6.0	22.4 ± 4.4	31.2 ± 3.9	26.2 ± 3.5
n-3	14.2 ± 5.8	13.6 ± 3.0	26.9 ± 5.0	21.5 ± 3.1
n-6	3.0 ± 1.5	3.3 ± 0.8	2.8 ± 0.6	2.3 ± 0.4

[a]Fatty acids showing a significant difference (regression analysis: $p < 0.05$) between animals fed *Isochrysis* sp. compared to *Chaetoceros calcitrans* throughout the life cycle

[b]Fatty acids that differ significantly (*t*-test: $p < 0.05$) from the respective values for *Isochrysis* sp. (Table 3)

Table 5. *Oikopleura dioica*. Fatty acid composition of *O. dioica* fed a standard food regime. Mean fatty acid compositions in the 3 growth phases of *O. dioica*. The full data set with samples every 24 h is given in Table C of Appendix 1. The major fatty acids are given as a percentage of total composition with standard deviation. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	*Oikopleura dioica*: standard food regime Phase 2	Phase 3	Phase 4
14:0[a]	5.3 ± 1.1	9.0 ± 2.1	7.9 ± 3.0
14:1n5[a]	0.3 ± 0.1	0.1 ± 0.1	0.1 ± 0.1
15:0[a]	1.5 ± 0.3	1.3 ± 0.3	1.3 ± 0.4
16:0[a]	14.0 ± 2.6	16.1 ± 2.3	15.7 ± 3.5
16:1n7[a]	14.2 ± 2.6	16.3 ± 2.4	15.9 ± 3.6
16:2n6[a]	1.5 ± 0.8	0.8 ± 0.4	0.6 ± 0.3
17:0[a]	0.5 ± 0.1	0.5 ± 0.1	0.5 ± 0.2
18:0	4.3 ± 1.2	3.7 ± 1.4	2.3 ± 0.9
18:1n7	1.3 ± 1.8	2.4 ± 1.3	1.9 ± 0.5
18:2n6[a]	1.3 ± 0.3	1.4 ± 0.3	2.0 ± 0.4
18:2n4[a]	1.8 ± 1.0	0.8 ± 0.6	0.6 ± 0.4
18:3n3[a]	2.3 ± 0.8	2.2 ± 0.7	2.6 ± 0.8
18:4n3[a]	2.8 ± 1.0	6.1 ± 2.0	6.8 ± 2.0
18:5n1	2.3 ± 1.1	1.3 ± 0.9	1.0 ± 0.6
20:0[a]	0.4 ± 0.1	0.4 ± 0.1	0.5 ± 0.2
20:1n9[a]	0.9 ± 0.3	0.6 ± 0.2	0.3 ± 0.1
20:4n6[a]	0.4 ± 0.1	0.4 ± 0.1	0.7 ± 0.1
20:5n3[a]	7.7 ± 1.2	7.2 ± 1.9	10.4 ± 2.5
21:5n3[a]	0.6 ± 0.2	1.1 ± 0.4	1.2 ± 0.4
22:5n6[a]	1.6 ± 1.1	0.9 ± 0.8	0.8 ± 0.6
24:0[a]	0.4 ± 0.2	0.4 ± 0.1	0.3 ± 0.1
22:6n3[a]	5.7 ± 1.8	6.9 ± 2.8	8.0 ± 2.6
Groups			
SFA	26.8 ± 4.5	31.1 ± 5.5	28.0 ± 4.7
MUFA	21.6 ± 3.9	22.7 ± 5.6	20.8 ± 3.7
PUFA	33.0 ± 4.0	31.7 ± 6.9	37.0 ± 4.4
n-3	19.7 ± 4.0	23.4 ± 7.3	29.3 ± 4.2
n-6	4.8 ± 1.5	3.5 ± 0.9	4.3 ± 0.8

[a]Fatty acids showing a significant difference (regression analysis: $p < 0.05$) between animals fed a standard compared to a limited food regime throughout the life cycle

modification to key fatty acids in the endocrine regulatory system. One such key fatty acid, EPA, is an important constituent of phospholipids in all animal tissues, especially the brain, and is the precursor of the PG_3 series of prostaglandins (Braden & Carroll 1986, Harwood 1994). Another fatty acid in this pathway, DHA is enriched in brain synapses and heart tissue, and is thought to have important roles during neural development of larvae (Gunstone & Herslof 2000, Valenzuela et al. 2004).

The fatty acid compositions of *Isochrysis* sp. and *Chaetoceros calcitrans* were significantly different from each other (Fig. 1), in general agreement with previous studies (Napolitano et al. 1990). There were a number of differences between the 2 algae at the level of individual fatty acids, but the most striking differences were in 16:1n7, EPA, DHA and 18C fatty acids. Many of the 18C fatty acids, as well as 16:1n7, are common in a variety of organisms, and it is intriguing to see the higher percentage of 18:2n6 and 18:3n3 in *Isochrysis* sp. (*t*-test; 18:2n6 and 18:3n3, $p < 0.001$) (Tables 3 & 4). These fatty acids are rapidly metabolized in animals as they are metabolites of the n-3 and n-6 family of essential fatty acids (Harwood 1994). The higher content of DHA in *Isochrysis* sp. and EPA in *C. calcitrans* is of interest as these fatty acids are known to be crucial for the development and survival of many marine animals (Støttrup & Attramadal 1992, Koven et al. 1993, Kraul et al. 1993, Lochmann & Gatlin 1993, Watanabe 1993, Whyte et al. 1994). The 2 algal species, thus, represent a characteristic nutritional difference between flagellates and diatoms, which might

Table 6. Fatty acid composition of *Oikopleura dioica* fed a limited food regime. Mean fatty acid compositions in the 3 growth phases of *O. dioica*. The full data set with samples every 24 h is given in Table D of Appendix 1. The major fatty acids are given as a percentage of total composition with standard deviation. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	*Oikopleura dioica*: limited food regime Phase 2	Phase 3	Phase 4
14:0[a]	4.6 ± 1.1	4.3 ± 0.9	4.1 ± 0.9
14:1n5[a]	0.3 ± 0.2	0.2 ± 0.1	0.2 ± 0.1
15:0[a]	1.5 ± 0.3	1.3 ± 0.5	1.3 ± 0.3
16:0[a]	15.4 ± 2.9	15.8 ± 3.8	16.5 ± 2.7
16:1n7[a]	15.6 ± 3.0	16.0 ± 3.9	16.8 ± 2.7
16:2n6[a]	1.3 ± 0.7	1.2 ± 0.7	0.8 ± 0.5
17:0[a]	0.6 ± 0.1	0.6 ± 0.2	0.6 ± 0.2
18:0	5.2 ± 1.5	5.1 ± 2.2	5.2 ± 2.4
18:1n7	2.0 ± 1.8	2.1 ± 1.7	2.8 ± 0.8
18:2n6[a]	1.2 ± 0.3	1.1 ± 0.4	1.6 ± 0.6
18:2n4[a]	1.5 ± 0.9	1.6 ± 0.9	0.6 ± 0.7
18:3n3[a]	2.0 ± 1.0	1.2 ± 0.5	1.7 ± 0.9
18:4n3[a]	2.0 ± 0.6	3.8 ± 1.8	5.5 ± 3.0
18:5n1	2.0 ± 1.1	2.2 ± 1.0	1.5 ± 0.8
20:0[a]	0.5 ± 0.1	0.5 ± 0.1	0.8 ± 0.2
20:1n9[a]	1.2 ± 0.3	0.8 ± 0.3	1.2 ± 0.5
20:4n6[a]	0.4 ± 0.1	0.3 ± 0.1	0.6 ± 0.2
20:5n3[a]	7.5 ± 2.4	6.7 ± 2.2	7.7 ± 3.6
21:5n3[a]	0.5 ± 0.2	0.5 ± 0.3	0.7 ± 0.3
22:5n6[a]	0.7 ± 0.8	1.7 ± 1.0	1.0 ± 0.9
24:0[a]	0.5 ± 0.2	0.4 ± 0.2	0.3 ± 0.1
22:6 n3[a]	4.9 ± 2.0	3.8 ± 2.0	5.4 ± 2.6
Groups			
SFA	28.7 ± 5.4	28.4 ± 6.9	29.1 ± 3.7
MUFA	25.2 ± 5.0	23.8 ± 3.6	24.3 ± 3.1
PUFA	28.4 ± 5.5	29.0 ± 7.6	32.3 ± 7.7
n-3	17.5 ± 5.3	16.7 ± 6.2	21.0 ± 5.4
n-6	3.6 ± 1.1	4.4 ± 1.4	4.1 ± 1.2

[a]Fatty acids showing a significant difference (regression analysis: $p < 0.05$) between animals fed a limited compared to a standard food regime throughout the life cycle

affect animals experiencing higher levels of each of these types of algae at different times of the year. Indeed, there are scattered studies of appendicularian abundance (Uye & Ichino 1995, Uye et al. 2000, Vargas et al. 2002, López-Urrutia et al. 2003, Tomita et al. 2003) that show peaks of *Oikopleura* density correlating with increased primary production, often beginning with a spring diatom bloom. However, although *O. dioica* clearly responds in its reproductive output to increased primary production (Troedsson et al. 2002), the regulation of *Oikopleura* population dynamics is more complex and also includes, for example, predation pressure, in addition to the quantity and quality of nutrition available.

The difference in fatty acid compositions of *Isochrysis* sp. and *Chaetoceros calcitrans* did indeed transfer into different compositions in *Oikopleura dioica* when fed a diet of only one or the other of these algae. Regression analysis on animals fed a diet of only

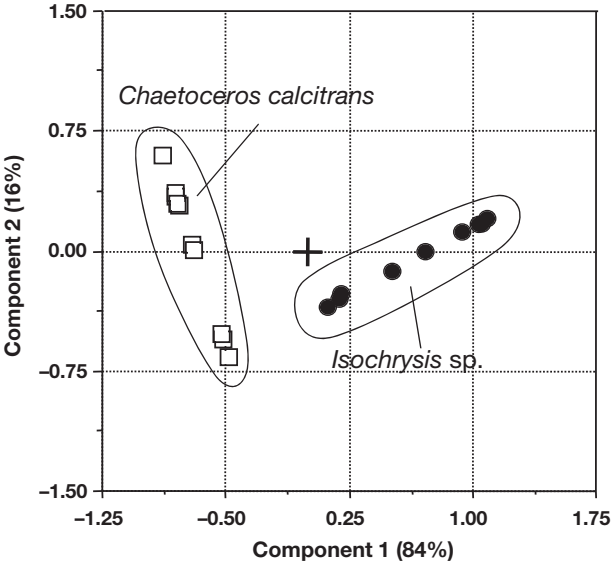

Fig. 1. Fatty acid compositions of algae. Two-dimensional principal component (2D-PC) plot of the fatty acid compositions of *Isochrysis* sp. (●) and *Chaetoceros calcitrans* (□). The percentage of the total variation among the groups along the 2 PCs is given on the respective axes. The symbols represent individual replicates. The 2 separated groups are circled

Isochrysis sp. or *C. calcitrans* did not show a significant difference (p[slope] = 0.3; p[elevation] = 0.9) in total quantity of fatty acids throughout development (Fig. 2A). As seen in the PC plot (Fig. 2B), however, animals aged 3 d and older (Phase 3) showed a clear difference in fatty acid composition. Approximately 47% of all fatty acids (Tables 3 & 4, Tables A & B in Appendix 1) showed a significant difference ($p < 0.05$) in a regression analysis. The 2 fatty acids characteristic of flagellates (DHA and 18:4n3) showed an increased level in *O. dioica* given only *Isochrysis* sp., while animals given only *C. calcitrans* had increased levels of EPA and 16:1n7, characteristic of diatom composition (Napolitano et al. 1990, Graeve et al. 1994, 1997, Falk-Petersen et al. 1998, Scott et al. 1999). The 2 metabolites of the n-3 (18:3n3) and the n-6 (18:2n6) synthetic cascade showed a significant accumulation in animals fed *Isochrysis* sp. (18:3n3: p[slope] < 0.001, p[elevation] < 0.001; 18:2n6: p[slope] = 0.31, p[elevation] < 0.001) (Tables 3 & 4). In *Isochrysis* sp., 18:2n6 and 18:3n3 represented 4.3 and 3.3%, respectively, of the fatty acid pool. In contrast, *C. calcitrans* showed corresponding values of 0.3 and 0.1%. These results agree with those found for herbivorous copepods (Graeve et al. 1997), the major component of marine zooplankton, where the fatty acid composition reflects that in the diet. The quality of microalgal fatty acid composition has been suggested to be more important for optimal growth and development than protein or carbohydrate compositions (Holland 1978, Webb & Chu 1983).

Table 7. *Oikopleura dioica*. Fatty acid compositions of *O. dioica* males and females under different dietary regimes. Composition of a subset of fatty acids from mature *O. dioica*. Full compositions are given in Table E of Appendix 1. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	*Isochrysis* sp.		*Chaetoceros calcitrans*		Standard		Limited	
	Male	Female	Male	Female	Male	Female	Male	Female
14:0	9.2 ± 0.8	9.0 ± 0.7	6.4 ± 1.4	7.2 ± 1.8	8.1 ± 0.9	8.8 ± 0.9	7.6 ± 1.5	7.4 ± 0.3
16:0	20.5 ± 0.9[a]	14.2 ± 0.8	16.8 ± 2.2	14.3 ± 1.4	15.0 ± 1.7	13.7 ± 0.8	18.5 ± 1.8[a]	14.7 ± 0.7
16:1n7	14.1 ± 1.1	13.3 ± 0.7	15.0 ± 3.4	15.3 ± 3.3	13.6 ± 0.7[a]	15.0 ± 1.1	15.3 ± 1.7	16.4 ± 1.0
18:0	3.7 ± 1.0[a]	1.9 ± 0.6	3.0 ± 1.0	2.3 ± 0.7	2.6 ± 0.3[a]	1.7 ± 0.2	2.3 ± 0.4[a]	1.8 ± 0.3
18:1n7	2.8 ± 0.3[a]	2.0 ± 0.3	1.9 ± 0.4[a]	1.3 ± 0.4	1.9 ± 0.3[a]	1.4 ± 0.1	1.8 ± 0.3[a]	1.3 ± 0.3
18:2n6	1.5 ± 0.5[a]	2.1 ± 0.1	1.2 ± 0.2	1.2 ± 0.2	1.3 ± 0.2[a]	1.7 ± 0.1	1.4 ± 0.1[a]	1.6 ± 0.1
18:3n3	2.1 ± 0.5[a]	3.3 ± 0.2	1.0 ± 0.3	1.2 ± 0.1	2.1 ± 0.5[a]	2.9 ± 0.2	1.7 ± 0.2[a]	2.3 ± 0.3
18:4n3	6.1 ± 1.5[a]	12.1 ± 1.0	3.9 ± 1.3	5.2 ± 0.9	6.3 ± 1.4[a]	11.0 ± 0.9	6.2 ± 1.0[a]	9.1 ± 1.0
20:1n7	0.1 ± 0.1[a]	0.2 ± 0.0	0.1 ± 0.0	0.1 ± 0.0	0.1 ± 0.0[a]	0.2 ± 0.0	0.1 ± 0.0[a]	0.2 ± 0.0
20:4n6	0.5 ± 0.1[a]	0.8 ± 0.1	0.5 ± 0.1	0.5 ± 0.1	0.6 ± 0.1	0.7 ± 0.1	0.5 ± 0.0	0.6 ± 0.1
20:5n3	6.5 ± 1.5[a]	9.9 ± 0.5	8.4 ± 1.5[a]	11.4 ± 2.1	8.1 ± 1.3[a]	11.0 ± 1.3	10.1 ± 2.7	12.3 ± 1.1
22:0	0.1 ± 0.1[a]	0.5 ± 0.1	0.2 ± 0.0[a]	0.3 ± 0.1	0.2 ± 0.1[a]	0.4 ± 0.1	0.1 ± 0.1[a]	0.4 ± 0.1
22:5n6	0.7 ± 0.2[a]	1.4 ± 0.3	–[a]	0.1 ± 0.1	0.6 ± 0.1[a]	0.8 ± 0.1	0.2 ± 0.1[a]	0.4 ± 0.1
22:6n3	4.5 ± 1.3[a]	9.0 ± 1.4	3.5 ± 1.1[a]	5.5 ± 1.2	4.6 ± 1.1[a]	7.5 ± 0.9	5.6 ± 0.9[a]	7.6 ± 0.4
Groups								
SFA	35.9 ± 1.6[a]	27.4 ± 1.2	28.7 ± 2.8	26.5 ± 2.4	27.9 ± 2.0	26.3 ± 1.2	30.5 ± 2.4[a]	26.3 ± 0.8
MUFA	18.3 ± 1.2[a]	16.6 ± 0.8	19.3 ± 3.6	18.8 ± 3.4	17.9 ± 1.1	17.9 ± 1.3	18.6 ± 1.7	19.4 ± 1.1
PUFA	25.1 ± 2.7[a]	40.2 ± 1.9	25 ± 3.2	28.8 ± 2.8	29.6 ± 2.6[a]	37.4 ± 1.9	29.0 ± 3.2	35.7 ± 1.6
n-3	20.2 ± 2.6[a]	34.9 ± 1.8	18.7 ± 2.5[a]	24.3 ± 2.6	22.8 ± 2.3[a]	32.9 ± 1.8	24.9 ± 3.1[a]	31.8 ± 1.6
n-6	3.4 ± 0.6[a]	4.6 ± 0.3	3.1 ± 0.4	3.5 ± 0.4	2.7 ± 0.3	2.9 ± 0.2	2.4 ± 0.2[a]	2.3 ± 0.2

[a]Values showing significant differences (*t*-test, $p < 0.05$) between males and females for a given food regime (shown beside male value only)

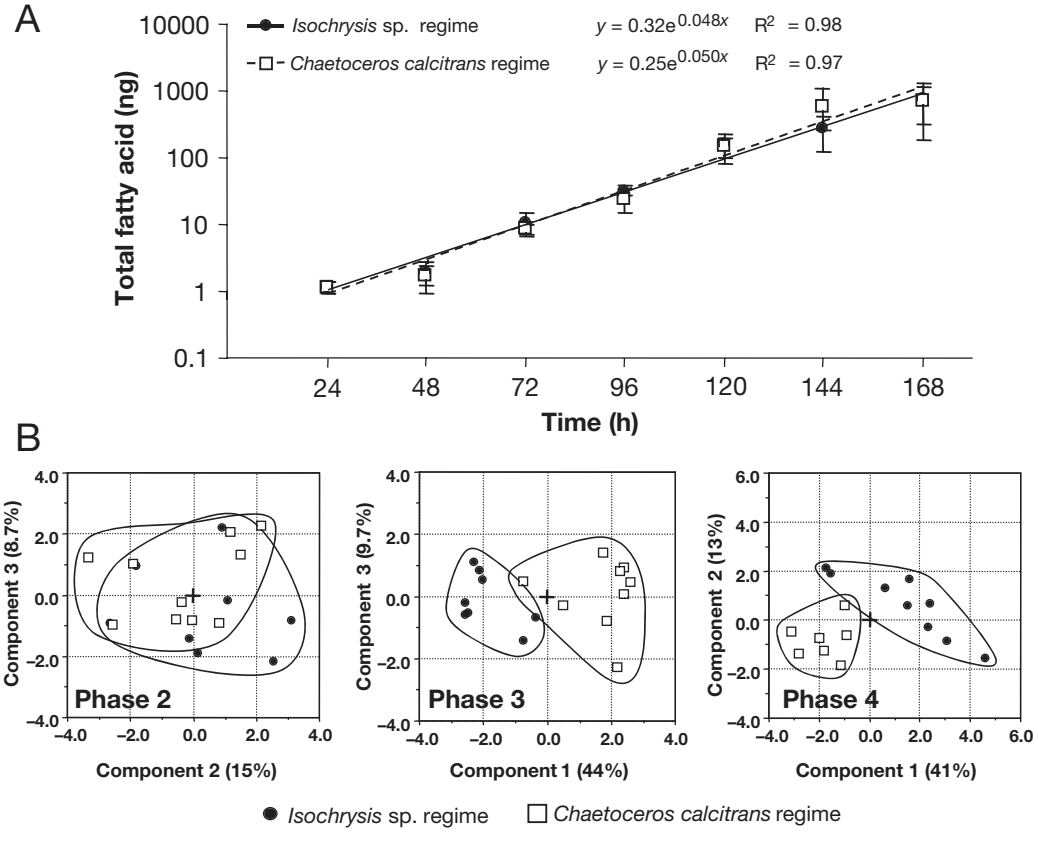

Fig. 2. *Oikopleura dioica*. Fatty acid compositions of *O. dioica* fed diets differing in quality of nutrition. (A) Log-transformed plot of total fatty acid content per individual *O. dioica* throughout the life cycle when animals were fed a standard food concentration diet of only *Isochrysis* sp. (●) versus a diet of only *Chaetoceros calcitrans* (□). Regression parameters are given and standard deviations are indicated by bars. (B) Two-dimensional principal component (2D-PC) plots of representative time points sampling fatty acid composition during phases 2, 3 and 4 (Troedsson et al. 2002) of *O. dioica* growth. The animals were separated into 2 groups after hatching and fed a diet of standard food concentration of *Isochrysis* sp. (●) or *C. calcitrans* (□). The percentage of the total variation among the groups along the 2 PCs is given on the respective axes. The symbols represent individual replicates. The 2 groups are circled

Table 8. *Oikopleura dioica*. Fatty acid composition of *O. dioica* fecal pellets under different dietary regimes. The major fatty acids are given as a percentage of total composition with standard deviation of the fecal pellets at the specified times. Full fatty acid compositions of fecal pellets are given in Table F of Appendix 1. Groups: percent proportion of 5 major groups of fatty acids

Fatty acid	Qualitative diet				Quantitative diet					
	Isochrysis sp.		*Chaetoceros calcitrans*		Standard			Limited		
	96 h	144 h	96 h	144 h	112 h	136 h	160 h	112 h	136 h	160 h
14:0	5.7 ± 1.7	7.1 ± 0.8	4.2 ± 1.5	2.2 ± 0.6	4.4 ± 1.5	7.3 ± 2.6	4.4 ± 1.5	1.8 ± 0.6	2.7 ± 1.0	2.8 ± 0.8
14:1n5	0.7 ± 0.3	0.7 ± 0.4	1.2 ± 0.4	0.9 ± 0.6	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.1	0.3 ± 0.2	0.4 ± 0.2
15:0	2.3 ± 0.3	1.8 ± 0.4	1.9 ± 0.6	1.6 ± 0.7	1.2 ± 0.6	1.6 ± 0.2	1.8 ± 0.7	0.8 ± 0.4	1.4 ± 0.4	1.7 ± 0.6
16:0	25.2 ± 3.2	19.0 ± 2.2	19.9 ± 7.7	13.3 ± 4.8	15.2 ± 4.4	22.2 ± 3.9	16.7 ± 4.5	11.1 ± 3.1	16.5 ± 3.5	18.6 ± 3.5
16:1n7	3.4 ± 1.1	2.4 ± 0.6	4.7 ± 3.0	2.0 ± 0.7	10 ± 11	4.2 ± 2.1	3.1 ± 1.6	1.7 ± 0.8	15.4 ± 4.5	2.3 ± 1.7
16:2n6	1.5 ± 0.6	1.1 ± 0.8	1.7 ± 0.9	0.9 ± 0.4	0.5 ± 0.5	0.3 ± 0.6	2.4 ± 0.5	–	–	–
17:0	0.6 ± 0.4	0.5 ± 0.2	0.7 ± 0.3	0.4 ± 0.3	0.8 ± 0.2	1.1 ± 0.3	1.1 ± 0.3	0.7 ± 0.3	0.9 ± 0.3	1.2 ± 0.3
18:0	13.5 ± 1.0	10.4 ± 1.3	8.6 ± 3.0	10.2 ± 3.3	9.1 ± 2.3	14.4 ± 5.6	8.8 ± 1.9	8.1 ± 1.9	10.5 ± 2.4	13.4 ± 2.8
18:1n9	2.3 ± 0.9	1.8 ± 0.6	4.2 ± 2.6	3.5 ± 2.8	3.7 ± 1.8	2.6 ± 1.4	4.7 ± 1.5	6.5 ± 1.2	6.8 ± 1.8	3.4 ± 1.3
18:1n7	5.3 ± 3.0	5.3 ± 1.5	3.4 ± 3.7	3.9 ± 3.6	4.5 ± 1.3	3.9 ± 0.8	5.2 ± 1.5	2.2 ± 1.5	2.9 ± 2.2	5.8 ± 1.8
18:2n6	0.8 ± 0.4	0.6 ± 0.3	0.6 ± 0.3	0.7 ± 0.6	0.8 ± 0.2	0.3 ± 0.3	0.5 ± 0.2	0.2 ± 0.2	0.2 ± 0.2	0.3 ± 0.1
18:2n4	2.2 ± 0.6	2.1 ± 0.5	3.3 ± 1.6	1.4 ± 0.9	1.3 ± 1.6	0.4 ± 0.8	–	3.6 ± 0.7	2.8 ± 0.8	1.0 ± 1.4
18:3n3	0.5 ± 0.1	0.5 ± 0.2	0.3 ± 0.1	0.3 ± 0.1	0.6 ± 0.3	0.3 ± 0.1	0.3 ± 0.1	0.2 ± 0.1	0.2 ± 0.2	0.2 ± 0.2
18:4n3	0.2 ± 0.3	1.3 ± 0.5	–	0.9 ± 0.3	1.5 ± 0.4	0.2 ± 0.2	–	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.3
18:5n1	2.8 ± 0.6	3.9 ± 1.0	3.8 ± 2.0	4.9 ± 1.8	2.8 ± 2.0	2.1 ± 0.8	4.6 ± 0.8	4.7 ± 1.2	3.6 ± 1.3	3.3 ± 1.1
20:0	0.7 ± 0.4	0.7 ± 0.7	0.6 ± 0.2	0.7 ± 0.9	0.5 ± 0.2	0.8 ± 0.2	1.0 ± 0.3	0.4 ± 0.1	0.6 ± 0.2	0.9 ± 0.2
20:1n9	2.1 ± 1.2	2.2 ± 1.0	2.0 ± 0.9	4.5 ± 3.4	1.5 ± 0.6	1.9 ± 1.0	0.9 ± 1.1	1.7 ± 0.5	1.9 ± 0.5	2.3 ± 0.5
20:4n6	0.2 ± 0.2	0.1 ± 0.1	0.1 ± 0.1	–	0.1 ± 0.3	0.2 ± 0.2	0.2 ± 0.2	–	–	0.3 ± 0.4
20:3n3	0.6 ± 0.8	0.6 ± 0.3	0.3 ± 0.4	0.5 ± 0.3	0.7 ± 0.4	0.5 ± 0.7	–	0.8 ± 0.2	1.1 ± 0.2	0.4 ± 0.4
20:5n3	2.7 ± 0.8	3.1 ± 1.8	3.2 ± 1.8	4.9 ± 1.3	1.9 ± 1.9	1.1 ± 1.5	0.3 ± 0.4	4.4 ± 1.4	3.4 ± 1.0	2.2 ± 2.1
22:0	–	0.4 ± 0.4	0.1 ± 0.2	0.4 ± 0.2	0.3 ± 0.2	0.3 ± 0.4	0.1 ± 0.2	0.3 ± 1.0	0.5 ± 0.2	0.3 ± 0.3
21:5n3	–	1.1 ± 1.0	0.4 ± 0.9	0.7 ± 1.0	0.3 ± 0.2	0.5 ± 0.2	0.2 ± 0.4	0.2 ± 0.1	0.3 ± 0.2	0.4 ± 0.3
22:5n6	–	0.1 ± 0.1	0.1 ± 0.2	0.2 ± 0.2	1.4 ± 1.6	0.8 ± 1.0	0.1 ± 0.1	3.5 ± 1.3	2.6 ± 1.4	1.3 ± 1.4
24:0	–	0.7 ± 0.9	0.1 ± 0.3	0.2 ± 0.3	0.5 ± 0.4	0.4 ± 0.5	–	0.5 ± 0.3	0.8 ± 0.5	0.5 ± 0.5
22:6n3	1.1 ± 0.4	0.3 ± 0.3	0.6 ± 0.5	0.2 ± 0.2	0.9 ± 0.4	0.8 ± 0.6	3.3 ± 0.8	–	–	1.1 ± 1.5
Groups										
SFA	48.1 ± 3.8	40.4 ± 3.0	36 ± 8.4	29 ± 6.0	32.1 ± 5.2	48 ± 7.3	33.9 ± 5.2	23.6 ± 3.9	33.9 ± 4.4	39.5 ± 4.6
MUFA	15.3 ± 3.8	14.4 ± 2.5	17 ± 5.7	16 ± 5.9	27 ± 11	20 ± 3.2	15.4 ± 2.9	14.1 ± 2.2	18.2 ± 5.6	18.6 ± 3.2
PUFA	14.4 ± 1.7	17.0 ± 2.9	17 ± 3.6	18.4 ± 3.3	17 ± 4.5	10.1 ± 2.8	18.1 ± 2.3	26.2 ± 2.7	20.3 ± 2.9	16 ± 4.1
n-3	5.1 ± 1.2	8.2 ± 2.5	5.2 ± 2.3	8.7 ± 2.5	6.0 ± 2.1	3.4 ± 1.8	4.2 ± 1.0	5.9 ± 1.4	5.2 ± 1.1	4.4 ± 2.7
n-6	2.5 ± 0.7	1.9 ± 0.9	2.6 ± 1.0	1.8 ± 0.7	2.8 ± 1.7	1.6 ± 1.2	5.2 ± 1.6	3.7 ± 1.3	2.8 ± 1.4	2.4 ± 1.8

Table 9. Instantaneous net lipid accumulation efficiency ($NLAE_i$) under standard and limited food conditions. IR_{FA}: fatty acids cleared from the water in 1 h; $\Delta G_{animals}$: net growth of soma and gonad fatty acid during 1 h; P_{FA}: fatty acids excreted as fecal pellets in 1 h. Further details are given in 'Materials and methods'

Food regime	IR_{FA} (ng FA h^{-1})	$\Delta G_{animals}$ (ng FA h^{-1})	P_{FA} (ng FA h^{-1})	NLAE (%)
Standard	12.6	1.0	2.8	10.0
Limited	3.9	0.6	2.0	32.0

The qualitative differences in appendicularians grazing on different food sources might influence growth and survival rates in organisms that prey on appendicularians. In marine species, the conversion of EPA to DHA is very limited (Kanazawa 1985), and it has been reported that DHA is superior to EPA as an essential fatty acid for growth and survival in marine fish larvae (Watanabe 1993). Indeed, the DHA level of appendicularians is significantly reduced when given a DHA-poor nutrient. Furthermore, there was a difference between individual fatty acids in animals fed *Isochrysis* sp. versus *Chaetoceros calcitrans* that correlated with the fatty acid differences in the algae (Tables 3 & 4), suggesting that *Oikopleura*, which transfers energy from micro-algae to fish, can also mediate nutritional value between trophic levels.

A regression analysis on animals fed a standard versus a limited food regime revealed significant differences (p[slope] = 0.18; p[elevation] < 0.05) in their respective total quantities of fatty acids throughout development (Fig. 3A). The 2 groups clearly separated in the multivariate analysis (Fig. 3B, Tables 5 & 6), where 66% of all fatty acids showed significant differences ($p < 0.05$). Separation between the 2 groups was observed continuously throughout the life cycle, and males separated from females at maturity (Fig. 4). The total fatty acid difference between animals fed standard and limited diets reflects the total body mass dif-

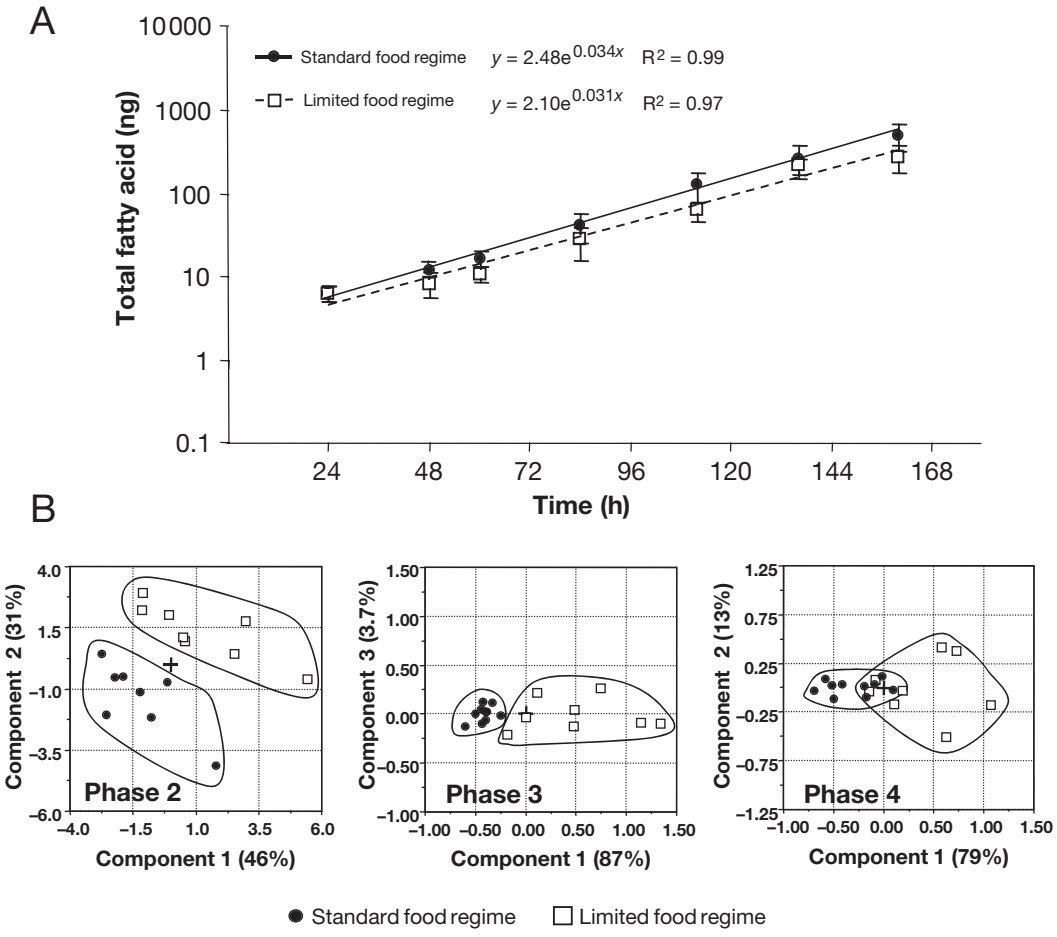

Fig. 3. *Oikopleura dioica*. Fatty acid compositions of *O. dioica* fed diets differing in quantity of nutrition. (A) Log-transformed plot of total fatty acid content per individual *O. dioica* throughout the life cycle when animals were fed a standard (●) versus a limited (□) food regime. Regression parameters are given and standard deviations are indicated by bars. (B) Two-dimensional principal component (2D-PC) plots of representative time points sampling fatty acid composition during phases 2, 3 and 4 of *O. dioica* growth. The animals were separated into 2 groups after hatching and fed a standard (●) or limited (□) diet. The percentage of the total variation among the groups along the 2 PCs is given on the respective axes. The symbols represent individual replicates. The 2 groups are circled

ferences resulting from the 2 regimes. The body mass difference in animals given a standard versus limited food regime was previously shown to reflect differences in reproductive output, as somatic growth appeared non-responsive to increased nutrition available (Troedsson et al. 2002). However, there was a difference (*t*-test, $p < 0.05$) in the total fatty acid quantities as early as 48 h post-fertilization, well before the reproductive organ showed significant growth. A possible explanation for increased fatty acid content during somatic growth is that *Oikopleura dioica* stores fatty acids as high-energy droplets. Indeed, Cima et al. (2002) showed that lipid storage occurs in the form of droplets, mainly in the right gastric lobe and vertical intestine. These droplets would have a minimal impact on overall trunk size, but would enhance survival and reproductive output.

The fatty acid composition in *Oikopleura dioica* was also sex-dependent. In a principal component analysis, the males and females were clearly separated from each other when either the quality or quantity of the food regimes was modified (Fig. 4). In females, there was a significant increase in some essential fatty acids required for larval growth and development (Table 7). Together with EPA and DHA, 18:3n3 and 18:2n6 were significantly up-regulated in females compared to males. Considering that at maturity the gonad accounts for ~50% of the total body size of the organism, this indicates storage of these essential fatty acids in oocytes, providing nutrition for larvae throughout organogenesis up to metamorphosis when the animal starts to actively feed. In females, the up-regulation of EPA and DHA was noteworthy (Table 7) because both are important in subsequent larval development.

Although animals experiencing the limited regime were restricted to a diet only 17% of the standard food regime, the total fatty acid content of individual fecal pellets did not show any significant difference (p[slope] = 0.19; p[elevation] = 0.57) (Fig. 5A). This agrees with the observation of López-Urrutia & Acuña (1999) that pellets are packed in the foregut, which empties as soon as pellets are formed. Pellets are then further transported through the digestive tract, and will therefore each contain the same quantity of food. On the other hand, they showed that gut passage time (GPT) varied with food concentration. This implies that the amount of fatty acids in excreted fecal pellets is independent of the quantity of food available for inges-

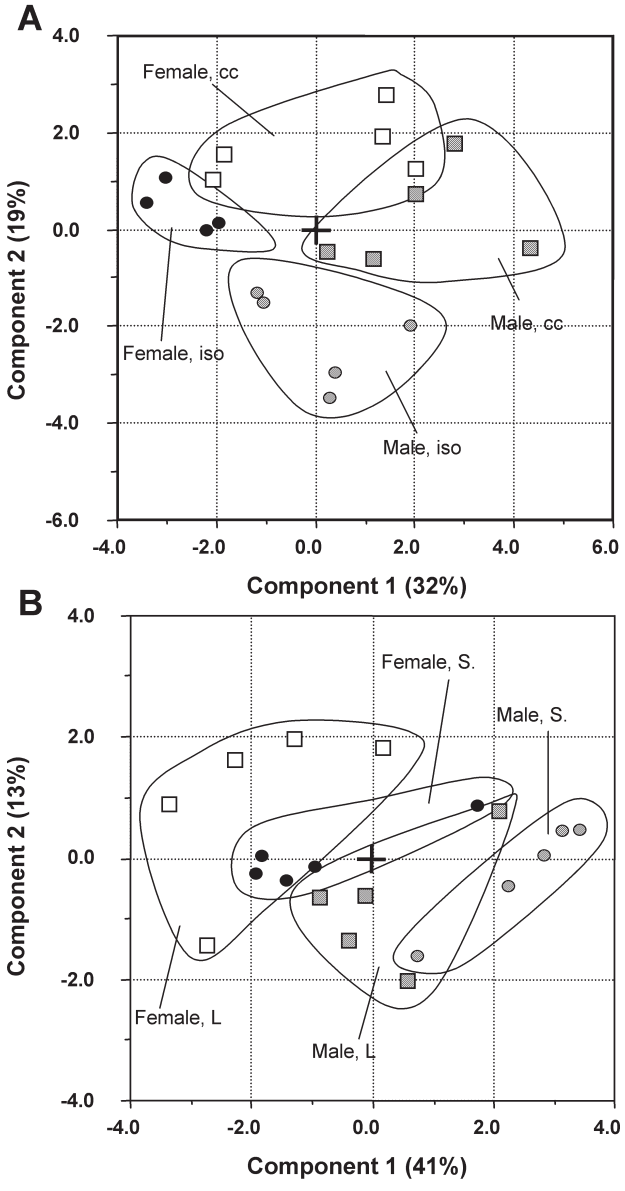

Fig. 4. *Oikopleura dioica*. Fatty acid compositions differ in *O. dioica* males and females. Two-dimensional principal component (2D-PC) plots of sexually mature animals. Plots show males and females in the respective food regimes. (A) Mature animals fed a diet of only *Isochrysis* sp. (iso) or only *Chaetoceros calcitrans* (cc). (B) Mature animals fed a standard (S) or limited food regime (L). Symbols represent individual replicates of mature animals. The sex as well as food regime groups are circled

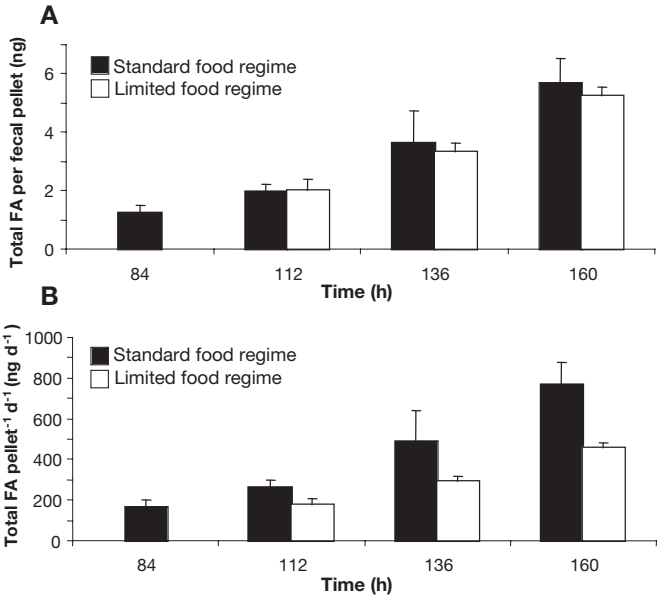

Fig. 5. *Oikopleura dioica*. Fatty acid (FA) content of fecal pellets throughout the life cycle of *O. dioica*. (A) Total quantity of FA per fecal pellet from 84 h (standard) and 112 h (limited) to maturation. Dark bars: fecal pellets from animals fed a standard food regime. White bars: fecal pellets from animals fed a limited food regime. (B) Total quantity of FA in fecal pellets over 24 h periods from 84 h (standard) and 112 h (limited) to maturation. Dark and white bars as in (A). Standard deviations are indicated

tion. It is instead the slower gut passage time that results in lower total excretion of fatty acids when food is scarce (Fig. 5B).

There was an apparent 3-fold difference in the net lipid accumulation efficiency (NLAE) of *Oikopleura dioica* in the standard versus limited food regime (Table 9). Possible explanations include (1) changes in animal behavior, some of which are linked to clearance rate (CR) (e.g. tail beat frequency and tail beat arrest), (2) increased clogging of filters with increasing food concentration, (3) plasticity of filter structure in response to altered food concentrations. Several studies have shown a nearly fixed CR for *O. dioica*, with only slight changes over very large ranges of food concentrations (Paffenhöfer 1975, King 1982, Bochdansky & Deibel 1999, Acuña & Kiefer 2000). Bochdansky & Deibel (1999) found that feeding effort was reduced in high food concentrations, although this factor was small and did not explain the slight decrease in CR. They argued that the discrepancy was due to clogging of filters by the algae. Selander & Tiselius (2003) showed that changes in tail beat frequency had some influence on CR with increasing food concentration, but the greatest effect in their model was unexplained residual error. They were, therefore, unable to rule out the clogging hypothesis.

If filter clogging was solely responsible for masking an equivalent NLAE between the standard and limited food regimes, rather than the result obtained in Table 9, this would imply that the number of particles trapped in the filters would account for approximately 50% of the standard food regime. With a house renewal rate of 0.25 h^{-1} at 15°C (C. Troedsson, J.L. Acuña, R. Skinees & E.M. Thompson unpubl. data), a discarded house

would then trap almost 20 000 algal cells. Examination of discarded houses indicates that this figure would be a significant overestimation. Although increased clogging of filters can be an important factor in partly explaining the apparent difference in NLAE, plastic response in the differential regulation of filter structures may also play a role (C. Troedsson, J.L. Acuña, R. Skinnes & E.M. Thompson unpubl. data). Feeding experiments on the fresh water filter feeding zooplankton *Daphnia* sp. showed discrepancies in the total energy budget between long-term and short-term experiments (Lampert 1977, Gliwicz 1990), where assimilation in short-term experiments was considerably higher. This was solved upon the discovery that *Daphnia* sp. responded to varying food concentrations by modifying their filtering screens (Lampert 1994). The difference in the NLAE between *Oikopleura dioica* given a standard versus a limited food regime might also be explained in a similar manner, through modification of filter structure to varying food concentrations available.

In conclusion, we have demonstrated that the fatty acid composition of *Oikopleura dioica* reflects dietary composition, in agreement with data on other zooplanktonic filter feeders. This suggests that the nutritional quality of primary production would be transferred to predators of *O. dioica*, and could have effects on their early ontogenetic stages (Støttrup & Attramadal 1992, Koven et al. 1993, Kraul et al. 1993, Lochmann & Gatlin 1993, Watanabe 1993, Whyte et al. 1994). It is also intriguing that some key fatty acids in endocrine signaling pathways are up-regulated as gametogenesis commences. There is a strong transition in allocation of resources to reproductive output versus somatic growth in *O. dioica*. Arrest of endocycling in the oikoplastic epithelium responsible for house production is concomitant with acceleration in growth of oocytes (Ganot & Thompson 2002). Exerting stress on animals prior to this point results in autophagy of the epithelium with a premature acceleration in oocyte differentiation (P. Ganot & E.M. Thompson unpubl. data). This suggests operation of a rapid inductive signal, switching between epithelial growth and house production on one hand, and oocyte maturation on the other. It will be of interest to determine whether some of the potential endocrine signaling pathways suggested by shifts in fatty acid profiles in this study play roles in such rapid switches in *O. dioica*.

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