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

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## 26 **Summary**

27 The free-living nematode *Panagrellus redivivus* can be mass produced in monoxenic solid  
28 culture on *Saccharomyces cerevisiae* and therefore could be useful as a live food for marine  
29 fish or crustacean larvae in the rapidly expanding aquaculture industry. However, this will  
30 depend on their lipid and fatty acid composition and so this was investigated in mass  
31 produced *P. redivivus* grown on *S. cerevisiae* in three different media. Live nematodes were  
32 also incubated with [1-<sup>14</sup>C]-labelled fatty acids and their desaturation and elongation  
33 determined. The combined results from the growth trials on different media and the  
34 metabolic studies with labelled fatty acids indicated the presence of  $\Delta 9$ ,  $\Delta 12$ ,  $\Delta 6$  and  $\Delta 5$  fatty  
35 acid desaturase activities, and elongase activities active towards C<sub>18</sub>, C<sub>16</sub> and shorter chain  
36 fatty acids. The presence of  $\Delta 15$ , and therefore the ability to produce n-3 polyunsaturated  
37 fatty acids, was suggested by the compositional data, but could not be conclusively  
38 established from metabolic studies.

39

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

41

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 branchiopod 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  $\geq 3$  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<sub>16</sub> and C<sub>18</sub> 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

93 with different [1-<sup>14</sup>C]-labelled fatty acids and their further metabolism by desaturation and  
94 elongation determined.

95

## 96 **Materials and Methods**

97

### 98 *Experimental Animals and Culture*

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

116

117 *Lipid extraction and lipid class composition*

118 Total lipid contents of nematodes and growth medium samples were determined  
119 gravimetrically after extraction by homogenization in chloroform/methanol (2:1, v/v)  
120 containing 0.01% butylated hydroxytoluene as antioxidant, basically according to Folch *et al.*  
121 (1957). Separation of lipid classes was performed by high-performance thin-layer  
122 chromatography. Approximately 10 µg of lipid extract was loaded as a 2 mm streak and the  
123 plate developed to two-thirds distance with methyl acetate/isopropanol/  
124 chloroform/methanol/0.25% aqueous KCl (25:25:25:10:9, by vol.). After drying, the plate  
125 was fully developed with isohexane/diethyl ether/acetic acid (85:15:1, by vol.). The classes  
126 were quantified by charring at 160°C for 15 min after spraying with 3% (w/v) aqueous cupric  
127 acetate containing 8% (v/v) phosphoric acid, followed by calibrated densitometry using a  
128 Shimadzu CS-9000 dual-wavelength flying spot scanner and a DR-13 recorder (Henderson  
129 and Tocher 1992).

130

131 *Fatty acid analysis*

132 Samples of total lipid (2 mg) were applied as 2 cm streaks to thin-layer chromatography  
133 plates, and polar lipids separated from neutral lipids using hexane/diethyl ether/acetic acid  
134 (90:10:1, by vol.) as developing solvent. The origin area corresponding to total polar lipids  
135 and the lane above the origin corresponding to total neutral lipids were scraped into  
136 stoppered glass test tubes for transmethylation directly on the silica (Christie, 1982). Fatty  
137 acid methyl esters of total lipid, total polar lipids, and total neutral lipids, purified as above,  
138 were prepared by acid-catalyzed transesterification using 2 ml of 1% H<sub>2</sub>SO<sub>4</sub> in methanol plus  
139 1 ml toluene as described by Christie (1982) and methyl esters extracted and purified as  
140 described previously (Tocher and Harvie, 1988). Fatty acid methyl esters were separated and  
141 quantified by gas-liquid chromatography (Fisons GC8600, Fisons Ltd., Crawley, U.K.) using  
142 a 30m x 0.32 mm capillary column (CP wax 52CB; Chrompak Ltd., London, U.K).

143 Hydrogen was used as carrier gas and temperature programming was from 50°C to 180°C at  
144 40°C/min and then to 225°C at 2°C/min. Individual methyl esters were identified by  
145 comparison to known standards and by reference to published data (Ackman, 1980).

146

#### 147 *Incubation of nematodes with [1-<sup>14</sup>C]-labelled fatty acids*

148 Live nematodes, containing juveniles (J1-4 stages) and adults, grown on *S. cerevisiae* in  
149 LLM, were resuspended in Medium 199 and dispensed in 4ml aliquots into 25cm<sup>2</sup> tissue  
150 culture flasks. Flasks were supplemented with 0.5 µCi (approximately 100µl) of a particular  
151 [1-<sup>14</sup>C]-labelled fatty acid added as a complex with fatty acid-free bovine serum albumin  
152 (FAF-BSA) prepared in medium 199 as described previously (Ghioni *et al.*, 1997). The  
153 specific fatty acids used were [1-<sup>14</sup>C]18:0, [1-<sup>14</sup>C]18:1 n-9, [1-<sup>14</sup>C]18:2 n-6, [1-<sup>14</sup>C]18:3 n-3,  
154 [1-<sup>14</sup>C]20:3 n-6, [1-<sup>14</sup>C]20:4 n-6 and [1-<sup>14</sup>C]20:5 n-3. The suspensions were mixed carefully  
155 and incubated at 20°C for 24h. After incubation the flasks were gently rocked and the  
156 suspension of live nematodes transferred to glass conical test tubes. Each flask was washed  
157 with 1 ml of ice-cold 1% FAF-BSA in Hanks' balanced salt solution added afterwards to the  
158 test tubes. The suspensions were centrifuged (400 x g for 2 min.; ~1200rpm), the  
159 supernatants decanted and the nematodes washed again with 5 ml 1% ice-cold Hanks'  
160 balanced salt solution containing FAF-BSA. After centrifugation the pellets were used for  
161 analyses of radiolabelled fatty acids.

162

#### 163 *Assay of fatty acyl desaturation/elongation activities*

164 Lipids were extracted from labelled nematode pellets using ice-cold chloroform/methanol  
165 (2:1, v/v) containing 0.01% (w/v) butylated hydroxytoluene essentially as described by Folch  
166 *et al.* (1957) and as described in detail previously (Tocher *et al.*, 1988). Total lipid was  
167 transmethylated and fatty acid methyl esters prepared as described above. The methyl esters  
168 were redissolved in 100 µl isohexane containing 0.01% butylated hydroxytoluene and

169 applied as 2.5 cm streaks to thin-layer chromatography plates impregnated by spraying with  
170 2 g silver nitrate in 20 ml acetonitrile and pre-activated at 110 °C for 30 min. Plates were  
171 fully developed in toluene/acetonitrile (95:5, v/v) (Wilson and Sargent, 1992).  
172 Autoradiography was performed with Kodak MR2 film for 6 days at room temperature.  
173 Areas of silica containing individual PUFA were scraped into scintillation mini-vials  
174 containing 2.5 ml of scintillation fluid (Ecoscint A, National Diagnostics, Atlanta, Georgia)  
175 and radioactivity determined in a TRI-CARB 2000CA scintillation counter (United  
176 Technologies Packard, U.K.). Results were corrected for counting efficiency and quenching  
177 of  $^{14}\text{C}$  under exactly these conditions.

178

#### 179 *Protein determination*

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

183

#### 184 *Materials*

185 [ $^{14}\text{C}$ ]18:0, [ $^{14}\text{C}$ ]18:1 n-9 [ $^{14}\text{C}$ ]18:2 n-6, [ $^{14}\text{C}$ ]18:3 n-3, [ $^{14}\text{C}$ ]20:3 n-6, [ $^{14}\text{C}$ ]20:4  
186 n-6 and [ $^{14}\text{C}$ ]20:5 n-3, all (50-55 mCi/mmol) were obtained from NEN (DuPont (U.K.)  
187 Ltd., Stevenage, U.K.). Hanks' balanced salt solution, HEPES buffer, FAF-BSA, butylated  
188 hydroxytoluene, and silver nitrate were obtained from Sigma Chemical Co. (Poole, U.K.).  
189 Thin-layer chromatography (20 cm x 20 cm x 0.25 mm) and high-performance thin-layer  
190 chromatography plates (10 cm x 10 cm x 0.15 mm), precoated with silica gel 60 (without  
191 fluorescent indicator) were obtained from Merck (Darmstadt, Germany). All solvents were  
192 HPLC grade and were obtained from Fisher Scientific U.K., Loughborough, England.

193



194 *Statistical analysis*

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

198

## 199 **Results**

200

### 201 *Fatty acid compositions of growth media components*

202 All nematodes were produced by culture on the yeast, *Saccharomyces cerevisiae*, but in three  
203 different media. The fatty acid composition of the basic yeast contained over 50%  
204 monounsaturated fatty acids, predominantly 18:1 n-9 and 16:1 n-7, nearly 18% saturated  
205 fatty acids, predominantly 16:0, and 32% PUFA composed of over 30% 18:2 n-6, and only  
206 1% 18:3 n-3 and virtually no HUFA (Table 1). The LLM medium contained a small amount  
207 of yeast extract which contained a similar level of monounsaturated fatty acids including  
208 13% 24:1 n-9, but a higher level of saturates and lower PUFA, than the yeast. The main  
209 PUFA in the yeast extract was 18:2 n-6 but there was 3.6% 20:3 n-6 and a small amount of  
210 20:5 n-3. The LOM medium contained oat flour (OAT) and sunflower oil (SFO), both of  
211 which were rich in 18:2 n-6, 40% and 67%, respectively, with the remaining fatty acids being  
212 18:1 n-9 and saturates, with virtually no n-3 PUFA (Table 1). The MTM medium was  
213 enriched with medium-chain triglycerides (MCT) which had over 42% 10:0 in a total of 54%  
214 saturates along with 30% 18:2 n-6 and 14% 18:1 n-9 (Table 1).

215

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

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

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

224

#### 225 *Effects of growth medium on fatty acid compositions of total lipid*

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

235 The proportion of saturated fatty acids in total fatty acids was slightly lower in nematodes  
236 grown on LOM, whereas it was significantly increased in nematodes grown in MTM,  
237 compared to growth in LLM (Table 3). Growth in both lipid-supplemented media resulted in  
238 the level of 16:0 exceeding that of 18:0 compared to nematodes grown in LLM. Saturated  
239 fatty acids after growth in MTM also resulted in higher proportions of shorter chain fatty  
240 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  
241 n-7 with decreased proportions of 18:0, 20:3 n-6, 20:4 n-6 and DMA. The proportion of 18:2  
242 n-6 and n-3PUFA in total fatty acids were not greatly affected by growth in MTM compared  
243 to growth in LLM (Table 3).

244

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 <sup>14</sup>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-<sup>14</sup>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-<sup>14</sup>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

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

275 However, approximately 5% of the radioactivity from each of [1-<sup>14</sup>C]18:2 n-6 or [1-<sup>14</sup>C]18:3  
276 n-3 was recovered in further desaturated fatty acid products. Incubation of *P. redivivus* with  
277 the radiolabelled HUFA resulted in 21%, 28% and 46% of the radioactivity from [1-  
278 <sup>14</sup>C]20:3 n-6, [1-<sup>14</sup>C]20:4 n-6 and [1-<sup>14</sup>C]20:5 n-3, respectively, being recovered as  
279 saturated, monounsaturated and shorter chain fatty acids (Table 5). Approximately, 6.5%,  
280 7.2% and 2.5% of the radioactivity from [1-<sup>14</sup>C]20:3 n-6, [1-<sup>14</sup>C]20:4 n-6 and [1-<sup>14</sup>C]20:5 n-3,  
281 respectively, was recovered in further desaturated products.

282

## 283 **Discussion**

284

285 The fatty acid composition of total lipid of *P. redivivus* grown on *S. cerevisiae* in LLM  
286 (Table 3) shows some interesting features such as 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9, the  
287 opposite to the situation in higher animals, and in the yeast on which it was grown in which  
288 16:0 and 18:1 n-9 both greatly exceeded 18:0 and 18:1 n-7, respectively. The composition of  
289 all the other media components were the same as the yeast in this respect (Table 1) which  
290 was most interesting, as 18:0 > 16:0 and 18:1 n-7 > 18:1 n-9 was consistently observed in the  
291 polar lipid fraction of *P. redivivus*, representative of the membrane lipids, irrespective of  
292 medium or diet. In contrast, the fatty acid composition of the neutral lipids did not show the  
293 same characteristic pattern in the saturated fatty acids (as 16:0 > 18:0) but 18:1 n-7 did  
294 exceed 18:1 n-9, although this could be changed by diet as shown with the LOM medium  
295 (oatmeal and sunflower oil) which was rich in 18:1 n-9. Therefore, the pattern of 18:0 > 16:0  
296 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  $\beta$ -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  $\beta$ -oxidation would remove the labelled  
308 carbon from the fatty acid in [1-<sup>14</sup>C]-labelled fatty acids. Thus, with [1-<sup>14</sup>C]18:0, over 4% of  
309 radioactivity was recovered in 16:1 n-7 indicating that 18:0 was also metabolised by  
310  $\beta$ -oxidation to produce <sup>14</sup>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<sub>18</sub> or C<sub>20</sub>) 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  $\beta$ -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-<sup>14</sup>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-<sup>14</sup>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-3 fatty 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-3 PUFA, 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-<sup>14</sup>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-<sup>14</sup>C]18:2 n-6 will be 18:3 n-6, the Δ6 product.

341 The data obtained from *P. redivivus* incubated with [1-<sup>14</sup>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-<sup>14</sup>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

349 amount of 20:4 n-6 suggesting its presence in the nematode was the result of  $\Delta 5$  action on  
350 20:3 n-6 either supplied directly or as a result of  $\Delta 6$  desaturation and elongation of 18:2 n-6.  
351 In comparison to  $\Delta 5$  activity, where supporting evidence for significant activity can be  
352 demonstrated, support for  $\Delta 6^*$  activity is lacking. The presence of 22:6 n-3 was not observed  
353 in *P. redivivus* under any conditions suggesting that the recovery of traces of radioactivity in  
354 the position corresponding to 22:6 n-3 was due to an unknown component or artifactual. The  
355 predominant n-3 fatty acid and, indeed, the most abundant fatty acid in *C. elegans*  
356 phospholipids grown on *E. coli* was 20:5 n-3, and no 22:6 n-3 was reported (Tanaka *et al.*,  
357 1996). Similarly, 20:5 n-3 was the most abundant C<sub>20</sub> HUFA in *Steinernema carpocapsae*  
358 when grown on artificial diet supplemented with lard or linseed oil, and no 22:6 n-3 was  
359 found (Fodor *et al.*, 1994).

360 The presence of a  $\Delta 12$  activity in an animal like *P. redivivus* is not without precedent. In an  
361 earlier study, a *Caenorhabditis elegans* cDNA encoding a  $\Delta 12$  fatty acid desaturase was  
362 identified and characterized (Peyou-Ndi *et al.*, 2000).

363 Although we found no unequivocal evidence for a  $\Delta 15$  (n-3) desaturase activity in *P.*  
364 *redivivus*, genes for all the fatty acid desaturases required to produce 20:5 n-3 and 20:4 n-6,  
365 including  $\Delta 9$ ,  $\Delta 12$ ,  $\Delta 15/n-3$ ,  $\Delta 6$  and  $\Delta 5$ , have been identified in the genome of *C. elegans*  
366 (Napier and Michaelson, 2001). The cDNAs for some of these *C. elegans* genes including the  
367 n-3 ( $\Delta 15$ ) (Spychalla *et al.*, 1997),  $\Delta 6$  (Napier *et al.*, 1998) and  $\Delta 5$  desaturases (Michaelson  
368 *et al.*, 1998; Watts and Browse, 1999) have been cloned and functionally characterized.  
369 Interestingly, the *C. elegans*  $\Delta 15$  desaturase was actually revealed to be an  $\omega 3$  desaturase as  
370 it desaturated both C<sub>18</sub> and C<sub>20</sub> n-6 substrates to the corresponding n-3 fatty acids (Spychalla  
371 *et al.*, 1997).

372 Increased levels of 14:0 and 16:0 in *P. redivivus* cultured in MTM is evidence for elongation  
373 of 10:0 through to 16:0. The presence of C<sub>18-20</sub> elongase activity was also clearly  
374 demonstrated by the recovery of significant amounts of radioactivity as 20:1 when *P.*

375 *redivivus* was incubated with [1-<sup>14</sup>C]18:0 or [1-<sup>14</sup>C]18:1 n-9. However, the elongase activity  
376 towards C<sub>20</sub> 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<sub>22</sub>  
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<sub>18</sub> PUFA with  
381 virtually no activity towards C<sub>20</sub> 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 C<sub>16</sub>: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<sub>16</sub> compared to C<sub>18</sub> 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 Δ<sub>9</sub>, Δ<sub>12</sub>, Δ<sub>6</sub> and Δ<sub>5</sub>  
399 fatty acid desaturase activities, and elongase activities active towards C<sub>18</sub>, C<sub>16</sub> and shorter  
400 chain fatty acids. The data suggested that elongation and Δ<sub>9</sub> desaturation were more active



401 towards C<sub>16</sub> than C<sub>18</sub> fatty acids, as the predominant saturated and monounsaturated fatty  
402 acids in *P. redivivus* were 18:0 and 18:1 n-7, rather than 16:0 and 18:1 n-9 normally found in  
403 higher animals. The fatty acid compositional data suggested that *P. redivivus* may have the  
404 ability to produce n-3PUFA *de novo*, indicating the presence of a  $\Delta$ 15 fatty acid desaturase,  
405 but this could not be conclusively established from the metabolic studies. Previous studies  
406 suggested that *P. redivivus* could be a useful live food for aquaculture, as it can be mass  
407 produced with fatty acid compositions specifically tailored to the particular aquaculture  
408 organism (Schlechtriem *et al.*, 2004a, b). However, the *de novo* production of n-3PUFA  
409 requires to be further investigated in order to determine whether tailoring can be achieved  
410 with purely plant-based products.

411

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418

419

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- 537 Wilson, R. & Sargent, J.R. (1992). High resolution separation of polyunsaturated fatty acids  
538 by argentation thin-layer chromatography. *Journal of Chromatography* 623, 403-407.

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

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



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

559

Fatty acid	LLM			LOM			MTM		
10:0	nd <sup>b</sup>			nd <sup>b</sup>			0.3	± 0.1	a
12:0	nd <sup>b</sup>			nd <sup>b</sup>			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 <sup>c</sup>			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

560

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

570

571 Results are means ± S.D. (n=3). Significance of differences between means were 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

Fatty acid	<b>[1-<sup>14</sup>C]18:0</b>		<b>[1-<sup>14</sup>C]18:1 n-9</b>		<b>[1-<sup>14</sup>C]18:2 n-6</b>		<b>[1-<sup>14</sup>C]18:3 n-3</b>	
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	<b>67.4</b>	± 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
			<b>[1-<sup>14</sup>C]20:3 n-6</b>		<b>[1-<sup>14</sup>C]20:4 n-6</b>		<b>[1-<sup>14</sup>C]20:5 n-3</b>	
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

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

587 **Figure legends**

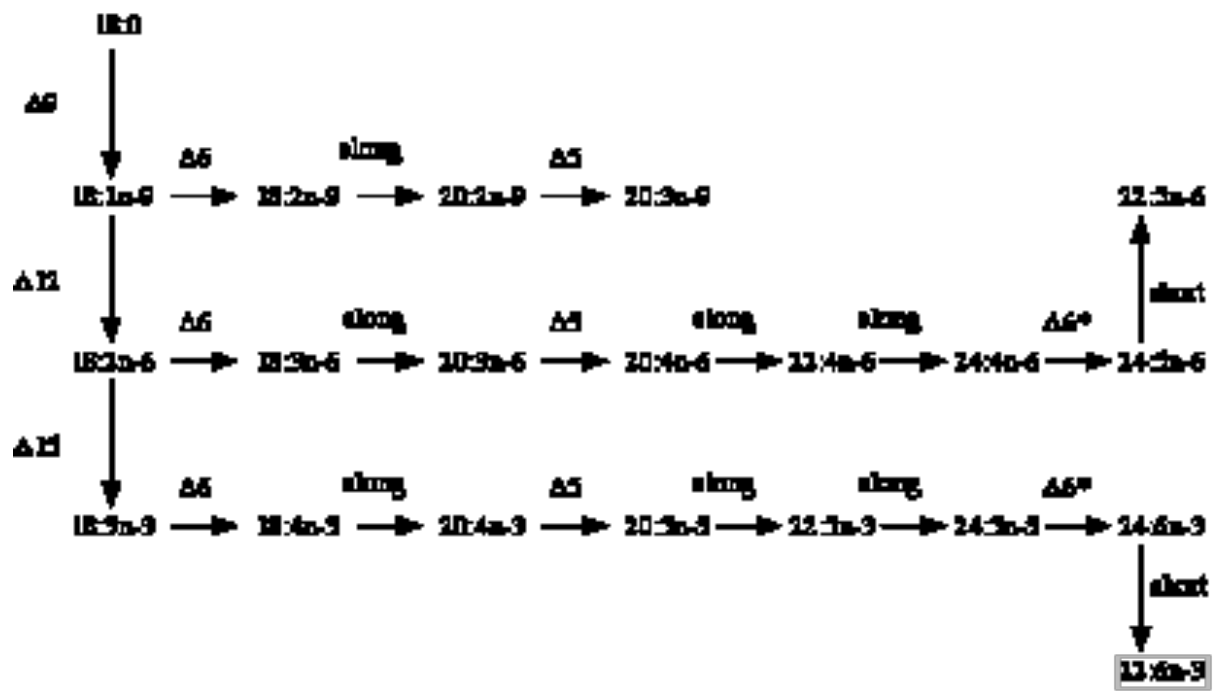
588

589 Figure 1. Pathways of biosynthesis of C<sub>20</sub> and C<sub>22</sub> HUFA from n-3, n-6 and n-9 C<sub>18</sub> 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<sub>24</sub> fatty acids may or may not be the same enzyme (Δ6) that acts  
596 on C<sub>18</sub> fatty acids.

597

598

598 Figure 1  
 599  
 600



601