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1 *Full Title:*

2 **Long chain polyunsaturated fatty acids (LC-PUFA) composition of fish sperm: nexus of dietary,**
3 **evolutionary, and biomechanical drivers**

4 *Running Title:*

5 **EcoEvoBio sperm**

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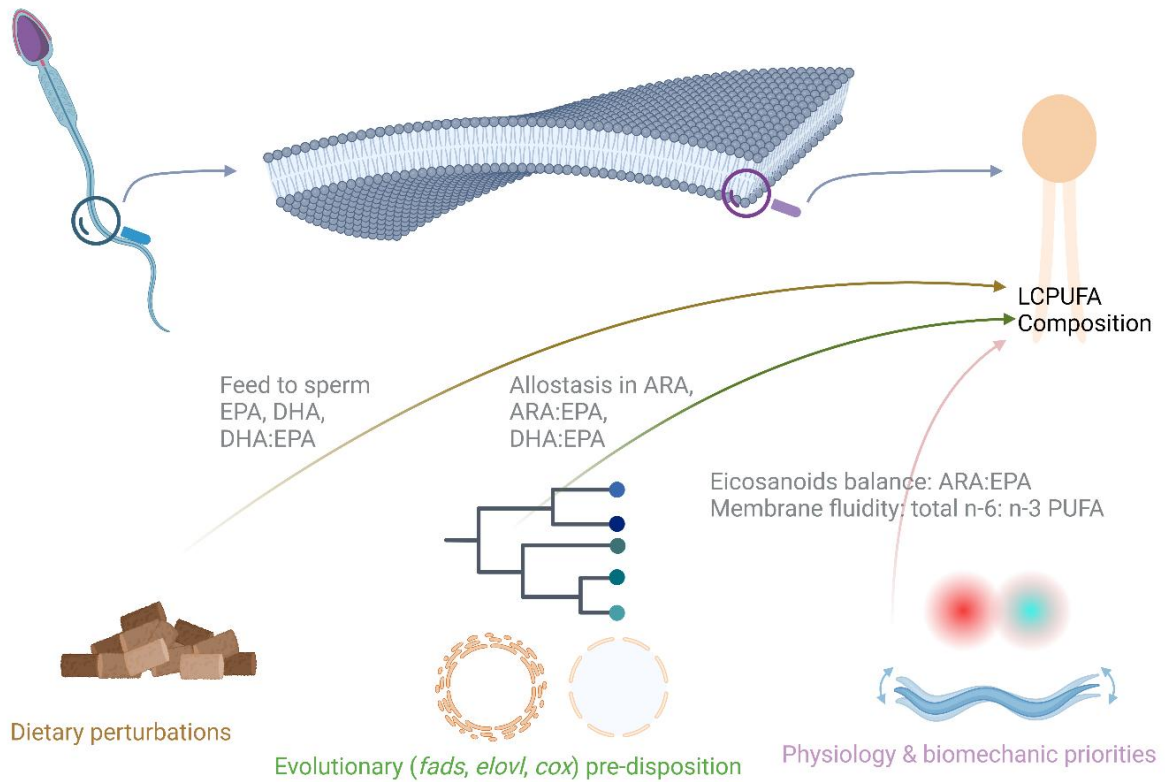
31

ABSTRACT

32 Long-chain polyunsaturated fatty acids (LC-PUFA) like arachidonic acid (ARA, 20:4n-6),
33 eicosapentaenoic acid (EPA, 20:5n-3), and docosahexaenoic acid (DHA, 22:6n-3) constitute one-third
34 to half of fish sperm lipids. Fish sperm is rich in phospholipid (PL) classes—primarily
35 phosphatidylcholine, phosphatidylethanolamine, and sphingomyelin. DHA is generally the most
36 abundant LC-PUFA in each PL class, followed by competition between ARA and EPA. While the
37 total n-6: n-3 PUFA ratio does not correlate significantly with sperm biomechanics, LC-PUFAs do.
38 DHA positively influences sperm biomechanics, while ARA and EPA may reduce motility duration
39 despite enhancing velocity. Fish sperm maintains a lower (≤ 1) total n-6 PUFA per unit of n-3 PUFA
40 but keep a higher (> 1) ARA per unit EPA. A weak dietary influence on sperm EPA and DHA exists
41 but not on ARA. The DHA: EPA ratio in fish sperm is often > 1 , though values < 1 occur. Certain
42 species cannot fortify DHA sufficiently during spermatogenesis, particularly in lineages diverging
43 through whole genome duplications. Fish genomes from Devonian, Cretaceous, or Jurassic eras show
44 sperm ARA: EPA ratios ≥ 1 , while Paleozoic or Triassic lineages show ratios ≤ 1 , likely due to
45 evolutionary shifts in prostaglandin pathways. DHA-rich PL bilayers provide unique packing and
46 fusogenic properties, with ARA/EPA-derived eicosanoids guiding sperm rheotaxis/chemotaxis,
47 modulated by DHA-derived resolvins. Docosapentaenoic acid (DPA, 22:5n-3) sometimes substitutes
48 for DHA in fish sperm. While testes biosynthesize PL and LC-PUFA de novo, some features may
49 carry over to sperm. As diet impacts sperm, sometimes limited corrective capacity risks unintended
50 selection in hatchery-bred fish. Knowledge gaps are discussed.

51 **Keywords:** Fish Sperm; Lipidomics; Reproductive Fitness; Evolutionary Adaptations; Motility
52 Mechanisms; Aquaculture Animal Nutrition.

53 GRAPHICAL ABSTRACT



54

55 1. Introduction (fish and fish sperm)

56 In terms of fatty acids composition, fish are what they eat [1], even though, to some extent, they have
57 undergone natural evolution of their fatty acid metabolism to counteract deficiencies or imbalances of
58 fatty acids of physiological importance [2, 3]. There is ample literature available on fatty acid
59 composition and metabolism in freshwater and marine fish and their dietary requirements at different
60 ontogeny stages [4, 5]. Also, literature regarding differences in fatty acid composition, abundance,
61 and variable capacity to synthesize polyunsaturated fatty acids (PUFA) *de novo*, also referred to as
62 fatty acid bioconversion, among freshwater and marine fish is available. Evolution of desaturase and
63 elongase enzymes and a series of promoters regulating their gene expression have been widely studied
64 [1, 3, 6]. At the whole fish level, the broad fatty acid metabolism, including anabolic and catabolic
65 processes, bioconversion, and deposition, has been abundantly researched [4, 7-10]. Fatty acids, in
66 addition to being an important energy source, are also fundamental structural components of the cell
67 membranes and, as such, contribute to cell flexibility, membrane fusion capacities, membrane
68 functionality, and integrity, and for free moving cells like sperm, their swimming capabilities. Sperm
69 is designed in a way that accomplishes its sole function, which is to swim, reach, and diffuse part of
70 its membrane (fertilize) with the egg to transfer nuclear materials. PUFA is known to improve
71 motility kinetics, [membrane fusion](#), and fertilization capacity of fish sperm [11, 12]. Although the
72 understanding of fatty acid [stoichiometry](#) on human sperm quality has been reviewed [13-15], such
73 understanding on evolutionarily primitive fish sperm is scarce [16].

74 In fish sperm, most of the energy required to sustain motility could be derived from pre-stored ATP
75 during the quiescent state of the spermatozoa [17-19]. The importance of stored glycogen in fish
76 spermatozoa is still not adequately described, but glucose and tricarboxylic acids are [well established](#)
77 [energy substrates](#) in fish sperm [20-23]. [Triacylglycerol](#) catabolism or β -oxidation of fatty acids is
78 used almost exclusively for the regeneration of ATP levels after motility has ended or intra-cellular
79 glucose, glycogen, or tricarboxylic acid reserves are depleted [22]. [Among fatty acids](#), the shorter-
80 length SFA [are easiest metabolic fuels](#) [24]. Compared to saturated fatty acids (SFA), the
81 mitochondrial β -oxidation of monounsaturated fatty acids (MUFA) and polyunsaturated fatty acids
82 (PUFA) [require 1-2 extra set of enzymes](#) [24]. [For example](#), mitochondrial preparations from rainbow
83 trout red muscle show 14:0 or 16:0 fatty acids, having ~100% β -oxidation rate (relative to pyruvate),
84 whereas PUFA (18:2n-6, 18:3n-3, 22:6n-3) have only 18-58% β -oxidation rate [25]. In fish sperm [too](#),
85 shorter chain (≤ 16) saturated fatty acids (14:0, 16:0) or monounsaturated fatty acids (16:1) [is reported](#)
86 [to be depleted](#) as metabolic fuel in [common carp and Eurasian perch sperm](#), compared to other fatty
87 acids [19, 26]. Post-motility shifts in the ARA: EPA ratio (*i.e.*, a marker for eicosanoid actions) and
88 DHA: EPA ratio has suggested the potential utilization of EPA as eicosanoid precursors or prone to β -
89 oxidation in [Eurasian perch sperm](#) [19]. The β -oxidation contributes energy [24], while [eicosanoids](#)
90 [\(lipid-based signaling molecules\)](#) modulate self-defense, inflammatory/ anti-inflammatory responses,
91 chemotaxis/ [sperm rheotaxis](#), and fertilization [27]. [Eicosanoids may influence sperm motility and](#)
92 [thus fertilization event in humans as well](#). Such as the series-2 and 3 prostaglandins (PG) containing
93 two and three double bonds, produced from 20:4n-6 (ARA) and 20:5n-3 (EPA), respectively. For
94 example, PGF1 α binds with high affinity to the calcium channel of sperm (CatSper), in human sperm
95 important for motility [28, 29]. Within hermaphrodite body (*Caenorhabditis elegans*), sperm-guiding
96 PG have been reported to guide and help fertilize egg, present within the same body [30]. Despite
97 carp and trout sperm proving a 'guided behavior' under exposure to ovarian fluids [31, 32], the
98 externally fertilizing fish sperm's self-guiding PG synthesis capabilities (under lack of ovarian fluid
99 PG exposure [28]) is a black box. This is mainly due to a lack of inter-disciplinary exchanges between
100 lipidologist, nutritionists and sperm biologists. In this review, we have created such inter-disciplinary
101 exchange.

102 The long chain (≥ 20 carbon) polyunsaturated fatty acids (LC-PUFA) in human sperm, which has a
103 long motility duration from several hours to day(s), a paradox exists between LC-PUFA's energetic
104 use versus non-energetic use [13, 14, 33]. Meanwhile, LC-PUFA in shortly motile fish sperm (limited
105 to minutes or < 1 minute [34]) most likely serves non-energetic purposes, as time is limited for
106 catabolism of LC-PUFA. Non-energetic purposes of sperm LC-PUFA, such as bio-mechanical
107 (structural and fluidity) and signaling (eicosanoids), are equally crucial for fertilization. Elevated
108 glycolysis or rapid burst of energy does not always ensure fertilization by fish sperm. The highest
109 swimming velocities in a fish sperm can be associated with reduced motility duration and poor
110 fertilization rates. Rather, the intra-cellular or seminal fluid lipid levels are correlated with the
111 fertilization capacity [35]. Fish spermatozoa contain abundant lipids (e.g., $17 \pm 2\%$ of fresh sperm),
112 characterized by high levels of phospholipids (approximately 60% of total lipid). LC-PUFA such as
113 docosahexaenoic acid (22:6n-3, DHA) can make up to $1/3^{\text{rd}}$ of fish sperm lipid and, together with
114 arachidonic acid (20:4n-6, ARA) and eicosapentaenoic acid (20:5n-3, EPA) can exceed half of fish
115 sperm lipid [19]. Our current review focuses on these typical fatty acid compositions, which are
116 [essential for structural fluidity, membrane fusion and eicosanoid responses](#) during swimming in an
117 external environment (mostly). The present review helps us to understand this in greater detail across
118 dietary treatments and fish phylogeny. We targeted the three most abundant LC-PUFA in fish sperm
119 for data exploration: ARA, EPA, and DHA (*hereinafter mentioned as LC-PUFA*). We have also
120 [considered the total omega-6 series PUFA \(n-6 PUFA\) and total omega-3 series PUFA \(n-3 PUFA\)](#)
121 [ratio](#). Our scientific questions were to understand: (1) [correlation of sperm LC-PUFA with sperm](#)
122 [biomechanics](#); (2) [dependency of fish sperm LC-PUFA, in its entirety or stoichiometrically, on fatty](#)
123 [acid composition of food](#), or (3) [if some sperm LC-PUFA does not have much plasticity to change and](#)
124 [independent of dietary effects](#). There, we explored if sperm LC-PUFA may have an evolutionary or
125 [genetic background](#).

126 **2. Methods of literature review and synthesis**

127 **2.1. Data collection and arrangement**

128 For data analysis, the keywords (“fish” OR “freshwater” OR “marine” OR “teleost”) AND (“sperm”
129 OR “spermatozoa” OR “semen” OR “milt”) AND (“fatty acid” OR “PUFA biosynthesis” OR “fads”
130 OR “elovl”) AND (“food” OR “fish feed”) were searched. Web of Science (WoS), Scopus,
131 ScienceDirect, and Google Scholar online databases were used for the literature search. Only articles
132 published in peer-reviewed journals were included in the present study. A total of 34 currently
133 available research articles reporting fish sperm fatty [acid composition](#) were obtained.

134 The following standards were set to select only relevant articles for the present study: (1) The articles
135 presenting fatty [acid composition](#) of either whole semen (spermatozoa including seminal fluid) or
136 centrifuged pellet (spermatozoa excluding seminal fluid) were included. Fatty [acid composition](#) of
137 only seminal fluid (without spermatozoa) was not considered for analysis [11]. (2) The articles
138 presenting fatty [acid composition](#) at only matured stage of spermatozoa were considered, immature or
139 maturing spermatozoa fatty [acid profile](#) were not covered. The data on the fatty [acid composition](#) of
140 testicular, immature, maturing spermatogonia, and spermatids were not selected [36]. (3) Articles
141 representing fatty [acid composition](#) from whole spermatozoa were considered. For example, a study
142 reporting only the fatty [acid composition](#) within the phospholipid fraction of spermatozoa was
143 excluded [12]. (3) Data from articles presenting the fatty acid data expressed as ‘percentage of total
144 lipids’ were collected. Fatty acid quantification data from non-WoS and non-Scopus indexed journals
145 were excluded [37]. Finally, 24 peer-reviewed research articles (published in the year 1991 to 2023)
146 were selected, and authors unpublished data on three fish species (*Esox lucius*, *Oreochormis niloticus*,
147 and *Sander lucioperca*) were supplemented to strengthen the dataset. Among all the studies, sperm

148 fatty acids with a carbon chain from 8 to 24 were reported. The fatty acids were detected using gas
149 chromatography/gas-liquid chromatography in all the included studies. The data used were fatty acids
150 methyl esters (FAME) share over total FAME, as generated by gas chromatography techniques and
151 reported in the publications as fatty acid profile. Some correction factors were applied after checking
152 methodology of individual studies. Only one study [11] had applied theoretical correction factors
153 despite using a standard (following a recommended practice [38-40]); its data were taken as it was
154 reported. In other studies that did not apply theoretical correction, corrections were applied following
155 a common approach. We corrected the reported FAME profiles for the flame ionization detector (FID)
156 response factor and methyl transformation [39]. Theoretical response correction factors (RCF) for FID
157 to convert to weight percent methyl ester, and molecular weight correction factors (MWF) to convert
158 to molar percent for some common fatty acids were used. The correction factors RCF and MWF used
159 for conversion of FAME to fatty acid profile [39] are tabulated in supplementary table T1. The change
160 in values were minute (clarified in supplementary data). All the experimental treatments given in
161 various studies such as the fatty acid composition of (quiescent/post motile sperm/ after exposure to
162 different dietary compositions/ aquaculture grown or wild-caught fish sperm/ sperm collected at
163 different periods after hormonal stimulation/ fresh or cryopreserved sperm) were included in
164 preliminary data collection. Wherever data was available as mean and standard deviation (mean±SD),
165 the data was populated as ‘mean’, ‘mean+1SD’, and ‘mean-1SD’. A range covered by one standard
166 deviation above the mean and one standard deviation below it includes about 68% of the observations
167 [41], also used in quality control range calculation in pathological labs (example). Instead of relying
168 on a single value (mean) from published literature, it also accounts for variability and gives some
169 upper and lower limits near the central tendency of the data (see, “68–95–99.7 rule”). Thus, capturing
170 data as close as possible to reality.

171 Additional studies that investigated specifically the phospholipid (PL) fractions in fish sperm, and
172 fatty acid composition within different PL classes were reviewed too [12, 42-48]. Their data is
173 presented separately. From all studies, CASA (computer-assisted semen analyses) parameters such as
174 mean curvilinear velocity (VCL) within 0 to 20 seconds post-activation, and reported motility
175 duration (in seconds) were collected to parameterize the “biomechanics” of fish sperm.

176 2.2. Characterization of dataset

177 Among all the selected articles, the data was extracted from 11 freshwater and four marine fish
178 species. Studied freshwater fish species were: hilsa shad (*Tenualosa ilisha*) 1x [49], sterlet (*Acipenser*
179 *ruthenus*) 1x [46], pikeperch (*Sander lucioperca*) 2x [50] including own data, common carp (*Cyprinus*
180 *carpio*) 2x [26, 46], Eurasian perch (*Perca fluviatilis*) 2x [19, 51], rainbow trout (*Oncorhynchus*
181 *mykiss*) 6x [11, 52-56], burbot (*Lota lota*) 3x [46, 57, 58], Arctic char (*Salvelinus alpinus*) 1x [59],
182 ocellate river stingray (*Potamotrygon motoro*) 2x [60, 61], northern pike (*Esox lucius*) 2x [46]
183 including own data and Nile tilapia (*Oreochromis niloticus*) 1x own data. The four studied marine fish
184 were European sea bass (*Dicentrarchus labrax*) 2x [42, 43, 62], European eel (*Anguilla anguilla*) 1x
185 [63], and Atlantic salmon (*Salmo salar*) 1x [64]. A detailed visualization in this regard can be found
186 in supplementary Figure S7.

187 To clarify the influence of fish origin and, therefore a potential impact of natural or artificial feeding
188 type on sperm quality, the selected articles were firstly screened for the origin of the fish (wild or
189 hatchery reared), and the fatty acid composition data of artificial feeding (in case of hatchery reared
190 fish) was collected. Among the cultured fish, the fatty acid composition of the feed was available for
191 *Anguilla anguilla*, *Oncorhynchus mykiss* [52, 53, 55, 56], *Lota lota* [57], *Perca fluviatilis* [51],
192 *Dicentrarchus labrax* [62], and *Oreochromis niloticus* (own data). The influence or change of fatty

193 **acid composition** from feed to sperm was investigated only from those sources whose paired data
194 (feed and sperm fatty acids composition) was available.

195 Whereas, the artificial feed fatty **acid composition** of *Acipenser ruthenus* [61], *Cyprinus carpio* [26,
196 46], *Esox lucius* [46] including own data, *Lota lota* [58], *Oncorhynchus mykiss* [11, 54], *Perca*
197 *fluviatilis* [65], *Potamotrygon motoro* [60, 61], *Salmo salar* [64], *Sander lucioperca* [50] including
198 own data, *Salvelinus alpinus* [59] and *Tenualosa ilisha* [49] was not reported. For all freshwater fish
199 species with unreported food data (*A. ruthenus*, *S. lucioperca*, *C. carpio*, *P. motoro*, *E. lucius*, and *S.*
200 *alpinus*), we assumed the probable ARA: EPA and DHA: EPA stoichiometries of most common
201 freshwater natural prey in temperate to sub-temperate freshwaters; as majority of those were
202 naturally-raised broodstock in such climatic region. The data was selectively compiled from the
203 literature [66-68], and from own data (Roy, K., Mraz, J., unpublished data). The food items included
204 plankton consortia ($\geq 200 \mu\text{m}$ mesh), zooplankton, macro-zoobenthos, aquatic insects, and freshwater
205 minnows. *S. salar* and *T. ilisha* were excluded from such analyses due to the complexity of predicting
206 their natural food base, as they were migratory (anadromous).

207 **2.3. Formulation of hypotheses**

208 Three independent hypotheses were used to frame the investigation:

209 *Hypothesis 1:* The hypothesis 1 is inspired by Ahlgren and colleagues [69]. Being an aquatic animal,
210 the total n-6: n-3 PUFA ratio of fish sperm is tightly conserved towards more preponderance of n-3
211 PUFA than n-6 PUFA. Mathematically, fish sperm n-6: n-3 PUFA ratio would be ≤ 1 irrespective of
212 dietary perturbations.

213 *Hypothesis 2:* The hypothesis 2 was inspired by Bell and colleagues [44], and Tocher [7], eicosanoids
214 precursor ratio ARA: EPA in fish sperm is preferably inclined towards highly bioactive
215 prostaglandins balance (high-bioactive $\text{PGE}_2 > \text{low-bioactive } \text{PGE}_3$). Mathematically, fish sperm
216 ARA: EPA ratio would be ≥ 1 for high bioactive eicosanoid balance irrespective of dietary
217 perturbations.

218 *Hypothesis 3:* The hypothesis 3 was inspired by the current understanding of human sperm and
219 multiple species comparisons [13, 14, 33, 70]. There is not much plasticity in sperm DHA to EPA
220 balance. DHA: EPA stoichiometry must always favor DHA over EPA in fish sperm due to the key
221 importance of DHA in sperm morphology, swimming, and membrane fusion reactions.
222 Mathematically, the DHA: EPA ratio would be ≥ 1 for sperm irrespective of dietary perturbations.

223 **2.4. Exploratory analyses on evolutionary/ genomic pre-disposition**

224 In addition to investigating dietary influences on hypothesis 2 and 3, we performed an **exploratory**
225 **analysis** to find out if the evolutionary timeline is relatable with stoichiometry (ratio) of certain LC-
226 PUFA in fish sperm. For this purpose, we considered DHA: EPA ratio and ARA: EPA ratio in fish
227 sperm. Then we considered the evolutionary divergence era of fish species from Hughes and
228 colleagues [71], including the occurrence of whole genome duplication events in fish from Berthelot
229 and colleagues [72]. In wild fish, with swimming habitat across different temperatures, pH, salinity
230 (osmolality), and ecological niches (surface, pelagic, benthic; with different hydrostatic pressure), the
231 interquartile range of body DHA: EPA ratio is from $\sim 1:1$ to $\sim 5:1$ (median $\sim 2:1$); recalculated from
232 published dataset [73]. This analogy can be drawn for fish sperm too, as it must swim. We assumed a
233 minimum threshold DHA: EPA ratio of 1 as a lower threshold for efficient swimming functions
234 (membrane fluidity). On the other hand, a lower than 1 ARA: EPA ratio in sperm was assumed as an
235 inclination towards having less bioactive or reduced pro-inflammatory eicosanoid balance [7, 27]. The

236 fatty acid ratios were arranged in descending order and the upper versus lower tails were
237 retrospectively evaluated using evolutionary knowledge. Era of divergence interpreted from the
238 graphical summarization provided by Hughes and colleagues [71].

239 **2.5. Data analysis framework and limitations**

240 All analysis and visualizations were done with a combination of GPT-4o Data Analyst and RStudio
241 2023.12.1 (Build 402) using packages ‘ggplot2’, ‘corrplot’, ‘e1071’, ‘dunn.test’, ‘FSA’. Paired fatty
242 acid profiles of feed and sperm were arranged species-wise, treatment-wise, and article-wise. First, a
243 2-tailed Pearson’s correlation test was performed at alpha level 0.05, and a correlogram was generated
244 using RStudio (library name: corrgram). A trend analysis using paired flows from feed to sperm was
245 done using a violin-boxplot (for artificially fed fishes) or histogram with kernel density estimate and
246 normal distribution fitting (for naturally fed fishes). A histogram of ARA: EPA and DHA: EPA ratios
247 in the sperm of natural temperate freshwater fish species was overlaid on the range of those FA
248 ratios expected in their natural food base to visually assess the skewness between the two. If a 2-
249 tailed and significant Pearson’s correlation coefficient is established, a bivariate linear regression
250 analysis was performed with feed fatty acid profile (independent variable) and sperm fatty acid profile
251 (dependent variable). For statistical tests of significant differences, the data distribution was first
252 determined by the Shapiro-Wilk normality test. For normally distributed data, one-way ANOVA test
253 was performed. For data not normally distributed, the Kruskal-Wallis rank sum test was performed.
254 All statistical tests were interpreted at a set alpha level of 0.05. The scope of limitations and
255 definitions of the analysis is declared in supplementary material.

256 **3. Distribution and drivers of fish sperm lc-pufa composition**

257 **3.1. General observations**

258 By the term LC-PUFA in this review, we include only ARA, EPA, and DHA. Together they make
259 from 1/3rd to half of fish sperm lipids (Fig S3-S5, Table 1). No dichotomy exists between freshwater
260 and marine fish, or external and internal fertilizing fish in terms of LC-PUFA. The DPA (22:5n-3) can
261 sometimes substitute DHA in fish sperm, as in the case of tilapia (own results). There were also cases
262 where both 22:5n-6 and 22:5n-3 substituting DHA was apparent in blunt snouted mullet, annular
263 seabream [48]. Fish species-wise sperm total n-6 PUFA (% of total FA), total n-3 PUFA (% of total
264 FA), ARA (% of total FA), EPA (% of total FA), and DHA (% of total FA) can be found in
265 supplementary Fig S1-S5. In our reviewed dataset, LC-PUFAs above DHA, such as 26:3 observed in
266 human sperm [15], were not encountered.

267 From reviewed data on fish sperm phospholipids (PL) in Table 1, phosphatidylcholine followed by
268 phosphatidylethanolamine or sometimes sphingomyelin are dominant PL classes in fish sperm.
269 Within each PL class, DHA was almost always the most predominant LC-PUFA followed by a
270 competition between ARA and EPA for predominance (Table 1). DiPUFA molecular species mainly
271 composed of LC-PUFAs dominate (65-72%) phosphatidylserine fraction of fish sperm membranes, as
272 in sea bass, Atlantic or chinook salmon [12]. In the next section, we will establish its implication on
273 sperm biomechanics.

274 **Table 1: Reported LC-PUFA share (% of total fatty acids) within phospholipid (PL), including PL classes distribution (% of total lipids) in fish**
 275 **sperm.** Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylserine (PS), Phosphatidylinositol (PI), Sphingomyelin (SM),
 276 Lysophosphatidylcholine (LPC), Diphosphatidylglycerol (DPG), Plasmalogenic phosphatidylcholine (PPC), Plasmalogenic phosphatidylethanolamine (PPE),
 277 data not available (-).

| Fish species (habitat) | Phospholipid (%) | 20:4n-6 (%) | 20:5n-3 (%) | 22:5n-3 (%) | 22:5n-6 (%) | 22:6n-3 (%) | Reference |
|---|------------------|-------------|-------------|-------------|-------------|-------------|-----------|
| <i>Dicentrarchus labrax</i> (marine) | Total | 0.2 | 9.3 | 1.9 | - | 37.3 | [42] |
| | | 0.2 | 9.3 | 2.0 | - | 37 | [43] |
| | PC (-) | 1.7-4.0* | 6.0-8.2* | 0.6-1.3* | 0.4-0.9* | 19.7-24.8* | [44] |
| | PE (-) | 2.3-5.2* | 4.5-7.7* | 1.1-2.8* | 0.8-1.3* | 45.7-48.2 | |
| | PS (-) | 2.5-5.1* | 1.9-4.3* | 3.7-6.1* | 1.1-2.0* | 68.2-74.3 | |
| | PI (-) | 21.3-31.7* | 5.0-17.7* | 0-0.5* | 0-0.4* | 2.8-13.1* | |
| <i>Cyprinus carpio</i> (freshwater) | PC (14.3%) | - | - | - | - | - | [46] |
| | PE (24.7%) | - | - | - | - | - | |
| | PS (9.0%) | - | - | - | - | - | |
| | PI (8.0%) | - | - | - | - | - | |
| | SM (7.1%) | - | - | - | - | - | |
| <i>Esox lucius</i> (freshwater) | PC (6.5%) | - | - | - | - | - | [45] |
| | PE (18.7%) | - | - | - | - | - | |
| | PS (7%) | - | - | - | - | - | |
| | PI (3.5%) | - | - | - | - | - | |
| | SM (5.5%) | - | - | - | - | - | |
| <i>Lota lota</i> (freshwater) | PC (12%) | - | - | - | - | - | [45] |
| | PE (19.4%) | - | - | - | - | - | |
| | PS (8%) | - | - | - | - | - | |
| | PI (4.3%) | - | - | - | - | - | |
| | SM (2.8%) | - | - | - | - | - | |
| <i>Potamotrygon motoro</i> (freshwater) | PC (-) | - | - | - | - | - | [45] |
| | PE (-) | - | - | - | - | - | |
| | PS (-) | - | - | - | - | - | |

| | | | | | | | |
|--|----------------|-----------|-----------|----------|----------|-----------|-------------------|
| | PI (2.8%) | - | - | - | - | - | |
| | SM (13%) | - | - | - | - | - | |
| <i>Oncorhynchus mykiss</i> (freshwater) | PC (46.5-54%) | 2.4-5.0* | 4.4-10.8* | 0.2-0.5* | 0.2-0.6* | 10.9-12.8 | [47] |
| | PE (2.5-33%) | 5.2-12.9* | 9.5-18.7* | 0.2-0.5* | 0.3-0.7* | 15-17.6 | |
| | PS (9.5-10.3%) | 3-7.1* | 3.5-2.7* | 0.9-1.8* | 0.4-1.4* | 23.4-25.9 | |
| | PI (2.5-4.5%) | 7.3-13.4* | 6.4-14.9* | 0.2-0.7 | 0.2-0.6* | 6.8-9.3 | |
| | SM (1-2.67%) | - | - | - | - | - | |
| | LPC (2.5-3.5%) | - | - | - | - | - | |
| | DPC (0.7-1.5%) | - | - | - | - | - | |
| <i>Scophthalmus maeoticus</i> (marine) | PC (42.3%) | 1.5-2.1 | 0.4-1.2 | 2.5-2.9 | 0.9-2.9 | 6-7.1 | [48] [#] |
| | PE (17.3%) | 3.2-4.7 | 1.1-1.5 | 2.4-2.9 | 2.0-3.8 | 9.2-21.6 | |
| | PS + PI (6.4%) | - | - | - | - | - | |
| | SM (6.8%) | - | - | - | - | - | |
| | PPC (12.4%) | - | - | - | - | - | |
| | PPE (14.8%) | - | - | - | - | - | |
| <i>Diplodus annularis</i> (marine) | PC (42.2%) | 4.3-5.7 | 9.7-14.2 | 0.7 | 1.0-2.2 | 0.9 | |
| | PE (20.2%) | 2.4-4.4 | 30.4-37.4 | 2.1-2.2 | 2.4-2.6 | 0.8 | |
| | PS + PI (3.8%) | - | - | - | - | - | |
| | SM (14.9%) | - | - | - | - | - | |
| | PPC (10.5%) | - | - | - | - | - | |
| | PPE (8.4%) | - | - | - | - | - | |
| <i>Mullus barbatus ponticus</i> (marine) | PC (25.5%) | 8.7-9.1 | 7.4-7.8 | 1.7 | 3.1-6.3 | 2.3 | |
| | PE (34.3%) | 6.7-8.2 | 5.9-6.3 | 2.6-4.1 | 1.2-2.5 | 3.1 | |
| | PS + PI (9.1%) | - | - | - | - | - | |
| | SM (8.6%) | - | - | - | - | - | |
| | PPC (6.6%) | - | - | - | - | - | |
| | PPE (15.9%) | - | - | - | - | - | |
| <i>Neogobius melanostomus</i> (marine) | PC (52.9%) | 0.7-2.2 | 1.5-8.3 | 3.0-6.1 | 1.0 | 7.2-8.7 | |
| | PE (22.6%) | 2.1-2.6 | 4.5 | 2.1-3.7 | 1.2-6.7 | 9.4-10.9 | |

| | | | | | | |
|---|----------------|-----------|----------|---------|---------|----------|
| | PS + PI (9.1%) | - | - | - | - | - |
| | SM (9.1%) | - | - | - | - | - |
| | PPC (5.1%) | - | - | - | - | - |
| | PPE (5.5%) | - | - | - | - | - |
| <i>Symphodus tinca</i> (marine) | PC (53.9%) | 2.0-3.5 | 1.0-11.7 | 1.2-2.3 | 1.0-1.4 | 4.4-5.8 |
| | PE (20.5%) | 3.5-4.9 | 1.4-6.8 | 2.1-4.4 | 2.6-4.3 | 7.5-14.2 |
| | PS + PI (5.0%) | - | - | - | - | - |
| | SM (9.3%) | - | - | - | - | - |
| | PPC (5.7%) | - | - | - | - | - |
| | PPE (5.6%) | - | - | - | - | - |
| <i>Oreochromis mossambicus</i> (freshwater) | PC (32.8%) | 6.3-13.1 | 0.4-1.3 | 1.3-1.7 | 0.8-2.5 | 1.8-2.3 |
| | PE (18.6%) | 7.1-22.9 | 0.7 | 1.0-2.9 | 2.5-6.2 | 2.8-4.3 |
| | PS (8.0%) | - | - | - | - | - |
| | PI (4.7%) | - | - | - | - | - |
| | SM (6.7%) | - | - | - | - | - |
| | PPC (11.3%) | - | - | - | - | - |
| | PPE (16.1%) | - | - | - | - | - |
| <i>Centrarchus macropterus</i> (freshwater) | PC (23.2%) | 9.9-11.1 | 3.5-5.4 | 1.8-2.1 | 3.9-4.0 | 2.2-7.6 |
| | PE (24.1%) | 13.1-17.3 | 1.9-6.4 | 5.7 | 1.6-2.1 | 14.8 |
| | PS (11.8%) | - | - | - | - | - |
| | PI (8.0%) | - | - | - | - | - |
| | SM (18.0%) | - | - | - | - | - |
| | PPC (6.3%) | - | - | - | - | - |
| | PPE (11.6%) | - | - | - | - | - |
| <i>Ictiobus cyprinellus</i> (freshwater) | PC (21.9%) | 11.1-13.2 | 0.6 | 0.5-0.9 | 2.3-2.7 | 4.6-4.9 |
| | PE (18.7%) | 14.8-15.9 | 0.3 | 0.5 | 4.5-5.2 | 9.2-10.0 |
| | PS (8.8%) | - | - | - | - | - |
| | PI (6.1%) | - | - | - | - | - |
| | SM (18.6%) | - | - | - | - | - |

| | | | | | | | |
|--|-------------|-----------|-----|---------|---------|------|--------------------|
| | PPC (15.0%) | - | - | - | - | - | |
| | PPE (9.2%) | - | - | - | - | - | |
| <i>Ctenopharyngodon idella</i> (freshwater) | PC (34.1%) | 17.1 | 8.4 | 0.6 | - | 3.2 | |
| | PE (25.8%) | 25.4 | 5.9 | - | 1.4 | 8.7 | |
| | PS (9.4%) | - | - | - | - | - | |
| | PI (0%) | - | - | - | - | - | |
| | SM (20.9%) | - | - | - | - | - | |
| | PPC (-) | - | - | - | - | - | |
| | PPE (5.4%) | - | - | - | - | - | |
| <i>Cyprinus carpio</i> (freshwater) | PC (30.4%) | 13.5-28.9 | - | - | 1.3-9.8 | 1.4 | |
| | PE (22.3%) | 6.7-40.6 | - | 1.6-9.8 | - | - | |
| | PS (5.4%) | - | - | - | - | - | |
| | PI (4.5%) | - | - | - | - | - | |
| | SM (10%) | - | - | - | - | - | |
| | PPC (5.8%) | - | - | - | - | - | |
| | PPE (9.6%) | - | - | - | - | - | |
| <i>Dicentrarchus labrax</i> (marine) | PC (-) | - | - | - | - | 1.7 | [12] ^{##} |
| | PE (-) | - | - | - | - | 10.9 | |
| | PS (-) | - | - | - | - | 50.2 | |
| <i>Salmo salar</i> (marine) | PC (-) | - | 0.3 | - | - | 1.3 | |
| | PE (-) | - | 0.5 | - | - | 3.3 | |
| | PS (-) | - | 1.8 | - | - | 24.1 | |
| <i>Onchorynchus tsawytscha</i> (marine) | PC (-) | - | 1.2 | 1.0 | - | 1.3 | |
| | PE (-) | - | 0.5 | 3.4 | - | 4.7 | |
| | PS (-) | - | 0.8 | 0.6 | - | 35.4 | |
| *Statistically significantly ($p \leq 0.01$) impacted by choice of food source [44, 47]. | | | | | | | |
| #Fatty acid data out of range (suspicious) compared to other literature [48]. | | | | | | | |
| ##Data are of DiPUFA molecular species, not single molecule [12]. | | | | | | | |

279 **3.2. Influence of LC-PUFA and total n6: n-3 PUFA ratio on fish sperm biomechanics**

280 Total n-6: n-3 PUFA ratio has no significant correlation with sperm biomechanics, but LC-PUFAs do.
 281 All LC-PUFAs are positively correlated with VCL, except EPA. The DHA is only LC-PUFA having a
 282 unanimous positive and statistically significant correlation to both VCL and motility duration. Results
 283 suggest DHA's positive influence on sperm biomechanics (discussed later), while both ARA and EPA
 284 involved in eicosanoids biology influence a reduced motility duration. Results can be found in Table
 285 2.

286 Present correlations affirm eicosanoids balance interact with motility duration of fish sperm, with a
 287 small positive role in VCL (Table 2). Similar observations in human sperm showed sperm motility
 288 was related to the balance between the content of 19-hydroxy PGE and 19-hydroxy PGF but not to
 289 their separate concentrations [74]. The balance is further visualized in fish sperm using a power
 290 regression (Fig 1).

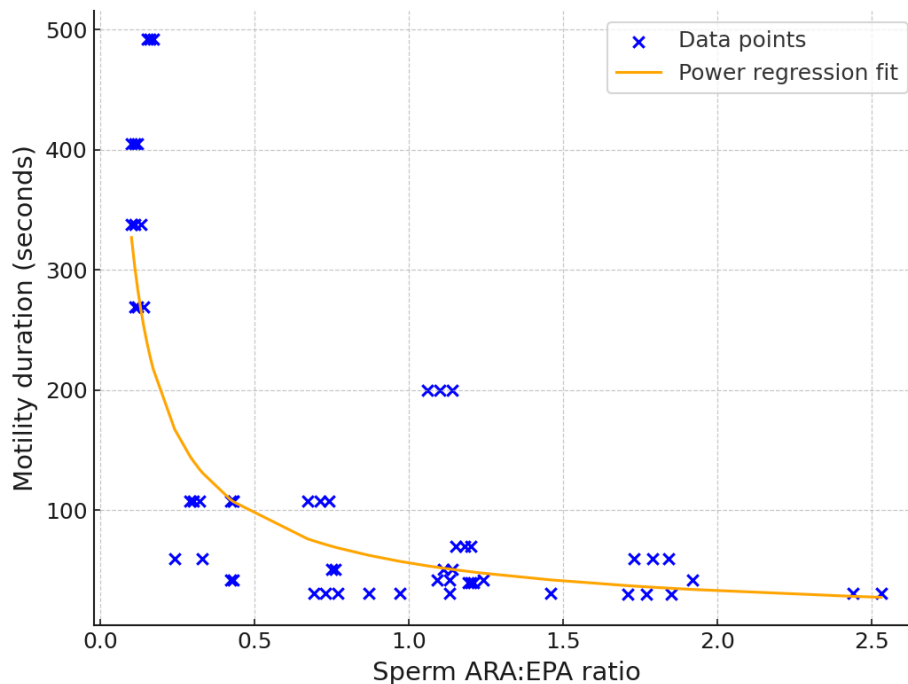
291 The eicosanoids balance of fish sperm is a black-box since the time of Bell and colleagues [44]. Not
 292 much is known in relation to fish sperm biomechanics, neither the right prostaglandin balance of
 293 fertile versus infertile males nor prostaglandin/ eicosanoid receptors present in fish sperm. There is a
 294 legacy of understanding in human sperm along those lines (e.g., [74-76]). Infertile men (n = 82) had
 295 significantly higher ARA: DHA and ARA: EPA ratios that were both negatively correlated with total
 296 sperm count, sperm motility, and sperm morphology [15]. Our correlation analysis shows sperm LC-
 297 PUFA's interaction with fish sperm biomechanics, especially the ARA: EPA ratio and DHA (Table
 298 2).

299 Using a 'spermatozoan spin-labeling' technique and without any CASA measurements or fertilization
 300 assays, it was argued that dietary lipids can alter the general fatty acids composition, but not fluidity,
 301 of trout sperm plasma membrane [47]. But such results are contradicted by recent literature that
 302 employ CASA or fertilization assays. For example, European eel sperm showed highest velocity and
 303 motility percentage (even at 30 seconds post activation) under dietary regime with highest LC-PUFA
 304 (ARA+ EPA+ DHA) clearly demonstrating enhanced biomechanics [77]. In internal fertilizing guppy,
 305 the importance of dietary LC-PUFA for share of paternity was observed [78] or motility duration [79].
 306 In zebrafish, the addition of phospholipids in the diet (especially phosphatidylethanolamine) caused
 307 higher total and progressive motility and higher velocities [80]. The fertilization, eyed egg stage, and
 308 hatching rates were lower from male fish fed n-3 PUFA deficient diet [56]. In the next section, we
 309 will establish the dietary connection with sperm LC-PUFA composition.

310 **Table 2: Pearson's 2-tailed correlation between selected PUFA parameters and sperm**
 311 **curvilinear velocity (VCL) or motility duration (MD).** Non-significant $p > 0.05$ (ns).

| Variable 1 | Variable 2 | Correlation coefficient | p-value |
|----------------------------------|------------|-------------------------|-------------|
| Total n6: n3 PUFA ratio | VCL | -0.139 | ns |
| | MD | -0.259 | ns (p=0.05) |
| 20:4n-6 (ARA) | VCL | 0.403 | p<0.001 |
| | MD | -0.666 | p<0.001 |
| 20:5n-3 (EPA) | VCL | 0.281 | ns |
| | MD | -0.604 | p<0.001 |
| 22:6n-3 (DHA) | VCL | 0.275 | p<0.01 |
| | MD | 0.515 | p<0.001 |
| ARA: EPA (eicosanoid actions) | VCL | 0.296 | p<0.01 |
| | MD | -0.625 | P<0.001 |

312



313

314 **Fig 1: Relationship of sperm eicosanoid actions (ARA: EPA ratio) and motility duration of fish**
 315 **sperm. Power regression (R^2 0.651, $p < 0.001$):** Motility duration (sec) = $56.209 \times (\text{ARA: EPA})^{-0.765}$

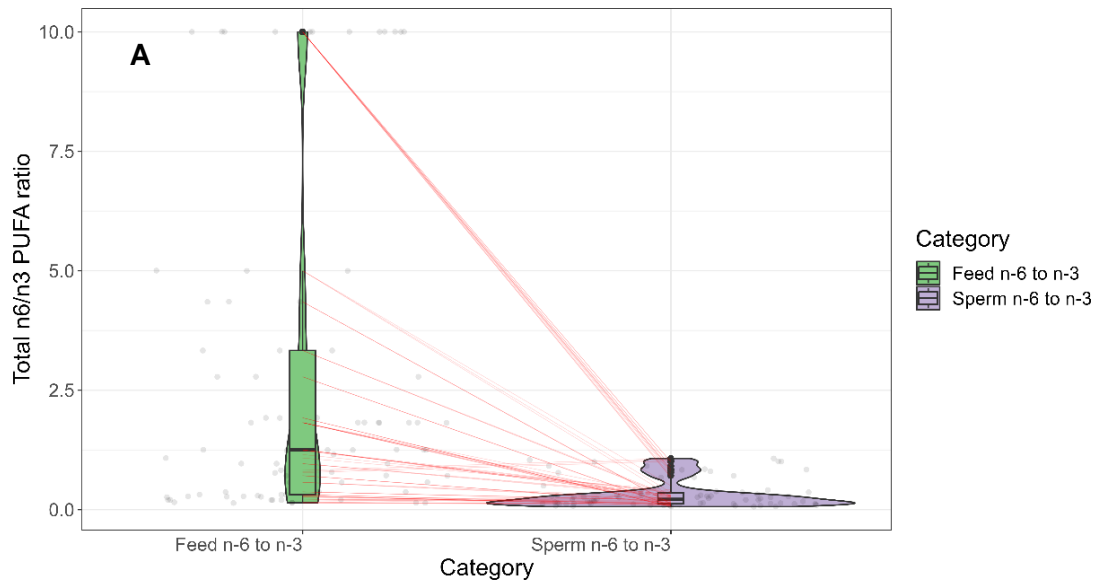
316 **3.3. Influence of dietary fatty acid profile on sperm fatty acid profile**

317 There are clear experimental evidence that choice of food source can significantly alter ($p \leq 0.01$) LC-
 318 PUFA composition in sea bass [44] or rainbow trout [47] sperm membrane phospholipids. Below is a
 319 further re-affirmation of such events based on reviewed data.

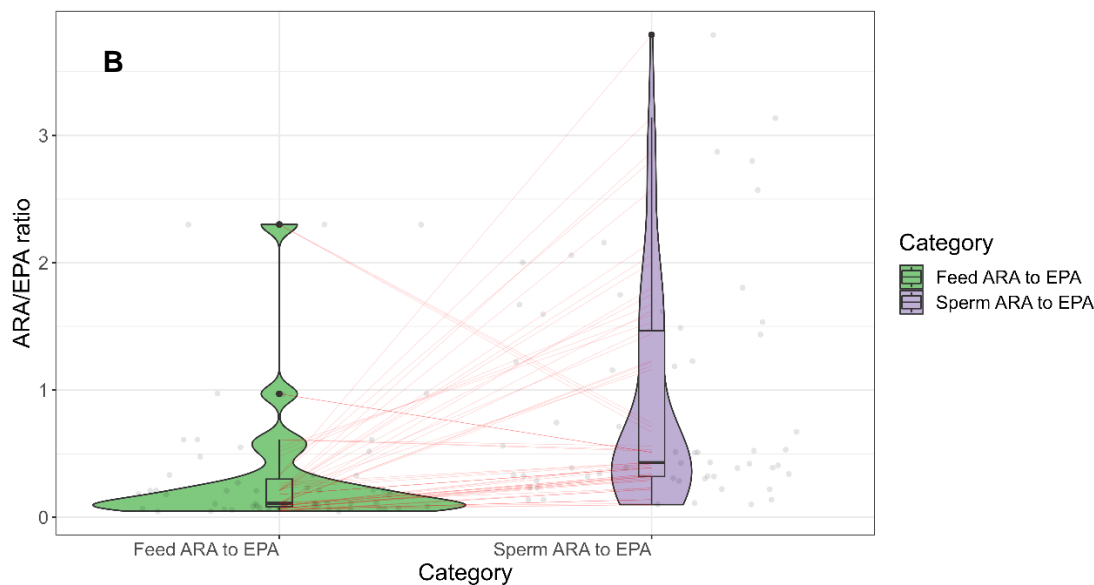
320 **3.3.1. Hypothesis 1**

321 Positive correlations were found between feed and sperm total n-6: n-3 PUFA ratio ($r \approx 0.6$, $p < 0.05$),
 322 also total n-6: n-3 PUFA ratio in feed with sperm ARA: EPA ratio ($r \approx 0.5$, $p < 0.05$). The correlogram
 323 can be found in supplementary material (Fig S6). In our paired dataset (feed and sperm), the total n-6:
 324 n-3 PUFA ratio ranged from 0.14 to 10 in feed, while total n-6: n-3 PUFA ratio in sperm (0.06-1.07)
 325 was significantly lower ($p < 0.01$; Fig 2A). It shows signs of homeostatic control in sperm, with
 326 regards to total n-6: n-3 PUFA ratio. On the contrary, the ARA: EPA ratio in sperm (0.1-3.79) was
 327 significantly higher ($p < 0.01$) than ARA: EPA ratio in feed (0.05 to 2.30) (Fig 2B). It shows signs of
 328 allostasis (defined as corrective measures) by sperm, with regards to ARA: EPA ratio to maintain a
 329 healthy or tolerable eicosanoids balance.

330 The hypothesis-1 holds true. Fish sperm tend to maintain lower (≤ 1 unit) total n-6 PUFA per unit of
 331 total n-3 PUFA (Fig 2A), but simultaneously prefer to maintain higher (> 1 unit) ARA per unit of EPA
 332 (Fig 2B). Thus, both homeostasis and allostasis mechanisms co-exist, depending on specific
 333 physiological or biomechanical needs (further discussed).



334



335

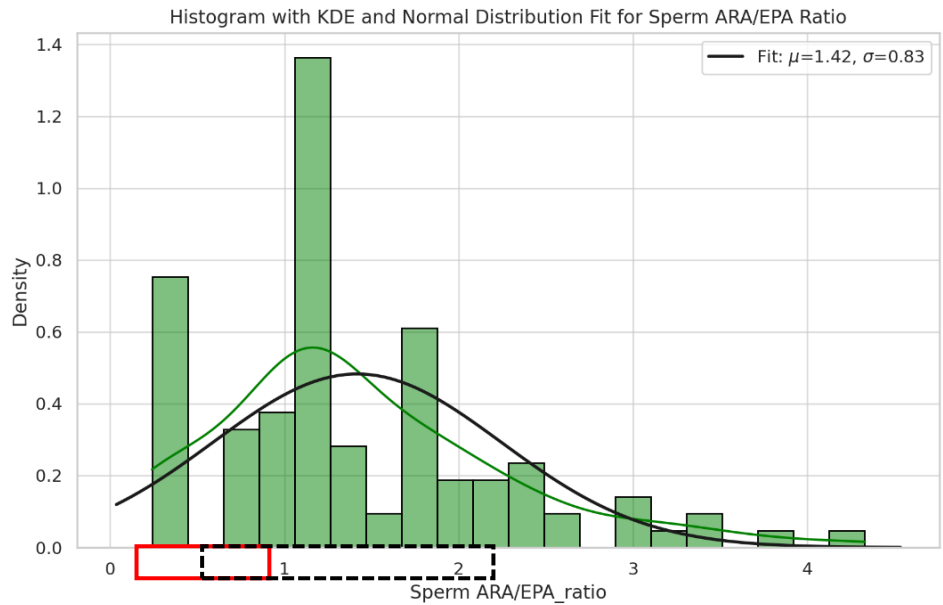
336 **Fig 2: Paired feed to sperm fatty acid ratio changes in artificially fed fish.** (A) total *n*-6: *n*-3
 337 PUFA decrease from feed to sperm. (B) ARA: EPA increases from feed to sperm. Red lines connect
 338 individual data points from feed to sperm. Downward lines indicate homeostatic control while upward
 339 lines indicate allostasis or corrective measures, perhaps happening during spermatogenesis.

340 3.3.2. Hypothesis 2

341 The dietary ARA: EPA ratio is not correlated ($p > 0.05$) with sperm ARA: EPA ratio (Fig S6). A case
 342 study on some temperate freshwater fish species in our dataset that grew on natural food suggests that
 343 the common ARA: EPA ratio in sperm is ≥ 1 (sperm ARA: EPA = 1.42 ± 0.83 ; Fig 3). The natural food
 344 base, comprising of plankton consortia ($\geq 200 \mu\text{m}$ mesh), zooplankton, benthic macro-zoobenthos,
 345 insects, smaller prey fish or minnows, can have ARA: EPA ratio from 0.05 to 0.72. The ARA: EPA
 346 ratio in wild fish sperm is higher than natural food (Fig 4). Same trend as artificially fed fish (Fig 2B).
 347 The hypothesis-2 holds true as well.

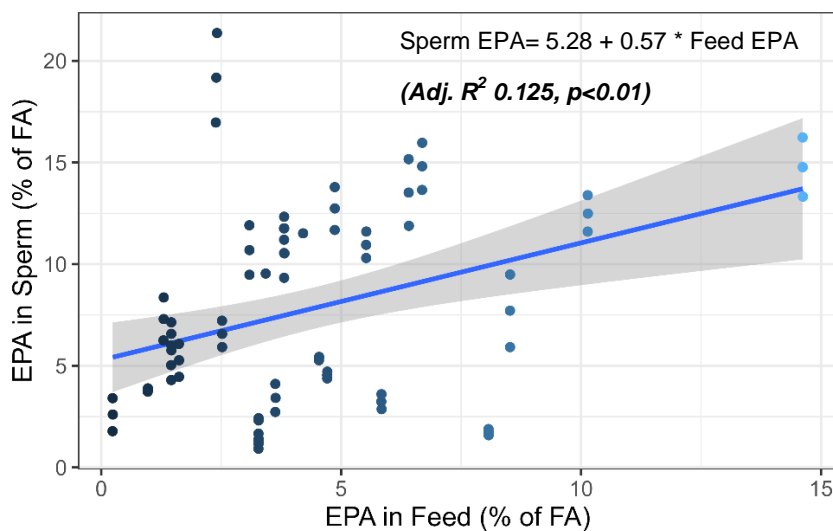
348 No relationship could be established between dietary and sperm ARA ($Adj R^2 0.02, p = 0.1489$), but a
 349 weak relationship exists between share of EPA in feed and sperm fatty acids ($Adj R^2 0.1254$,

350 $p < 0.001$). The relationship predicts per unit increase in dietary EPA, there is a 0.57 unit or 57%
 351 increase in sperm EPA (Fig 4). In an experiment with salmon, the fate of dietary EPA being deposited
 352 was close to 50% of what was fed, in diets that contained only EPA or EPA+DHA above minimum
 353 requirements. Rest was lost by β -oxidation and further bioconversion [81]. Whether the same
 354 bioenergetic partitioning of EPA happens during spermatogenesis too is presently unknown, but the
 355 prediction hints a close alignment.



356

357 **Fig 3: Histogram of natural freshwater fish sperm ARA: EPA ratio, with kernel density**
 358 **estimate (KDE; green curve) and normal distribution fit (black curve).** Solid red box on X-axis
 359 indicates ARA: EPA ratio range in natural food base (0.05-0.72). Dashed black box on X-axis
 360 indicates ARA: EPA ratio range in wild fish sperm (1.42 ± 0.83). KDE is a non-parametric method to
 361 estimate the probability density function of a random variable.



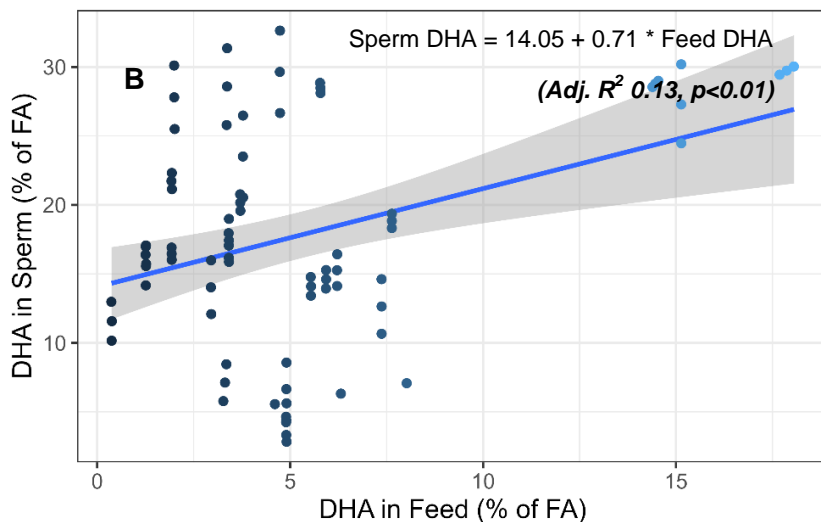
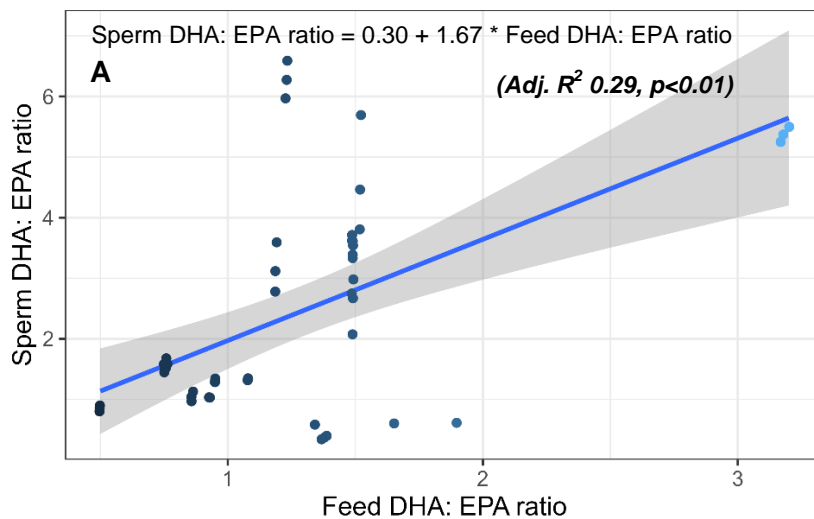
362

363 **Fig 4: Relationship between EPA share in dietary fatty acids and EPA share in sperm fatty**
 364 **acids.** Sperm EPA = $5.28 + 0.57 * \text{Feed EPA}$ ($\text{Adj. } R^2 0.125, p < 0.01$). Dark to lighter blue dots imply high
 365 to low FA quantity in feed.

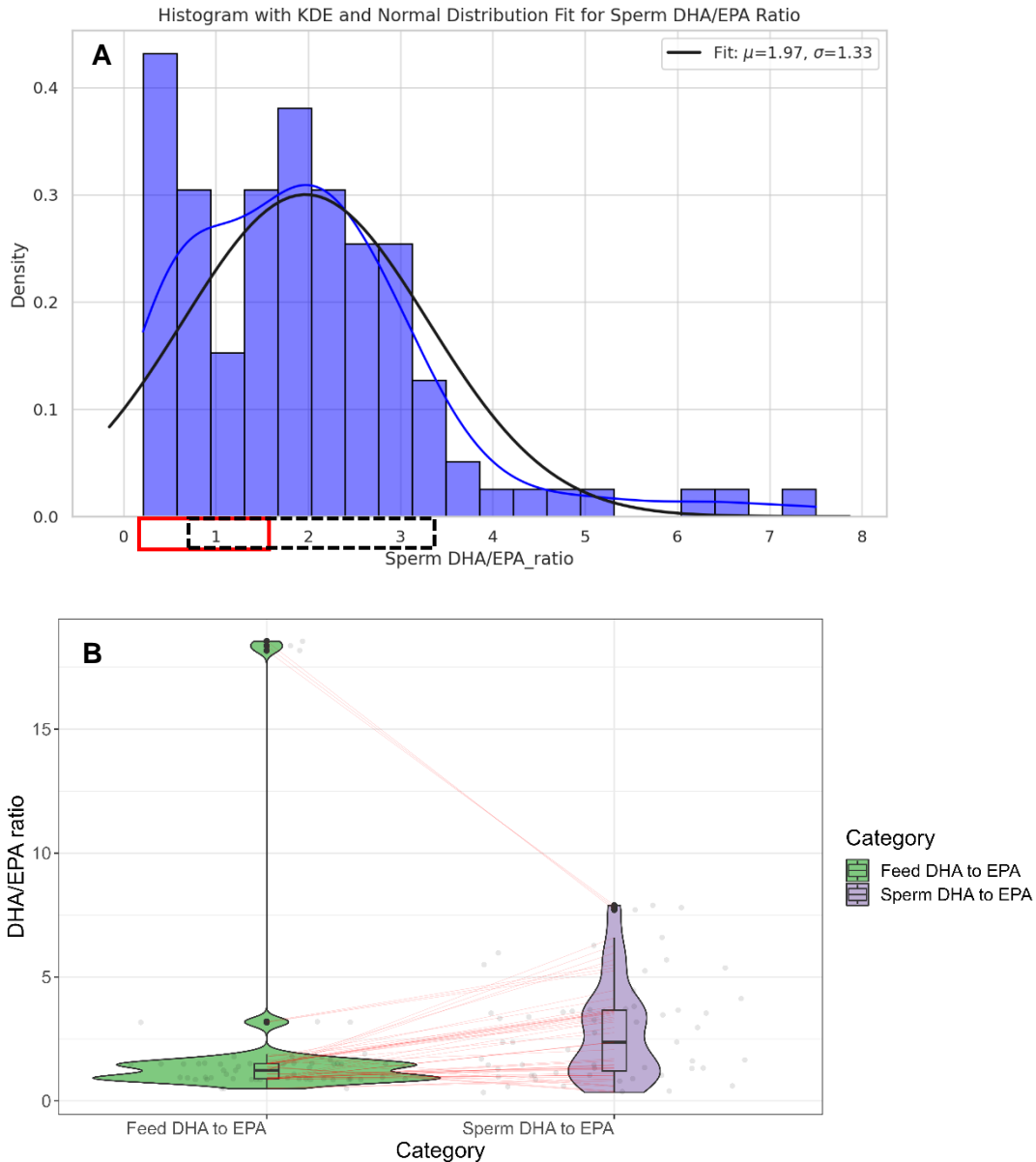
366 3.3.3. Hypothesis 3

367 The dietary DHA: EPA ratio is correlated ($r \approx 0.6$, $p < 0.05$) with sperm DHA: EPA ratio (Fig S6). A
368 weak but statistically significant relationship exist between dietary and sperm DHA: EPA ratio (Adj
369 R^2 0.2963, $p < 0.001$). For every 1 unit increase in dietary DHA: EPA, we recorded a 1.67 unit increase
370 in sperm DHA: EPA (Fig 5A). In artificially fed fish, sperm DHA: EPA ratio is mostly greater than
371 feed DHA: EPA ratio (Fig 6B). But there are cases, shown by almost straight red lines (Fig 6B), the
372 sperm or feed DHA: EPA ratio mimic each other. In wild fish, for example our case study on some
373 temperate freshwater fish species grazed on natural food (DHA: EPA ratio range 0.1-1.6), the sperm
374 DHA: EPA ratio is 1.97 ± 1.33 . Meaning sperm DHA: EPA ratio can be commonly > 1 , with possibility
375 of values < 1 too (Fig 6A). Thus, hypothesis-3 holds true, with some genetic limitations. There are fish
376 species that cannot sufficiently endogenously fortify DHA during spermatogenesis (next section).

377 A weak relationship exists between share of DHA in feed and sperm fatty acids (Adj R^2 0.127,
378 $p < 0.001$). The relationship predicts per unit increase in dietary DHA, there is a 0.71 unit or 71%
379 increase in sperm DHA (Fig 5B). In an experiment with salmon, the fate of dietary DHA being
380 deposited was almost 100% of what was fed. The reason being DHA being too important or complex
381 for catabolism [81]. Such research is not present in fish sperm, but the prediction hints at a close
382 alignment.



385 **Fig 5: (A) Relationship between dietary and fish sperm DHA: EPA ratio**, Sperm DHA: EPA ratio
 386 = $0.30 + 1.67 * \text{Feed DHA: EPA ratio}$ (*Adj. R*² 0.29, *p*<0.01) **(B) Relationship between DHA share**
 387 **in dietary fatty acids and DHA share in sperm fatty acids**. Sperm DHA = $14.05 + 0.71 * \text{Feed}$
 388 **DHA** (*Adj. R*² 0.13, *p*<0.01). Dark to lighter dots imply high to low FA quantity in feed.



389

390

391 **Fig 6: (A) Histogram of natural freshwater fish sperm ARA: EPA ratio, with kernel density**
 392 **estimate (KDE; blue curve) and normal distribution fit (black curve).** Solid red box on X-axis
 393 indicates ARA: EPA ratio range in natural food (0.1-1.6). Dashed black box on X-axis indicates
 394 ARA: EPA ratio range in wild fish sperm (1.97 ± 1.33). KDE is a non-parametric method to estimate
 395 the probability density function of a random variable. **(B) Paired feed to sperm DHA: EPA ratio**
 396 **changes in artificially fed fish.**

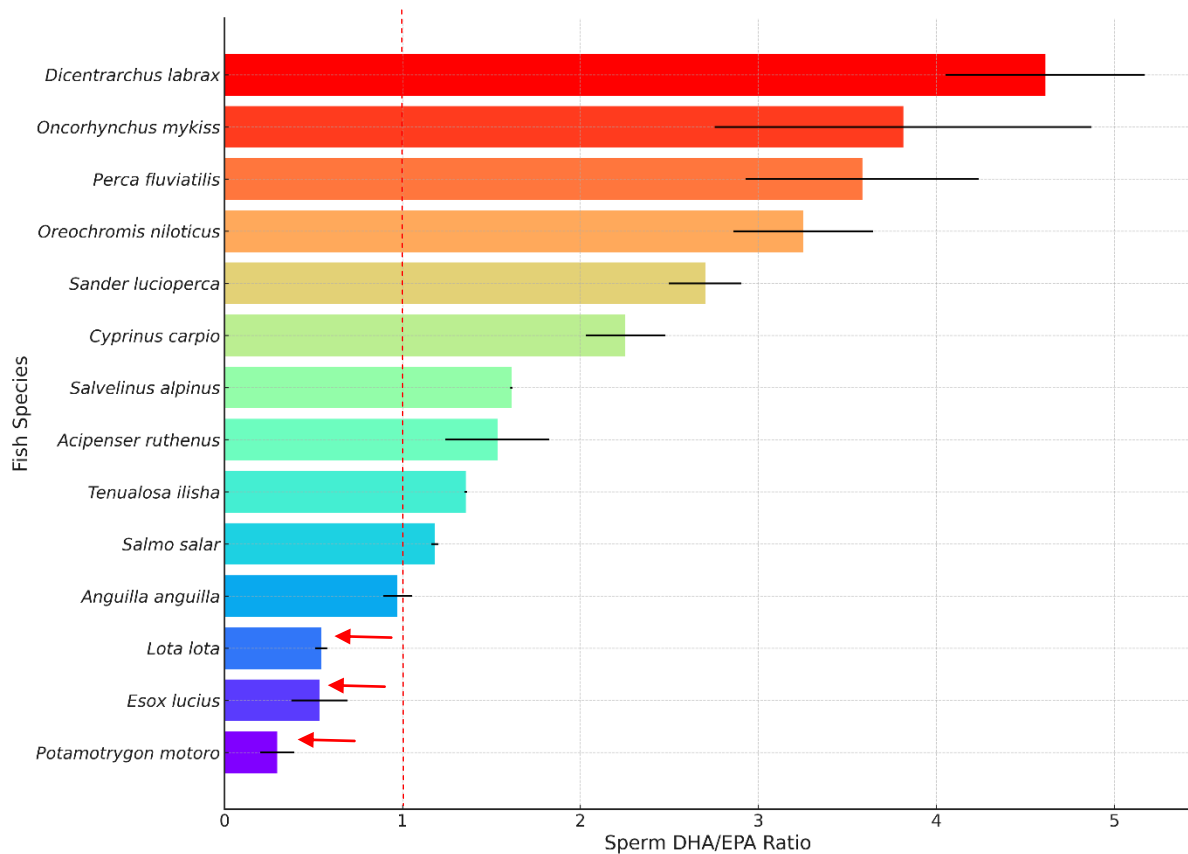
397 It is worth mentioning that the weak relationships in Fig 4, 5A, and 5B are derived from a limited
 398 dataset, with different diets and across different fish species. The relationship although weak cannot
 399 be overlooked, as fish sperm phospholipids data reviewed in Table 2 has shown significant effect of

400 diets altering fatty acids composition. Future controlled experiments with graded dietary treatments of
 401 EPA, DHA, and DHA: EPA and their deposition in sperm need to be conducted, to ascertain the true
 402 correlation. The inter-relationships in Fig 4, 5A, and 5B are quite weak, and it may account for other
 403 governing factors that are not explained. Among such factors could be the evolutionary and genetic
 404 pre-disposition, which will be presented below.

405 3.4. Evolutionary connection to LC-PUFA stoichiometries in fish sperm

406 3.4.1. DHA-to-EPA stoichiometry

407 Fig 7 shows three species, *Lota lota*, *Esox lucius*, and *Potamotrygon motoro*, having sperm DHA:
 408 EPA ratio below 1 (threshold). Those below threshold represent some uniquely diverged fish lineages.
 409 The ocellate river stingray (*P. motoro*) is an elasmobranchii but uniquely displaced to freshwaters
 410 before the third-round whole genome duplication event (3R-WGD). Burbot (*L. lota*) is a unique
 411 descendant of marine cod lineage into freshwaters. The Northern pike (*E. lucius*) is a uniquely
 412 diverged sister group from freshwater salmonids. They were uniquely diverged during the fourth-
 413 round whole genome duplication event (4R-WGD). The DHA fortification seems limited or
 414 inefficient in these species (Fig 7), neither DPA substituted for DHA in their sperm (see data).



415
 416 **Fig 7: Sperm DHA: EPA ratio of different fish species in our dataset.** The red line is a
 417 demarcation of sperm DHA: EPA ratio greater than or less than 1. Red arrow (←) indicates uniquely
 418 diverged fish lineages during 3R- or 4R-WGD events.

419 3.4.2. ARA-to-EPA stoichiometry

420 In Table 3, fish species are arranged in descending order of their sperm ARA: EPA ratio (median) and
 421 matched with their era of evolutionary divergence. The fish genomes that diverged in the Devonian,
 422 Cretaceous, or Jurassic eras seem to have sperm ARA: EPA ratio ≥ 1 . While fish genomes that

423 diverged in the Paleozoic or Triassic era seem to have sperm ARA: EPA ratio ≤ 1 . There is one
 424 exception of *D. labrax* (European seabass), a marine Perciformes genome diverged in the Cretaceous
 425 era, having sperm ARA: EPA ratio < 1 ; unlike freshwater Perciformes genome or others in Cretaceous
 426 era (Table 3).

427 **Table 3: Median sperm ARA: EPA ratio of different fish species and their evolutionary**
 428 **timeline.** Era of divergence interpreted from the graphical summarization in Hughes and colleagues
 429 [71]. The red line is a demarcation of sperm ARA: EPA ratio above or below 1 (rounded-off).

| Fish Species | Sperm ARA: EPA | Order | Era of divergence |
|------------------------------|----------------|------------------|-------------------|
| <i>Acipenser ruthenus</i> | 2.570 | Acipenseriformes | Devonian |
| <i>Oreochromis niloticus</i> | 2.030 | Cichliformes | Cretaceous |
| <i>Potamotrygon motoro</i> | 1.930 | Myliobatiformes | Devonian |
| <i>Lota lota</i> | 1.710 | Gadiformes | Cretaceous |
| <i>Sander lucioperca</i> | 1.435 | Perciformes | Cretaceous |
| <i>Cyprinus carpio</i> | 1.190 | Cypriniformes | Jurassic |
| <i>Perca fluviatilis</i> | 0.970 | Perciformes | Cretaceous |
| <i>Esox lucius</i> | 0.930 | Esociformes | Paleozoic |
| <i>Anguilla anguilla</i> | 0.470 | Anguilliformes | Triassic |
| <i>Salvelinus alpinus</i> | 0.370 | Salmoniformes | Paleozoic |
| <i>Oncorhynchus mykiss</i> | 0.290 | Salmoniformes | Paleozoic |
| <i>Salmo salar</i> | 0.260 | Salmoniformes | Paleozoic |
| <i>Tenualosa ilisha</i> | 0.130 | Clupeiformes | Triassic |
| <i>Dicentrarchus labrax</i> | 0.020 | Perciformes | Cretaceous |

430

431 **4. Basis of fish sperm lc-pufa composition**

432 **4.1. Biomechanics perspective**

433 When it comes to sperm PUFA composition, the total n-6: n-3 PUFA ratio is less than 1, be it aquatic
 434 [82] or terrestrial animals, including humans [83], irrespective of dietary fatty acid supply. Similar to
 435 our observations in fish, the total n-6: n-3 PUFA ratio in boar (*Sus scrofa*) sperm was consistently
 436 below 1 (0.14-0.23) despite a wide variability in dietary total n-6: n-3 PUFA ratio (0.05-29.6) across
 437 four different diets [84]. Total n-6: n-3 PUFA ratio in human spermatozoa and sperm quality
 438 parameters such as motility, viability, morphology, and total spermatozoa were significantly inversely
 439 correlated ($r \approx -0.31$ to -0.58 , $p < 0.01$), clearly suggesting sperm performances being improved by a
 440 lower n-6: n-3 PUFA ratio. Fatty acid composition in sperm membrane of normozoospermic human
 441 male subjects had total n-6: n-3 PUFA ratio lower than 1 (0.77 ± 0.05 , $p < 0.05$), compared to
 442 oligozoospermic (1.17 ± 0.14 , $p > 0.05$), asthenozoospermic (0.9 ± 0.06 , $p > 0.05$), and
 443 oligoasthenozoospermic human male subjects (1.41 ± 0.2 , $p > 0.05$) which had sperm total n-6: n-3
 444 PUFA ratio equal to or higher than 1 [85]. Our present analysis suggests that this may be true for fish
 445 as well. In rainbow trout, fed with a diet deficient in total n-3 fatty acids, lower sperm motility was
 446 observed [56]. Some earlier discussions by Dzyuba and colleagues postulated that fish sperm
 447 phospholipid bilayer containing more n-6 PUFA than n-3 PUFA to be softer and more deformable
 448 (\approx higher flagellar beating in three-dimensional space) [60], but we observed no correlation between
 449 total n-6: n-3 PUFA ratio and biomechanics of sperm (Table 1).

450 Changes in the phospholipid class composition during sperm maturation have not been investigated in
 451 fish [12], but from the reviewed data it seems PC, PE, and SM (Table 2) are the dominant

452 phospholipid class in mature spermatozoa of fish. As previously described, the phospholipids from all
453 sperm samples were very unsaturated with almost all molecular species from PE, PS, and PI
454 containing at least one polyunsaturated acyl chain [12]. The same could be corroborated by our
455 reviewed data across species, as the sum of total PUFA in sperm, irrespective of n-3 or n-6 series (Fig
456 S1-S2), is $\geq 50\%$ of all fatty acids, with 1/3rd or up to half of all PUFAs being the sum of ARA, EPA
457 and DHA (Fig S3-S5). We have considered these three as the main LC-PUFA (ARA, EPA, DHA) in
458 sperm. Among the three, DHA is the only LC-PUFA that showed a unanimous positive correlation
459 with both VCL and motility duration simultaneously, whereas either EPA or ARA did not. Across the
460 spectrum DHA also seems to be present in high proportions, likely due to its combined/ synergistic
461 impact on both physiology and biomechanics. First, although DHA-specific lipid mediators are not
462 described in fish sperm, DHA is traditionally believed to be ‘not a substrate’ of eicosanoids synthesis
463 in fish sperm [44]. Whereas recent research suggests otherwise that resolvin D1 (RvD1), produced by
464 DHA, exerts anti-inflammatory and pro-resolving activities in sperm [86]. DHA-derived specialized
465 pro-resolving mediators such as resolvins and protectins are recently identified molecules that can
466 orchestrate the timely resolution of inflammation [87, 88]. DHA and DHA-derived mediators have
467 even been described to be an ‘analgesic’ [89], so its high presence in sperm is worth investigating
468 beyond just structural significance. Second, the structural significance of DHA is huge for sperm
469 phospholipid bilayer membranes. The best description in this regard is given by Bell and colleagues
470 [12]. In the bilayer, DHA showed a high tendency to link with itself (DHA×DHA) and other LC-
471 PUFA (DHA×EPA, DHA×ARA, DHA×DPA) giving ‘DiPUFA’ molecular speciation in sperm
472 membranes of seabass, Atlantic or chinook salmon. [12]. Once esterified into phospholipids, DHA has
473 been demonstrated to significantly alter many basic properties of membranes including acyl chain
474 order and “fluidity”, phase behavior, elastic compressibility, permeability, fusion, flip-flop and
475 protein activity [90]. DHA’s interaction with other membrane lipids, particularly cholesterol [90],
476 may play a prominent role in modulating sperm membrane fluidity and “fusogenic properties”
477 (discussed below). The fish sperm, besides having phospholipids, have a fair share of cholesterol too,
478 with cholesterol-to-phospholipid ratio of 0.3 [47]. Under such circumstances, a DHA dominated
479 phospholipid bi-layer membrane gives the most optimal biomechanical properties to sperm to swim
480 and fuse with egg, with n-3 series DPA being the closest substitute in some cases/ species (discussed
481 later).

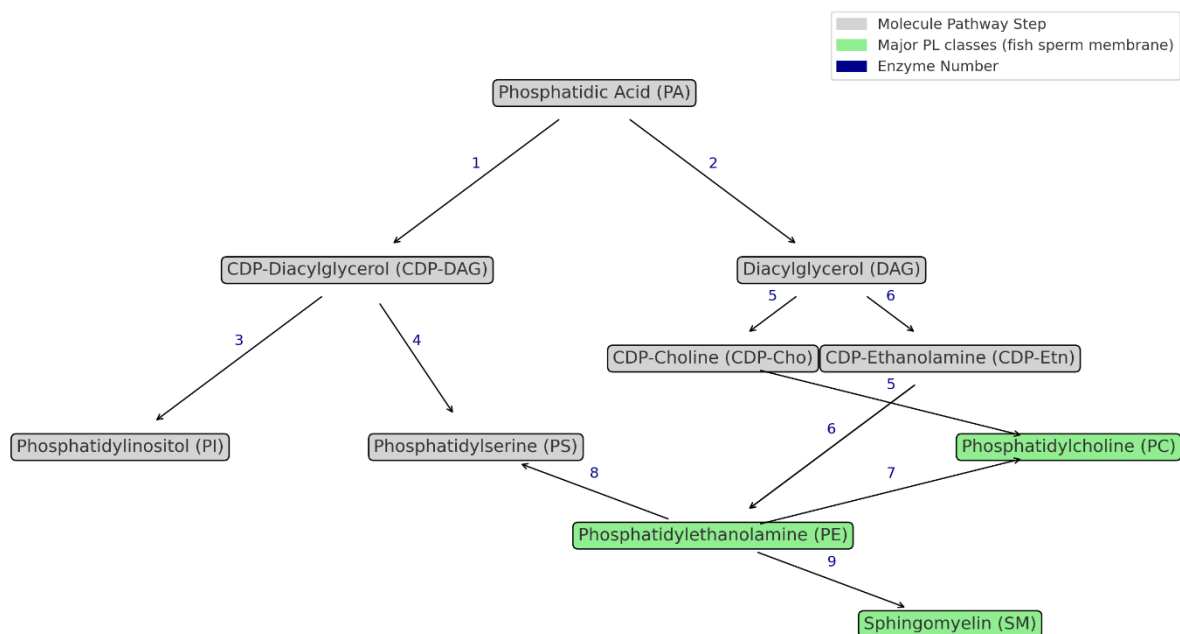
482 The relationships between membrane composition (*i.e.*, fatty acid unsaturation and acyl chain length,
483 cholesterol, phospholipids, and proteins) and the physical parameter often referred to as "membrane
484 fluidity" have been historically discussed [91-93]. Many reports now demonstrate that DHA-
485 containing bilayers behave differently from bilayers containing the other major membrane PUFA,
486 such as ARA [90, 94]. We postulate ARA’s (20:4n-6) role in fish sperm is mostly limited to
487 inflammatory eicosanoids synthesis. Its 22-carbon chain length elongated and desaturated product (*i.e.*,
488 22:5n-6) is known not to be as efficient as 22:5n-3 or 22:6n-3 in biomembrane functions [95, 96].
489 Among features, in particular, DHA dominated membrane bi-layers reportedly [90, 94]: (a) increases
490 the area/headgroup (e.g, lateral surface area for sperm swimming); (b) increases permeability to water
491 and other solutes (e.g., enable osmoregulation, ion-channel function of sperm); (c) increases acyl
492 chain flexibility (e.g., sperm flagellar beating and waveform function), and; (d) increases packing free
493 volume, drives H_{II} phase, induces negative curvature strain and may enhance formation of lateral
494 domains (e.g., all relevant while fusing with egg).

495 ***4.2. Dietary perspective***

496 It is now well established that the apparent digestibility of LC-PUFA (ARA, EPA, DHA) is generally
497 highest of all practical fatty acids, increasing with increased unsaturation, and possibly aided by

498 selective uptake of these essential fatty acids. The digestion, emulsification, absorption and transport
499 of dietary lipids in fish is well described by Turchini and colleagues [97]. The lipid digestion starts
500 with lingual and/or gastric lipases, partitioning lipid droplets into smaller vesicles that are easier to
501 digest once entering the intestinal system. After leaving the stomach, or the anterior part of the
502 intestine in stomach-less fish, lipid is usually solubilized by bile salts and pancreatic lipases (e.g.,
503 pancreatic triacylglycerol lipase, bile-stimulated triacylglycerol lipase, and phospholipase A₂) and
504 other lipases secreted by enterocytes to further reduce droplet size and increase lipolytic efficiency.
505 Following primary digestion, the partially digested lipids, acylglycerols (DAG, 1,3- and 2-MAG),
506 lyso-phospholipids (lyso-PL), free fatty acids (FFA), and cholesterol will then reconstitute into
507 smaller disc-shaped mixed micelles. Once delivered to the surface of enterocytes, they are absorbed
508 across the enterocyte membrane, by membrane-associated proteins, and transported to subcellular
509 compartments (endoplasmic reticulum) or cytosol by fatty acid transport proteins (FATP) [97]. Fish
510 seem to express a variety of FATP in the intestine. Furthermore, due to the gene duplications that
511 have taken place in fish, more variants of FATP are found in fish (genes *fatp 1-6*, including their α or
512 β variants) [97]. After absorption into enterocytes, the products (lyso-PL, FFA, acylglycerols,
513 cholesterol) go on their way to target tissues such as testes, via lipoprotein synthesis and exocytosed
514 to the blood as mature chylomicrons containing high-, low- or very low- density lipoproteins (HDL,
515 LDL, VLDL). The n-3 LC-PUFA (EPA, DHA) are mostly embedded on HDL, while n-6 LC-PUFA
516 (ARA) is embedded on LDL or VLDL [97]. The pre-ponderance of main intestinal and blood
517 lipoproteins in fish differs by evolution too. For example, in primitive fishes like class
518 Chondrichthyes, the major blood lipoprotein appears VLDL-like [98] and relatively high levels of
519 VLDL/LDL are also seen in primitive ray-finned fish, like eels [99]. Higher ray finned fish, and
520 especially teleost, have HDL as the prime lipoprotein [98, 100, 101]. Whether the differences
521 hypothesized in sperm ARA: EPA ratio by evolutionary period (Table 3) could be due to dichotomy
522 in LC-PUFA export mechanisms or eicosanoid preference, merits investigation.

523 *De novo* synthesis of PL is largely dependent on the amount and type of dietary lipid, with PL and
524 TAG having different contributions to endogenous PL re-synthesis [102]. It is believed that the
525 principal precursor of PL synthesis is dietary PL, with lyso-PL and FFA taken up by enterocytes and
526 re-acylated to reform PL [97], in testes for example. After exporting the main products, FFA, lyso-PL
527 and acylglycerols, to target sites such as testicular cells, the triacylglycerol (TAG) and PL re-synthesis
528 begins in endoplasmic reticulum. The most common PL classes in fish sperm membrane (PC, PE,
529 SM) is reconstructed as follows (Fig 8), based on biosynthetic pathways for the major phospholipid
530 classes described [97]. Interestingly, PC can be synthesized from multiple routes (possibilities) while
531 PE offers multiple possibilities to synthesize other PL classes in sperm. This peculiar distribution can
532 be compared to having a population with the majority universal donors with O blood group (the PE),
533 as well as universal recipients with AB blood group (the PC) offering the largest flexibility. It is
534 presently unknown whether such distribution of PL classes in fish sperm is intentional to allow
535 maximum flexibility during spermatogenesis process. In Atlantic salmon, an increase in total testis
536 content of fatty acids and PL, and a shift to higher PL composition was observed in maturing males,
537 concomitant with increases in mRNA levels for genes involved in FA uptake and synthesis, and
538 production of LC-PUFA and PL [103]. Testis fatty acid composition of gilthead seabream fed fish oil
539 or blended vegetable oil diets showed significant differences. Vegetable oils decreased/ halved share
540 of LC-PUFA (ARA, EPA, DHA) within total PUFA in testis (which was somewhat maintained
541 constant due to homeostasis) [104].



542

543 **Fig 8: Biosynthetic pathways for the major phospholipid (PL) classes in fish testes or during**
 544 **spermatogenesis.** Numbers indicate enzymes: (1) CDP-DAG synthases, (2) PA-phosphatases, (3) PI
 545 synthase, (4) PS synthase, (5) CDP-choline: DAG phosphotransferase, (6) CDP-ethanolamine: DAG
 546 phosphotransferase, (7) PE methyltransferase, (8) PS decarboxylase, (9) SM synthase, (10) PE N-
 547 methyltransferase, and (11) PS synthases via base exchange. The green boxes constitute the majority
 548 PL classes in fish sperm (Table 2). The map is re-drawn from Turchini and colleagues [97].

549 The ARA: EPA ratio in sperm could, in theory, be influenced by diet, as it has been discussed
 550 previously by Bell and colleagues [44] that improper ARA: EPA ratio in hatchery fish feed may have
 551 implications on broodstock gamete quality and unknown repercussions on larval vitality. For
 552 example, in goldfish testis, it was shown that n-6 LC-PUFA (ARA) stimulates testosterone
 553 production, whereas n-3 LC-PUFA (EPA) may function as inhibitory regulators of steroid production
 554 [105]. Our analysis suggests that ARA concentration, as well as ARA: EPA ratio in sperm may be
 555 quite resilient to dietary manipulation. There are some **allostasis/ corrective measures to achieve the**
 556 **right n-6: n-3 PUFA ratio and ARA: EPA balance in fish sperm**, independent of dietary perturbations.
 557 Rather, sperm EPA and DHA could be influenced directly by dietary levels. This is even true at the
 558 fish level, as fillet EPA+DHA reserves are synchronous with the richness of EPA+DHA in feed [1,
 559 106]. However, from the dietary supply, EPA can be more prone to β -oxidation and further
 560 bioconversion, whereas DHA is almost non-catabolized in fish and is preferentially deposited and
 561 retained [81]. The same was corroborated in our analysis.

562 DHA is a very long chain n-3 PUFA, which is perhaps the most important ‘structural’ fatty acid for
 563 gametes [107], and more important than EPA. DHA’s central role has been established in human and
 564 other animal spermatozoa by several authors [13, 14, 33, 70]. While DHA in human spermatozoa
 565 could be positively correlated ($r= 0.26-0.67, p<0.01$) with motility, viability, morphology, and total
 566 spermatozoa count, such correlation could not be found for EPA [85]. Our analysis suggests DHA is
 567 stoichiometrically dominating over EPA in fish sperm and in most fish species. DHA is more
 568 abundant than EPA in sperm, even when the food base has the opposite composition (DHA < EPA).
 569 Systematic reviews of randomized controlled clinical trials show that DHA supplementation treats
 570 male infertility in human subjects by improving fertility markers in semen [108, 109]. Beirão and
 571 colleagues [110] reported that the sperm quality of *Solea senegalensis* improved through an enriched

572 DHA diet, specifically the sperm curvilinear velocity and share of progressive sperm. Once it is a very
573 long chain (≥ 22) PUFA such as DHA, it is likely to have a direct effect on sperm membrane fluidity,
574 as shown in human sperm [14, 33]. In a study by Bell and colleagues [12], it appeared that
575 docosapentaenoic acid (22:5n-3, DPA) can substitute functionally for DHA within sperm
576 phospholipid classes of two salmonids. In fact, large amounts of DPA were found in sperm from
577 rabbit (*Oryctolagus cuniculus*), boar (*S. scrofa*), rat (*Rattus rattus*), stallion (*Equus ferus*), and rooster
578 (*Gallus domesticus*), together with lower amounts of DHA [12]. The sperm of Nile tilapia, blunt
579 snouted mullet, annular seabream in our dataset showed such peculiarity of a high DPA share, with
580 lower share of DHA. It is interesting that a simple elimination of one double bond from DHA
581 producing DPA (but omega-6; 22:5n-6) results in the loss of several behavioral features in animals as
582 concluded by Stillwell and Wassall [90], whereas, multiple species of fish or animal substitute DHA
583 by DPA (but omega-3; 22:5n-3). The biomechanical and physiological function of sperm membrane
584 may remain uncompromised by such substitution. The n-3 DPA can form and substitute for some D-
585 series resolvins, otherwise produced by DHA [88].

586 Sperm ARA: EPA ratio is an index of good sperm quality in human [33]. The ARA-to-EPA
587 stoichiometry in cellular membranes determines eicosanoid actions [7, 27], such as in fish sperm [44].
588 Our analysis suggests that the stoichiometry of ARA to EPA in fish sperm is not completely malleable
589 by dietary perturbations; in fact, it may be physiologically regulated. The ARA produces pro-
590 inflammatory eicosanoids that are biologically more active (potent), such as 2-series prostaglandins
591 (PGE_2). The EPA produces reduced pro-inflammatory or anti-inflammatory eicosanoids that are
592 biologically less active (potent), such as 3-series prostaglandins (PGE_3 ; biologically less active) [7].
593 Our analysis showed that ARA is selectively preserved among n-6 PUFAs in fish sperm, but not all
594 fish species have ARA > EPA in sperm. Although eicosanoids and prostaglandins have not been
595 studied in fish sperm in detail, during motility, the ARA: EPA ratio (high activity: low activity
596 eicosanoids) in fish sperm may change significantly, but not DHA: EPA ratio (both low bioactive
597 eicosanoid pre-cursors) [19]. The results indicate there could be a deliberate ARA: EPA balancing in
598 fish sperm, independent of diet and related to species-specific eicosanoids biology or tolerance/
599 detection limits of eicosanoids action (inflammatory, anti-inflammatory responses, chemotaxis, etc.)
600 [27] on prostaglandin receptors on sperm membrane [76], presently unknown in fish.

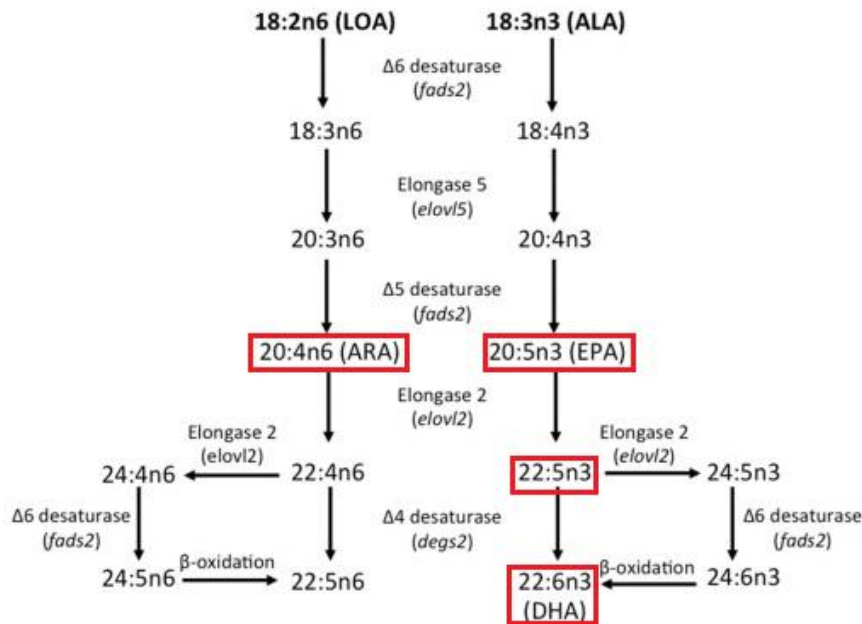
601 ***4.3. Evolutionary and physiological perspective***

602 As proved in the rabbit or stallion testis/ epididymis [111, 112], fish testes could be an active site of
603 fatty acid metabolism. Especially *de-novo* LC-PUFA (ARA, EPA, DHA) biosynthesis by desaturation
604 and elongation, as active as in other PL-rich tissue such as brain [65, 113]. The presence of enzymes
605 ($\Delta 4$, $\Delta 5$, and $\Delta 6$ desaturases, along with elongase-2, elongase-5) necessary for ARA, EPA, and DHA
606 synthesis in fish testes, primary spermatocytes/ spermatogonia (diploid cell) have not been widely
607 studied. However, a recent transcriptomic profiling of fish spermatozoa (haploid from diploid cell)
608 hinted at their existence [114]. During spermatogenesis in eel, the EPA, ARA and DHA were reported
609 to be fortified in testes relative to other fatty acids, although it was hypothesized as transported from
610 liver, and not synthesized on-site [115, 116]. In Atlantic salmon testis, an increased expression of
611 genes for elongation/desaturation to DHA was observed in sexually maturing males relative to
612 immature fish. Besides, there was concomitant increases in phosphatidic acid, a backbone of PL (Fig
613 8), in maturing males compared to immature fish [103]. This coincidence must be towards
614 spermatogenesis, as DHA is a major LC-PUFA and PL is a major lipid class in fish sperm. It is hence
615 suggested that fish testes are a major site of *de-novo* LC-PUFA biosynthesis.

616 It seems [that the fish sperm itself](#) has preserved both lipid catabolism and anabolism functions [117],
617 much like human spermatozoa [118], albeit primitive spermatozoan design or motility duration
618 limited to minutes in fish [16, 34]. Fish sperm has enzymatic capacity for glycolysis, triglyceride, and
619 phospholipid catabolism, as well as triglyceride synthesis [22]. Fatty acid synthase (FAS) activity
620 responsible for initiating *de-novo* fatty acids synthesis is present. The FAS activity is comparable to
621 that of phospholipase, pyruvate kinase, and glucose-6-phosphate dehydrogenase activities in fish
622 sperm [22]. Transcriptomic study in haploid fish spermatozoa recently [114] revealed most enriched
623 genes in fish sperm could be related to fatty acid biosynthesis, namely elongases *elovl1*, *elovl5*, *elovl6*
624 (but no desaturases, *fads*, or *scd* reported). Compared to haploid germ cells, fish sperm could have
625 several-fold upregulated mRNAs pertaining to eicosanoids, namely arachidonate lipoxigenase
626 synthesis (*alox15b*); leukotrienes, thromboxane, or prostaglandin too, mentioned in supplementary
627 data of Castro-Arnau and colleagues [114]. This evidence indicates that haploid fish spermatozoa may
628 share partly the capabilities of diploid somatic cells, which can adjust the fatty [acid composition](#) of its
629 membrane as or when necessary, like epithelioma papillosum cells (EPC) or liver cells in fish [119,
630 120]. [Recently, eicosanoid actions in Eurasian perch sperm, as it ascended from basal metabolism](#)
631 [\(pre-activation quiescent state\) to active metabolism \(post-activation exhaustion\), was also](#)
632 [hypothesized \[19\]](#). Our results of feed to sperm changes in ARA: EPA and DHA: EPA
633 stoichiometries corroborate the presence of such bio-machineries. An absence of desaturases in the
634 transcriptome of spermatozoa of the marine carnivorous fish [114] could be related to marine habitat,
635 as they thrive on an EPA+DHA rich diet with no real need for endogenous bioconversion [3, 4]. A
636 similar transcriptomic study on spermatozoa of a freshwater fish species is needed in the future, to
637 determine if probe *elovls* and *fads*, *scd* are present. As freshwaters are DHA deficient, there is a
638 general need for its endogenous bioconversion to reach physiologically relevant levels [67, 69].
639 However, our results show, not all freshwater species may engage in allostasis (corrections) of DHA
640 in sperm, relative to food base. Some species, such as northern pike, could simply reflect in sperm
641 what it eats in ponds/ lakes [121].

642 It may be related to the loss of certain genetic repertoire at cellular levels to endogenously
643 biosynthesize or do bioconversion of DHA [2, 3]. The 3R WGD (third round whole genome
644 duplication) event occurred around 320-350 mya, leading to the emergence of teleost [122], from
645 marine and cartilaginous ancestors. Before 3R WGD event, there was a similar divergence from
646 marine cartilaginous fishes who had both *fads1* and *fads2* [3] to freshwater cartilaginous fishes such
647 as riverine sting rays (e.g., *Potamotrygon* sp.) that may have partly retained *fads1* or *fads2* [72, 123].
648 In our analysis, the early-diverged freshwater elasmobranchii *P. motoro* (ocellate river stingray),
649 which evolved before the 3R WGD event, shows a weakened DHA biosynthesis capacity or DHA:
650 EPA ratio in sperm. The 4R WGD event (fourth-round whole genome duplication), that happened
651 around 100 mya [122], could be related to *Salmo salar*, *Oncorhynchus mykiss*, *Lota lota*, and *Esox*
652 *lucius*. After 4R WGD, fishes either retained (e.g., rainbow trout) or completely lost (e.g., northern
653 pike, burbot) DHA synthesis capacity [2, 3, 124]. After 4R WGD, Northern pike or burbot became a
654 uniquely diverged genome from a sister group of freshwater Salmoniformes or marine Gadiformes;
655 living in freshwaters [71, 72]. The loss of efficient DHA biosynthesis machinery in Northern pike
656 have been discussed before [125]. In these species, absence of $\Delta 5$ desaturase [126], absence of $\Delta 4$
657 desaturase forcing partial DHA synthesis via less efficient Sprecher pathway [127], presence of
658 monofunctional $\Delta 5$ *fads2* forcing partial DHA synthesis only from C20 precursors, or loss of *elovl2*
659 functions (inability to elongate C20 or C22 substrates for DHA biosynthesis) have weakened DHA
660 synthesis capacity [2, 3, 124]. Irrespective of a loss in desaturation capacity, a single-step elongated
661 product of EPA, 20:5n-3 (i.e., DPA, 22:5n-3) have the potential to substitute DHA within fish sperm
662 phospholipid [12], [as observed in some fish species in this review \(tilapia, blunt snouted mullet,](#)

663 annular seabream). However, a loss of *elovl2* in such fish lineages would prevent such substitution
 664 from happening too. Figure 9 presents a general LC-PUFA biosynthesis pathway, which may be
 665 active in fish testes or sperm.



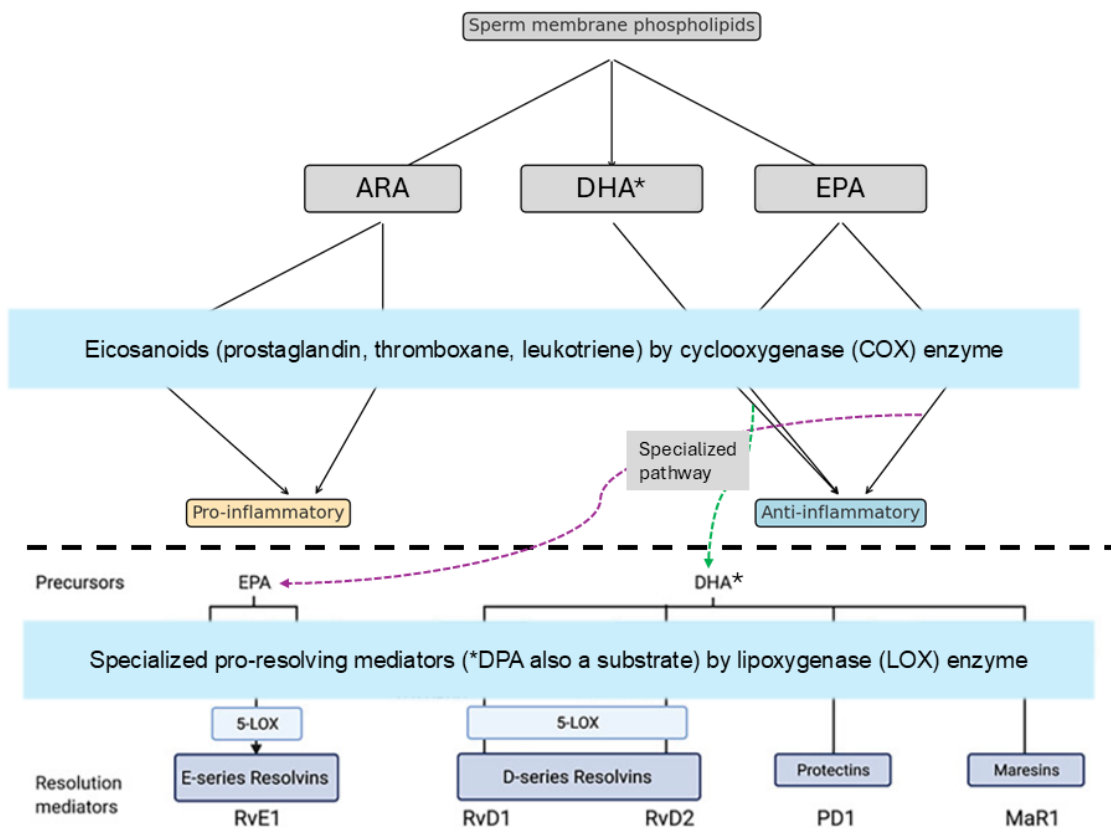
666

667 **Fig 9: Biosynthetic pathways for the major LC-PUFA (ARA, EPA, DHA or DPA) in fish testes**
 668 **or during spermatogenesis.** Major sperm LC-PUFA are highlighted in red box. Above arrows, the
 669 enzymes and genes (in parenthesis) are mentioned, based on a general understanding [97, 113].

670 In wild fish with swimming habitat across different gradients of temperature, pH, salinity/ osmolality,
 671 and ecological niches or hydrostatic pressure, the interquartile range (IR) of whole-body DHA: EPA
 672 ratio is from ~1:1 to ~5:1 (median ~2:1); recalculated from published dataset [73]. This analogy can
 673 be drawn for swimming fish sperm in external or internal environment too. Our observed range of
 674 sperm DHA: EPA ratio was identical to IR of whole-body DHA: EPA by Gladyshev and colleagues
 675 [73]. There were several species having DHA: EPA stoichiometry in sperm below the median of 2 or
 676 even worse <1. An analysis is yet to be done on how missing DHA in sperm impacts swimming
 677 behavior [34] in those species relative to others. Our analysis shows the DHA: EPA stoichiometry in
 678 fish sperm may have an evolutionary/ genetic pre-disposition to engage or not engage in allostasis or
 679 corrective measures from food to sperm. Molecular modeling studies have suggested that DHA
 680 favored conformations (angle-iron or helix) which give phospholipids containing DHA unusual
 681 packing properties. Or, DHA dominated phospholipid fractions give unstable lipid bilayers with
 682 fusogenic properties [12]. The former aspect (packing properties) could be important for sperm
 683 flagellar waveform [34], while the latter (fusogenic properties) may be important for promoting fusion
 684 between the plasma membrane of fish eggs and sperm, as discussed by Bell and colleagues [12]. DHA
 685 has the smallest volume change with the temperature of any fatty acid, thus important in preserving a
 686 thermal adaptation of sperm biomembrane across temperatures [12].

687 In fish sperm, it has been hypothesized that ARA-derived PGE₂ serve more important functions than
 688 EPA-derived PGE₃ [44], and linked with acrosome reactions [75]. In male fish, such prostaglandins
 689 are linked with chemotaxis for opposite sexes [128]; same may be speculated for sperm seeking an
 690 egg. Our analysis suggests, in addition to influences of feed/ diet, Paleozoic and Triassic era diverged
 691 genomes of fish, may be potentially inclined towards lower amounts of highly bioactive eicosanoids

692 in sperm (*i.e.*, ARA-derived PGE₂ < EPA-derived PGE₃). Whereas, the argument by Bell and
693 colleagues that sperm may prefer more of highly bioactive and pro-inflammatory eicosanoids [44]
694 may hold true for fish genomes diverged in Devonian, Jurassic or Cretaceous era. For example, in the
695 Triassic era, diverged fish genome such as European eel [63, 115], diets with high ARA induced
696 medium milt volumes and high sperm motility, but diets with higher EPA induced ‘remarkable’
697 volumes of milt with high sperm motility. It is to be noted that like *fads* and *elovl* gene repertoire
698 (discussed above), the cyclooxygenase (*cox*) gene sequences too show a complicated history of
699 duplications and losses [129]. The *cox* produces prostaglandins in animals. In our reviewed data, we
700 observed that some fish sperm function at higher ARA: EPA ratio, while some function at lower
701 ARA: EPA ratio, showing a potential dichotomy in choice of eicosanoids or prostaglandin synthesis
702 pathways. It may also mean a competition between rate-limiting *cox-1* and *cox-2* enzymes for a
703 common substrate (ARA or EPA; [7]) in a species-specific manner, in fish sperm, which is currently
704 an unexplored territory. However, both isoforms (*cox-1*, *cox-2*) can be expressed constitutively in the
705 testes [88]. In simplistic terms, *cox-1* is the enzyme responsible for basal, constitutive prostaglandin
706 synthesis, whereas *cox-2* is important in various inflammatory and “induced” (e.g., induced breeding)
707 settings [27]. Havird and colleagues [129] hinted at a functional diversification of *cox-2a* and *cox-2b*
708 genes before cyprinids genome diverged, with a possible loss of *cox-2a* in some derived teleosts
709 during that time. The previous era before cyprinids genome emerged (around Jurassic era) was Triassic
710 era and some derived teleost genome during this era could have been clupeids and eel-like fishes [71].
711 Some fish genomes diverged during paleozoic era [71] had also documented possible loss in *cox-1a*
712 gene in some species of the order Salmoniformes [129]. Not much is known in this field, see
713 discussions [129], and our exploratory analyses justifies further scientific investigation with
714 spermatozoa as a model. Recent research has demonstrated an altered behavior of fish sperm under
715 exposure to ovarian fluid. For example, guiding or promoting the spermatozoon to find its way to the
716 egg [31, 32], enhanced sperm motility, prolonged sperm longevity [130], or activation from a
717 prolonged hibernation [131]. This is an indication that eicosanoids or prostaglandin receptors must
718 exist in fish sperm too, as in human or *C. elegans* enabling sperm rheotaxis/ chemotaxis (see
719 introduction). In fact, eicosanoids could be the main drive stimuli, besides ATP, in fish sperm that
720 must swim in external aquatic environment, without immediate direct access to ovarian fluid always
721 (except internal-fertilizing fish). Current research on fish sperm rheotaxis or biomechanics (exposed
722 to ovarian fluid; [31, 32, 130, 131]) discuss theories limited to salinity, osmotic pressure/ osmolality,
723 pH, membrane ion-channel functions, ion, energy, proteins, amino acids and sugar uptake, etc. But an
724 alternating mechanism where ARA-derived eicosanoids cause inflammation, and EPA/DPA/DHA-
725 derived specialized pro-resolving mediators (SPMs; [88]) work to resolve it, cannot be overlooked.
726 All n-3 LC-PUFA in fish sperm (EPA, DHA) is involved in SPM, including n-3 DPA which can
727 substitute DHA in production of D-series resolvins (e.g., RvDn-3DPA [88]). Hence, lipid mediators
728 and eicosanoid biology of fish sperm (Fig 10) is suggested as a new area of research [65].



729

730 **Fig 10: Hypothesized lipid-mediators in fish sperm membrane modulating inflammation and its**
 731 **resolution during motility.** The map is based on Harwood [88], Oliver and colleagues [132].

732 **5. Conclusion**

733 This review shows that LC-PUFAs are not merely structural components of fish sperm, but
 734 biomechanical, physiological cornerstones too. For enabling a smooth function or behavior of sperm,
 735 a fish ejaculating in an external water environment and not in ovarian fluid, must have some auto-
 736 guidance capabilities modulated by eicosanoids balance. A lower ARA: EPA ratio enables searching
 737 longer (motility duration) perhaps, as a higher preponderance of inflammatory eicosanoids or ARA
 738 presence may induce swelling/ inflammation that is not very comfortable to move. Yet some fish
 739 sperm function at higher ARA: EPA ratio, while some function at lower ARA: EPA ratio, showing a
 740 dichotomy in choice of eicosanoids or prostaglandin synthesis pathways. In classic fish sperm
 741 literature, a lot of emphasis has been on biophysics or osmolality-induced swelling of sperm, due to
 742 water intake or malfunctioning of membrane ion channels or depleted energy/ substrates, resulting in
 743 cessation of motility. But the immunological aspects of fish sperm motility such as eicosanoids
 744 modulating inflammation or anti-inflammation are not explored.

745 Negative association of ARA with sperm motility duration but not VCL could be related to an
 746 example of a swollen finger which is movable, but poses some resistance to move, caused by some
 747 local inflammation by higher ARA-oxidized eicosanoids. Conversely, higher DHA (or at times
 748 substituted by DPA) may enable faster searching and fertilizing, as associated positively with both
 749 VCL and motility duration. Additionally, the dietary influences show highest conservation of DHA in
 750 sperm over EPA or ARA, being the only LC-PUFA that allow highest membrane packaging/ re-
 751 packaging/ fusion capabilities, least change to biophysical stress (thermal-pressure conditions), and

752 not usually subjected to catabolism or inflammatory eicosanoid production. The fact that diets can
753 influence them significantly, and some fish genomes (lineages) cannot undertake sufficiently
754 corrective measures during spermatogenesis, needs careful consideration. Especially in hatchery-bred
755 fish for aquaculture or released to natural waters, undesirable man-made selection (trade-off with
756 natural selection) or downstream consequences to progeny may go unchecked/ overlooked due to
757 usually high *libido* and sperm count in male fish, compared to female fish where research is more
758 concentrated.

759 6. References

- 760 [1] Xu, H., G. M. Turchini, D. S. Francis, M. Liang, T. S. Mock, A. Rombenso, and Q. Ai. 2020. Are
761 fish what they eat? A fatty acid's perspective. *Progress in Lipid Research*: 101064.
- 762 [2] Castro, L. F., D. R. Tocher, and O. Monroig. 2016. Long-chain polyunsaturated fatty acid
763 biosynthesis in chordates: Insights into the evolution of Fads and Elovl gene repertoire. *Prog Lipid*
764 *Res* **62**: 25-40.
- 765 [3] Monroig, O., A. C. Shu-Chien, N. Kabeya, D. R. Tocher, and L. F. C. Castro. 2022. Desaturases
766 and elongases involved in long-chain polyunsaturated fatty acid biosynthesis in aquatic animals:
767 From genes to functions. *Prog Lipid Res* **86**: 101157.
- 768 [4] Tocher, D. R. 2010. Fatty acid requirements in ontogeny of marine and freshwater fish.
769 *Aquaculture Research* **41**: 717-732.
- 770 [5] Glencross, B. D. 2009. Exploring the nutritional demand for essential fatty acids by
771 aquaculture species. *Reviews in Aquaculture* **1**: 71-124.
- 772 [6] Xie, D., C. Chen, Y. Dong, C. You, S. Wang, O. Monroig, D. R. Tocher, and Y. Li. 2021.
773 Regulation of long-chain polyunsaturated fatty acid biosynthesis in teleost fish. *Prog Lipid Res* **82**:
774 101095.
- 775 [7] Tocher, D. R. 2003. Metabolism and functions of lipids and fatty acids in teleost fish. *Reviews*
776 *in Fisheries Science* **11**: 107-184.
- 777 [8] Tocher, D. R. 2015. Omega-3 long-chain polyunsaturated fatty acids and aquaculture in
778 perspective. *Aquaculture* **449**: 94-107.
- 779 [9] Turchini, G. M., B. E. Torstensen, and W. K. Ng. 2009. Fish oil replacement in finfish nutrition.
780 *Reviews in Aquaculture* **1**: 10-57.
- 781 [10] Turchini, G. M., J. A. Conlan, J. A. Emery, F. Norambuena, T. O. Akanbi, and D. S. Francis.
782 2024. The melting point of dietary fatty acids is a key regulator of omega-3 fatty acid metabolism in
783 Atlantic salmon. *Aquaculture* **578**.
- 784 [11] Lahnsteiner, F., N. Mansour, M. A. McNiven, and G. F. Richardson. 2009. Fatty acids of
785 rainbow trout (*Oncorhynchus mykiss*) semen: Composition and effects on sperm functionality.
786 *Aquaculture* **298**: 118-124.
- 787 [12] Bell, M. V., J. R. Dick, and C. Buda. 1997. Molecular speciation of fish sperm phospholipids:
788 large amounts of dipolyunsaturated phosphatidylserine. *Lipids* **32**: 1085-1091.
- 789 [13] Esmaili, V., A. H. Shahverdi, M. H. Moghadasian, and A. R. Alizadeh. 2015. Dietary fatty
790 acids affect semen quality: a review. *Andrology-Us* **3**: 450-461.
- 791 [14] Collodel, G., C. Castellini, J. C. Lee, and C. Signorini. 2020. Relevance of Fatty Acids to Sperm
792 Maturation and Quality. *Oxid Med Cell Longev* **2020**: 7038124.
- 793 [15] Craig, L. B., R. S. Brush, M. T. Sullivan, M. T. Zavy, M. P. Agbaga, and R. E. Anderson. 2019.
794 Decreased very long chain polyunsaturated fatty acids in sperm correlates with sperm quantity and
795 quality. *J Assist Reprod Gen* **36**: 1379-1385.
- 796 [16] Mattei, X. 1991. Spermatozoon Ultrastructure and Its Systematic Implications in Fishes.
797 *Canadian Journal of Zoology* **69**: 3038-3055.
- 798 [17] Rahi, D., B. Dzyuba, M. Xin, Y. Cheng, and V. Dzyuba. 2020. Energy pathways associated with
799 sustained spermatozoon motility in the endangered Siberian sturgeon *Acipenser baerii*. *J Fish Biol*
800 **97**: 435-443.

801 [18] Rahi, D., B. Dzyuba, T. Policar, O. Malinovskyi, M. Rodina, and V. Dzyuba. 2021. Bioenergetic
802 Pathways in the Sperm of an Under-Ice Spawning Fish, Burbot (*Lota lota*): The Role of Mitochondrial
803 Respiration in a Varying Thermal Environment. *Biology (Basel)* **10**.

804 [19] Roy, D. R., K. Roy, A. Tomcala, J. Matousek, J. Mraz, and V. Stejskal. 2024. Fatty acid and
805 energy (ATP-related compounds) metabolism of Eurasian perch (*Perca fluviatilis*) sperm: Endogenous
806 metabolic strategy. *Aquaculture* **579**.

807 [20] Lahnsteiner, F., R. A. Patzner, and T. Weismann. 1992. Monosaccharides as energy resources
808 during motility of spermatozoa in *Leuciscus cephalus* (Cyprinidae, Teleostei). *Fish Physiol Biochem*
809 **10**: 283-289.

810 [21] Dreanno, C., F. o. Seguin, J. Cosson, M. Suquet, and R. Billard. 2000. ¹H-NMR and ³¹P-NMR
811 analysis of energy metabolism of quiescent and motile turbot (*Psetta maxima*) spermatozoa. *Journal*
812 *of Experimental Zoology* **286**: 513-522.

813 [22] Lahnsteiner, F., R. A. Patzner, and T. Weismann. 1993. Energy resources of spermatozoa of
814 the rainbow trout *Oncorhynchus mykiss* (Pisces, Teleostei). *Reprod Nutr Dev* **33**: 349-360.

815 [23] Lahnsteiner, F., T. Weismann, and R. A. Patzner. 1997. Aging processes of rainbow trout
816 semen during storage. *Prog Fish Cult* **59**: 272-279.

817 [24] Houten, S. M., S. Violante, F. V. Ventura, and R. J. Wanders. 2016. The Biochemistry and
818 Physiology of Mitochondrial Fatty Acid beta-Oxidation and Its Genetic Disorders. *Annu Rev Physiol*
819 **78**: 23-44.

820 [25] Kiessling, K. H., and A. Kiessling. 1993. Selective Utilization of Fatty-Acids in Rainbow-Trout
821 (*Oncorhynchus-Mykiss* Walbaum) Red Muscle Mitochondria. *Can J Zool* **71**: 248-251.

822 [26] Dadras, H., S. Sampels, A. Golpour, V. Dzyuba, J. Cosson, and B. Dzyuba. 2017. Analysis of
823 common carp *Cyprinus carpio* sperm motility and lipid composition using different in vitro
824 temperatures. *Anim Reprod Sci* **180**: 37-43.

825 [27] Funk, C. D. 2001. Prostaglandins and leukotrienes: advances in eicosanoid biology. *Science*
826 **294**: 1871-1875.

827 [28] Lishko, P. V., I. L. Botchkina, and Y. Kirichok. 2011. Progesterone activates the principal Ca⁺
828 channel of human sperm. *Nature* **471**: 387-+.

829 [29] Strünker, T., N. Goodwin, C. Brenker, N. D. Kashikar, I. Weyand, R. Seifert, and U. B. Kaupp.
830 2011. The CatSper channel mediates progesterone-induced Ca⁺ influx in human sperm. *Nature* **471**:
831 382-+.

832 [30] Tiwary, E., M. H. Hu, and J. K. Prasain. 2021. Sperm-Guiding Unconventional Prostaglandins
833 in
834 : Synthesis and Signaling. *Metabolites* **11**.

835 [31] Kholodnyy, V., B. Dzyuba, M. Rodina, H. Bloomfield-Gadêlha, M. Yoshida, J. Cosson, and S.
836 Boryshpolets. 2021. Does the Rainbow Trout Ovarian Fluid Promote the Spermatozoon on Its Way to
837 the Egg? *International Journal of Molecular Sciences* **22**.

838 [32] Kholodnyy, V., K. Shibata, A. T. Beken, B. Dzyuba, H. Bloomfield-Gadêlha, J. Cosson, and S.
839 Boryshpolets. 2022. Common carp spermatozoa performance is significantly affected by ovarian
840 fluid. *Aquaculture* **554**.

841 [33] Collodel, G., E. Moretti, D. Noto, F. Iacoponi, and C. Signorini. 2020. Fatty Acid Profile and
842 Metabolism Are Related to Human Sperm Parameters and Are Relevant in Idiopathic Infertility and
843 Varicocele. *Mediators Inflamm* **2020**: 3640450.

844 [34] Cosson, J. 2010. Frenetic activation of fish spermatozoa flagella entails short-term motility,
845 portending their precocious decadence. *J Fish Biol* **76**: 240-279.

846 [35] Lahnsteiner, F., B. Berger, T. Weismann, and R. A. Patzner. 1998. Determination of semen
847 quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal plasma parameters,
848 and spermatozoal metabolism. *Aquaculture* **163**: 163-181.

849 [36] Baeza, R., I. Mazzeo, M. Vílchez, V. Gallego, D. Peñaranda, L. Pérez, and J. Asturiano. 2014.
850 Effect of thermal regime on fatty acid dynamics in male European eels (*Anguilla anguilla*) during
851 hormonally-induced spermatogenesis. *Aquaculture* **430**: 86-97.

852 [37] Mehdinejad, N., V. Taghizadeh, and M. R. Imanpour. 2013. The correlation between semen
853 fatty acids with spermatological parameters in Iranian sturgeon (*Acipenser persicus*) brood stocks.
854 *Global Veterinaria* **11**: 23-29.

855 [38] Ackman, R. G. 1972. The analysis of fatty acids and related materials by gas-liquid
856 chromatography. *Progress in the Chemistry of Fats and other Lipids* **12**: 165-284.

857 [39] Christie, W. W., and X. Han. 2010. Lipid Analysis – Isolation, Separation, Identification and
858 Lipidomic Analysis (4th edition) The Oily Press and Woodhead Publishing Limited, Bridgwater,
859 England.

860 [40] Ackman, R. G. 2002. The gas chromatograph in practical analyses of common and
861 uncommon fatty acids for the 21st century. *Anal Chim Acta* **465**: 175-192.

862 [41] Andrade, C. 2020. Understanding the Difference Between Standard Deviation and Standard
863 Error of the Mean, and Knowing When to Use Which. *Indian J Psychol Med* **42**: 409-410.

864 [42] Martínez-Páramo, S., P. Diogo, J. Beirão, M. Dinis, and E. Cabrita. 2012. Sperm lipid
865 peroxidation is correlated with differences in sperm quality during the reproductive season in
866 precocious European sea bass (*Dicentrarchus labrax*) males. *Aquaculture* **358**: 246-252.

867 [43] Martínez-Páramo, S., P. Diogo, M. Dinis, M. Herráez, C. Sarasquete, and E. Cabrita. 2012. Sea
868 bass sperm freezability is influenced by motility variables and membrane lipid composition but not
869 by membrane integrity and lipid peroxidation. *Animal reproduction science* **131**: 211-218.

870 [44] Bell, M. V., J. R. Dick, M. Thrush, and J. C. Navarro. 1996. Decreased 20:4n-6/20:5n-3 ratio in
871 sperm from cultured sea bass, *Dicentrarchus labrax*, broodstock compared with wild fish.
872 *Aquaculture* **144**: 189-199.

873 [45] Engel, K. M., V. Dzyuba, A. Ninhaus-Silveira, R. Verissimo-Silveira, D. Dannenberger, J.
874 Schiller, C. Steinbach, and B. Dzyuba. 2020. Sperm Lipid Composition in Early Diverged Fish Species:
875 Internal vs. External Mode of Fertilization. *Biomolecules* **10**.

876 [46] Engel, K. M., S. Sampels, B. Dzyuba, P. Podhorec, T. Policar, D. Dannenberger, and J. Schiller.
877 2019. Swimming at different temperatures: The lipid composition of sperm from three freshwater
878 fish species determined by mass spectrometry and nuclear magnetic resonance spectroscopy.
879 *Chemistry and physics of lipids* **221**: 65-72.

880 [47] Labbe, C., G. Maise, K. Muller, A. Zachowski, S. Kaushik, and M. Loir. 1995. Thermal
881 acclimation and dietary lipids alter the composition, but not fluidity, of trout sperm plasma
882 membrane. *Lipids* **30**: 23-33.

883 [48] Drokin, S. I. 1993. Phospholipids and fatty acids of phospholipids of sperm from several
884 freshwater and marine species of fish. *Comparative Biochemistry and Physiology Part B:*
885 *Comparative Biochemistry* **104**: 423-428.

886 [49] Das, B. K., D. Sadhukhan, N. Chakraborty, S. Ganguly, and A. K. Sahoo. 2023. An insight into
887 milt antioxidant, fatty acid, amino acid composition and testis histology of an anadromous
888 euryhaline fish *Tenualosa ilisha*, Ham. 1822 for its conservation and aquaculture perspectives.
889 *Aquaculture* **576**: 739832.

890 [50] Herrera, F., S. Boryshpolets, J. Mraz, J. Knowles, and O. Bondarenko. 2022. Pikeperch
891 (*Sander lucioperca*) spermatozoa motility and volume regulation under different osmotic and ionic
892 conditions. *Fish Physiology and Biochemistry* **48**: 899-910.

893 [51] Henrotte, E., V. Kaspar, M. Rodina, M. Psenicka, O. Linhart, and P. Kestemont. 2010. Dietary
894 n-3/n-6 ratio affects the biochemical composition of Eurasian perch (*Perca fluviatilis*) semen but not
895 indicators of sperm quality. *Aquaculture research* **41**: e31-e38.

896 [52] Köprücü, K., M. E. Yonar, and S. Özcan. 2015. Effect of dietary n-3 polyunsaturated fatty
897 acids on antioxidant defense and sperm quality in rainbow trout (*Oncorhynchus mykiss*) under
898 regular stripping conditions. *Animal reproduction science* **163**: 135-143.

899 [53] Labbe, C., M. Loir, S. Kaushik, and G. Maise. 1993. The influence of both rearing
900 temperature and dietary lipid origin on fatty acid composition of spermatozoan polar lipids in
901 rainbow trout (*Oncorhynchus mykiss*). Effect on sperm cryopreservation tolerance.

902 [54] Liu, L., A. Ciereszko, S. Czesny, and K. Dabrowski. 1997. Dietary ascorbyl monophosphate
903 depresses lipid peroxidation in rainbow trout spermatozoa. *Journal of Aquatic Animal Health* **9**: 249-
904 257.

905 [55] Pustowka, C., M. A. McNiven, G. F. Richardson, and S. P. Lall. 2000. Source of dietary lipid
906 affects sperm plasma membrane integrity and fertility in rainbow trout *Oncorhynchus mykiss*
907 (Walbaum) after cryopreservation. *Aquaculture Research* **31**: 297-305.

908 [56] Vassallo-Agius, R., T. Watanabe, G. Yoshizaki, S. Satoh, and Y. Takeuchi. 2001. Quality of eggs
909 and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and its effects on the
910 lipid and fatty acid components of eggs, semen and livers. *Fisheries Science* **67**: 818-827.

911 [57] Blecha, M., B. Dzyuba, S. Boryshpolets, Y. Horokhovatskyi, H. Dadras, O. Malinovskyi, S.
912 Sampels, and T. Policar. 2018. Spermatozoa quality and sperm lipid composition in intensively
913 cultured and wild burbot (*Lota lota*). *Animal reproduction science* **198**: 129-136.

914 [58] Dadras, H., T. Policar, D. Loginov, S. Boryshpolets, Y. Loginova, S. Sampels, A. Sotnikov, V.
915 Kholodnyy, J. Sterba, and M. Xin. 2021. Relationship of motility activation to lipid composition,
916 protein profile, and swelling rate of burbot *Lota lota* spermatozoon following change of temperature
917 and osmolality. *Frontiers in marine science* **8**: 736087.

918 [59] Mansour, N., F. Lahnsteiner, M. A. McNiven, G. F. Richardson, and C. S. Pelletier. 2011.
919 Relationship between fertility and fatty acid profile of sperm and eggs in Arctic char, *Salvelinus*
920 *alpinus*. *Aquaculture* **318**: 371-378.

921 [60] Dzyuba, V., S. Sampels, A. Ninhaus-Silveira, M. Kahanec, R. Veríssimo-Silveira, M. Rodina, J.
922 Cosson, S. Boryshpolets, M. Selinger, and J. Sterba. 2019. Sperm motility and lipid composition in
923 internally fertilizing ocellate river stingray *Potamotrygon motoro*. *Theriogenology* **130**: 26-35.

924 [61] Engel, K. M., V. Dzyuba, A. Ninhaus-Silveira, R. Veríssimo-Silveira, D. Dannenberger, J.
925 Schiller, C. Steinbach, and B. Dzyuba. 2020. Sperm lipid composition in early diverged fish species:
926 internal vs. external mode of fertilization. *Biomolecules* **10**: 172.

927 [62] Asturiano, J. F., L. A. Sorbera, M. Carrillo, S. Zanuy, J. Ramos, J. C. Navarro, and N. Bromage.
928 2001. Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed two PUFA-
929 enriched experimental diets: a comparison with males fed a wet diet. *Aquaculture* **194**: 173-190.

930 [63] Butts, I. A., R. Baeza, J. G. Stottrup, M. Kruger-Johnsen, C. Jacobsen, L. Perez, J. F. Asturiano,
931 and J. Tomkiewicz. 2015. Impact of dietary fatty acids on muscle composition, liver lipids, milt
932 composition and sperm performance in European eel. *Comp Biochem Physiol A Mol Integr Physiol*
933 **183**: 87-96.

934 [64] Díaz, R., M. Lee-Estevez, J. Quiñones, K. Dumorné, S. Short, P. Ulloa-Rodríguez, I.
935 Valdebenito, N. Sepúlveda, and J. G. Farías. 2019. Changes in Atlantic salmon (*Salmo salar*) sperm
936 morphology and membrane lipid composition related to cold storage and cryopreservation. *Animal*
937 *reproduction science* **204**: 50-59.

938 [65] Rahi Roy, D., K. Roy, A. Tomcala, J. Matousek, J. Mraz, and V. Stejskal. 2024. Fatty acid and
939 energy (ATP-related compounds) metabolism of Eurasian perch (*Perca fluviatilis*) sperm: Endogenous
940 metabolic strategy. *Aquaculture* **579**: 740183.

941 [66] Scharnweber, K., F. Chaguaceda, and P. Eklov. 2021. Fatty acid accumulation in feeding types
942 of a natural freshwater fish population. *Oecologia* **196**: 53-63.

943 [67] Pilecky, M., M. Mathieu-Resuge, L. Závorka, L. Fehlinger, K. Winter, D. Martin-Creuzburg, and
944 M. J. Kainz. 2022. Common carp obtain omega-3 long-chain polyunsaturated fatty acids via dietary
945 supply and endogenous bioconversion in semi-intensive aquaculture ponds. *Aquaculture* **561**:
946 738731.

947 [68] Roy, K., P. Dvorak, Z. Machova, and J. Mraz. 2023. Nutrient footprint versus EPA + DHA
948 security in land-locked regions-more of local pond farmed, imported marine fish or fish oil capsules?
949 *NPJ Sci Food* **7**: 48.

950 [69] Ahlgren, G., T. Vrede, and W. Goedkoop. 2009. Fatty acid ratios in freshwater fish,
951 zooplankton and zoobenthos—are there specific optima? *In* *Lipids in aquatic ecosystems*. Springer.
952 147-178.

953 [70] Ollero, A., R. D. Powers, and J. G. Alvarez. 2000. Variation of docosahexaenoic acid content
954 in subsets of human spermatozoa at different stages of maturation: Implications for sperm
955 lipoperoxidative damage. *Mol Reprod Dev* **55**: 326-334.

956 [71] Hughes, L. C., G. Orti, Y. Huang, Y. Sun, C. C. Baldwin, A. W. Thompson, D. Arcila, R. R.
957 Betancur, C. Li, L. Becker, N. Bellora, X. Zhao, X. Li, M. Wang, C. Fang, B. Xie, Z. Zhou, H. Huang, S.
958 Chen, B. Venkatesh, and Q. Shi. 2018. Comprehensive phylogeny of ray-finned fishes (Actinopterygii)
959 based on transcriptomic and genomic data. *Proc Natl Acad Sci U S A* **115**: 6249-6254.

960 [72] Berthelot, C., F. Brunet, D. Chalopin, A. Juanchich, M. Bernard, B. Noel, P. Bento, C. Da Silva,
961 K. Labadie, A. Alberti, J. M. Aury, A. Louis, P. Dehais, P. Bardou, J. Montfort, C. Klopp, C. Cabau, C.
962 Gaspin, G. H. Thorgaard, M. Boussaha, E. Quillet, R. Guyomard, D. Galiana, J. Bobe, J. N. Volff, C.
963 Genet, P. Wincker, O. Jaillon, H. Roest Crolius, and Y. Guiguen. 2014. The rainbow trout genome
964 provides novel insights into evolution after whole-genome duplication in vertebrates. *Nat Commun*
965 **5**: 3657.

966 [73] Gladyshev, M. I., N. N. Sushchik, A. P. Tolomeev, and Y. Y. Dgebuadze. 2018. Meta-analysis of
967 factors associated with omega-3 fatty acid contents of wild fish. *Reviews in Fish Biology and Fisheries*
968 **28**: 277-299.

969 [74] Svanborg, K., C. Gottlieb, E. Bendvold, and M. Bygdeman. 1989. Variation in, and Inter-
970 Relationship between, Prostaglandin Levels and Other Semen Parameters in Normal Men. *Int J*
971 *Androl* **12**: 411-419.

972 [75] Breitbart, H., Y. Shalev, S. Marcus, and M. Shemesh. 1995. Modulation of prostaglandin
973 synthesis in mammalian sperm acrosome reaction. *Hum Reprod* **10**: 2079-2084.

974 [76] Mercado, E., M. Villalobos, R. Dominguez, and A. Rosado. 1978. Differential binding of PGE-1
975 and PGF-2alpha to the human spermatozoa membrane. *Life Sci* **22**: 429-436.

976 [77] Butts, I. A. E., G. S. Hilmarsdóttir, V. Zadmajid, V. Gallego, J. G. Stottrup, C. Jacobsen, M.
977 Krüger-Johnsen, S. N. Politis, J. F. Asturiano, L. K. Holst, and J. Tomkiewicz. 2020. Dietary amino acids
978 impact sperm performance traits for a catadromous fish,
979 reared in captivity. *Aquaculture* **518**.

980 [78] Rahman, M. M., C. Gasparini, G. M. Turchini, and J. P. Evans. 2014. Experimental reduction in
981 dietary omega-3 polyunsaturated fatty acids depresses sperm competitiveness. *Biol Lett* **10**.

982 [79] Rahman, M. M., G. M. Turchini, C. Gasparini, F. Norambuena, and J. P. Evans. 2014. The
983 expression of pre- and postcopulatory sexually selected traits reflects levels of dietary stress in
984 guppies. *PLoS One* **9**: e105856.

985 [80] Diogo, P., G. Martins, P. Gavaia, W. Pinto, J. Dias, L. Cancela, and S. Martínez-Páramo. 2015.
986 Assessment of nutritional supplementation in phospholipids on the reproductive performance of
987 zebrafish, (Hamilton, 1822). *Journal of Applied Ichthyology* **31**: 3-9.

988 [81] Emery, J. A., F. Norambuena, J. Trushenski, and G. M. Turchini. 2016. Uncoupling EPA and
989 DHA in Fish Nutrition: Dietary Demand is Limited in Atlantic Salmon and Effectively Met by DHA
990 Alone. *Lipids* **51**: 399-412.

991 [82] de Vivar, M. E. D., E. V. Zárate, T. Rubilar, L. Epherra, M. G. Avaro, and M. A. Sewell. 2019.
992 Lipid and fatty acid profiles of gametes and spawned gonads of *Arbacia dufresnii* (Echinodermata:
993 Echinoidea): sexual differences in the lipids of nutritive phagocytes. *Mar Biol* **166**.

994 [83] Aksoy, Y., H. Aksoy, K. Altinkaynak, H. R. Aydin, and A. Ozkan. 2006. Sperm fatty acid
995 composition in subfertile men. *Prostaglandins Leukot Essent Fatty Acids* **75**: 75-79.

996 [84] Lin, Y., X. Cheng, J. Mao, D. Wu, B. Ren, S. Y. Xu, Z. F. Fang, L. Q. Che, C. M. Wu, and J. Li.
997 2016. Effects of different dietary n-6/n-3 polyunsaturated fatty acid ratios on boar reproduction.
998 *Lipids Health Dis* **15**: 31.

999 [85] Martinez-Soto, J. C., J. Landeras, and J. Gadea. 2013. Spermatozoa and seminal plasma fatty
1000 acids as predictors of cryopreservation success. *Andrology-Us* **1**: 365-375.

1001 [86] Signorini, C., E. Moretti, D. Noto, L. Micheli, R. Ponchia, and G. Collodel. 2022. Fatty Acid
1002 Oxidation and Pro-Resolving Lipid Mediators Are Related to Male Infertility. *Antioxidants-Basel* **11**.

1003 [87] Kohli, P., and B. D. Levy. 2009. Resolvins and protectins: mediating solutions to
1004 inflammation. *Brit J Pharmacol* **158**: 960-971.

1005 [88] Harwood, J. L. 2023. Polyunsaturated Fatty Acids: Conversion to Lipid Mediators, Roles in
1006 Inflammatory Diseases and Dietary Sources. *International Journal of Molecular Sciences* **24**.

1007 [89] Zhang, L. L., N. Terrando, Z. Z. Xu, S. S. Bang, S. E. Jordt, W. Maixner, C. N. Serhan, and R. R.
1008 Ji. 2018. Distinct Analgesic Actions of DHA and DHA-Derived Specialized Pro-Resolving Mediators on
1009 Post-operative Pain After Bone Fracture in Mice. *Front Pharmacol* **9**.

1010 [90] Stillwell, W., and S. R. Wassall. 2003. Docosahexaenoic acid: membrane properties of a
1011 unique fatty acid. *Chem Phys Lipids* **126**: 1-27.

1012 [91] Stubbs, C. D., and A. D. Smith. 1984. The modification of mammalian membrane
1013 polyunsaturated fatty acid composition in relation to membrane fluidity and function. *Biochim*
1014 *Biophys Acta* **779**: 89-137.

1015 [92] Quinn, P. J., F. Joo, and L. Vigh. 1989. The role of unsaturated lipids in membrane structure
1016 and stability. *Prog Biophys Mol Biol* **53**: 71-103.

1017 [93] Hazel, J. R., and E. E. Williams. 1990. The Role of Alterations in Membrane Lipid-Composition
1018 in Enabling Physiological Adaptation of Organisms to Their Physical-Environment. *Progress in Lipid*
1019 *Research* **29**: 167-227.

1020 [94] Mitchell, D. C., and B. J. Litman. 2001. Modulation of Receptor Signaling by Phospholipid Acyl
1021 Chain Composition. *In Fatty Acids: Physiological and Behavioral Functions*. D. I. Mostofsky, S. Yehuda,
1022 and N. Salem, editors. Humana Press, Totowa, NJ. 23-40.

1023 [95] Ward, G., J. Woods, M. Reyzer, and N. Salem. 1996. Artificial rearing of infant rats on milk
1024 formula deficient in n-3 essential fatty acids: A rapid method for the production of experimental n-3
1025 deficiency. *Lipids* **31**: 71-77.

1026 [96] Eldho, N. V., S. E. Feller, S. Tristram-Nagle, I. V. Polozov, and K. Gawrisch. 2003.
1027 Polyunsaturated docosahexaenoic vs docosapentaenoic acid - Differences in lipid matrix properties
1028 from the loss of one double bond. *J Am Chem Soc* **125**: 6409-6421.

1029 [97] Turchini, G. M., D. S. Francis, Z.-Y. Du, R. E. Olsen, E. Ringø, and D. R. Tocher. 2022. Chapter 5
1030 - The lipids. *In Fish Nutrition (Fourth Edition)*. R. W. Hardy and S. J. Kaushik, editors. Academic Press.
1031 303-467.

1032 [98] Babin, P. J., and J. M. Vernier. 1989. Plasma lipoproteins in fish. *Journal of Lipid Research* **30**:
1033 467-489.

1034 [99] Ando, S., A. Tachibana, S. Yamada, and H. Kishimura. 2005. Apolipoprotein complexity in
1035 Japanese eel
1036 :: Truncated apolipoprotein A-I and apolipoprotein A-I-like protein in plasma lipoproteins. *Biosci*
1037 *Biotech Bioch* **69**: 2258-2262.

1038 [100] Kjær, M. A., A. Vegusdal, G. M. Berge, T. F. Galloway, M. Hillestad, Å. Krogdahl, H. Holm, and
1039 B. Ruyter. 2009. Characterisation of lipid transport in Atlantic cod (*Gadus morhua*) when fasted and
1040 fed high or low fat diets. *Aquaculture* **288**: 325-336.

1041 [101] Kondo, H., K. Morinaga, R. Misaki, M. Nakaya, and S. Watabe. 2005. Characterization of the
1042 pufferfish
1043 apolipoprotein multigene family. *Gene* **346**: 257-266.

1044 [102] Tocher, D. R., E. Å. Bendiksen, P. J. Campbell, and J. G. Bell. 2008. The role of phospholipids
1045 in nutrition and metabolism of teleost fish. *Aquaculture* **280**: 21-34.

1046 [103] Bøgevik, A. S., E. S. Hayman, M. T. Bjerke, J. E. Dessen, K. A. Rørvik, and J. A. Luckenbach.
1047 2020. Phospholipid and LC-PUFA metabolism in Atlantic salmon
1048 testes during sexual maturation. *Plos One* **15**.

1049 [104] Wassef, E. A., O. M. Wahbi, and S. H. Shalaby. 2012. Effects of dietary vegetable oils on liver
1050 and gonad fatty acid metabolism and gonad maturation in gilthead seabream (
1051) males and females. *Aquaculture International* **20**: 255-281.

1052 [105] Wade, M. G., G. Vanderkraak, M. F. Gerrits, and J. S. Ballantyne. 1994. Release and
1053 Steroidogenic Actions of Polyunsaturated Fatty-Acids in the Goldfish Testis. *Biol Reprod* **51**: 131-139.

1054 [106] Sprague, M., J. R. Dick, and D. R. Tocher. 2016. Impact of sustainable feeds on omega-3 long-
1055 chain fatty acid levels in farmed Atlantic salmon, 2006-2015. *Sci Rep* **6**: 21892.

1056 [107] Pickova, J., A. Kiessling, A. Pettersson, and P. C. Dutta. 1999. Fatty acid and carotenoid
1057 composition of eggs from two nonanadromous Atlantic salmon stocks of cultured and wild origin.
1058 *Fish Physiology and Biochemistry* **21**: 147-156.

1059 [108] Gonzalez-Ravina, C., M. Aguirre-Lipperheide, F. Pinto, D. Martin-Lozano, M. Fernandez-
1060 Sanchez, V. Blasco, E. Santamaria-Lopez, and L. Candenas. 2018. Effect of dietary supplementation
1061 with a highly pure and concentrated docosahexaenoic acid (DHA) supplement on human sperm
1062 function. *Reprod Biol* **18**: 282-288.

1063 [109] Falsig, A. L., C. S. Gleerup, and U. B. Knudsen. 2019. The influence of omega-3 fatty acids on
1064 semen quality markers: a systematic PRISMA review. *Andrology-Us* **7**: 794-803.

1065 [110] Beirao, J., E. Cabrita, F. Soares, M. P. Herráez, and M. T. Dinis. 2008. Cellular damage in
1066 spermatozoa from wild-captured as detected by two different assays:: comet analysis and Annexin
1067 V-Fluorescein staining. *Journal of Applied Ichthyology* **24**: 508-513.

1068 [111] Gautier, C., D. Scarlet, R. Ertl, I. Walter, M. Wulf, C. Nagel, J. Aurich, and C. Aurich. 2020.
1069 Expression of enzymes involved in polyunsaturated fatty acid synthesis in the stallion testis and
1070 epididymis. *Reprod Fert Develop* **32**: 851-861.

1071 [112] Castellini, C., S. Mattioli, E. Moretti, E. Cotozzolo, F. Perini, A. Dal Bosco, C. Signorini, D. Noto,
1072 G. Belmonte, E. Lasagna, G. Brecchia, and G. Collodel. 2022. Expression of genes and localization of
1073 enzymes involved in polyunsaturated fatty acid synthesis in rabbit testis and epididymis. *Scientific*
1074 *Reports* **12**.

1075 [113] Fadhlou, M., F. Pierron, and P. Couture. 2018. Temperature and metal exposure affect
1076 membrane fatty acid composition and transcription of desaturases and elongases in fathead minnow
1077 muscle and brain. *Ecotoxicology and Environmental Safety* **148**: 632-643.

1078 [114] Castro-Arnau, J., F. Chauvigne, J. Gomez-Garrido, A. Esteve-Codina, M. Dabad, T. Alioto, R. N.
1079 Finn, and J. Cerda. 2022. Developmental RNA-Seq transcriptomics of haploid germ cells and
1080 spermatozoa uncovers novel pathways associated with teleost spermiogenesis. *Sci Rep* **12**: 14162.

1081 [115] Baeza, R., I. Mazzeo, M. C. Vilchez, V. Gallego, D. S. Penaranda, L. Perez, and J. F. Asturiano.
1082 2015. Relationship between sperm quality parameters and the fatty acid composition of the muscle,
1083 liver and testis of European eel. *Comp Biochem Physiol A Mol Integr Physiol* **181**: 79-86.

1084 [116] Baeza, R., I. Mazzeo, M. C. Vilchez, V. Gallego, D. S. Peñaranda, L. Pérez, and J. F. Asturiano.
1085 2014. Effect of thermal regime on fatty acid dynamics in male European eels (
1086) during hormonally-induced spermatogenesis. *Aquaculture* **430**: 86-97.

1087 [117] Terner, C., and G. Korsh. 1963. The Biosynthesis of Fatty Acids of Glycerides and
1088 Phosphatides by Isolated Spermatozoa of the Rainbow Trout. *J Cell Comp Physiol* **62**: 251-256.

1089 [118] Minassian, E. S., and C. Terner. 1966. Biosynthesis of lipids by human and fish spermatozoa.
1090 *Am J Physiol* **210**: 615-618.

1091 [119] Tocher, D. R., and J. R. Dick. 1999. Polyunsaturated fatty acid metabolism in a cell culture
1092 model of essential fatty acid deficiency in a freshwater fish, carp (*Cyprinus carpio*). *Fish Physiology*
1093 *and Biochemistry* **21**: 257-267.

1094 [120] Livermore, R. C., and J. R. Hazel. 1988. Acylation of lysophosphatidylcholine in liver
1095 microsomes of thermally acclimated trout. *Am J Physiol* **255**: R923-928.

1096 [121] Desvillettes, C., G. Bourdier, and J. C. Breton. 1994. Lipid Class and Fatty-Acid Composition of
1097 Planktivorous Larval Pike Esox-Lucius Living in a Natural Pond. *Aquatic Living Resources* **7**: 67-77.

1098 [122] Pasquier, J., C. Cabau, T. Nguyen, E. Jouanno, D. Severac, I. Braasch, L. Journot, P. Pontarotti,
1099 C. Klopp, J. H. Postlethwait, Y. Guiguen, and J. Bobe. 2016. Gene evolution and gene expression after
1100 whole genome duplication in fish: the PhyloFish database. *Bmc Genomics* **17**: 368.

1101 [123] Zhou, J., A. Liu, F. He, Y. Zhang, L. Shen, J. Yu, and X. Zhang. 2023. Draft Genome of White-
1102 blotched River Stingray Provides Novel Clues for Niche Adaptation and Skeleton Formation.
1103 *Genomics Proteomics Bioinformatics* **21**: 501-514.

1104 [124] Oboh, A., N. Kabeya, G. Carmona-Antonanzas, L. F. C. Castro, J. R. Dick, D. R. Tocher, and O.
1105 Monroig. 2017. Two alternative pathways for docosahexaenoic acid (DHA, 22:6n-3) biosynthesis are
1106 widespread among teleost fish. *Sci Rep* **7**: 3889.

1107 [125] Carmona-Antonanzas, G., D. R. Tocher, J. B. Taggart, and M. J. Leaver. 2013. An evolutionary
1108 perspective on Elovl5 fatty acid elongase: comparison of Northern pike and duplicated paralogs from
1109 Atlantic salmon. *BMC Evol Biol* **13**: 85.

1110 [126] Henderson, R. J., M. T. Park, and J. R. Sargent. 1995. The desaturation and elongation of
1111 (14)C-labelled polyunsaturated fatty acids by pike (*Esox lucius* L.) in vivo. *Fish Physiol Biochem* **14**:
1112 223-235.

1113 [127] Buzzi, M., R. J. Henderson, and J. R. Sargent. 1997. The biosynthesis of docosahexaenoic acid
1114 [22:6(n-3)] from linolenic acid in primary hepatocytes isolated from wild northern pike. *J Fish Biol* **51**:
1115 1197-1208.

1116 [128] Li, C. Y., K. Lawrence, J. Merlo-Coyne, and S. A. Juntti. 2023. Prostaglandin F(2alpha) drives
1117 female pheromone signaling in cichlids, revealing a basis for evolutionary divergence in olfactory
1118 signaling. *Proc Natl Acad Sci U S A* **120**: e2214418120.

1119 [129] Havird, J. C., M. M. Miyamoto, K. P. Choe, and D. H. Evans. 2008. Gene duplications and
1120 losses within the cyclooxygenase family of teleosts and other chordates. *Mol Biol Evol* **25**: 2349-
1121 2359.

1122 [130] Wang, X. Y., Q. H. Liu, L. Zhou, Z. C. Song, and J. Li. 2023. Effect of ovarian fluid on sperm
1123 performance in teleost with internal and external fertilization strategies (vol 206, pg 189, 2023).
1124 *Theriogenology* **209**: 234-234.

1125 [131] Rusco, G., M. Di Iorio, S. Esposito, E. Antenucci, A. Roncarati, and N. Iaffaldano. 2023. The
1126 Use of Ovarian Fluid as Natural Fertilization Medium for Cryopreserved Semen in Mediterranean
1127 Brown Trout: The Effects on Sperm Swimming Performance. *Vet Sci* **10**.

1128 [132] Oliver, E., F. McGillicuddy, C. Phillips, S. Toomey, and H. M. Roche. 2010. Postgraduate
1129 Symposium The role of inflammation and macrophage accumulation in the development of obesity-
1130 induced type 2 diabetes mellitus and the possible therapeutic effects of long-chain
1131 -3 PUFA. *P Nutr Soc* **69**: 232-243.

1132