Incorporation and metabolism of fatty acids by desaturation and elongation in the nematode, *Panagrellus redivivus*.

Christian Schlechtriem[1*], Douglas R. Tocher[2], James R. Dick[2] and Klaus Becker[1]

[1]Department of Animal Nutrition and Aquaculture, Institute for Animal Production in the Tropics and Subtropics, University of Hohenheim (480b), D-70593 Stuttgart, Germany.
Inst480@uni-hohenheim.de

[2]Institute of Aquaculture, University of Stirling, Stirling FK9 4LA, Scotland
d.r.tocher@stir.ac.uk

*Author for correspondence: Christian.Schlechtriem@ec.gc.ca

Summary

The free-living nematode *Panagrellus redivivus* can be mass produced in monoxenic solid culture on *Saccharomyces cerevisiae* and therefore could be useful as a live food for marine fish or crustacean larvae in the rapidly expanding aquaculture industry. However, this will depend on their lipid and fatty acid composition and so this was investigated in mass produced *P. redivivus* grown on *S. cerevisiae* in three different media. Live nematodes were also incubated with [1-^{14}C]-labelled fatty acids and their desaturation and elongation determined. The combined results from the growth trials on different media and the metabolic studies with labelled fatty acids indicated the presence of Δ9, Δ12, Δ6 and Δ5 fatty acid desaturase activities, and elongase activities active towards C_{18}, C_{16} and shorter chain fatty acids. The presence of Δ15, and therefore the ability to produce n-3 polyunsaturated fatty acids, was suggested by the compositional data, but could not be conclusively established from metabolic studies.

Keywords: live food, mass produced nematodes, fatty acid metabolism,

41 During their early stages of development, many fish and crustacean species important for
42 marine aquaculture rely on live food organisms (Sargent et al., 1995). The most commonly
43 used live food is the brine shrimp Artemia salina, a small branchipod crustacean representing
44 approximately 40% of the total aquaculture demand for live feeds for early stages (Lavens
45 and Sorgeloos, 2000). Following one of the worst harvests of recent times from the premier
46 source of Artemia cysts, the Great Salt Lakes in the United States, cyst production was barely
47 sufficient to satisfy the increasing demand of the rapidly growing aquaculture industry
48 (Sorgeloos et al., 2001). The lack of potential alternatives to Artemia may become an
49 obstacle to a further increase of aquaculture production especially in developing countries. It
50 has been shown that the free-living nematode Panagrellus redivivus is a suitable food for fish
51 (Kahan and Appel, 1975; Kahan et al., 1980) and crustacean larvae (Biedenbach et al., 1989;
52 Kumlu and Fletcher, 1997; Kumlu et al., 1998; Wilkenfeld et al., 1984). Although nematodes
53 have been proven to be an excellent food source, their use has not become widespread due to
54 problems involved in mass production. However, a low-cost technology for the mass
55 production of *P. redivivus* on solid medium was recently described by Ricci et al. (2003).
56 The nutritional value of nematodes can be influenced by the culture medium. For instance,
57 lipid content and fatty acid composition of nematodes can be modified by adding lipid
58 components to the culture medium (Rouse *et al.*, 1992; Kumlu *et al.*, 1998; Schlechtriem *et*
59 *al*., 2004a,b). *P. redivivus* cultured on simple oat-based medium were found to feed mainly
60 upon the yeast growing on the medium and possibly on the breakdown products of oats.
61 Lipids extracted from such nematodes contained highly unsaturated fatty acids (HUFA, fatty
62 acids having carbon chain lengths of $\geq C_{20}$ and with ≥ 3 double bonds; n-x signifying the
63 position of the double bond from the methyl end of the molecule) like arachidonic (20:4 n-6)
64 and eicosapentaenoic acid (20:5 n-3) although no C_{20} HUFA were found either in oats or in
65 the yeast (Sivapalan and Jenkins, 1966; Schlechtriem *et al*., 2004b). Similar results were
66 obtained by Lower *et al.* (1970) who cultured *P. redivivus* axenically (bacteria-free) on an

67 aqueous medium composed of heated liver extract and soy-peptone yeast. Therefore, *P.*
68 *redivivus* appear to possess fatty acid desaturase and elongase activities necessary to
69 synthesize several HUFAs from shorter chain fatty acid precursors, as previously described
70 for the free-living nematodes *Caenorhabditis elegans* (Hutzell and Krusberg, 1982; Watts
71 and Browse, 2002) and *Turbatrix aceti* (Rothstein and Götz, 1968; Fletcher and Krusberg,
72 1973). Thus, the fatty acid pattern of the nematodes is influenced by the fatty acid
73 composition of the culture medium and the nematodes' capability to synthesize HUFAs. In
74 this way, both factors also influence the nutritive value of *P. redivivus* as live food for first
75 feeding fish larvae. For instance, arachidonic acid and eicosapentaenoic acid are important as
76 structural components of membrane glycerolipids and as precursors of families of signalling
77 molecules including prostaglandins, thromboxanes, and leukotrienes in fish (Sargent et al.,
78 2002; Tocher, 2003). To assess the effect of endogenous HUFA biosynthesis on the fatty
79 acid composition of *P. redivivus,* the pathway of HUFA synthesis and the complement of
80 fatty acid desaturase/elongase enzymes must be elucidated. In addition, it was not clear from
81 the studies above whether *P. redivivus* was able to directly synthesise polyunsaturated fatty
82 acids (PUFA, fatty acids with two or more double bonds) *de novo*, that is, produce 18:2 n-6
83 from 18:1 n-9.

84 In the present study, the effect of different culture media on the lipid content, lipid class
85 composition and fatty acid composition of mass produced *P. redivivus* was examined. The
86 nematodes were grown on yeast, *Saccharomyces cerevisiae*, in three different media. One
87 medium represented a low lipid medium with little added lipid. The other two media
88 contained, respectively, very high 18:2 n-6 (66.8% of total fatty acids in sunflower oil), to
89 investigate PUFA metabolism, and high medium chain saturates (10:0, 42.1% of total fatty
90 acids in MCT oil) to investigate effects on C_{16} and C_{18} metabolism. Differences in the fatty
91 acid composition of total polar and neutral lipids were analysed. To further investigate the
92 pathway of PUFA and HUFA biosynthesis in *P. redivivus*, live nematodes were incubated

with different [1-^{14}C]-labelled fatty acids and their further metabolism by desaturation and elongation determined.

Materials and Methods

Experimental Animals and Culture

The free-living nematode *Panagrellus redivivus* was provided by Dr. Manuele Ricci (BioTecnologie B.T. S.r.l., Pantalla di Todi, 06050, PG, Italy). Nematodes were mass produced in monoxenic solid culture (single microorganism: *Saccharomyces cerevisiae*) according to Ricci *et al.* (2003). Three different culture media were used: 1) Low lipid medium (LLM) containing 86.8% saline solution (0.8% sea salt; Tetra Marin), 1.3 peptone from soybean meal (Fluka, 70178), 0.8% yeast extract (Fluka, 70161), 10.8% wheat starch (Sigma, S-5127) and 0.2% Glucose (Riedel de Haen 16301). 2) Lipid-enriched oat-based medium (LOM; Schlechtriem et al. 2004b) consisting of 82.4% saline solution, 16.4% oat flour (Kölln), and 1.2% sunflower oil (Thomy, Germany). 3) Low lipid medium enriched with medium-chain triglycerides (MTM) consisting of 85.4% saline solution, 1.3% peptone from soybean meal, 0.8% yeast extract, 10.7% wheat starch, 0.2% glucose and 1.6% medium-chain triglycerides (Heess Stuttgart, 4605). Three batches of nematodes were produced per medium. After twelve days of incubation at 25°C nematodes were separated from the medium by filtering them through a coarse sieve, covered with cotton discs for manual milk filtration, which was placed in a petri-dish filled with water. Clean nematodes were obtained by passing the residue through a 105 µm plankton net to remove the yeast cells and remaining particles of the medium.

Lipid extraction and lipid class composition

Total lipid contents of nematodes and growth medium samples were determined gravimetrically after extraction by homogenization in chloroform/methanol (2:1, v/v) containing 0.01% butylated hydroxytoluene as antioxidant, basically according to Folch *et al.* (1957). Separation of lipid classes was performed by high-performance thin-layer chromatography. Approximately 10 μg of lipid extract was loaded as a 2 mm streak and the plate developed to two-thirds distance with methyl acetate/isopropanol/ chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.). After drying, the plate was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The classes were quantified by charring at 160^{o}C for 15 min after spraying with 3% (w/v) aqueous cupric acetate containing 8% (v/v) phosphoric acid, followed by calibrated densitometry using a Shimadzu CS-9000 dual-wavelength flying spot scanner and a DR-13 recorder (Henderson and Tocher 1992).

Fatty acid analysis

Samples of total lipid (2 mg) were applied as 2 cm streaks to thin-layer chromatography plates, and polar lipids separated from neutral lipids using hexane/diethyl ether/acetic acid (90:10:1, by vol.) as developing solvent. The origin area corresponding to total polar lipids and the lane above the origin corresponding to total neutral lipids were scraped into stoppered glass test tubes for transmethylation directly on the silica (Christie, 1982). Fatty acid methyl esters of total lipid, total polar lipids, and total neutral lipids, purified as above, were prepared by acid-catalyzed transesterification using 2 ml of 1% H_2SO_4 in methanol plus 1 ml toluene as described by Christie (1982) and methyl esters extracted and purified as described previously (Tocher and Harvie, 1988). Fatty acid methyl esters were separated and quantified by gas-liquid chromatography (Fisons GC8600, Fisons Ltd., Crawley, U.K.) using a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K).

Hydrogen was used as carrier gas and temperature programming was from 50°C to 180°C at 40°C/min and then to 225°C at 2°C/min. Individual methyl esters were identified by comparison to known standards and by reference to published data (Ackman, 1980).

Incubation of nematodes with [1-^{14}C]-labelled fatty acids

Live nematodes, containing juveniles (J1-4 stages) and adults, grown on *S. cerevisiae* in LLM, were resuspended in Medium 199 and dispensed in 4ml aliquots into 25cm^2 tissue culture flasks. Flasks were supplemented with 0.5 μCi (approximately 100μl) of a particular [1-^{14}C]-labelled fatty acid added as a complex with fatty acid-free bovine serum albumin (FAF-BSA) prepared in medium 199 as described previously (Ghioni *et al.*, 1997). The specific fatty acids used were [1-^{14}C]18:0, [1-^{14}C]18:1 n-9, [1-^{14}C]18:2 n-6, [1-^{14}C]18:3 n-3, [1-^{14}C]20:3 n-6, [1-^{14}C]20:4 n-6 and [1-^{14}C]20:5 n-3. The suspensions were mixed carefully and incubated at 20°C for 24h. After incubation the flasks were gently rocked and the suspension of live nematodes transferred to glass conical test tubes. Each flask was washed with 1 ml of ice-cold 1% FAF-BSA in Hanks' balanced salt solution added afterwards to the test tubes. The suspensions were centrifuged (400 x g for 2 min.; ~1200rpm), the supernatants decanted and the nematodes washed again with 5 ml 1% ice-cold Hanks' balanced salt solution containing FAF-BSA. After centrifugation the pellets were used for analyses of radiolabelled fatty acids.

Assay of fatty acyl desaturation/elongation activities

Lipids were extracted from labelled nematode pellets using ice-cold chloroform/methanol (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene essentially as described by Folch *et al.* (1957) and as described in detail previously (Tocher *et al.*, 1988). Total lipid was transmethylated and fatty acid methyl esters prepared as described above. The methyl esters were redissolved in 100 μl isohexane containing 0.01% butylated hydroxytoluene and

applied as 2.5 cm streaks to thin-layer chromatography plates impregnated by spraying with 2 g silver nitrate in 20 ml acetonitrile and pre-activated at 110 ^{o}C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent, 1992). Autoradiography was performed with Kodak MR2 film for 6 days at room temperature. Areas of silica containing individual PUFA were scraped into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia) and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching of ^{14}C under exactly these conditions.

Protein determination

Protein concentration in nematode suspensions was determined according to the method of Lowry *et al.* (1951) after incubation with 0.4 ml of 0.25% (w/v) SDS/1M NaOH for 45 min at 60^{o}C.

Materials

[1-^{14}C]18:0, [1-^{14}C]18:1 n-9 [1-^{14}C]18:2 n-6, [1-^{14}C]18:3 n-3, [1-^{14}C]20:3 n-6, [1-^{14}C]20:4 n-6 and [1-^{14}C]20:5 n-3, all (50-55 mCi/mmol) were obtained from NEN (DuPont (U.K.) Ltd., Stevenage, U.K.). Hanks' balanced salt solution, HEPES buffer, FAF-BSA, butylated hydroxytoluene, and silver nitrate were obtained from Sigma Chemical Co. (Poole, U.K.). Thin-layer chromatography (20 cm x 20 cm x 0.25 mm) and high-performance thin-layer chromatography plates (10 cm x 10 cm x 0.15 mm), precoated with silica gel 60 (without fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were HPLC grade and were obtained from Fisher Scientific U.K., Loughborough, England.

Statistical analysis

Data recorded as percentages were arcsine-transformed to ensure a normal distribution and subjected to analysis of variance (ANOVA). Duncan's Multiple Range Test (DMRT) was used to identify differences among treatment means ($P<0.05$) (STATISTICA 5.1 software).

Results

Fatty acid compositions of growth media components

All nematodes were produced by culture on the yeast, *Saccharomyces cerevisiae*, but in three different media. The fatty acid composition of the basic yeast contained over 50% monounsaturated fatty acids, predominantly 18:1 n-9 and 16:1 n-7, nearly 18% saturated fatty acids, predominantly 16:0, and 32% PUFA composed of over 30% 18:2 n-6, and only 1% 18:3 n-3 and virtually no HUFA (Table 1). The LLM medium contained a small amount of yeast extract which contained a similar level of monounsaturated fatty acids including 13% 24:1 n-9, but a higher level of saturates and lower PUFA, than the yeast. The main PUFA in the yeast extract was 18:2 n-6 but there was 3.6% 20:3 n-6 and a small amount of 20:5 n-3. The LOM medium contained oat flour (OAT) and sunflower oil (SFO), both of which were rich in 18:2 n-6, 40% and 67%, respectively, with the remaining fatty acids being 18:1 n-9 and saturates, with virtually no n-3 PUFA (Table 1). The MTM medium was enriched with medium-chain triglycerides (MCT) which had over 42% 10:0 in a total of 54% saturates along with 30% 18:2 n-6 and 14% 18:1 n-9 (Table 1).

Effects of growth media on lipid content and lipid class composition of *P. redivivus*

The lipid content of *P. redivivus* grown in media supplemented with lipid was significantly increased in comparison to nematodes grown in the low lipid medium with the order being

MTM > LOM > LLM (Table 2). The increased lipid content was accompanied by increased proportions of triacylglycerol and total neutral lipid although the percentage of triacylglycerol in nematodes grown in MTM was lower than that of nematodes grown in LOM despite having a higher lipid content suggesting an increase in the absolute amount of polar lipids in *P. redivivus* grown in MTM (Table 2).

Effects of growth medium on fatty acid compositions of total lipid

Saturated fatty acids constituted 14.7% of the fatty acid content of *P. redivivus* grown on yeast in the low lipid medium, with 18:0 exceeding 16:0, and around 24% monounsaturated fatty acids, mainly 18:1 n-7 (12.4%) and 18:1 n-9 (7.6%) (Table 3). However, around 44% of all fatty acids were n-6PUFA, specifically 13.8% 18:2 n-6, 13.4% 20:3 n-6 and 15.8% 20:4 n-6, with 8% n-3PUFA, mainly 20:5 n-3, giving an n-6/n-3 ratio of 5.5. The remaining fatty chains (9%) were dimethyl acetals (DMA) produced as a result of transmethylation of the ether-linked chains in the *sn-1* position plasmalogen phospholipids. Growth in LOM resulted in significantly increased proportions of 18:2 n-6 and 18:1 n-9 with concomitantly decreased proportions of 18:1 n-7, 20:3 n-6, 20:4 n-6, n-3PUFA and dimethylacetals (Table 3).

The proportion of saturated fatty acids in total fatty acids was slightly lower in nematodes grown on LOM, whereas it was significantly increased in nematodes grown in MTM, compared to growth in LLM (Table 3). Growth in both lipid-supplemented media resulted in the level of 16:0 exceeding that of 18:0 compared to nematodes grown in LLM. Saturated fatty acids after growth in MTM also resulted in higher proportions of shorter chain fatty acids in total fatty acids, including 14:0, 12:0 and 10:0, but also 18:1 n-7, 18:1 n-9 and 16:1 n-7 with decreased proportions of 18:0, 20:3 n-6, 20:4 n-6 and DMA. The proportion of 18:2 n-6 and n-3PUFA in total fatty acids were not greatly affected by growth in MTM compared to growth in LLM (Table 3).

245 *Effects of growth medium on fatty acid compositions of polar and neutral lipids*

246 The polar lipid of *P. redivivus* grown in LLM contained higher proportions of n-6PUFA and
247 n-3PUFA and lower proportions of saturated and monounsaturated fatty acids compared to
248 neutral lipids (Table 4). Growth in LOM increased the proportion of 18:2 n-6 in both polar
249 and neutral lipids whereas the increased 18:1 n-9 was only observed in neutral lipids. The
250 proportions of 20:3 n-6, 20:4 n-6 and 20:5 n-3 and total n-3PUFA were reduced in both polar
251 and neutral lipids in nematodes grown in LOM compared to growth in LLM (Table 4). In
252 contrast, the fatty acid composition of polar lipids of nematodes grown in MTM were
253 relatively unaffected by growth in MTM compared to growth in LLM (Table 4). However,
254 the proportions of saturated and monounsaturated fatty acids were increased, and those of n-6
255 and n-3PUFA decreased, in *P. redivivus* grown in MTM compared to growth in LLM
256 (Table 4).

257 Consistent features observed, irrespective of growth medium, were that 18:0 exceeded 16:0
258 in polar lipids, whereas the opposite was the case in neutral lipids, and similarly 18:1 n-7
259 always exceeded 18:1 n-9 in polar lipids whereas this ratio was affected by medium
260 composition (diet) in neutral lipids (Table 4). DMA were only found in polar lipids,
261 reflecting their origin from ether-linked phospholipid classes and their levels were relatively
262 unaffected by growth medium.

263

264 *Metabolism of ^{14}C-labelled fatty acids in P. redivivus*

265 Total lipid was extracted from *P. redivivus* after incubation with radiolabelled fatty acids.
266 Approximately 25% of the radioactivity from [1-^{14}C]18:0 was recovered in other fatty acid
267 fractions with approximately 15% recovered as monounsaturated fatty acids (16:1, 18:1 and
268 20:1) and 10% recovered as polyunsaturated fatty acids, predominantly 18:2 n-6 (Table 5).
269 The primary fate of radioactivity from [1-^{14}C]18:1 n-9 was as 18:2 n-6 although
270 radioactivity was recovered in other PUFA as well as the elongation product 20:1 and

saturated (18:0/16:0) and shorter chain fatty acids (16:1 n-7). After incubation of *P. redivivus* with [1-^{14}C]18:2 n-6 or [1-^{14}C]18:3 n-3, the recovery of radioactivity was primarily in saturated and monounsaturated, including shorter chain, fatty acids, totaling around 27% and 47%, respectively (Table 5).

However, approximately 5% of the radioactivity from each of [1-^{14}C]18:2 n-6 or [1-^{14}C]18:3 n-3 was recovered in further desaturated fatty acid products. Incubation of *P. redivivus* with the radiolabelled HUFA resulted in 21%, 28% and 46% of the readioactivity from [1-^{14}C]20:3 n-6, [1-^{14}C]20:4 n-6 and [1-^{14}C]20:5 n-3 , respectively, being recovered as saturated, monounsaturated and shorter chain fatty acids (Table 5). Approximately, 6.5%, 7.2% and 2.5% of the radioactivity from 1-^{14}C]20:3 n-6, [1-^{14}C]20:4 n-6 and [1-^{14}C]20:5 n-3, respectively, was recovered in further desaturated products.

Discussion

The fatty acid composition of total lipid of *P.redivivus* grown on *S. cerevisiae* in LLM (Table 3) shows some interesting features such as 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9, the opposite to the situation in higher animals, and in the yeast on which it was grown in which 16:0 and 18:1 n-9 both greatly exceeded 18:0 and 18:1 n-7, respectively. The composition of all the other media components were the same as the yeast in this respect (Table 1) which was most interesting, as 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9 was consistently observed in the polar lipid fraction of *P. redivivus*, representative of the membrane lipids, irrespective of medium or diet. In contrast, the fatty acid composition of the neutral lipids did not show the same characteristic pattern in the saturated fatty acids (as 16:0 > 18:0) but 18:1 n-7 did exceed 18:1 n-9, although this could be changed by diet as shown with the LOM medium (oatmeal and sunflower oil) which was rich in 18:1 n-9. Therefore, the pattern of 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9 are inherent features of *P. redivivus*. This appears to extend to

297 related nematodes such as *Caenorhabditis elegans,* which also shows this pattern (Hutzell
298 and Krusberg, 1982; Tanaka *et al.*, 1996). In the former study, *C. elegans* were grown on a
299 liver extract/yeast extract/soy peptone medium that was devoid of 18:1 n-7, although 18:0
300 exceeded 16:0, whereas in the latter study, *C. elegans* were grown on *E.coli* in which 18:1 n-
301 7 was the predominant monoene, but 16:0 and not 18:0 was the main saturated fatty acid, but
302 in both cases *C. elegans* maintained the 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9 pattern despite
303 the "dietary" influence.
304 The data obtained from the experiments with radioactively labelled fatty acids clearly show
305 that a major fate of each of the fatty acids was β-oxidation. This is the explanation for the
306 radioactivity that was recovered in fatty acids with shorter chain lengths, or more saturated,
307 than the labelled substrate fatty acid as one round of β-oxidation would remove the labelled
308 carbon from the fatty acid in [1-^{14}C]-labelled fatty acids. Thus, with [1-^{14}C]18:0, over 4% of
309 radioactivity was recovered in 16:1 n-7 indicating that 18:0 was also metabolised by
310 β-oxidation to produce ^{14}C-labelled acetyl–CoA which was recycled by fatty acid synthase
311 (FAS), or an elongase, producing labelled 16:0 (although this could not be resolved from
312 18:0 by the chromatographic procedure) which was subsequently desaturated to 16:1 n-7. It
313 was noteworthy that within a chain length (C_{18} or C_{20}) the amount of radioactivity recovered
314 as recycled fatty acid products increased with increasing unsaturation of the labelled
315 substrate fatty acid suggesting that *P. redivivus* may prefer to β-oxidise PUFA compared to
316 saturated and monounsaturated fatty acids. However, it could be that lower amounts of
317 labelled acetyl-CoA were recycled with 18:0 or 18:1 n-9 than with PUFA as the data are
318 expressed as percentages of recovered fatty acids. Although differences in recycling cannot
319 be discounted, recoveries were generally comparable between the different fatty acids
320 suggesting that it cannot be a full explanation.
321 The data clearly suggest that *P. redivivus* express a wide range of desaturation and elongation
322 activities. In interpreting the data in the present study, it is necessary to be familiar with the

323 pathways for synthesis of HUFA and the range of desaturases that are commonly found in
324 either plants or animals (see Fig.1; note that this figure is not representative of any one
325 species). Production of labelled 18:1 n-9 from [1-^{14}C]18:0 and of 16:1 n-7, presumably
326 arising by desaturation of 16:0, labelled as a result of recycling (see above) indicates Δ9
327 desaturase activity. Production of 18:2 n-6 from [1-^{14}C]18:1 n-9 indicates significant Δ12
328 desaturase activity. The presence of a Δ15 (or n-3) desaturase activity (responsible for the
329 production of 18:3 n-3 from 18:2 n-6 in plants) is more difficult to establish as it is not
330 possible to distinguish between 18:3 n-3 (Δ15 product) and 18:3 n-6 (a Δ6 desaturase
331 product) and the same applies to all similar pairs such as 20:3 n-6/20:3 n-3 or 20:4 n-6/20:4
332 n-3. The fatty acid composition data obtained from the growth media studies suggested that
333 *P. redivivus* may express Δ15 desaturase activity as significant amounts of n-3fatty acids,
334 especially 20:5 n-3, were present despite these being very low in the growth media.
335 However, growth in LOM, containing very high 18:2 n-6, did not result in increased levels of
336 18:3 n-3 or n-3PUFA, with the proportion of 20:5 n-3 significantly decreasing in all lipid
337 classes. In contrast, the presence of Δ6 desaturase activity is strongly suggested by the
338 recovery of radioactivity as 18:4 n-3 when *P. redivivus* was incubated with [1-^{14}C]18:3 n-3
339 suggesting that at least some of the radioactivity recovered in 18:3 when *P. redivivus* was
340 incubated with [1-^{14}C]18:2 n-6 will be 18:3 n-6, the Δ6 product.
341 The data obtained from *P.redivivus* incubated with [1-^{14}C]18:3 n-3 were also consistent with
342 the presence of a Δ5 desaturase activity. This is because there is no known pathway for
343 conversion of 18:3 n-3 into n-6 fatty acids such as 20:4 n-6 or 22:5 n-6 and it is unlikely for
344 the amounts of radioactivity recovered in these fractions to have arisen solely from recycling.
345 Thus, the radioactivity recovered from [1-^{14}C]18:3 n-3 is almost certain to be as 20:4 n-3 and
346 20:5 n-3 indicating the presence of Δ5 desaturase (20:4 n-3 to 20:5 n-3). However, the best
347 evidence for significant Δ5 activity is the high level of 20:4 n-6 in *P. redivivus* grown on
348 *S. cerevisiae* in LLM as neither the yeast nor the yeast extract contained any significant

amount of 20:4 n-6 suggesting its presence in the nematode was the result of Δ5 action on 20:3 n-6 either supplied directly or as a result of Δ6 desaturation and elongation of 18:2 n-6. In comparison to Δ5 activity, where supporting evidence for significant activity can be demonstrated, support for Δ6* activity is lacking. The presence of 22:6 n-3 was not observed in *P. redivivus* under any conditions suggesting that the recovery of traces of radioactivity in the position corresponding to 22:6 n-3 was due to an unknown component or artifactual. The predominant n-3 fatty acid and, indeed, the most abundant fatty acid in *C. elegans* phospholipids grown on *E. coli* was 20:5 n-3, and no 22:6 n-3 was reported (Tanaka *et al.*, 1996). Similarly, 20:5 n-3 was the most abundant C_{20} HUFA in *Steinernema carpocapsae* when grown on artificial diet supplemented with lard or linseed oil, and no 22:6 n-3 was found (Fodor *et al.*, 1994).

The presence of a Δ12 activity in an animal like *P. redivivus* is not without precedent. In an earlier study, a *Caenorhabditis elegans* cDNA encoding a Δ12 fatty acid desaturase was identified and characterized (Peyou-Ndi *et al.*, 2000).

Although we found no unequivocal evidence for a Δ15 (n-3) desaturase activity in *P. redivivus*, genes for all the fatty acid desaturases required to produce 20:5 n-3 and 20:4 n-6, including Δ9, Δ12, Δ15/n-3, Δ6 and Δ5, have been identified in the genome of *C. elegans* (Napier and Michaelson, 2001). The cDNAs for some of these *C.elegans* genes including the n-3 (Δ15) (Spychalla *et al.*, 1997), Δ6 (Napier *et al.*, 1998) and Δ5 desaturases (Michaelson *et al.*, 1998; Watts and Browse, 1999) have been cloned and functionally characterized. Interestingly, the *C. elegans* Δ15 desaturase was actually revealed to be an ω3 desaturase as it desaturated both C_{18} and C_{20} n-6 substrates to the corresponding n-3 fatty acids (Spychalla *et al.*, 1997).

Increased levels of 14:0 and 16:0 in *P. redivivus* cultured in MTM is evidence for elongation of 10:0 through to 16:0. The presence of $C_{18\text{-}20}$ elongase activity was also clearly demonstrated by the recovery of significant amounts of radioactivity as 20:1 when *P.*

375 *redivivus* was incubated with [1-^{14}C]18:0 or [1-^{14}C]18:1 n-9. However, the elongase activity
376 towards C_{20} fatty acids would not appear to be as high as towards shorter chain substrates.
377 This is confirmed by the fatty acid composition data that showed very little evidence for C_{22}
378 fatty acids in *P. redivivus* under any conditions. An enzyme catalyzing the elongation of fatty
379 acids, ELO-1, has been cloned and functional characterised from the nematode *C. elegans*,
380 and heterologous expression in yeast showed it was predominantly active on C_{18} PUFA with
381 virtually no activity towards C_{20} PUFA (Beaudoin *et al.*, 2000, Watts and Browse, 2002).
382 ELO-1 functions together with ELO-2 a predicted *C. elegans* enzyme with fatty acid
383 elongation activity apparently towards C16:0 (Kniazeva *et al*. 2003).
384 The data from the metabolic studies using radiolabelled fatty acids can perhaps help to
385 explain the characteristic "normal" fatty acid composition of *P. redivivus* with 18:0 > 16:0
386 and 18:1n7 > 18:1 n-9 as discussed above. A possible explanation is that the desaturase and
387 elongase enzymes are more efficient with C_{16} compared to C_{18} fatty acids, and therefore,
388 16:0, produced by fatty acid synthetase, is readily desaturated to 16:1 n-7 and elongated to
389 18:0 and that subsequent elongation of 16:1 n-7 to 18:1 n-7 is more efficient than
390 desaturation of 18:0 to 18:1 n-9. The result of these differential activities being that 18:0 and
391 18:1 n-7 tend to accumulate more than 16:0 and 18:1 n-9. Growth in medium such as LOM,
392 with very high 18:1 n-9 from oatmeal and sunflower oil, increases triacylglycerol rich in 18:1
393 n-9, thus reversing the ratio of 18:1 n-9 to 18:1 n-7 in neutral lipid. In contrast, MTM is
394 characterized by high saturated fatty acids, particularly 10:0 which is presumably efficiently
395 elongated to 16:0 in *P. redivivus*, greatly increasing 16:0 and reversing the 18:0 to 16:0 ratio,
396 but also increasing production of 18:1 n-7 through conversion of 16:0 to 16:1 n-7 to 18:1 n-7
397 and so 18:1 n-7 still exceeds in both polar and neutral lipid in *P. redivivus* grown in MTM.
398 In summary the results have indicated the presence in *P. redivivus* of Δ9, Δ12, Δ6 and Δ5
399 fatty acid desaturase activities, and elongase activities active towards C_{18}, C_{16} and shorter
400 chain fatty acids. The data suggested that elongation and Δ9 desaturation were more active

towards C_{16} than C_{18} fatty acids, as the predominant saturated and monounsaturated fatty acids in *P. redivivus* were 18:0 and 18:1 n-7, rather than 16:0 and 18:1 n-9 normally found in higher animals. The fatty acid compositional data suggested that *P. redivivus* may have the ability to produce n-3PUFA *de novo*, indicating the presence of a Δ15 fatty acid desaturase, but this could not be conclusively established from the metabolic studies. Previous studies suggested that *P. redivivus* could be a useful live food for aquaculture, as it can be mass produced with fatty acid compositions specifically tailored to the particular aquaculture organism (Schlechtriem *et al*., 2004a, b). However, the *de novo* production of n-3PUFA requires to be further investigated in order to determine whether tailoring can be achieved with purely plant-based products.

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539 Table 1. Fatty acid composition (percentage of total fatty acid by weight) of lipid-containing
540 ingredients of media for culture of the nematode *Panagrellus redivivus*.
541

Fatty acid/treatment	Yeast	YE	SFO	OAT	MCT
8:0	nd	nd	nd	nd	0.9
10:0	nd	nd	nd	nd	42.1
12:0	nd	nd	nd	nd	2.8
14:0	0.6	1.9	0.1	0.2	0.2
15:0	0.2	1.3	tr	nd	0.3
16:0	13.8	19.1	6.0	16.3	5.6
18:0	3.0	5.4	4.3	1.5	1.9
20:0	nd	0.5	0.2	0.1	nd
Total saturated	17.7	28.2	10.7	18.1	53.7
16:1 n-9	nd	1.9	nd	nd	nd
16:1 n-7	13.4	5.4	0.1	0.2	0.2
18:1 n-9	33.9	24.0	21.4	38.8	14.4
18:1 n-7	1.4	1.5	nd	nd	0.4
20:1 n-11	nd	nd	0.1	nd	nd
20:1 n-9	0.6	1.9	0.1	0.8	0.2
20:1 n-7	nd	0.2	nd	nd	nd
22:1	0.3	2.2	0.7	0.1	nd
24:1 n-9	0.6	13.1	0.1	0.2	0.6
Total monounsaturated	50.3	50.3	22.4	40.1	15.8
18:2 n-6	30.4	14.6	66.8	40.4	29.9
18:3 n-6	nd	0.7	nd	nd	nd
20:2 n-6	nd	0.4	nd	nd	nd
20:3 n-6	0.3	3.6	nd	nd	0.4
20:4 n-6	nd	0.3	nd	nd	nd
Total n-6 PUFA	30.7	19.6	66.8	40.4	30.3
18:3 n-3	1.2	0.8	0.1	1.3	0.2
18:4 n-3	nd	0.3	nd	nd	nd
20:4 n-3	nd	0.2	nd	0.0	nd
20:5 n-3	0.1	0.5	tr	0.1	nd
22:6 n-3	nd	nd	nd	nd	nd
Total n-3 PUFA	1.3	1.9	0.1	1.4	0.2
Total PUFA	32.0	21.5	66.9	41.8	30.5
n-6/n-3	23.6	10.3	668.0	28.9	151.5

542

543 Results are means of duplicate analyses. Yeast, *Saccharomyces cerevisiae*; YE, yeast extract;
544 SFO, sunflower oil; OAT, oat flour; MCT, medium-chain tryglyerides; nd, not detected;
545 PUFA, polyunsaturated fatty acids.
546

546 Table 2. Lipid content (mg lipid/ g dry mass) and lipid class compositions (percentage of total
547 lipid) of *Panagrellus redivivus* grown on different culture media
548

	LLM		LOM		MTM	
Lipid content	13.3 ± 0.8	c	23.0 ± 0.4	b	29.0 ± 1.0	a
Lipid class						
Choline phosphoglycerides	19.7 ± 0.0	a	8.3 ± 0.1	c	15.8 ± 0.8	b
Ethanolamine phosphoglycerides	20.9 ± 0.6	a	12.4 ± 0.2	c	15.4 ± 0.6	b
Serine phosphoglycerides	6.6 ± 0.2	a	3.1 ± 0.1	c	4.5 ± 0.1	b
Inositol phosphoglycerides	4.6 ± 0.1	a	3.1 ± 0.1	c	3.9 ± 0.1	b
PG/CL/PA	6.0 ± 0.3	a	0.1 ± 0.1	c	4.8 ± 0.4	b
Sphingomyelin	3.3 ± 0.1	a	1.6 ± 0.1	c	2.3 ± 0.0	b
Total polar	61.0 ± 0.3	a	28.6 ± 0.4	c	46.7 ± 1.5	b
Total neutral	39.0 ± 0.3	c	71.4 ± 0.4	a	53.3 ± 1.5	b
Sterol	12.7 ± 0.6	a	10.3 ± 0.4	b	3.3 ± 0.2	c
Triacylglycerol	22.7 ± 0.5	c	56.4 ± 1.0	a	45.4 ± 1.3	b
Free fatty acids	1.2 ± 0.6	a	1.0 ± 0.2	a	0.9 ± 0.8	a
Sterol esters	2.4 ± 0.1	b	3.7 ± 0.3	a	3.6 ± 0.4	a

549
550
551 Results are means ± S.D. (n=3). Significance of differences between means were determined
552 by one-way ANOVA followed, where appropriate, by Tukey´s multiple comparison test as
553 described in the Materials and Methods. Values within a row with a different superscript letter
554 are significantly different ($P<0.05$). LLM, low lipid medium; LOM, lipid enriched oat based
555 medium; MTM, medium chain triglyceride medium; PG, phosphatidylglycerol; CL,
556 cardiolipin; PA, phosphatidic acid;.
557

Table 3. Fatty acid composition (percentage of total fatty acids) of *Panagrellus redivivus* grown on different culture media.

Fatty acid	LLM		LOM		MTM	
10:0	nd[b]		nd[b]		0.3 ± 0.1	[a]
12:0	nd[b]		nd[b]		0.9 ± 0.1	[a]
14:0	0.9 ± 0.0	[b]	0.4 ± 0.0	[c]	3.6 ± 0.2	[a]
15:0	0.1 ± 0.0	[a]	0.1 ± 0.0	[a]	0.1 ± 0.1	[a]
16:0	6.1 ± 0.2	[b]	6.2 ± 0.1	[b]	8.5 ± 0.5	[a]
18:0	7.0 ± 0.1	[a]	4.6 ± 0.1	[b]	4.7 ± 0.3	[b]
20:0	0.3 ± 0.0	[a]	0.2 ± 0.0	[b]	0.1 ± 0.0	[b]
24:0	0.3 ± 0.0	[a]	0.1 ± 0.0	[b]	0.3 ± 0.1	[a]
Total saturated	14.7 ± 0.2	[b]	11.6 ± 0.2	[c]	18.6 ± 1.2	[a]
16:1 n-7	2.0 ± 0.1	[b]	2.0 ± 0.0	[b]	4.6 ± 0.1	[a]
18:1 n-9	7.6 ± 0.3	[c]	15.3 ± 0.2	[a]	10.5 ± 0.8	[b]
18:1 n-7	12.4 ± 0.3	[b]	4.7 ± 0.1	[c]	20.1 ± 0.6	[a]
20:1 n-9	0.4 ± 0.1	[a]	0.4 ± 0.0	[a]	0.2 ± 0.0	[b]
20:1 n-7	0.4 ± 0.0	[b]	0.1 ± 0.0	[c]	0.5 ± 0.0	[a]
22:1	0.7 ± 0.0	[a]	0.4 ± 0.0	[b]	0.1 ± 0.0	[c]
24:1 n-9	0.3 ± 0.3	[a]	0.1 ± 0.0	[a]	0.1 ± 0.1	[a]
Total monoenes	23.9 ± 0.2	[b]	23.0 ± 0.2	[b]	36.2 ± 1.1	[a]
18:2 n-6	13.8 ± 0.1	[b]	45.4 ± 0.1	[a]	13.1 ± 0.3	[c]
18:3 n-6	0.7 ± 0.0	[c]	1.1 ± 0.0	[a]	1.0 ± 0.1	[b]
20:2 n-6	0.8 ± 0.0	[b]	2.6 ± 0.0	[a]	0.4 ± 0.0	[c]
20:3 n-6	13.4 ± 0.1	[a]	4.4 ± 0.1	[c]	9.0 ± 0.3	[b]
20:4 n-6	15.8 ± 0.1	[a]	5.6 ± 0.1	[c]	10.3 ± 0.4	[b]
Total n-6PUFA	44.4 ± 0.2	[b]	59.1 ± 0.3	[a]	33.7 ± 0.9	[c]
18:3 n-3	0.3 ± 0.0	[b]	1.1 ± 0.0	[a]	0.2 ± 0.0	[c]
18:4 n-3	0.1 ± 0.0	[a]	0.1 ± 0.0	[a]	0.1 ± 0.0	[b]
20:4 n-3	0.3 ± 0.0	[a]	0.1 ± 0.0	[c]	0.2 ± 0.0	[b]
20:5 n-3	7.2 ± 0.2	[a]	1.9 ± 0.1	[c]	6.1 ± 0.1	[b]
22:6 n-3	nd		nd		nd	
Total n-3PUFA	8.0 ± 0.2	[a]	3.2 ± 0.1	[c]	6.6 ± 0.1	[b]
16:0DMA	0.4 ± 0.0	[a]	0.1 ± 0.0	[c]	0.3 ± 0.0	[b]
18:0DMA	8.4 ± 0.4	[a]	3.0 ± 0.1	[b]	4.6 ± 1.7	[b]
18:1DMA	0.2 ± 0.0	[a]	nd[c]		0.1 ± 0.0	[b]
Total DMA	9.0 ± 0.4	[a]	3.1 ± 0.1	[b]	5.0 ± 1.7	[b]
Total PUFA	52.5 ± 0.3	[b]	62.3 ± 0.4	[a]	40.3 ± 1.0	[c]
n-6/n-3	5.5 ± 0.2	[b]	18.4 ± 0.5	[a]	5.1 ± 0.1	[b]

Results are means ± S.D. (n=3). Significance of differences between means were determined by one-way ANOVA followed, where appropriate, by Tukey's multiple comparison test as

563 described in the Materials and Methods. Values within a row with a different superscript letter
564 are significantly different ($P < 0.05$). nd, not detected; LLM, lipid-free medium; LOM, lipid
565 enriched oat based medium; MTM, medium-chain triglyceride medium; nd, not detected;
566 PUFA, polyunsaturated fatty acids; DMA, dimethylacetals.
567

567 Table 4. Fatty acid compositions (percentage of total fatty acids) of total polar and total
568 neutral lipids from *Panagrellus redivivus* grown on different culture media.
569

	Polar lipids			Neutral lipids		
	LLM	LOM	MTM	LLM	LOM	MTM
10:0	nd	nd	nd	nd	nd	0.3 ± 0.1 [a]
12:0	nd	nd	nd	nd	nd	1.6 ± 0.1 [a]
14:0	0.3 ± 0.0 [b]	0.3 ± 0.0 [b]	0.8 ± 0.0 [a]	2.7 ± 0.1 [b]	0.5 ± 0.0 [c]	6.6 ± 0.2 [a]
15:0	0.1 ± 0.0 [a]	0.0 ± 0.0 [b]	0.1 ± 0.0 [ab]	0.3 ± 0.0 [a]	0.1 ± 0.0 [c]	0.2 ± 0.0 [b]
16:0	4.1 ± 0.0 [b]	4.3 ± 0.1 [b]	4.6 ± 0.2 [a]	10.4 ± 0.3 [b]	6.5 ± 0.1 [c]	13.2 ± 0.4 [a]
18:0	8.4 ± 0.4 [b]	10.4 ± 0.1 [a]	7.9 ± 0.2 [b]	3.8 ± 0.4 [a]	2.7 ± 0.0 [b]	2.5 ± 0.1 [b]
20:0	0.3 ± 0.0 [a]	0.3 ± 0.0 [ab]	0.3 ± 0.0 [b]	0.1 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.1 [a]
22:0	0.6 ± 0.0 [a]	0.7 ± 0.2 [a]	0.5 ± 0.1 [a]	0.2 ± 0.1 [a]	0.2 ± 0.0 [a]	0.1 ± 0.1 [b]
Total saturated	13.8 ± 0.5 [b]	16.0 ± 0.3 [a]	14.1 ± 0.4 [b]	17.6 ± 0.6 [b]	10.2 ± 0.1 [c]	24.4 ± 0.9 [a]
16:1 n-9	0.1 ± 0.0 [a]	0.1 ± 0.0 [b]	0.1 ± 0.0 [c]	0.4 ± 0.0 [a]	0.4 ± 0.0 [a]	0.4 ± 0.1 [a]
16:1 n-7	1.2 ± 0.0 [a]	0.6 ± 0.0 [c]	1.0 ± 0.1 [b]	4.3 ± 0.1 [b]	2.5 ± 0.0 [c]	8.2 ± 0.0 [a]
18:1 n-9	5.2 ± 0.1 [a]	4.8 ± 0.1 [ab]	4.5 ± 0.4 [b]	16.8 ± 0.4 [b]	18.6 ± 0.1 [a]	16.8 ± 0.6 [b]
18:1 n-7	10.9 ± 0.5 [b]	5.2 ± 0.1 [c]	14.2 ± 0.0 [a]	20.9 ± 0.4 [b]	4.8 ± 0.0 [c]	28.0 ± 0.1 [a]
20:1 n-9	0.3 ± 0.0 [a]	0.3 ± 0.0 [a]	0.2 ± 0.1 [a]	0.7 ± 0.0 [a]	0.5 ± 0.0 [b]	0.3 ± 0.0 [c]
20:1 n-7	0.4 ± 0.0 [a]	0.2 ± 0.0 [b]	0.4 ± 0.0 [a]	0.7 ± 0.0 [b]	0.1 ± 0.0 [c]	0.8 ± 0.0 [a]
22:1	0.2 ± 0.1 [a]	0.1 ± 0.2 [a]	0.1 ± 0.1 [a]	0.5 ± 0.1 [a]	0.1 ± 0.0 [b]	0.1 ± 0.1 [b]
24:1 n-9	0.2 ± 0.1 [a]	0.2 ± 0.1 [a]	0.1 ± 0.1 [a]	nd	nd	nd
Total monoenes	18.5 ± 0.7 [b]	11.5 ± 0.3 [c]	20.7 ± 0.5 [a]	44.3 ± 0.9 [b]	26.9 ± 0.2 [c]	54.5 ± 0.8 [a]
18:2 n-6	13.7 ± 0.3 [c]	25.8 ± 0.2 [a]	14.7 ± 0.1 [b]	16.8 ± 0.4 [b]	52.0 ± 0.1 [a]	10.9 ± 0.5 [c]
18:3 n-6	0.6 ± 0.0 [b]	0.6 ± 0.0 [b]	0.9 ± 0.0 [a]	1.1 ± 0.1 [a]	1.2 ± 0.0 [a]	0.8 ± 0.1 [b]
20:2 n-6	0.7 ± 0.0 [b]	2.7 ± 0.0 [a]	0.5 ± 0.0 [b]	0.9 ± 0.0 [b]	2.4 ± 0.0 [a]	0.4 ± 0.0 [c]
20:3 n-6	15.7 ± 0.1 [a]	11.4 ± 0.2 [c]	14.7 ± 0.0 [b]	5.0 ± 0.3 [a]	1.8 ± 0.0 [c]	2.5 ± 0.2 [b]
20:4 n-6	17.0 ± 0.3 [a]	12.6 ± 0.2 [c]	15.1 ± 0.3 [b]	8.1 ± 0.5 [a]	2.9 ± 0.0 [c]	3.6 ± 0.3 [b]
22:4 n-6	0.2 ± 0.1 [a]	0.1 ± 0.1 [a]	0.1 ± 0.1 [a]	0.0 ± 0.1 [a]	nd[a]	nd[a]
Total n-6 PUFA	48.0 ± 0.3 [b]	53.2 ± 0.4 [a]	46.0 ± 0.4 [c]	32.0 ± 1.1 [b]	60.2 ± 0.2 [a]	18.3 ± 1.1 [c]
18:3 n-3	0.3 ± 0.0 [b]	0.5 ± 0.0 [a]	0.2 ± 0.0 [c]	0.8 ± 0.4 [b]	1.4 ± 0.1 [a]	0.5 ± 0.1 [b]
18:4 n-3	0.2 ± 0.0 [b]	0.2 ± 0.0 [a]	0.1 ± 0.0 [c]	0.0 ± 0.1 [a]	0.1 ± 0.0 [a]	0.1 ± 0.1 [a]
20:3 n-3	nd	nd	nd	0.4 ± 0.1 [a]	0.1 ± 0.0 [b]	nd[c]
20:4 n-3	0.4 ± 0.0 [a]	0.3 ± 0.0 [b]	0.5 ± 0.1 [a]	0.2 ± 0.0 [a]	0.1 ± 0.0 [a]	0.1 ± 0.1 [a]
20:5 n-3	6.8 ± 0.3 [b]	3.9 ± 0.0 [c]	7.9 ± 0.2 [a]	4.0 ± 0.3 [a]	1.1 ± 0.0 [c]	2.1 ± 0.3 [b]
22:6 n-3	nd	nd	nd	nd	nd	nd
Total n-3 PUFA	7.8 ± 0.3 [b]	4.9 ± 0.1 [c]	8.7 ± 0.3 [a]	5.3 ± 0.5 [a]	2.8 ± 0.1 [b]	2.8 ± 0.2 [b]
16:0DMA	0.3 ± 0.0 [b]	0.1 ± 0.0 [c]	0.4 ± 0.0 [a]	nd	nd	nd
18:0DMA	11.5 ± 1.0 [b]	14.2 ± 0.4 [a]	9.9 ± 0.3 [c]	0.8 ± 0.5 [a]	nd[b]	nd[b]
18:1DMA	0.2 ± 0.0 [a]	0.0 ± 0.1 [b]	0.2 ± 0.0 [a]	nd	nd	nd
Total DMA	12.0 ± 1.0 [b]	14.4 ± 0.5 [a]	10.5 ± 0.3 [c]	0.8 ± 0.5 [a]	nd[b]	nd[b]
Total PUFA	55.8 ± 0.4 [b]	58.1 ± 0.5 [a]	54.7 ± 0.7 [b]	37.4 ± 1.2 [b]	68.0 ± 0.3 [a]	21.1 ± 1.3 [c]
n-3/n-6	6.2 ± 0.2 [b]	10.8 ± 0.2 [a]	5.3 ± 0.2 [c]	6.0 ± 0.6 [b]	21.8 ± 0.8 [a]	6.5 ± 0.2 [b]

570

571 Results are means ± S.D. (n=3). Significance of differences between meanswere determined
572 by one-way ANOVA followed, where appropriate, by Tukey´s multiple comparison test as

573 described in the Materials and Methods. Values within a row with a different superscript letter
574 are significantly different ($P<0.05$). LLM, low lipid medium; LOM, lipid enriched oat based
575 medium; MTM, medium chain triglyceride medium; DMA, dimethyl acetals; nd, not detected;
576 PUFA, polyunsaturated fatty acids.
577

Table 5. Metabolism of ^{14}C-labelled fatty acids by *Panagrellus redivivus*

Fatty acid	[1-^{14}C]18:0	[1-^{14}C]18:1 n-9	[1-^{14}C]18:2 n-6	[1-^{14}C]18:3 n-3
18:0 (16:0)	74.9 ± 2.8	6.7 ± 1.0	11.9 ± 0.5	18.5 ± 0.7
16:1 n-7	4.2 ± 1.1	2.0 ± 1.6	7.0 ± 0.4	11.3 ± 0.3
18:1 n-9	6.0 ± 0.4	67.4 ± 2.0	8.5 ± 0.1	12.7 ± 0.2
20:1	5.4 ± 2.1	3.9 ± 0.9	2.1 ± 0.2	2.0 ± 0.4
18:2 n-6	4.7 ± 0.1	14.4 ± 1.1	62.5 ± 1.2	1.4 ± 0.3
20:2 n-6	0.9 ± 0.2	1.8 ± 1.1	2.6 ± 0.3	0.7 ± 0.1
18:3(n-6/n-3)	0.6 ± 0.1	1.2 ± 0.1	1.2 ± 0.9	46.6 ± 1.4
18:4 n-3	0.2 ± 0.0	0.1 ± 0.0	0.5 ± 0.3	1.7 ± 0.1
20:3(n-6/n-3)	1.9 ± 0.3	1.3 ± 0.3	2.1 ± 0.3	2.0 ± 0.3
20:4 (n-6/n-3)	0.5 ± 0.1	0.7 ± 0.1	0.8 ± 0.3	1.6 ± 0.2
20-5 n-3/22:5(n-3/n-6)	0.2 ± 0.0	0.3 ± 0.1	0.4 ± 0.4	0.6 ± 0.0
22:6 n-3 ?	0.4 ± 0.1	0.3 ± 0.0	0.2 ± 0.1	0.8 ± 0.1

	[1-^{14}C]20:3 n-6	[1-^{14}C]20:4 n-6	[1-^{14}C]20:5 n-3
Saturated fatty acids	9.1 ± 1.4	10.2 ± 0.3	18.7 ± 1.2
16:1	6.4 ± 1.1	8.6 ± 1.3	12.3 ± 0.3
18:1	4.4 ± 1.4	6.7 ± 1.2	11.5 ± 1.0
18:2 n-6	1.1 ± 0.2	1.0 ± 0.4	1.2 ± 0.2
20:3(n-6/n-3)	72.5 ± 3.4	1.9 ± 0.2	0.9 ± 0.3
20:4(n-6/n-3)	4.0 ± 0.5	64.4 ± 1.1	2.1 ± 0.8
20:5 n-3/22:5(n-6/n-3)	1.1 ± 0.1	4.3 ± 0.5	50.8 ± 0.0
22:6 n-3 ?	1.4 ± 0.7	2.9 ± 0.6	2.5 ± 1.4

The data represent the amount of radioactivity recovered in each of the fatty acid fractions indicated in column one after incubation of *Panagrellus redivivus* for 24 hr with the [1-^{14}C]-labelled fatty acids as indicated. Results are reported as a percentage of the total radioactivity recovered and are means ± S.D. of three separate experiments. The percentage of radioactivity recovered unmetabolised (i.e. as the supplemented fatty acid) is highlighted in bold in each column.

587 **Figure legends**

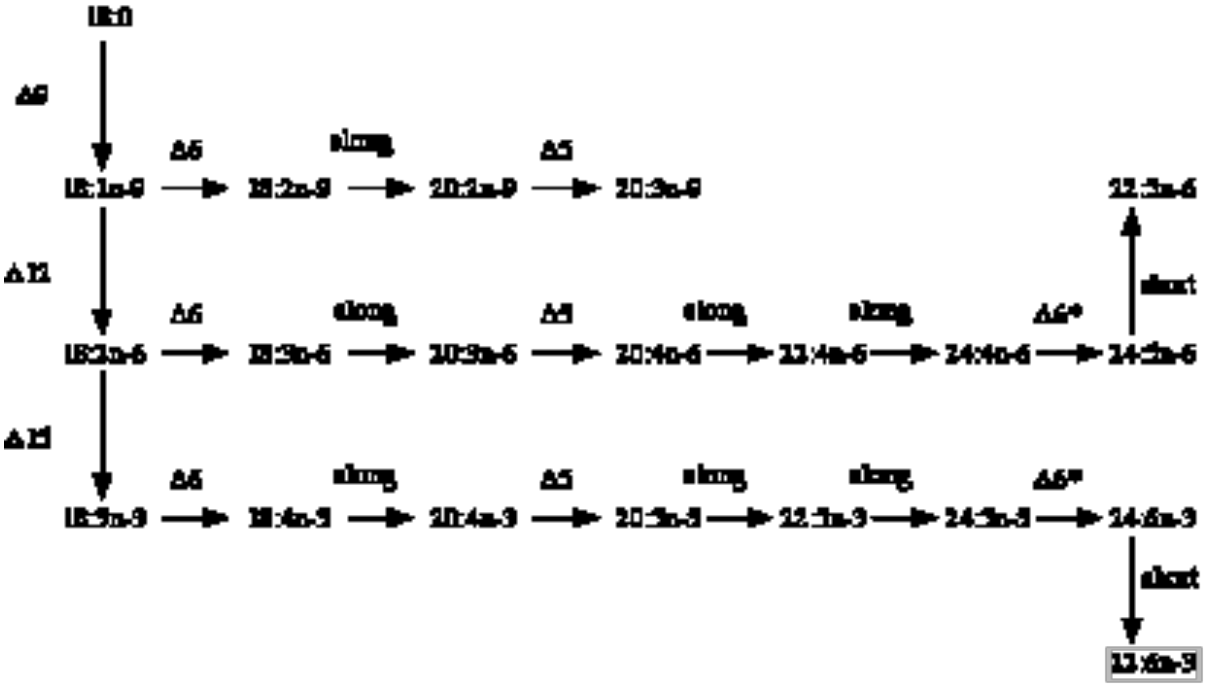
588

589 Figure 1. Pathways of biosynthesis of C_{20} and C_{22} HUFA from n-3, n-6 and n-9 C_{18} precursors
590 as determined in rat liver (Sprecher *et al.*, 1995) and rainbow trout hepatocytes (Buzzi *et al.*,
591 1996, 1997). Δ5, Δ6, Δ6*, Δ9, Δ12, Δ15, Fatty acyl desaturases; Elong, Fatty acyl elongases;
592 Short, chain shortening. Δ9 desaturase is found in all animals and plants whereas Δ12 and Δ15
593 desaturases are generally only found in plants and some lower animals and so 18:2 n-6 and
594 18:3 n-3 are "essential" fatty acids (EFA) for higher animals including mammals and fish.
595 The Δ6* enzyme acting on C_{24} fatty acids may or may not be the same enzyme (Δ6) that acts
596 on C_{18} fatty acids.

597

598

598 Figure 1
599
600



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