

1 **Effect of salinity on the biosynthesis of n-3 long-chain polyunsaturated fatty**
2 **acids in silverside *Chirostoma estor*.**

3

4 **Fonseca-Madrigal J¹., Pineda-Delgado D¹., Martínez-Palacios C¹., Rodríguez C².,**
5 **Tocher D.R³.**

6

7 ¹ Laboratorio de Acuicultura, Instituto de Investigaciones Agropecuarias y Forestales,
8 UMSNH, Morelia, Michoacán, México.

9 ² Departamento de Biología Animal (U.D.I. Fisiología Animal), Facultad de Biología,
10 Universidad de La Laguna, La Laguna, Tenerife, España.

11 ³ Institute of Aquaculture, University of Stirling, U.K.

12

13 Corresponding author: Jorge Fonseca-Madrigal, Laboratorio de Acuicultura, Instituto
14 de Investigaciones Agropecuarias y Forestales, UMSNH. Av. San Juanito Itzícuarro
15 S/N, Col. San Juanito Itzícuarro, C.P. 58330, Morelia, Michoacán, México.

16 fonseca.madrigal@gmail.com

17

18

19 **Key Words:** *Chirostoma estor*, *Menidia estor*, long-chain polyunsaturated fatty acids,
20 biosynthesis, desaturation, elongation, salinity

21

22

22 **Abstract**

23

24 The genus *Chirostoma* (silversides) belongs to the family Atherinopsidae, which
25 contains around 150 species, most of which are marine. However, Mexican silverside
26 (*Chirostoma estor*) is one of the few representatives of freshwater atherinopsids and
27 is only found in some lakes of the Mexican Central Plateau. However, studies have
28 shown that *C. estor* has improved survival, growth and development when cultured in
29 water conditions with increased salinity. In addition, *C. estor* displays an unusual fatty
30 acid composition for a freshwater fish with high docosahexaenoic acid (DHA) :
31 eicosapentaenoic acid (EPA) ratios. Freshwater and marine fish species display very
32 different essential fatty acid metabolism and requirements and so the present study
33 investigated long-chain polyunsaturated fatty acid (LC-PUFA) biosynthesis to
34 determine the capacity of *C. estor* for endogenous production of EPA and DHA, and
35 the effect that salinity has on these pathways. Briefly, *C. estor* were maintained at
36 three salinities (0, 5 and 15 ppt) and the metabolism of ¹⁴C-labelled 18:3n-3
37 determined in isolated hepatocyte and enterocyte cells. The results showed that *C.*
38 *estor* has the capacity for endogenous biosynthesis of LC-PUFA from 18-carbon fatty
39 acid precursors, but that the pathway was essentially only active in saline conditions
40 with virtually no activity in cells isolated from fish grown in freshwater. The activity of
41 the LC-PUFA biosynthesis pathway was also higher in cells isolated from fish at 15
42 ppt compared to fish at 5 ppt, The pathway was around 5-fold higher in hepatocytes
43 compared to enterocytes, although the majority of 18:3n-3 was converted to 18:4n-3
44 and 20:4n-3 in hepatocytes whereas the proportions of 18:3n-3 converted to EPA
45 and DHA were higher in enterocytes. The data were consistent with the hypothesis

46 that conversion of EPA to DHA could contribute, at least in part, to the generally high
47 DHA:EPA ratios observed in the tissue lipids of *C. estor*.

48

48 **Introduction**

49

50 Mexican silverside (*Chirostoma estor*, also reported as *Menidia estor*) from lake
51 Pátzcuaro is one of the most valued freshwater fish in Mexico. The species, locally
52 known as “pez blanco”, has greatly influenced the cultural environment and economy
53 of the native people of the region. The species is now endangered because of a
54 range of factors including over-fishing, environmental degradation of the lake and
55 introduction of exotic species (Martínez-Palacios et al. 2008). However, silverside is
56 a species with high potential for aquaculture commanding a good price in regional
57 markets (\$40-80 USD kg⁻¹) (Martínez-Palacios et al. 2008). Recently, there have
58 been efforts to preserve the species through aquaculture techniques (Martínez-
59 Palacios et al. 2002, 2003, 2004, 2006, 2007). The genus *Chirostoma* (silversides)
60 belongs to the family Atherinopsidae, which contains around 150 species, most of
61 which are marine. However, *C. estor* is one of the few representatives of freshwater
62 atherinopsids and is only found in some freshwater lakes of the Mexican Central
63 Plateau. Therefore, although silverside is a freshwater species, it shares many
64 characteristics in common with marine Atherinopsids because of their common
65 ancestry (Barbour 1973). Thus, aquaculture of Mexican silverside *C. estor* involves
66 the transfer of the fish to different salinities over the whole cycle of production from
67 incubation of the eggs to juvenile development. Specifically, silverside has better
68 growth and survival when cultured in saline conditions (Martínez-Palacios et al.
69 2004).

70 . *C. estor* is also considered to be a carnivorous species and so their essential fatty
71 acid (EFA) requirements were expected to be more similar to that of a marine
72 carnivorous species than that of a typical freshwater fish (Martínez-Palacios et al.

73 2008). It has been reported that most freshwater fish studied, in contrast to the
74 marine species studied, have the ability to elongate and desaturate 18-carbon
75 polyunsaturated fatty acids (PUFA) (18:2n-6, linoleic acid/LOA and 18:3n-3 α -
76 linolenic acid/ALA) to long-chain PUFA (LC-PUFA) of 20 carbons (20:4n-6,
77 arachidonic acid/ARA and 20:5n-3, eicosapentanoic acid/EPA) and 22 carbons
78 (22:6n-3, docosahexaenoic acid/DHA) (Tocher 2010). Thus, it is generally assumed
79 that LOA and ALA can satisfy EFA requirements for freshwater species but ARA,
80 EPA and DHA are the required EFA for marine species (Sargent et al. 1995a).
81 However, the feeding habits of fish may also be determinants of precise EFA
82 requirements: carnivorous fish obtain the biologically active LC-PUFA directly from
83 their diet and consequently they now have only a low ability to desaturate and
84 elongate 18-carbon fatty acids whereas herbivorous fish have higher levels of C₁₈
85 PUFA and lower LC-PUFA in their diet and so have retained the ability to convert C₁₈
86 PUFA to LC-PUFA (Sargent et al. 1999). If the assertion that *C. estor* may have
87 characteristics of a marine species is correct, then ARA, EPA and DHA would need
88 to be included in the diet to satisfy their nutritional requirements.

89 There is scarce information of the lipid and fatty acid compositions and metabolism
90 in *C. estor* (Palacios et al. 2007). Wild fish contained high levels of DHA (20 - 32% of
91 total fatty acids) but surprisingly low levels of EPA (1 - 3%) in contrast with the fatty
92 acid profile found in samples of zooplankton, its natural diet (12% DHA, 13% EPA)
93 (Martínez-Palacios et al. 2003). There are two possible explanations for these
94 findings; firstly, that *C. estor* selectively accumulates DHA preferentially over other
95 fatty acids such as EPA depending on its own physiological requirements, or
96 secondly, that this species has the capacity to convert EPA and/or other n-3 series
97 fatty acids to DHA (Tocher 2003). The second explanation is also supported by the

98 presence of DHA in larvae fed rotifers with a low DHA / high ALA composition
99 (Martínez-Palacios et al. 2006).

100 Salinity has been shown to affect lipid and fatty acid composition in salmonids
101 although many effects occurred in advance of seawater transfer during parr-smolt
102 transformation (Bendiksen et al. 2003; Peng et al. 2003). It was also shown that the
103 activity of the LC-PUFA synthesis pathway was regulated by environmental cues in
104 Atlantic salmon (*Salmo salar*) and peaked around seawater transfer and was
105 considerably lower during the seawater phase (Bell et al. 1997; Tocher et al. 2000).
106 These changes in activity reflected changes in the expression of fatty acyl
107 desaturase genes in freshwater and seawater phases (Zheng et al. 2005). There are
108 also several studies reporting fatty acid compositions in fish reared at different
109 salinities (Cordier et al. 2002; Kheriji et al. 2003; Haliloglu et al. 2004; Martinez-
110 Alvarez et al 2005; Dantagnan et al. 2007; Navarro et al. 2009; Xu et al. 2010; Hunt
111 et al., 2011). Results are not consistent with increased associated with both reduced
112 (Cordier et al. 2002; Kheriji et al. 2003) and increased (Xu et al. 2010; Hunt et al.
113 2011) levels of LC-PUFA including EPA and DHA. In contrast, the effects of salinity
114 on lipid and fatty acid biochemistry and metabolism have been little studied in non-
115 salmonid fish although the modulation of $\Delta 6$ fatty acyl desaturase in teleosts was
116 recently reviewed, with the effects of salinity again being variable (Vagner and
117 Santigosa 2011). However, the expression of $\Delta 6$ desaturase was higher in liver of
118 rabbitfish (*Siganus canaliculatus*) and red sea bream (*Pagrus major*) reared at lower
119 salinity (10-15 ppt) compared to fish reared at higher salinity (32-33 ppt) (Li et al
120 2008; Sarker et al 2011).

121 The present study aims to investigate the two issues of salinity preference and
122 LC-PUFA metabolism in *C. estor* to determine if there is a relationship between them.

123 The basic hypothesis investigated was that LC-PUFA synthesis in *C. estor* will be
124 influenced by ambient salinity, and that the improved performance at increased
125 salinity will be related to this interaction between salinity and LC-PUFA biosynthesis.
126 The specific objectives were to determine the pathways and activities of LC-PUFA
127 biosynthesis in *C. estor* in order to elucidate potential mechanisms underpinning its
128 uncommon fatty acid profile and if the pathway reflects its marine ancestry, and to
129 determine if salinity affects the biosynthesis of LC-PUFA in a way that can explain
130 the apparent preference of *C. estor* for saline conditions (Martínez-Palacios et al.
131 2004).

132

133 **Materials and methods**

134 *Experimental fish*

135

136 Forty-five juvenile silverside (*Chirostoma estor*) of average initial weight around 50g
137 were obtained from a research production plant (UMSNH, Michoacan, Mexico). Fish
138 were maintained in glass-fiber tanks of 40cm high x 60cm diameter and 100L
139 capacity with constant aeration and temperature control ($25 \pm 0.4^\circ\text{C}$). All the
140 experimental units were maintained in a 12:12 dark:light photoperiod. All fish were
141 fed a standard commercial pellet feed (see Palacios et al. 2007), and every three
142 days tanks were siphoned and 30% of the water was renewed in order to maintain
143 high water quality with dissolved oxygen, nitrites, nitrates, pH, and total ammonia
144 monitored at 3-day intervals. The experimental design consisted of three salinity
145 treatments: freshwater (0 ppt; i.e. < 0.05), 5 ppt and 15 ppt of salinity, each in
146 triplicate with 5 fish per tank (15 per treatment). Different salinities were obtained by
147 using artificial seawater (Instant Ocean Synthetic sea salt, Aquarium Systems) and

148 UV filtered ground water. Fish were fed a diet consisting of *Artemia franciscana* and
149 a commercial feed (1:3) four times per day over 15 days prior to experimentation.

150

151 *Preparation of isolated hepatocytes and enterocytes*

152

153 With some modifications, the method for the preparation of isolated hepatocytes and
154 enterocytes established for salmonids was followed (Bell et al. 1997; Tocher et al.
155 1997, 2002). Briefly, six fish from each salinity treatment (two per tank) were
156 sacrificed with an overdose of benzocaine (50-60mg L⁻¹) to minimize stress (Ross et
157 al. 2007) and the livers and intestinal tracts dissected immediately. The livers and
158 intestines of two fish (i.e. per tank) were pooled for each sample so that there were 3
159 liver and 3 intestinal samples per treatment. The gall bladder was removed carefully
160 from the liver, the main blood vessels trimmed, and the liver perfused via the hepatic
161 vein with solution A (calcium and magnesium-free Hanks balanced salt solution
162 (HBSS) containing 10 mM HEPES) to clear blood from the tissue. The liver was
163 chopped finely and about 0.5 g was taken and incubated with shaking in 20 ml of
164 solution A containing 0.1% (w/v) collagenase in a temperature controlled incubator at
165 25 °C for 45 min. Digested liver tissue was filtered through 100 µm nylon gauze and
166 the cells collected by centrifugation at 300 x g for 2 min. The cell pellet was washed
167 with 20 ml of solution A containing 1% w/v fatty acid-free bovine serum albumin
168 (FAF-BSA) and re-centrifuged. The washing was repeated with a further 20 ml of
169 solution A without FAF-BSA. The hepatocytes were resuspended in 10 ml of Medium
170 199 containing 10 mM HEPES. One hundred µl of cell suspension was mixed with
171 400 µl of the vital stain, Trypan Blue, and hepatocytes counted and viability assessed
172 using a haemocytometer.

173 With relatively minor modification, the above method was used to isolate
174 enterocyte-enriched preparations from *C. estor* intestine as described previously for
175 caecal enterocytes from salmon (Fonseca-Madrigal et al. 2006). Briefly, entire
176 intestinal tracts were dissected, cleaned of adhering adipose tissue, and luminal
177 contents rinsed away with solution A before being chopped finely and incubated with
178 0.1% (w/v) collagenase as above. The digested intestinal tissue was filtered through
179 100 µm nylon gauze and the cells collected, washed, resuspended in medium (as
180 above), and viability checked as for hepatocytes. The enriched enterocyte
181 preparation was predominantly enterocytes although some secretory cells were also
182 present.

183 Viability of both isolated cell preparations was > 95% at isolation and decreased by
184 less than 5% over the period of the incubation. One hundred µl of the hepatocyte and
185 enterocyte suspensions were retained for protein determination according to the
186 method of Lowry et al. (1951) after incubation with 0.4 ml of 0.25% (w/v) SDS/1M
187 NaOH for 45 min at 60 °C.

188

189 *Assay of hepatocyte and enterocyte fatty acyl desaturation/elongation activities*

190

191 Six ml of each hepatocyte or enterocyte suspension were dispensed into 25 cm²
192 tissue culture flasks and incubated at 20°C for 2h with 0.3 µCi (~ 1 µM) [1-¹⁴C]18:3n-
193 3 or [1-¹⁴C]20:5n-3, added as complexes with FAF-BSA in phosphate buffered saline
194 as described previously (Ghioni et al. 1997). After incubation, the cell suspensions
195 were transferred to glass conical test tubes and centrifuged at 500 x g for 2 min. The
196 supernatants were discarded and the cell pellets washed with 5 ml of ice-cold
197 HBSS/FAF-BSA. The supernatant was carefully discarded and total lipid extracted

198 from the cell pellets using ice-cold chloroform/methanol (2:1, v/v) containing 0.01%
199 (w/v) BHT as described in detail previously (Tocher and Harvie 1988). Fatty acid
200 methyl esters (FAME) were prepared from total lipid by acid-catalyzed
201 transesterification using 2 ml of 1% H₂SO₄ in methanol plus 1 ml toluene as
202 described by Christie (1993), and FAME extracted and purified as described
203 previously (Tocher and Harvie 1988). The methyl esters were redissolved in 100 µl
204 isohexane containing 0.01% BHT and applied as 2.5 cm streaks to TLC plates
205 impregnated by spraying with 2 g silver nitrate in 20 ml acetonitrile and pre-activated
206 at 110°C for 30 min. Plates were fully developed in toluene/acetonitrile (95:5, v/v)
207 (Wilson and Sargent 1992) and autoradiography performed with Kodak MR2 film for
208 6 days at room temperature. Areas of silica containing individual PUFA were scraped
209 into scintillation mini-vials containing 2.5 ml of scintillation fluid (Ultima Gold, Perkin
210 Elmer, Monterrey, Mexico) and radioactivity determined in a scintillation β-counter
211 (Beckman LS Analyzer, Beckman Coulter de Mexico SA, Mexico City).

212

213 *Statistical analysis*

214

215 All the data are presented as means ± SD (n = 3) and all statistical analyses were
216 performed using S-Plus 2000 Professional Release 2 (MathSoft, Inc., Cambridge,
217 MA, USA). The effects of salinity on LC-PUFA synthesis was analyzed by one-way
218 ANOVA followed, where appropriate, by Tukey's post-test to determine significant
219 differences between individual treatments (Zar 1999).

220

221 *Materials*

222 [1-¹⁴C]18:3n-3 and [1-¹⁴C]20:5n-3 (50-55 mCi/mmol) were obtained from American
223 Radiolabeled Chemicals Inc. (St. Louis, MO, USA). HBSS, Medium 199, HEPES
224 buffer, collagenase (type IV), FAF-BSA, BHT, silver nitrate and all solvents (HPLC
225 grade) were obtained from Sigma Chemical Co. (St. Louis, MO, USA.). Thin-layer
226 chromatography (TLC) plates, precoated with silica gel 60 (without fluorescent
227 indicator) were obtained from Merck (Whitehouse Station, NJ, USA).

228

229 **Results**

230

231 *Desaturation and elongation of ALA, [1-¹⁴C]18:3n-3*

232

233 Irrespective of tissue, activity of the LC-PUFA synthesis pathway from 18:3n-3 was
234 very low in fish maintained in freshwater. Increasing salinity resulted in significantly
235 increased LC-PUFA synthesis in both hepatocytes and enterocytes as measured by
236 the recovery of radioactivity in the summed desaturated products (18:4, 20:4, 20:5,
237 22:5 and 22:6) of [1-¹⁴C]18:3n-3 (Fig.1). In both cell types, the rate of LC-PUFA
238 synthesis was highest in fish cultured at 15 ppt salinity, with rates of 0.41 ± 0.10 and
239 0.09 ± 0.04 pmol/h/mg protein in hepatocytes and enterocytes, respectively. These
240 values were 50- and 5-fold higher in hepatocytes and enterocytes, respectively, than
241 the activity observed in fish in freshwater. In both tissues, LC-PUFA synthesis at the
242 5 ppt salinity was intermediate between the activities in freshwater and 15 ppt salinity
243 with values of 0.13 ± 0.01 and 0.06 ± 0.01 pmol/h/mg protein in hepatocytes and
244 enterocytes, respectively. The LC-PUFA synthesis activity was 2.3- and 4.6-fold
245 higher in hepatocytes than in enterocytes at 5 and 15 ppt, respectively (Fig.1). The
246 rank order for recovery of radioactivity in desaturated products of 18:3n-3 was 18:4

247 >20:4 >22:6 >22:5 > 20:5 in hepatocytes (Fig. 2) whereas in enterocytes it was 20:4
248 >20:5 > 22:6 > 22:5 > 18:4 (Fig 3). In hepatocytes, recovery of radioactivity in DHA
249 exceeded that recovered in EPA, with the recovery of radioactivity in EPA and DHA
250 combined amounting to around 25% of the total radioactivity recovered (Fig. 2). In
251 contrast around 50% of total radioactivity recovered in enterocytes was as EPA and
252 DHA combined. Furthermore, in enterocytes, the recovery of radioactivity in EPA
253 increased, and that in DHA decreased, with increasing salinity (Fig. 3).

254

255 *Desaturation and elongation of EPA, [1-¹⁴C]20:5n-3*

256

257 As with [1-¹⁴C]18:3n-3, desaturation/elongation activity towards EPA in tissues from
258 fish maintained in freshwater was very low and increasing salinity significantly
259 increased desaturation/elongation activity in both hepatocytes and enterocytes as
260 measured by the recovery of radioactivity in the summed products (22:5 and 22:6) of
261 [1-¹⁴C]20:5n-3 metabolism (Fig. 4). However, in contrast to LC-PUFA synthesis from
262 [1-¹⁴C]18:3n-3, the activity in hepatocytes was similar in fish at both 5 and 15 ppt
263 salinity with values of 0.37 ± 0.16 and 0.36 ± 0.15 pmol/h/mg protein, respectively. In
264 enterocytes, highest activity was obtained in fish at 5 ppt salinity, with a value of 0.13
265 ± 0.01 pmol/h/mg protein compared to 0.05 ± 0.02 in fish reared at 15 ppt (Fig. 4).
266 Similar to the data obtained with [1-¹⁴C]18:3n-3, the LC-PUFA synthesis activity from
267 [1-¹⁴C]20:5n-3 was 2.9- and 7.0-fold higher in hepatocytes than in enterocytes from
268 fish reared at 5 ppt and 15 ppt, respectively (Fig.4). There was also a significant
269 difference in the products of [1-¹⁴C]20:5n-3 metabolism between hepatocytes and
270 enterocytes irrespective of treatment. The rank order for recovery of radioactivity in
271 products of 20:5n-3 metabolism was 22:6 >22:5 in hepatocytes, with the recovery of

272 radioactivity in DHA increasing with salinity with percentages of 57%, 65% and 78%
273 at 0, 5 and 15 ppt, respectively (Fig. 5). In enterocytes, the recovery of radioactivity in
274 22:5 exceeded the recovery in 22:6, with approximately 25%, 10% and 37% of
275 radioactivity recovered in DHA at 0, 5 and 15 ppt salinity, respectively (Fig 6).

276

277 **Discussion**

278

279 The primary objectives of the present work were to establish the extent and activity of
280 the LC-PUFA synthesis pathway in enterocytes and hepatocytes of *C. estor* and,
281 furthermore, to determine whether these activities were influenced by salinity. The
282 results demonstrated that both hepatocytes and enterocytes of *C. estor* displayed
283 physiologically relevant activities of LC-PUFA synthesis from ALA, particularly in
284 saline conditions. Thus, the values in hepatocytes and enterocytes from *C. estor* at
285 15 ppt salinity (0.41 and 0.09 pmol/h/mg protein, respectively) were lower than those
286 obtained in Atlantic salmon hepatocytes and enterocytes (0.9 and 1.2 pmol.h/mg
287 protein, respectively) (Zheng et al 2005), but higher than those obtained in
288 hepatocytes from the marine teleost Atlantic cod (*Gadus morhua*) (0.02 pmol/h/mg
289 protein) and similar to values from cod enterocytes (0.15 pmol/h/mg protein) (Tocher
290 et al. 2006). In addition, interest in the LC-PUFA synthesis pathway in *C. estor* is
291 partly due in its tissue fatty acid composition that, unusually for a freshwater species,
292 shows a very high DHA:EPA ratio (Martínez-Palacios et al. 2006). In marine and
293 freshwater fish tissue DHA:EPA ratios are most commonly in the range of 1:1 to 2:1
294 although ratios lower than this are also found in some species, particularly in
295 Southern oceans (Sargent et al. 1989). In contrast, *C. estor* has a fatty acid profile
296 with a DHA:EPA ratio that can vary from 10:1 to 20:1 (Martínez-Palacios et al. 2006).

297 This is generally unusual, even in marine fish, and very uncommon in freshwater
298 species (Ackman 1980). A few marine species show high DHA:EPA ratios, most
299 notable tuna species that can display ratios between 4 and 11, depending upon
300 tissue and species (Tocher 2003). In tuna the high tissue DHA:EPA ratios appear to
301 be due to generally higher DHA levels combined with relatively low EPA levels
302 (Tocher 2003). However, in *C. estor*, it appears that the main cause of the high
303 DHA:EPA ratios is the latter factor, that is, relatively low EPA levels in tissues, rather
304 than exceptionally high DHA (Martínez-Palacios et al. 2006). The present study has
305 confirmed that enterocytes and, especially, hepatocytes of *C. estor* demonstrated
306 significant DHA synthesis from EPA, particularly in saline conditions. Therefore, the
307 data are consistent with the hypothesis that conversion of EPA to DHA, particularly in
308 the liver, but also in the intestine, could contribute, at least in part, to the generally
309 high DHA:EPA ratios observed in the tissue lipids of *C. estor* (Martínez-Palacios et
310 al. 2006).

311 Previously, intestine and pyloric caeca were shown to be tissue sites of substantial
312 LC-PUFA biosynthesis in salmonids (Atlantic salmon and trout) (Fonseca-Madriral et
313 al. 2005, 2006), and this is why the capacity of enterocytes in *C. estor* for LC-PUFA
314 production was also investigated in the present study. The present study has
315 demonstrated that intestine in *C. estor* had the capability for LC-PUFA biosynthesis
316 but at significantly lower level than liver. The lower capacity of intestine for LC-PUFA
317 biosynthesis in *C. estor* in comparison to salmonids could be related to the feeding
318 habits of the species as *C. estor* is a zooplanktivorous fish with a short intestinal tract
319 and agastric digestive system (1:0.7 size of fish:size of intestine) with no pyloric
320 caeca (Martínez-Palacios et al. 2006). This is, of course, completely different to the
321 digestive tract of carnivorous species such as salmonids, which have a considerably

322 longer digestive system including a stomach and multiple caeca (Olsen and Ringø
323 1997). The results therefore suggest that the enterocytes in the much smaller
324 intestinal tract in *C. estor* and planktonivorous fish in general may not express the
325 same range of activities for processing absorbed nutrients as carnivorous fish
326 species, and may be focused more on the digestive and absorption roles. For
327 example, the activity of the LC-PUFA biosynthesis pathway is an order of magnitude
328 lower in enterocytes from *C. estor* compared to enterocytes from Atlantic salmon
329 (Zheng et al. 2005). Hepatocytes from *C. estor* showed much higher LC-PUFA
330 synthesis activity, which is expected due to the liver generally being the most
331 important organ in fatty acid and lipid metabolism in most fish species (Henderson
332 1996; Grum et al. 2002; Tocher 2003; Fonseca-Madrigal et al. 2005, 2006).

333 Studies on the development of *C. estor* aquaculture showed that this species
334 displays improved survival, growth and development when cultured in water
335 conditions with increased salinity (Martinez-Palacios et al. 2004). Generally, egg
336 fertilization and incubation as well as many physiological processes including lipid
337 metabolism are dependent on, or influenced by, salinity (Bœuf and Payan 2001). For
338 example, changes in the fatty acid composition of tissue lipids associated with
339 changes in salinity have been reported previously in a number of fish species
340 including guppy (*Poecilia reticulata*) (Daikoku et al. 1982), milkfish (*Chanos chanos*)
341 (Borlogan and Benítez 1992) and turbot (*Psetta maxima*) (Tocher et al. 1994, 1995).

342 These adaptations in response to salinity include altered proportions of total
343 phospholipids and individual phospholipid classes, as well as changes in fatty acid
344 composition including levels of LC-PUFA and n-3/n-6 PUFA ratio. However, the data
345 are variable depending upon species and whether low or high salinity is the actual
346 challenge for that species. For instance, in marine fish, reduced salinity increased

347 percentages of DHA and ARA in mullet (*Mugil cephalus*) (Kheriji et al. 2003), but
348 reduced proportions of EPA and DHA in Japanese sea bass (*Lateolabrax japonicus*)
349 and European sea bass (*Dicentrarchus labrax*) (Xu et al. 2010; Hunt et al. 2011).

350 In the present study, there was a clear relationship between the salinity of the water
351 the fish were maintained and synthesis of LC-PUFA, independent of cell type, with
352 higher LC-PUFA synthesis activity in cells of fish cultured in water with higher salinity
353 compared to fish cultured in freshwater. However, it is perhaps more appropriate and
354 noteworthy to highlight the fact that the activity of the LC-PUFA synthesis pathway
355 was very low in freshwater. This was actually the most unusual feature of the
356 pathway in *C. estor*, rather the activities observed at higher salinity. Clearly, there
357 was very little activity in freshwater and this was largely unprecedented as all the
358 freshwater fish species examined to date have generally shown appreciable LC-
359 PUFA synthesis activity (Tocher 2010), such that 18:3n-3 and/or 18:2n-6 can satisfy
360 their essential fatty acid requirements (NRC 2011).

361 The adaptation processes in response to a saline environment are primarily a series
362 of physiological changes involved osmoregulation, the regulation of ion balances
363 between the external medium and the corporal fluids (Morgan 1997; Laiz-Carrión et
364 al. 2004). Many of adaptations depend upon membrane processes and so changes
365 in lipid and, especially, fatty acid metabolism can be linked to the capacity of the fish
366 to adapt to salinity through changes in lipid and fatty acid compositions of
367 membranes that, in turn, affect membrane-associated proteins (receptors, enzymes
368 etc). Therefore, the influence of salinity on LC-PUFA production may be related to
369 the osmoregulatory response required for adaptation to higher salinity. The effects of
370 salinity on fatty acid compositions have been investigated (Tocher et al. 1994, 1995)
371 and the effects of salinity on LC-PUFA synthesis in hepatocytes have been indirectly

372 investigated in studies on the process of smoltification in Atlantic salmon (Bell et al.
373 1997; Tocher et al. 2000, 2002). In a trial investigating LC-PUFA synthesis in both
374 hepatocytes and enterocytes in farmed salmon, a peak of LC-PUFA production
375 occurred around the time the fish were transferred from freshwater to seawater, with
376 synthetic activity declining rapidly in the seawater phase to minimum levels (Tocher
377 et al. 2002). Although the effects of salinity on lipid and fatty acid biochemistry and
378 metabolism have been little studied in non-salmonid fish, the expression of $\Delta 6$ fatty
379 acyl desaturase in liver of the marine teleosts, rabbitfish and red sea bream, was
380 higher in fish maintained at low salinity compared to fish reared at high salinity (Li et
381 al 2008; Sarker et al 2011). This association between salinity and LC-PUFA
382 biosynthesis observed in fish was one of the factors underpinning the hypothesis
383 tested in the present study and the specific objectives were developed in this context.
384 However, the precise links between salinity changes, and LC-PUFA synthesis fatty
385 acid composition in fish including *C. estor* require further investigation.

386 Irrespective of the precise mechanistic links, the results presented, showing very low
387 levels of activity in fish reared in freshwater and increased capacity for LC-PUFA
388 synthesis essentially in hepatocytes as salinity increased may be related with the fact
389 that this species displays better growth performance and development when cultured
390 in saline water. At the most simplistic level, increased capability for endogenous
391 synthesis of the biologically and physiologically essential LC-PUFA would be
392 potentially beneficial to the fish in comparison to the situation in freshwater where the
393 pathways appear almost totally suppressed. Therefore, it is tempting to speculate
394 that the differing activity of the LC-PUFA synthesis pathway is an underpinning factor
395 on the effect of salinity on growth performance of *C. estor*. However, it is not so clear
396 how the effect of salinity on the activity of LC-PUFA synthesis pathway in *C. estor*

397 relates to current knowledge of LC-PUFA and environmental salinity or to discuss of
398 the possible marine origin of this species. Marine species generally have a reduced
399 ability to produce LC-PUFA compared to freshwater species (Tocher 2010). This has
400 been explained as a possible evolutionary adaptation to the generally higher levels of
401 DHA in the marine environment (Sargent et al. 1995b), and so marine species have
402 had less evolutionary pressure to retain the ability to endogenously produce LC-
403 PUFA; in contrast, freshwater food webs are generally characterized by lower levels
404 of DHA (Sargent et al. 1995b) and so evolutionary pressure for endogenous
405 production of LC-PUFA has been retained in freshwater species (Tocher 2010).
406 Therefore, this hypothesis would suggest it would be more advantageous for *C.*
407 *estor* to have higher LC-PUFA biosynthesis in freshwater where the supply of EPA
408 and, especially, DHA would likely be lower.

409 Although the data obtained to date with over 30 species, generally support this
410 hypothesis linking LC-PUFA levels and, especially, DHA levels in the different food
411 webs to evolutionary pressure for endogenous production of LC-PUFA, there are
412 several potential confounding factors including precise feeding habit of different
413 species (herbivorous vs. carnivorous/piscivorous) as well as phylogenetic issues. For
414 instance, defining fish species simply as marine or freshwater is often not ideal
415 considering the large number of euryhaline and diadromous species. Furthermore,
416 the effect of feeding habit can also be generalized with the ability for endogenously
417 LC-PUFA biosynthesis being retained in herbivorous fish, but not in omnivorous,
418 carnivorous, or piscivorous fish. As alluded to above, most fish species studied to
419 date could fit either of these generalizations (environment or feeding habit). However,
420 recent studies have contributed directly to this debate. A feeding study with rabbitfish,
421 *Siganus canaliculatus*, which consumes benthic algae and seagrasses and is thus a

422 rare example of a herbivorous marine fish, suggested that it was able to
423 biosynthesize EPA and DHA (Li et al. 2008). Very recently, it was shown that
424 rabbitfish possess all the fatty acyl desaturase activities required for endogenous
425 synthesis of LC-PUFA (Li et al. 2010). These data suggest that trophic level and/or
426 feeding habit are indeed important factors associated with or determining a species'
427 ability for endogenous LC-PUFA synthesis.

428 The genus *Chirostoma* (silversides also known as *Menidia*) belongs to the family
429 Atherinopsidae, which contains around 150 species, most of which are marine. Thus,
430 *C. estor* is among the few representatives of totally freshwater atherinopsids and,
431 although it is only found in some lakes of the Mexican Central Plateau, it shares
432 common ancestry with marine Atherinopsids (Barbour 1973). However, the
433 evolutionary pathway for *C. estor* is not entirely clear (Barbour 1973), although
434 relationships determined by classical phylogeny can give clues to evolutionary history
435 (Nelson 2006). Significant advances in determining the molecular mechanisms of LC-
436 PUFA biosynthesis in fish have been made in the last decade with the cloning and
437 functional characterization of fatty acyl desaturases and elongases from many fish
438 including freshwater, diadromous and marine species (Tocher 2010). Phylogenetic
439 analyses of the desaturase and elongase sequences have revealed some insights
440 into the possible evolutionary history of LC-PUFA biosynthesis in fish species
441 (Hastings et al. 2001; Zheng et al. 2004, 2009; Morais et al. 2009). The phylogenetic
442 sequence analysis generally reflected classical phylogeny, and grouped fish
443 desaturases in three distinct clusters (Leaver et al 2008). The Ostariophysii (common
444 carp and zebrafish), the Salmoniformes (trout and salmon), and the Acanthopterygia
445 (tilapia, sea bream, turbot, stickleback and medaka), with the cod
446 (Paracanthopterygii) branching from the Acanthopterygia line. However, many

447 questions still remain (Leaver et al. 2008; Li et al. 2010; Monroig et al. 2010) and *C.*
448 *estor* represent an interesting species to study in this respect. The interesting
449 ancestry, pattern of LC-PUFA biosynthesis activity and the effects of salinity, which
450 conflicts with the existing paradigm, make *C. estor* a choice candidate for molecular
451 studies with the isolation, cloning and characterization of fatty acyl desaturases and
452 elongases being important goals for future studies.

453 The results of the present study have provided data that contribute to our
454 understanding of the unusual fatty acid profile found in tissues of *C. estor* indicating
455 that it could be explained, at least partly, by endogenous metabolic activity resulting
456 in elongation and desaturation of EPA to DHA. Moreover, with respect to the well-
457 known beneficial effect of n-3 LC-PUFA on human health, it is noteworthy that the
458 results demonstrate that the increased ambient salinity used as part of the
459 management of this species in aquaculture farming should positively affect the
460 nutritional quality of the flesh in terms of fatty acid composition. However, a complete
461 understanding of fatty acid metabolism in *C. estor* requires further more extensive
462 analysis to determine the potential roles of selective β -oxidation, acylation and
463 incorporation of fatty acids into lipid classes, and lipid and fatty acid transport
464 between tissues.

465

466 **Acknowledgements**

467

468 Dr. Jorge Fonseca-Madrugal was supported by CONACyT, Mexico, with the research
469 grant #61117. Dr. Rodríguez was supported by project CTM2006-14279-C02-02-
470 MAR from Spanish MEC.

471

472

473 **References**

474

475 Ackman RG (1980) Fish lipids, part 1. In: Connell JJ (ed) *Advances in Fish Science*
476 *and Technology*. Fishing News Books, Farnham, pp 87-103

477 Barbour CD (1973) The systematics and evolution of the genus *Chirostoma*
478 Swainson (Pisces: Atherinidae). *Tulane Stud Zool Bot* 18:97-141

479 Bell JG, Tocher DR, Farndale BM, Cox DI, McKinney RW, Sargent JR (1997) The
480 effect of dietary lipid on polyunsaturated fatty acid metabolism in Atlantic salmon
481 (*Salmo salar*) undergoing parr-smolt transformation. *Lipids* 32:515-525

482 Bendiksen EA, Arnesen AM, Jobling M (2003) Effects of dietary fatty acid profile and
483 fat content on smolting and seawater performance in Atlantic salmon (*Salmo*
484 *salar* L.) *Aquaculture* 225:149-163

485 Bœuf G, Payan P (2001) How should salinity influences growth? *Comp Biochem*
486 *Physiol* 130C:411-423

487 Borlongan IG, Benitez LV (1992) Lipid and fatty acid composition of milkfish (*Chanos*
488 *chanos* Forsskal) grown in fresh water and seawater. *Aquaculture* 104:79-89

489 Christie WW (1993) Preparation of derivatives of fatty acids for chromatographic
490 analysis. In: Christie WW (ed) *Advances in Lipid Methodology – Two*. The Oily
491 Press, Dundee, pp 69-111

492 Cordier M, Brichon G, Weber J-M, Zwingelstein G (2002) Changes in fatty acid
493 composition of phospholipids in tissues of farmed sea bass (*Dicentrarchus*
494 *labrax*) during an annual cycle. Roles of environmental temperature and salinity.
495 *Comp Biochem Physiol* 133B:281-288

496 Daikoku T, Yano I, Masui M (1982) Lipid and fatty acid compositions and their
497 changes in the different organs and tissues of guppy, *Poecilia reticulata* on sea
498 water adaptation. *Comp Biochem Physiol* 73A:167-174

499 Dantagnan H, Borquez AS, Valdebenito IN, Salgado IA, Serrano EA, Izquierdo MS
500 (2007) Lipid and fatty acid composition during embryo and larval development of
501 puye *Galaxias maculatus* Jenyns, 1842, obtained from estuarine, freshwater and
502 cultured populations. *J Fish Biol* 70:770-781

503 Fonseca-Madrigal J, Bell JG, Tocher DR (2006) Nutritional and environmental
504 regulation of the synthesis of highly unsaturated fatty acids and fatty-acid
505 oxidation in Atlantic salmon (*Salmo salar* L.) enterocytes and hepatocytes. *Fish*
506 *Physiol Biochem* 32:317-328

507 Fonseca-Madrigal J, Karalazos PJ, Campbell PJ, Bell JG, Tocher DR (2005)
508 Influence of dietary palm oil on growth, tissue fatty acid compositions, and fatty
509 acid metabolism in liver and intestine in rainbow trout (*Oncorhynchus mykiss*).
510 *Aquacult Nutr* 11:241-250

511 Ghioni C, Tocher DR, Sargent JR (1997) The effect of culture on morphology, lipid
512 and fatty acid composition, and polyunsaturated fatty acid metabolism of
513 rainbow trout (*Oncorhynchus mykiss*) skin cells. *Fish Physiol. Biochem.*
514 16:499-513.

515 Grum DE, Drackley JK, Clark JH (2002) Fatty acid metabolism in liver of dairy cows
516 fed supplemental fat and nicotinic acid during an entire lactation. *J Dairy Sci*
517 85:3026-3034

518 Haliloglu HI, Bayir A, Sirkecioglu AN, Aras NM, Atamanalp M (2004) Comparison of
519 fatty acid composition in some tissues of rainbow trout (*Oncorhynchus mykiss*)
520 living in seawater and freshwater. *Fd Chem* 86:55-59

521 Hastings N, Agaba M, Tocher DR, Leaver MJ, Dick JR, Sargent JR, Teale AJ (2001)
522 A vertebrate fatty acid desaturase with $\Delta 6$ and $\Delta 5$ activities. Proc Natl Acad Sci
523 USA 98:14304–14309

524 Henderson RJ (1996) Fatty acid metabolism in freshwater fish with particular reference
525 to polyunsaturated fatty acids. Arch Anim Nutr 49:5-22

526 Hunt AO, Oxkan FE, Engin K, Tekelioglu N (2011) The effects of freshwater rearing
527 on the whole body and muscle tissue fatty acid profile of the European sea bass
528 (*Dicentrarchus labrax*). Aquacult. Internat 19:51-61

529 Kheriji S, El Cafsi M, Masmoudi W, Castell JD, Romdhane MS (2003) Salinity and
530 temperature effects on the lipid composition of mullet sea fry (*Mugil cephalus*,
531 Linne, 1758). Aquacult Internat 11:571-582

532 Laiz-Carrión R, Sangiao-Alvarellos S, Guzmán JM, Martín del Río MP, Míguez
533 JM, Soengas JL, Mancera JM (2004) Energy metabolism in fish tissues related
534 to osmoregulation and cortisol action. Fish Physiol Biochem 27:179-188

535 Leaver MJ, Bautista JM, Björnsson T, Jönsson E. Krey G, Tocher DR, Torstensen
536 BE (2008) Towards fish lipid nutrigenomics: current state and prospects for fin-
537 fish aquaculture. Rev Fisheries Sci 16(S1):71-92.

538 Li Y, Hu C, Zheng Y, Xia X, Xu W, Wang S, Chen W, Sun Z, Huang J (2008) The
539 effects of dietary fatty acids on liver fatty acid composition and delta 6-
540 desaturase expression differ with ambient salinities in *Siganus canaliculatus*.
541 Comp Biochem Physiol 151B:183–190

542 Li Y, Monroig Ó, Zhang L, Wang S, Zheng X, Dick JR, You C, Tocher DR (2010)
543 Vertebrate fatty acyl desaturase with $\Delta 4$ activity. Proc Natl Acad Sci USA
544 107:16840-16845

545 Lowry OH, Rosebrough NJ, Farr AL, Randall RJ (1951) Protein measurement with
546 the folin phenol reagent. J Biol Chem 193:265-275

547 Martínez-Alvarez RM, Sanz A, Gracia-Gallego M, Domezain A, Domezain J,
548 Carmona R, de ValleOstos-Garrido M, Morales AE (2005) Adaptive branchial
549 mechanisms in the sturgeon *Acipenser naccarii* during acclimation to saltwater.
550 Comp Biochem Physiol 141A:183-190

551 Martínez-Palacios CA, Ambriz-Cervantes L, Ríos-Duran MG, Jauncey KJ, Ross LG
552 (2007) Dietary protein requirement of juvenile Mexican Silverside (*Chirostoma*
553 *estor estor* Jordan 1879), a stomachless zooplanktophagous fish. Aquaculture
554 Nutr 13304-310

555 Martínez-Palacios CA, Barriga-Tovar E, Taylor JF, Ríos-Duran MG, Ross LG (2002)
556 Effect of temperature on growth and survival of *Chirostoma estor estor*, Jordan
557 1879, monitored using a simple video technique for remote measurement of
558 length and mass of larval and juvenile fishes. Aquaculture 209:369-377

559 Martínez-Palacios CA, Comas Morte J, Tello-Ballinas JA, Toledo-Cuevas M, Ross
560 LG 2004. The effects of saline environments on survival and growth of eggs and
561 larvae of *Chirostoma estor estor* Jordan 1879 (Pisces: Atherinidae). Aquaculture
562 238:509-522

563 Martínez-Palacios CA, Racotta IS, Ríos-Duran MG, Palacios E, Toledo-Cuevas M,
564 Ross LG (2006) Advances in applied research for the culture of Mexican
565 silversides (*Chirostoma*, Atherinopsidae). BIOCELL 30:137-148

566 Martínez-Palacios CA, Ríos-Duran MG, Campos-Mendoza A, Toledo-Cuevas M,
567 Aguilar-Valdez MA, Ross LG (2003) "Desarrollo tecnológico alcanzado en el
568 cultivo del pez blanco de Pátzcuaro" In:"Historia y avances del cultivo del

569 pescado blanco” Instituto Nacional de la Pesca. Secretaría de Agricultura,
570 Ganadería, Desarrollo Rural, Pesca y Alimentación. México.

571 Martínez-Palacios CA, Ríos-Duran MG, Fonseca-Madrigal J, Toledo-Cuevas M,
572 Sotelo Lopez A, Ross LG (2008) Developments in the nutrition of *Menidia estor*
573 Jordan 1880. *Aquacult Res* 39:738-747

574 Monroig Ó, Zheng X, Morais S, Leaver MJ, Taggart JB, Tocher DR (2010) Multiple
575 fatty acyl desaturase (FAD) genes in Atlantic salmon: Cloning and functional
576 expression of cDNAs confirm presence of three $\Delta 6$ FADs. *Biochim Biophys*
577 *Acta* 1801:1072-1081

578 Morais S, Monroig O, Zheng X, Leaver MJ, Tocher DR (2009) Highly unsaturated
579 fatty acid synthesis in Atlantic salmon: Isolation of genes of fatty acyl elongases
580 and characterisation of ELOVL5- and ELOVL2-like elongase cDNAs. *Mar*
581 *Biotechnol* 11:627–639

582 Morgan JD (1997) Energetic aspects of osmoregulation in fish. Doctoral Thesis. The
583 University of British Columbia, Canada.

584 National Research Council (2011) Nutrient Requirements of Fish and Shrimp.
585 National Academic Press, Washington, DC.

586 Navarro JC, Perez MJ, Acosta NG, Viciano E, Varo I, Urena R, Torreblanca A,
587 Rodriguez C. (2009) The fatty acid profiles of polar and neutral lipids from
588 enterocytes and hepatocytes of gilthead seabream (*Sparus aurata*) cultured at
589 two salinities: A chemometric approach. *Comp Biochem Physiol* 154A:S20

590 Nelson JS (2006) *Fishes of the World*, 4th Edition. Wiley, New York.

591 Olsen RE, Ringø E (1997) Lipid digestibility in fish: A review. *Rec Res Dev Lipids*
592 *Res* 1:199–265.

593 Palacios E, Racotta IS, Apaicio B, Arjona O, Martínez-Palacios CA (2007) Lipid
594 classes and fatty acids during embryogenesis of captive and wild silverside
595 (*Chirostoma estor estor*) from Pátzcuaro Lake. Fish Physiol Biochem. 33:81-91.

596 Peng J, Larondelle Y, Pham D, Ackman RG, Rollin X (2003) Polyunsaturated fatty
597 acid profiles of whole body phospholipids and triacylglycerols in anadromous
598 and landlocked Atlantic salmon (*Salmo salar* L.) fry. Comp Biochem Physiol
599 134B:335-348

600 Ross LG, Sánchez-Blanco J, Martínez-Palacios CA, Racotta IS, Toledo-Cuevas M
601 (2007) Anesthesia, sedation and transportation of juvenile *Menidia estor*
602 (Jordan) using benzocaine and hypothermia. Aquacult Res 38:909-917

603 Sargent JR, Bell JG, Bell MV, Henderson RJ, Tocher DR (1995a) Requirement
604 criteria for essential fatty acids. J Appl Ichthyol 11:183-198

605 Sargent JR, Bell JG, Bell MV, Henderson RJ, Tocher DR (1995b) Dietary origins and
606 functions of long-chain (n-3) polyunsaturated fatty acids in marine fish. J Mar
607 Biotechnol 3:26-28

608 Sargent JR, Bell JG, McEvoy L, Tocher DR, Estevez A (1999) Recent developments
609 in the essential fatty acid nutrition of fish. Aquaculture 177:191-199.

610 Sargent JR, Henderson RJ, Tocher DR (1989) The lipids. In: Halver JE (ed) Fish
611 Nutrition, 2nd edn. Academic Press, San Diego, pp. 154-219

612 Sarkar MA, Yamamoto YH, YutakaSarker MSA, Miwa M, Yoshizaki G, Satoh S
613 (2011) Influences of low salinity and dietary fatty acids on fatty acid composition
614 and fatty acid desaturase and elongase expression in red sea bream *Pagrus*
615 *major*. Fisheries Sci. 77:385-396

616 Tocher DR (2003) Metabolism and functions of lipids and fatty acids in teleost fish.
617 Rev Fisheries Sci 11:107-184.

618 Tocher DR (2010) Fatty acid requirements in ontogeny of marine and freshwater fish.
619 Aquacult Res 41:717-732

620 Tocher DR, Harvie DG (1988) Fatty acid compositions of the major
621 phosphoglycerides from fish neural tissues; (n-3) and (n-6) polyunsaturated fatty
622 acids in rainbow trout (*Salmo gairdneri*) and cod (*Gadus morhua*) brains and
623 retinas. Fish Physiol Biochem 5:229-239

624 Tocher DR, Bell JG, Dick JR, Sargent JR (1997) Fatty acyl desaturation in isolated
625 hepatocytes from Atlantic salmon (*Salmo salar*): Simulation by dietary borage oil
626 containing γ -linolenic acid. Lipids 32:1237-1247

627 Tocher DR, Bell JG, Henderson RJ, McGhee F, Mitchell D, Morris PC (2000)
628 Polyunsaturated fatty acid metabolism in Atlantic salmon (*Salmo salar*)
629 undergoing parr-smolt transformation and the effects of dietary linseed and
630 rapeseed oils. Fish Physiol Biochem 23:59-73

631 Tocher DR, Castell JD, Dick JR, Sargent JR (1994) Effects of salinity on the growth
632 and lipid composition of Atlantic salmon (*Salmo salar*) and turbot
633 (*Scophthalmus maximus*) cells in culture. Fish Physiol Biochem 13:451-461

634 Tocher DR, Castell JD, Dick JR, Sargent JR (1995) Effects of salinity on the fatty acid
635 compositions of total lipid and individual glycerophospholipid classes of Atlantic
636 salmon (*Salmo salar*) and turbot (*Scophthalmus maximus*) cells in culture. Fish
637 Physiol Biochem 14:125-137

638 Tocher DR, Fonseca-Madrigal J, Bell JG, Dick JR Henderson RJ, Sargent, JR (2002)
639 Effects of diets containing linseed oil on fatty acid desaturation and oxidation in
640 hepatocytes and intestinal enterocytes in Atlantic salmon (*Salmo salar*). Fish
641 Physiol Biochem 26:157-170

642 Tocher DR, Zheng X, Schlechtriem C, Hastings N, Dick JR, Teale AJ (2006) Highly
643 unsaturated fatty acid synthesis in marine fish: Cloning, functional
644 characterization, and nutritional regulation of fatty acyl Δ 6 desaturase of Atlantic
645 cod (*Gadus morhua* L.). *Lipids* 41:1003-1016

646 Vagner M, Santigosa E (2011) Characterization and modulation of gene expression
647 and enzymatic activity of Δ -6 desaturase in teleosts: A review *Aquaculture*
648 315:131-143

649 Wilson R, Sargent JR (1992) High resolution separation of polyunsaturated fatty
650 acids by argentation thin-layer chromatography. *J Chromatogr* 623:403-407

651 Xu J, Yan B, Teng Y, Lou G, Lu Z (2010) Analysis of nutrient composition and fatty
652 acid profiles of Japanese sea bass *Lateolabrax japonicus* (Cuvier) reared in
653 seawater and freshwater. *J Fd Comp Anal* 23:401-405

654 Zar JH (1999) *Biostatistical Analysis* 4th Edition; Prentice-Hall; Upper Saddle River,
655 New Jersey.

656 Zheng X, Seilliez I, Hastings N, Tocher DR, Panserat S, Dickson CA, Bergot P, Teale
657 AJ (2004) Characterization and comparison of fatty acyl Δ 6 desaturase cDNAs
658 from freshwater and marine teleost fish species. *Comp Biochem Physiol*
659 139B:269–279

660 Zheng X, Torstensen BE, Tocher DR, Dick JR, Henderson RJ, Bell JG (2005)
661 Environmental and dietary influences on highly unsaturated fatty acid
662 biosynthesis and expression of fatty acyl desaturase and elongase genes in
663 liver of Atlantic salmon (*Salmo salar*). *Biochim Biophys Acta* 1734:13–24

664 Zheng X, Ding Z, Xu Y, Monroig O, Morais S, Tocher DR 2009. Physiological roles of
665 fatty acyl desaturase and elongase in marine fish: Characterisation of cDNAs of

666 fatty acyl Δ 6 desaturase and Elovl5 elongase of cobia (*Rachycentron canadum*).

667 Aquaculture 290:122–131

668

669

669

670 Legends to Figures:

671

672 FIG. 1. Total LC-PUFA biosynthesis (desaturation/elongation) activity in hepatocytes
673 and enterocytes of *C. estor* cultured at different salinities. Results are means \pm S.D.
674 ($n = 3$) and represent the rate of conversion ($\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$) of $[1-^{14}\text{C}]18:3\text{n-3}$
675 to all desaturated products (sum of radioactivity recovered as 18:4n-3, 20:4n-3,
676 20:5n-3, 22:5n-3 and 22:6n-3). Columns assigned to a specific cell type with different
677 superscript letters are significantly different as determined by one-way ANOVA
678 followed by the Tukey post test ($P < 0.05$).

679

680 FIG. 2. Individual fatty acid products of the desaturation and elongation of $[1-$
681 $^{14}\text{C}]18:3\text{n-3}$ in *C. estor* hepatocytes. Results are means \pm S.D. ($n = 3$) and represent
682 the rate of production ($\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$) of individual fatty acids as determined
683 by the recovery of radioactivity in each fatty acid fraction. Columns referring to a
684 specific fatty acid having different superscript letters are significantly different as
685 determined by one-way ANOVA followed by the Tukey post test ($P < 0.05$).

686

687 FIG. 3. Individual fatty acid products of the desaturation and elongation of $[1-$
688 $^{14}\text{C}]18:3\text{n-3}$ in *C. estor* enterocytes. Results are means \pm S.D. ($n = 3$) and represent
689 the rate of production ($\text{pmol}\cdot\text{h}^{-1}\cdot\text{mg protein}^{-1}$) of individual fatty acids as determined
690 by the recovery of radioactivity in each fatty acid fraction. Columns referring to a
691 specific fatty acid having different superscript letters are significantly different as
692 determined by one-way ANOVA followed by the Tukey post test ($P < 0.05$).

693

694 FIG. 4. Production of desaturation/elongation products from labeled EPA in
695 hepatocytes and enterocytes of *C. estor* cultured at different salinities. Results are
696 means \pm S.D. (n= 3) and represent the rate of conversion ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of
697 $[1-^{14}\text{C}]$ 20:5n-3 to metabolised products (sum of radioactivity recovered as 22:5n-3
698 and 22:6n-3). Columns representing a specific cell type with different superscript
699 letters are significantly different as determined by one-way ANOVA followed by the
700 Tukey post test ($P < 0.05$).

701
702 FIG. 5. Individual fatty acid products of the desaturation and elongation of $[1-^{14}\text{C}]$
703 20:5n-3 in *C. estor* hepatocytes. Results are means \pm S.D. (n = 3) and represent the
704 rate of production ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of individual fatty acids as determined by
705 the recovery of radioactivity in each fatty acid fraction. Columns referring to a specific
706 fatty acid having different superscript letters are significantly different as determined
707 by one-way ANOVA followed by the Tukey post test ($P < 0.05$).

708
709 FIG. 6. Individual fatty acid products of the desaturation and elongation of $[1-^{14}\text{C}]$
710 20:5n-3 in *C. estor* enterocytes. Results are means \pm S.D. (n = 3) and represent the
711 rate of production ($\text{pmol.h}^{-1}.\text{mg protein}^{-1}$) of individual fatty acids as determined by
712 the recovery of radioactivity in each fatty acid fraction. Columns referring to a specific
713 fatty acid having different superscript letters are significantly different as determined
714 by one-way ANOVA followed by the Tukey post test ($P < 0.05$).

715

716











