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**Fish sperm motility assessment as a tool for aquaculture
research, a historical approach**

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31 **Abstract**

32 Fish sperm motility is nowadays considered the best quality biomarker for fish
33 spermatozoa, and sperm motion parameters from more than 300 fish species have been
34 already reported throughout 1500 scientific articles covering a wide range of topics, from
35 molecular to ecology issues. Within these topics, *i*) sperm storage (involving both the use
36 of chilled-storage protocols for short-term periods, and sperm cryopreservation
37 techniques for long-term storage), *ii*) sperm physiology (fathom in the spermatozoa
38 activation process and the whole propulsion machinery of the sperm cells), and *iii*)
39 broodstock management (covering aspects such as rearing conditions, dietary
40 requirements or hormonal induction treatments), have been the most studied fields
41 through the evaluation of fish sperm motion, enhancing many aspects of management
42 practices in fish farms. In addition, other aquaculture and ecological topics such as *iv*) the
43 knowledge of the breeding cycle of the species, *v*) the phenomenon of the sperm
44 competition, and *vi*) ecotoxicological studies for the evaluation of aquatic environments,
45 have also been approached from the evaluation of sperm motion performance.

46 To sum up, fish sperm motility assessment can serve as a potential tool for aquaculture
47 and ecological purposes, covering key topics of fundamental and applied research. This
48 review gives an overview of the major research areas in which fish sperm motility have
49 been applied successfully.

50

51 **Keywords:**

52 Spermatozoa; velocity; sperm quality; kinetic, CASA

53 **1. Introduction**

54 Fish are the most diverse and numerous group of vertebrates, and a wide diversity of
55 reproductive strategies can be found both in freshwater and marine species (Bone *et al.*
56 1995). However, most of them share a common fertilization mode, the external
57 fertilization, in which gametes both from males and females are released into the aquatic
58 environment (Crowe & Russell 2009). Up to this point, fish spermatozoa remain
59 completely quiescent in the spermiduct, and they became motile once are released to the
60 external medium by an hyper- or hypo-osmotic shock, depending on seawater or
61 freshwater species (Morisawa 2008). In this context, sperm activation will be an essential
62 requirement in the pursuit of female gametes, and a wide range of factors of both external
63 and internal origin will determine the fertilization success.

64 It is reasonable to imagine that fertilization success will depend on gamete quality and,
65 focusing in males, it makes sense to define and understand what gamete (sperm) quality
66 means. From a biological standpoint, sperm quality could be defined as the ability of the
67 spermatozoa to exploit their swimming ability for reaching and fertilize the oocyte
68 (Fauvel *et al.* 2010). Therefore, any quantifiable sperm trait directly correlated with the
69 fertilization success could be potentially used as a sperm quality biomarker. A relatively
70 high number of sperm quality biomarkers have been reported over the years in several
71 fish species (Rurangwa *et al.* 2004). These sperm biomarkers have so far been
72 documented in scientific papers, and several traits of the fish/sperm itself such as
73 osmolality, pH and chemical composition of the seminal plasma (Alavi *et al.* 2004);
74 enzymatic activity (Burness *et al.* 2005); ATP concentration (Dzyuba & Cosson 2014);
75 spermatocrit and sperm density (Sørensen *et al.* 2013); sperm motility (Ottesen *et al.*
76 2009); or sperm morphology and ultrastructure (Ciereszko *et al.* 2015) have been linked
77 to the ability of sperm to fertilize the ova. Although some of these parameters are
78 relatively easy to score and commonly used (spermatocrit, viability and subjective
79 motility); others need sophisticated laboratory analyses (biochemical analyses),
80 expensive equipment (objective and quantitative motility) or availability of eggs
81 (fertilisation success).

82

83 **2. Sperm motility as a sperm quality biomarker**

84 Although it is a set of sperm characteristics that contribute to determining sperm quality,
85 sperm motility is currently considered the best sperm quality biomarker in fish (Suquet *et*

86 *al.* 2010; Boryshpolets *et al.* 2013; Mylonas *et al.* 2016). In fact, high correlations have
87 been reported between sperm motility and fertilization and hatching rates in some fish
88 species (Gage *et al.* 2004; Gasparini *et al.* 2010; Gallego *et al.* 2013a). Although sperm
89 motility could loosely defined as the capacity of spermatozoa to move or not once
90 released into the activation media, a deeper comprehensive description would also
91 involve other kinetic features such as spermatozoa velocity, progressiveness, linearity,
92 etc. In this sense, the method or technique chosen by the researcher for assessing the
93 sperm movement will generate the degree of depth of understanding of the motion pattern.
94 Nowadays, sperm motility evaluation can be done by two different ways in the laboratory:
95 i) the subjective way, in which an experienced technician (or not) make an evaluation of
96 sperm motility through a simple observation under the microscope; and ii) the objective
97 way, in which sophisticated software integrate the successive positions of the heads of
98 moving spermatozoa in consecutive frames of video records to calculate the trajectories
99 and their characteristics. Subjective evaluation method has been the most used technique
100 to appraise sperm motility over the history, but some problems have emerged from this
101 method. First drawback is focused the own limitation of human eye, through which we
102 can only provide a coarse evaluation of sperm quality by motility criteria assessing classes
103 in terms of percent of motile sperm and motility duration. In addition, this type of
104 evaluation will depend on the observer's experience and several aspects such as sperm
105 density, sperm velocity, drift, etc. can cause over- or underestimated readings. Therefore,
106 the low reproducibility of this subjective motility evaluation, which can result in
107 variations of 30 to 60% from the same sample, often makes it difficult to interpret and
108 compare the results intra- and inter-labs (Verstegen *et al.* 2002; Rosenthal *et al.* 2010).
109 On the other hand, the gradual appearance of Computer Assisted Sperm Analysis (CASA)
110 systems has made possible to estimate a higher number of sperm motion parameters not
111 given by subjective evaluation, providing the scientific community with new useful tools
112 to be applied in multidisciplinary studies. These systems, which are the evolution of
113 multiple photomicrography exposure and video-micrography techniques for spermatozoa
114 track, represent an objective, sensitive and accurate technique for obtaining sperm kinetic
115 features (Kime *et al.* 2001). CASA systems were first used in the 70's in mammalian
116 sperm, and only in the 90's modern CASA systems have been adapted for fish
117 spermatozoa studies (Perchec *et al.* 1995; Toth *et al.* 1995; Christ *et al.* 1996). The
118 differences on the size and the biology of fish and mammalian spermatozoa may explain
119 the delay in the release of adequate tools for the measurement of sperm motility in fish.

120 To date, these systems have been used and validated in a wide range of animal groups
121 such as marine invertebrates (Riesco *et al.* 2017), birds (Lüpold *et al.* 2009), marine
122 mammals (Montano *et al.* 2012), reptiles (Tourmente *et al.* 2011) or even insects (Al-
123 Lawati *et al.* 2009). It is noteworthy that most of the parameters evaluated by CASA
124 systems have been correlated positively with spermatozoa fertilization potential, thus
125 CASA is a very useful tool for assessing sperm quality in fish reproduction research
126 (Tuset *et al.* 2008; Gallego *et al.* 2013a).

127

128 **3. Fish sperm motility: a useful tool for multidisciplinary studies**

129 The first scientific reports focused on fish sperm motility date from a century ago (Gee
130 1916). From this date, new articles about this topic were reported sporadically until 60s,
131 and became continuous but still scarcely until 80s. Nevertheless, a marked and continuous
132 increase of scientific contributions were detected from 90's to present, and we can
133 currently find more than 1500 publications using fish sperm motility as a research tool
134 through a wide range of topics: from ecology to molecular issues (Figure 1 and 2).

135 To date, sperm motion parameters from 340 fish species belonging to different families
136 have been already studied. However, only a few species (~30) represent more than 50%
137 of published papers, of which salmonids, cyprinids and sturgeons are the most studied
138 families. In this context, scientists have devoted much more time to study freshwater than
139 seawater species, so the flood information is five-fold bigger in freshwater fish. Here we
140 present an overview of the state of the art about the most developed research areas in
141 which sperm motility have been applied successfully.

142

143 **3.1 Sperm physiology**

144 Sperm physiology has centred the use of spermatozoa motion as a research tool. In fact,
145 first studies using fish sperm motility were carried out on this topic at the beginning of
146 the last century, assessing the sperm behaviour of rainbow trout on different activation
147 media conditions. Since then, this research field has shown a continuous increase over
148 the years, and more than 400 sperm motility-physiology articles have been published
149 (Figure 2).

150 Fish sperm activation process has been the key subject within this area, and learning about
151 the process by which spermatozoa begin to move has been the main goal of fish
152 physiology. In this context, three general pathways through which sperm becomes motile

153 have been discovered and reported in teleost fish (Morisawa 2008): *i*) marine fish
154 spermatozoa become motile by an hyperosmotic shock; *ii*) spermatozoa of freshwater fish
155 become motile by an hypoosmotic shock; and *iii*) spermatozoa from salmonids and
156 sturgeons become motile by a low environment K^+ concentration. Although these three
157 spermatozoa activation models are widely accepted by the scientific community and they
158 can be tested even by subjective motility evaluation, in-depth motion analysis through
159 CASA systems have contributed to describe deeply these activation pathways (Gallego
160 *et al.* 2013b; Pérez *et al.* 2016). Nowadays, novel research lines about the effect of
161 absence/presence of certain ions in the activation medium on the sperm kinetics
162 parameters are emerging both in freshwater and seawater species (Dietrich *et al.* 2007;
163 Vílchez *et al.* 2016, 2017)

164 Another important topic on this research area has focused on the relationship between the
165 composition of the seminal plasma and sperm motility. The literature reveals many data
166 about the ionic composition in different species belonging to different families, but the
167 correlation between these seminal plasma compositions and the sperm the motility has
168 been investigated in only a few species. Regarding cyprinids, a positive relationship
169 between Na^+ and sperm motility was reported both in common carp (*Cyprinus carpio*)
170 and common bleak (*Alburnus alburnus*), while K^+ had a negative correlation with sperm
171 motion parameters (Lahnsteiner *et al.* 1996; Bozkurt *et al.* 2009). Regarding salmonids,
172 Ca^{2+} was significantly correlated with the sperm fertilization capacity in rainbow trout
173 (*Oncorhynchus mykiss*), estimating optimum levels from 0.8 to 1.2 mmol/L for carrying
174 out fertilization trials (Lahnsteiner *et al.* 1998). In Atlantic salmon (*Salmo salar*), Na^+ and
175 K^+ levels were correlated with fertilization rates (Aas *et al.* 1991). Regarding marine fish
176 we can find mixed results: while in the European eel (*Anguilla anguilla*) $[K^+]$ increased
177 and $[Ca^{2+}]$ and $[Mg^{2+}]$ showed a progressive reduction as sperm quality improved
178 (Asturiano *et al.* 2004); in Atlantic cod, $[Ca^{2+}]$ showed significant and positive
179 relationship with sperm motion parameters in several months during the breeding season
180 (Butts *et al.* 2011).

181 Moreover, the propulsion machinery of spermatozoa has been another research focus
182 within sperm physiology studies. In this regard, scientists have tried to find correlations
183 between sperm motility and other factors such as spermatozoa flagella or its power
184 source, the energetic metabolites. Although several studies have reported a sharp decrease
185 of ATP once motility start in a wide range of teleost species (Perchec *et al.* 1995; Butts
186 *et al.* 2010), it is not an easy task to find significant correlations between ATP content

187 and spermatozoa velocities or motilities. Sperm ATP levels have been correlated with
188 motility, velocity and/or fertilizing ability in two salmonid species like rainbow trout
189 (Lahnsteiner *et al.* 1998) or chinook salmon, *Oncorhynchus tshawytscha* (Bencic *et al.*
190 1999); and even in some marine species like sea bass, *Dicentrarchus labrax* (Zilli *et al.*
191 2004). In contrast, no correlations between ATP and sperm motility were found in species
192 as common bleak, *Alburnus alburnus* (Lahnsteiner *et al.* 1996); bluegill, *Lepomis*
193 *macrochirus* (Burness *et al.* 2005); or Atlantic cod, *Gadus morhua* (Butts *et al.* 2010).
194 Actually, it is known that ATP alone is not a strong indicator of sperm motility by itself,
195 and other metabolites such as ADP or CrP should be taken into account (Dzyuba *et al.*
196 2017). In this context, the macroenergetic phosphates content have been recently proposed
197 as a biomarker for semen quality (Hatef *et al.* 2013; Cabrita *et al.* 2014), but it is
198 noteworthy that this parameter could be only used in a species-specific context.

199

200 **3.2 Sperm storage**

201 Sperm storage, by either short-, medium- or long-term period, has been the most
202 investigated field using sperm motility as a research tool. More than 500 scientific
203 publications reporting kinetic spermatozoa parameters have contributed for discover and
204 improving sperm storage protocols in a large number of fish species (Figure 2).
205 Nowadays, these techniques show a high number of potential applications, ranging from
206 ecology to aquaculture perspective.

207

208 **3.2.1 Chilled storage**

209 Short- and medium-term storage methods, also known as chilled- or cold-storage
210 protocols, aim to preserve the sperm integrity and quality over several days, weeks or
211 even months. The main applications of these protocols are focused in aquaculture issues,
212 allowing the improvement of broodstock management mainly through the
213 synchronization in the gamete production of male and females. Temperatures of 0 °C
214 (melting ice) to 4 °C are the most widely used as they are easy to reach and easy to
215 regulate. Besides, these low temperatures reduce bacteria growth, so this may explain
216 why temperatures below 6 °C are always reported as better than higher temperatures
217 (Bobe & Labbe 2009). In addition to the temperature, other elements such as the type of
218 extender, the dilution ratio (sperm:extender), or the environmental conditions become
219 essential factors in order to achieve a successful sperm storage. Given that these factors
220 are usually species-specific, a large number of storage protocols can be found through the

221 literature. The Table 1 summarizes studies on short- and medium-term storage protocols
222 applied to several fish species during the last 30 years, and showing the best results
223 obtained in terms of sperm motility.

224 Regarding short-term storage, great results have been obtained in the key aquaculture
225 families such as Salmonidae, with more than 50% of motility after 7 and 14 days in
226 rainbow trout (Ubilla *et al.* 2015) and Atlantic salmon (Parodi *et al.* 2017), respectively;
227 Cyprinidae, keeping good quality samples (>70%) in common carp and perch (*Perca*
228 *fluviatilis*) during the first week of storage; Acipenseridae, with more than 50% of
229 motility after 7 days in Siberian sturgeon (Shaliutina *et al.* 2013); and marine fish species,
230 where spermatozoa still retained some motility after 30 days storage both in Atlantic cod
231 (*G. morhua*) and haddock (*Melanogrammus aeglefinus*) (DeGraaf & Berlinsky 2004).

232 In addition to chilled storage, sperm super cooling method (-2 to -5 °C) has been sparingly
233 used in fish species. Within this technique, the use of different substances like
234 cryoprotectants avoid the ice crystallization, keeping a proper membrane integrity on
235 spermatozoa. There are only a few reports of just sub-zero storage on fish sperm: rainbow
236 trout semen was stored at -2 °C for at least 23 days without loss of fertilizing power (Stoss
237 & Refstie 1983), and Atlantic salmon sperm stored for 21-28 days at -4.5 °C was able to
238 fertilize 80-90% of eggs (Truscott & Idler 1968). However, this method requires a very
239 strict control of the temperature to avoid ice crystallization if temperature decreases or
240 cryoprotectant toxicity if temperature increases (Bobe & Labbe 2009).

241

242 3.2.2 Cryopreservation

243 Cryopreservation is a long-term storage technique that apply extreme temperatures for
244 keeping viable spermatozoa, most common is -196 °C in liquid nitrogen. At these low
245 temperatures all biological activity stops, so this process is able to preserve and store
246 sperm cells over long periods, from days to years. Therefore, enforcements from this
247 technique evolved from aquaculture purposes (broodstock management, genetic
248 improvement programs, species-specific reproductive problems, etc.) to ecology goals,
249 as cryobanking of genetic resources from endangered species

250 To date, sperm of more than 200 fish species haven been successfully cryopreserved and
251 techniques of thawed sperm management have been established for freshwater and
252 marine fish species (Tsai & Lin 2012). Although some previous manuscripts have
253 reviewed fish sperm cryopreservation subject (Suquet *et al.* 2000; Kopeika *et al.* 2007;
254 Cabrita *et al.* 2010; Asturiano *et al.* 2017; Martínez-Páramo *et al.* 2017), Table 2

255 summarizes the best results reached on fish sperm cryopreservation on the most important
256 fish families for aquaculture. This table includes the cryoprotectants used (and their
257 concentrations), focusing on pre- and post-thaw motility values obtained for each species.
258 Freshwater species has been the most studied group, and specific protocols have been
259 established for salmonids, sturgeons, carps and catfishes. Regarding Salmonidae, great
260 results have been obtained in the key aquaculture species such as rainbow trout (*O.*
261 *mykiss*), brown trout (*S. trutta*), and Atlantic salmon (*S. salar*); and post-thaw motilities
262 higher than 60% have been reported using methanol and sugars as a rule for
263 cryopreservation protocols on this fish family (Horváth *et al.* 2015).

264 In sturgeons, whose spermatozoa have an acrosome, cryopreservation process can induce
265 some deleterious effects on this structure (Billard *et al.* 2004), and post-thaw motility can
266 be compromised in species such as beluga (*Huso huso*), starlet (*Acipenser ruthenus*), or
267 Siberian sturgeon (*Acipenser baeri*). However, the addition of amino acids to the
268 cryopreservation medium (usually 10% methanol) can noticeably improve post-thaw
269 sperm quality, reaching motility values around 80% in Persian sturgeon (*A. persicus*)
270 (Aramli *et al.* 2016a).

271 In relation to cyprinids, different types of cryoprotectants such as methanol, dimethyl-
272 sulfoxide (DMSO) or glycerol have been successfully applied. DMSO provided great
273 results in silver carp (*Hypophthalmichthys molitrix*) and java barb (*Barbus gonionotus*),
274 showing post-thaw motilities higher than 80%; while methanol and glycerol provided
275 good results in tench (*P. fluviatilis*) and grass carp (*Ctenopharyngodon idella*),
276 respectively. Surprisingly, the best results in cyprinids have been reported in common
277 carp (*C. carpio*) using only 15% egg yolk as external cryoprotectant, and reaching post-
278 thaw motility values close to fresh sperm values (~90%).

279 Marine species have received much less attention than freshwater species for the
280 development of cryopreservation protocols, and many of these research efforts have been
281 made during the present century. Although DMSO has been the most used cryoprotectant
282 in marine fish, providing remarkable results in sparids and flatfish species (see Table 2),
283 other cryoprotectants such as methanol, glycerol or egg yolk have been successfully and
284 recently applied in other marine species in which DMSO was the common cryoprotectant
285 (Asturiano *et al.* 2017). In this context, new trials using methanol have provided better
286 results than DMSO-protocols in European eel (*A. anguilla*), and notably post-thaw
287 motility values close to 50% have been currently reported in this species (Herranz-
288 Jurdado, pers. comm., 2017).

289 Finally, successful results have been also published in fish species with internal
290 fertilization. In green swordtail (*Xiphophorus helleri*), a simply and useful protocol based
291 on glycerol (14%) as cryoprotectant provided post-thaw motilities as high as 77% at
292 10 min after thawing. Moreover, if sperm was immediately diluted after thawing, protocol
293 was be able to retained motility values for as long as 8 days when stored at 4 °C (Huang
294 *et al.* 2004). Lower post-thaw motilities have been reported in other close species such as
295 *Xiphophorus couchianus* and *Xiphophorus variatus* (35 and 37%, respectively) (Yang *et*
296 *al.* 2009, 2012).

297 To sum up, methods for sperm freezing have progressed during the last decades, and the
298 assessment of fish sperm motility has been consolidated as a very useful tool for
299 evaluating the validity of cryopreservation protocols. However, new techniques are
300 emerging in order to provide in-depth information on the negative effects of freezing-
301 thawing processes (DNA fragmentation, changes in protein profile, etc.), so fish sperm
302 cryopreservation becomes an interesting research for studying the impact of
303 cryopreservation process through new emerging tools of sperm quality analysis (Cabrita
304 *et al.* 2014; Martínez-Páramo *et al.* 2017).

305

306 **3.3 Broodstock management**

307 Broodstock management involves a large number of topics that have the common goal of
308 enabling a captive group of fish to undergo reproductive maturation and fertilization
309 success. In this context, spermatozoa motion will have an essential role to achieve this
310 goal, and the effect of different factors such as the environmental conditions, diet
311 composition, type of gamete collection or the use of hormonal treatments, could be tested
312 through sperm motility assessment.

313

314 **3.3.1 Environmental conditions**

315 In many cases, reproduction of fish in captivity can be controlled or modified exclusively
316 by the use of environmental factors: *i*) water temperature, *ii*) photoperiod and/or *iii*)
317 salinity. When these environmental factors are not optimal, reproductive dysfunctions
318 compromise male gametogenesis and, therefore, sperm quality (Mylonas *et al.* 2010).

319 There are little reports on sperm quality changes in response to broodstock rearing
320 temperature. In river lamprey (*Lamprea fluviatilis*), male reproductive performance was
321 compared under three temperatures (7, 10 and 14 °C). Temperature had a significant
322 effect on the quantity and quality of sperm produced: 70% of males held at 10 °C and

323 14 °C did not spermiate, while males held at 7 °C produced samples with more than 80%
324 of sperm motility (Cejko *et al.* 2016). In Siberian sturgeon (*A. baeri*), sperm production
325 performance was tested at four temperatures (10, 12.5, 15 and 17.5 °C), and the
326 significantly highest spermatozoa motilities (>65%) were also obtained with the lowest
327 one (Williot *et al.* 2000). However, regarding European eel (*A. anguilla*), in which three
328 temperatures were tested (10, 15 and 20 °C), the warmest thermal treatment (20 °C)
329 showed the best results in all the sperm production parameters (volume, density) as well
330 as the maximum values total motility (>75%) (Gallego *et al.* 2012). All these data remark
331 that temperature seems to be a species-specific factor: while cold-water species need low
332 temperatures for showing the good quality sperm, warm-water species need high
333 temperatures in order to achieve proper sperm motility values. In fact, temperatures above
334 or below the optimum range can adversely reduce gamete quality, or even stop the onset
335 and progression of spermiation (Migaud *et al.* 2013).

336 Photoperiod is involved on the regulation of annual reproductive rhythms in many teleost
337 fish, and photothermal programs are commonly used in fish farms in order to advance or
338 delay the gamete production (Bromage *et al.* 1993). In rainbow trout (*O. mykiss*), the
339 combination of a long photoperiod (18L:6D, 4 months) followed by a short-one (18D:6L,
340 3 months) was able to generate a high percentage of spermiating males (~80%) during
341 the out-of spawning season. However, the sperm motility in this experimental group was
342 lower than in the control group maintained with natural photoperiod (70 and 83%,
343 respectively) (Atasever & Bozkurt 2015). In turbot (*S. maximus*), sperm collected in
344 males submitted to a contracted cycle (compressed to 6 months) presented a significantly
345 higher sperm motion parameters at first stripping than that recorded in males submitted
346 to natural photoperiod (Suquet *et al.* 1992). In shortfin silverside fish (*Chirostoma*
347 *humboldtianum*), males were induced to reproduction through a 81-day artificial
348 photothermal compressed cycle, showing similar sperm motility values (81±7%) than in
349 natural conditions (Blancas-Arroyo *et al.* 2004). Therefore, artificial photoperiods
350 become a useful tool in commercial hatcheries, advancing the sperm production and
351 causing similar motility values than those reached using natural photo-cycles.

352 Water salinity is the less studied environmental factor, and several trials suggest that
353 gamete maturation and final spawning can take place across a wide range of rearing
354 salinities in several species (Lee *et al.* 1992; Bani *et al.* 2016). Although the effect of
355 salinity has been mainly studied regarding sperm activation process, there are only a few
356 reports about the effect or rearing salinity on breeders and the quality of gametes that they

357 produce. In this context, motility parameters of sperm activated with two activation media
358 (seawater and a sucrose solution) were compared in blackchin tilapia (*Sarotherodon*
359 *melanotheron heudelotii*) reared in freshwater (FW; 0‰), seawater (SW; 35‰), and
360 hypersaline water (HW; 70‰). Results showed that for FW fish, sperm motility was high
361 and varied little between individual fish for both activation media. In contrast,
362 spermatozoa of SW and HW fish were significantly less active in sucrose solution than
363 in synthetic SW, with substantial differences between individual fish (Legendre *et al.*
364 2016). In Mozambique tilapia (*Oreochromis mossambicus*), regulation of sperm motility
365 seems to be modulated during acclimation of the fish from freshwater (FW) to seawater
366 (SW), being independent of extracellular Ca²⁺ in FW and dependent in SW. In this sense,
367 sperm of SW tilapia showed motility even in a hypertonic environment, whereas sperm
368 of FW tilapia were not motile (Morita *et al.* 2004). In another euryhaline species, such as
369 European eel (*A. anguilla*), spermatozoa produced from males induced both in freshwater
370 and seawater could be activated (10–90% motility) in SW solution. Since there is no
371 significant difference between motility of freshwater and seawater spermatozoa, authors
372 assume that the freshwater rearing of males is no limiting factor in the artificial
373 propagation of this endangered species (Müller *et al.* 2005). All these results show that in
374 euryhaline species, spermatozoa can present a wide plasticity regarding activation media,
375 reaching suitable motility values regardless of medium in which they are released.

376

377 3.3.2 Dietary requirements

378 Broodstock nutrition is a key factor controlling gonadal development and gamete quality
379 in fish, and diet composition can affect reproduction success and offspring survival
380 (Izquierdo *et al.* 2001). Although there are many publications linking up the dietary with
381 reproduction success, scarce reports are able to link directly the broodstock diet with the
382 kinetic characteristic of spermatozoa. In this sense, Table 3 summarizes studies on this
383 topic over the last years.

384 Fatty acid composition of broodstock diet has been identified as major dietary factor that
385 determine sperm quality, mainly due to carnivore fish are not able to synthesize certain
386 fatty acids. In general, both freshwater and seawater species need PUFA (polyunsaturated
387 fatty acids) or HUFA in the diet (Izquierdo *et al.* 2001), and the enrichment of dietary
388 broodstock with these fatty acids can generate a substantial improvement in sperm
389 motility parameters (see Table 3). Beirão *et al.* (2015) reported that sperm quality of
390 Senegalese sole (*Solea senegalensis*) improved through the enriched docosahexaenoic

391 acid (DHA) diet, specifically the sperm velocity (VCL) and the percentage of progressive
392 sperm. In European eel (Butts *et al.* 2015; Baeza *et al.* 2015), diets with high levels of
393 arachidonic acid (ARA) induced medium milt volumes and high sperm motilities, while
394 diets with higher percentage of eicosapentaenoic acid (EPA) induce remarkable volumes
395 of milt and also high sperm motilities. In freshwater fish like rainbow trout (*O. mykiss*),
396 some breeders fed with a diet deficient in essential fatty acids (n-3) showed a lower sperm
397 motility than breeders fed with a control diet (Vassallo-Agius *et al.* 2001); while in other
398 trial carried out in rainbow trout, fish fed with a properly HUFA/PUFA ratio showed the
399 highest semen motility percentage and duration than other treatments (Hajiahmadian *et*
400 *al.* 2016). In aquarium species such as zebrafish (*Danio rerio*) and guppy (*Poecilia*
401 *reticulata*), fatty acid composition of broodstock diet also provided an improvement on
402 sperm quality parameters. In guppy, Rahman *et al.* (2015) reported significant main
403 effects of PUFAs on sperm viability and weak but significant interacting effects of both
404 nutrients on sperm motility time, evidencing PUFAs as critical determinants of sperm
405 quality. In zebrafish (*D. rerio*), the addition of phospholipids (fatty acids linked to
406 phosphate group) in the diet caused great results in sperm quality of breeders, which was
407 revealed by higher total and progressive motility and higher velocities than sperm from
408 males fed with control diet (Diogo *et al.* 2015).

409 Regarding vitamins, which are also components that the fish cannot synthesize, their
410 addition to the diet often means an improvement of gamete quality. Vitamin C (ascorbic
411 acid) and D have been the most used vitamins for improving broodstock diet, and positive
412 effect on sperm motility have been reported in several species. In Nile tilapia, animals fed
413 with vitamin C-diet showed higher motility values ($54.9 \pm 8.9\%$) than fish from control
414 group ($22.3 \pm 19.4\%$) (Sarmiento *et al.* 2017). In rainbow trout (*O. mykiss*), the highest
415 motility rate was recorded in fish fed with a vitamin E enriched diet (94.5%), while the
416 lowest motility was detected in the control group (62.2%) (Ciereszko & Dabrowski 1995,
417 2000). The addition of vitamin C and /or E to the diet also enhanced sperm motility in
418 goldfish (Kashani *et al.* 2011; Kashani & Imanpoor 2012), African catfish (Dada 2012)
419 and Senegalese sole (Beirão *et al.* 2015).

420

421 3.3.3 Hormonal induction

422 Hormonal therapies for the enhancement of spermiation and sperm production have been
423 tried and employed in fish research and aquaculture. These hormonal protocols are often
424 used in two scenarios: *i*) in some species in which sperm production exist during rearing

425 conditions, but sperm performance (volume, motility density, etc.) is not good enough for
426 hatchery operations; and *ii*) in some fish species in which it is impractical or even
427 impossible to simulate the environmental factors of the breeding process (i.e., spawning
428 migration, depth, etc.), so gonadal maturation does not occur in captivity (Mylonas *et al.*
429 2016). In both situations, hormonal stimulation could provide several advantages for the
430 aquaculture industry, and breeding males can be able to produce more sperm of higher
431 quality for a longer period, avoiding hatchery problems such as the gamete
432 synchronization or limitation.

433 The injection of pituitary extracts (PE) from mature fish into breeders was the first method
434 used to control reproductive function in aquaculture handling, and has been used widely
435 in a variety of species, especially cyprinids (Mylonas *et al.* 2010). For example, in
436 common bream (*Abramis brama*), males treated with bream PE (2.5 mg kg⁻¹) or carp PE
437 (2 mg kg⁻¹) showed higher sperm volume and motility than control males (Kucharczyk
438 *et al.* 1997). In common carp (*C. carpio*), CPE (2 mg kg⁻¹) treatment led to 100%
439 spermiation males compared to only 25% in the control group, and sperm quality were
440 also improved by the hormonal treatment (Vazirzadeh *et al.* 2016). In pikeperch (*Sander*
441 *luciperca*), males treated with CPE produced sperm with higher motility (67.5–86.7%)
442 than control group (Falahatkar & Poursaeid 2014). However, in other cyprinid species
443 such as in dace (*Leuciscus leuciscus*), crucian carp (*Carassius carassius*), or even
444 common carp (*C. carpio*), there were no statistical differences between control and
445 hormone-treated groups (Cejko *et al.* 2012, 2013). In South American fishes, a single or
446 multiple injection of CPE usually did not improve the spermatozoa motion performance,
447 but CPE treatment was able to increase sperm volume and decrease sperm density,
448 facilitating the sperm handling steps over cryopreservation protocols in these species
449 (Viveiros & Godinho 2009). Similar results have been reported in Siluridae, and different
450 catfish species such as European catfish (*Silurus glanis*), African catfish (*Clarias*
451 *gariepinus*), or Amazon catfish (*Leiarius marmoratus*), that did not show statistical
452 differences in sperm quality parameters between control and hormone-treated groups
453 (Linhart *et al.* 2004; Araújo *et al.* 2014).

454 Gonadotropin (GTH) preparations of mammalian origin (ovine, mare or human) stage
455 another technique for inducing or enhancing spermiation in some fish species belonging
456 from different families. In cyprinids, notable results have been reported in several species
457 using these hormonal therapies. In pikeperch (*S. luciperca*), males treated with hCG
458 (human chorionic gonadotropin) produced sperm with higher motility (67.5–86.7%) than

459 control group (Falahatkar & Poursaeid 2014). In goldfish (*Carassius auratus*), hCG
460 treatment was able for inducing 100% of spermiation males (n=10), which showed a
461 motility about 80% (Targońska & Kucharczyk 2011). In common bream (*A. brama*),
462 males treated with hCG showed higher motility (54%) than control males (22%)
463 (Kucharczyk *et al.* 1997). However, if there is a genus in which this hormone has
464 generated great results, this is the genus *Anguilla*. In European eel (*A. anguilla*), three
465 different GTHs (hCG, hCG_{rec} and PMSG) were tested on the induction of maturation on
466 eel males. Regarding motion performance, hCG_{rec} treatment generated the highest values
467 throughout most weeks of treatment, reaching maximums of 70% of total motility, and
468 keeping spermiating males until the 20th week of the treatment (Gallego *et al.* 2012). In
469 relation to Japanese eel (*Anguilla japonica*), repeated weekly injections of hCG provide
470 the onset of spermiation at 5th injection, and the percent motility of spermatozoa remained
471 at approximately 70% from 9 to 14th injection (Ohta *et al.* 1996). It is important to note
472 that the results obtained by GTHs in these species are particularly relevant for the
473 scientific community, mainly due to eels (*Anguilla* spp.) are not able to mature
474 spontaneously in captivity.

475 Recently, studies have examined the production and use of recombinant (re) GtHs of
476 piscine origin, which have been successfully produced for zebrafish (*D. rerio*), channel
477 catfish (*Ictalurus punctatus*), goldfish (*C. auratus*), Japanese eel (*A. japonica*), European
478 seabass (*D. labrax*), Senegalese sole (*S. senegalensis*), cinnamon clownfish (*Amphiprion*
479 *melanopus*), and European eel (*A. anguilla*) (reviewed by Mylonas *et al.*, 2017). However,
480 although the *in vitro* effect of these hormones was relatively good in most of these species
481 (stimulating both FSH and LH receptors, steroids production, etc.), specific reGtHs had
482 little *in vivo* effect. In fact, first full spermatogenesis and spermiation has only been
483 achieved in one species: the European eel (Peñaranda, pers. comm., 2015). Although the
484 sperm quality was variable and not all the spermiating males produced samples with high
485 sperm quality, some sperm samples reached motilities $\geq 50\%$, densities around
486 7×10^9 cells ml⁻¹ and sperm volumes of approximately 0.4 ml (Peñaranda, pers. comm.,
487 2015).

488 Finally, gonadotropin-releasing hormone agonists (GnRHa), administered by injections
489 or controlled-release delivery systems, become the last technique for inducing or
490 enhancing spermiation in some fish species. GnRHa treatments offers some important
491 advantages in comparison to GTH treatments, such as *i*) GnRHa are less species-specific
492 due to the high structural similarity of native GnRHs among fishes, and *ii*) this technique

493 decrease considerably the handling stress generated by the repetitive manipulations of
494 breeders. In cyprinids such as barbel (*Barbus barbus*), common carp (*C. carpio*), or
495 crucian carp (*C. carassius*), hormonal stimulation by GnRH α did not have a significant
496 influence on the CASA parameters (motility and velocity indicators), which were shown
497 to be similar in hormonally stimulated groups and control groups (Cejko *et al.* 2014, 2015;
498 Cejko & Kucharczyk 2015). However, in marine species GnRH α implants have provided
499 great results in some species such as Atlantic halibut (*Hippoglossus hippoglossus*), where
500 sperm motility was enhanced in males treated with a high dose of GnRH α (25 μ g/kg)
501 compared to controls (Vermeirssen *et al.* 2004); in Senegalese sole (*S. senegalensis*),
502 where sperm motility produced by GnRH α -treated males was enhanced by 2-fold with
503 respect to controls; or in yellowtail flounder (*Pleuronectes ferrugineus*), where
504 percentage of motile sperm activated was higher in the high dose GnRH α treatment (90%)
505 than the control fish (20%). However, GnRH α implants did not have any effect on sperm
506 motility in other seawater species such as meagre (*Argyrosomus regius*), European
507 seabass (*D. labrax*) or bluefin tuna (*Thunnus thynnus*) (Rainis *et al.* 2003; Mylonas *et al.*
508 2016).

509 To sum up, even when hormonal treatments (CPEs, GTHs or GnRHs) can be useful to
510 enhance sperm production (overall in terms of volume) in aquaculture fish, hormonal
511 therapies usually do not affect sperm motion performance. In general, hormonal
512 treatments only provide successful results just in cases where fish species fail to spermiate
513 naturally or produce very small volumes of high-density sperm.

514

515 3.3.4 Gamete collection

516 Once the gametes have been produced naturally or thanks to the application of hormonal
517 or environmental treatments, it is time to gamete collection. Although at first glance it
518 may seem like a simple process, gamete collection often become a delicate task that can
519 affect negatively the gamete quality.

520 First step for recollecting gametes is usually the anesthetizing of breeders, which
521 obviously involves the use of different types of anaesthetics for minimizing fish stress.
522 Although most of aquaculture species does not present negative effect of anaesthetics at
523 gamete level, some exceptions can be found on the literature. First report in brook trout
524 (*Salvelinus fontinalis*) showed that tricaine (MS222) affected the motility duration of trout
525 sperm at concentrations as low as 19 mg/L (Allison 1961). In rainbow trout (*O. mykiss*),
526 despite the percentage of motile spermatozoa was also unaffected by the type of

527 anaesthetic or concentration used, the duration of motility decreased as anaesthetic
528 concentration increased (Wagner *et al.* 2002; Dietrich *et al.* 2005). These results
529 suggested anaesthesia have a moderate effect on total sperm motility values but, by
530 contrast, can affect significantly the duration of sperm movement.

531 Moreover, the method of obtaining gametes is also of great importance in order to avoid
532 urine contamination, which can negatively affect sperm characteristics and quality
533 (Lavens *et al.* 1996). Collection of fish sperm can be carried out by different techniques
534 such as *i*) the traditional procedure (manual stripping), *ii*) using a catheter, or *iii*) taking
535 out the testes, which involves killing the animal. Although traditional stripping procedure
536 has been the most widely technique for collecting sperm, it also presents a high risk of
537 urine contamination. In this context, some recent studies carried out in salmonids and
538 cyprinids report excellent sperm motility results collecting sperm samples with a catheter.
539 For example, in Caspian brown trout (*S. trutta caspius*), sperm samples collected with a
540 catheter were characterized by higher spermatozoa motility (~80%) than the sperm
541 collected via stripping (~60%) (Aramli *et al.* 2016b). In pikeperch (*S. lucioperca*), the
542 results were even more conclusive, and motility rate of sperm collected with a catheter
543 was 73%, whereas the motility rate of sperm collected with a syringe (manual stripping)
544 did not exceed 35% (Sarosiek *et al.* 2016). Therefore, in both species, catheter was proven
545 to effectively reduce the contamination of sperm with urine and was the best technique to
546 collect sperm samples.

547 Last method involves the collection of sperm samples directly from testes (post-mortem
548 samples), and can be applied both in aquaculture, research or field topics (Rosenberg
549 1983; Aoki *et al.* 1997). In this context, Dietrich *et al.* (2005) reported that sperm collected
550 from testes of rainbow trout (*O. mykiss*) at different post-mortem times did not show
551 significant differences respect the control groups in sperm motility values (>90%) over
552 the first hour. In dace (*L. leuciscus*), spermatozoa collected from testicles showed same
553 motility values and lower initial velocities than sperm collected from the sperm duct
554 (Kowalski *et al.* 2012). In Indian catfish (*Heteropneustes fossilis*), sperm samples
555 collected from specimens stored during 240 days at -20 °C showed an incredible motility
556 of 96.4% (Koteeswaran & Pandian 2002). However, even this post-mortem sperm
557 reached fertilization rates about 93%, hatching rates were extremely low (~2%) in
558 comparison to the control group (~98%).

559 In order to maximize the amount of available sperm produced by broodstock males, the
560 possibility of repeat sperm collections (sequential stripping) in a short-time period has

561 been extensively studied is several species. In Persian sturgeon (*A. persicus*), Alavi et al.
562 (2006) reported multiple collections (x3) at different times within the 48 h after hormone
563 injection. Despite total volume collected over multiple stripping was remarkable,
564 significant differences were found in the percentage of motile spermatozoa between the
565 two first collections (80-90%) and the 3rd collection. In sterlet (*A. ruthenus*), multiple
566 stripping method (every 3 h; from 12 to 66 h after hormone injection; 9 collections in
567 total) yielded larger volumes (>80 mL) than a single collection did. In addition, except
568 for the 1st and 7th stripping, sperm motility was extremely high during all the stripping
569 process, with values closed to 95%.

570

571 3.3.5 Biotechnology and genetic engineering

572 In the last few years, biotechnology and genetic engineering have contributed greatly to
573 fish culture through the application of novel techniques such as chromosome
574 manipulation, transgenesis, etc. (Foresti 2000). Thanks to these methods, it has been
575 possible to produce triploid, tetraploid, haploid, gynogenetic or androgenetic fish for
576 improving the production on fish farms. However, these types of techniques involves
577 from small to large changes in the genetic material of cells, which can produce a negative
578 impact on gamete quality (Pandian & Koteeswaran 1998).

579 Gynogenesis consist in the production of offspring with the genes of the mother only, and
580 has been successfully applied in a large number of fish species. Due to this technique
581 requires the inactivation of the male genome by exposure the spermatozoa to either
582 ultraviolet (UV) or gamma (Γ) rays, sperm motility assessment should be an essential step
583 after irradiation process. Regarding sturgeons, UV exposure has significant impacts on
584 the sperm motility. In Siberian sturgeon (*A. baerii*), spermatozoa revealed high sensitivity
585 to UV irradiation, with complete loss of motility after homogeneous UV irradiation at
586 doses above 200 mJ/cm² (Lebeda *et al.* 2014). Zhang et al. (2011) reported similar results
587 in this species, with significant effects of UV exposure on sperm kinetic parameters such
588 as total motility, velocity, and motility time. With regard to Salmonidae, irradiated sperm
589 of rainbow trout (*O. mykiss*) showed approximately 60% of motility after a 20 minutes
590 exposure to UV irradiation, became activated and maintained progressive movements for
591 at least 15 seconds duration (Goryczko *et al.* 1991). Reduction in spermatozoon activation
592 with UV exposure has also been described for other freshwater species such as silver barb
593 (*P. gonionotus*), catfish (*I. punctatus*) or common tench (*Tinca tinca*) (Goudie *et al.* 1995;
594 Pongthana *et al.* 1995; Nowosad *et al.* 2014).

595 In marine fish, gynogenesis is widely extended in several aquaculture species, in which
596 female production become more profitable than males. In European seabass (*D. labrax*),
597 exposure of sperm to UV light ($\geq 15000 \text{ erg mm}^{-2}$) reduced the amount of motile
598 spermatozoa, without affecting the duration of motility in the spermatozoa that remained
599 motile (Felip *et al.* 1999). In turbot (*S. maximus*), a dose-dependent effect of UV light on
600 sperm motility was found. The dose at which both the amount of motile sperm and the
601 duration of sperm motility was reduced to 50% of the original value (ID-50) was 28000
602 erg/mm^2 (Piferrer *et al.* 2004). UV exposure also generated a decreased of sperm motility
603 values in several finfish species such as Atlantic halibut (*H. hipoglossus*), Southern
604 flounder (*P. lethostigma*), or Japanese halibut (*P. olivaceus*) (Luckenbach *et al.* 2004;
605 You *et al.* 2008).

606 Polyploidy can be defined as the condition for having one or more additional chromosome
607 sets with respect to the number most frequently found in nature for a given species
608 (Piferrer *et al.* 2009). Although polyploidy can be easily induced in some relevant
609 aquaculture species, polyploid organisms can also spontaneously appear in both wild and
610 cultured populations. Some reports have shown the sperm performance of polyploid fish
611 (mainly triploids), and Table 4 summarizes these results. Within triploidy, disparate
612 results about sperm quality have been reported in several species. To begin with, no
613 spermatozoa production has been reported in triploid males of European sea bass (*D.*
614 *labrax*), turbot (*S. maximus*), gilthead sea bream (*Sparus aurata*), and Arctic charr (*S.*
615 *alpinus*) (reviewed by Piferrer *et al.* 2009). Sperm production, but with sperm motility
616 values low or close to zero has been reported in Prussian carp (*C. gibello*), yellowtail
617 flounder (*L. ferruginea*) and pond loach (*Misgurnus anguillicaudatus*) (Manning *et al.*
618 2004; Flajšhans *et al.* 2008; Fujimoto *et al.* 2008). However, triploid specimens of some
619 fish species are able to produce spermatozoa with high motility and velocity rates, as
620 occur in rosy bitterling (*R. ocellatus*), common tench (*T. tinca*) or cod (*G. morhua*)
621 (Kawamura *et al.* 1999; Peruzzi *et al.* 2009; Pšenička *et al.* 2010). Although usually the
622 triploidy confers genetic sterility, in some species spermatozoa from triploid males could
623 carry out egg activation leading to non-viable aneuploid embryos, generating a genetic
624 impact in fish population (Piferrer *et al.* 2009).

625

626 **3.4 Breeding cycle**

627 Sperm motility assessment has become a useful tool for studying several aspects of fish
628 ecology. To date, more than 100 scientific publications reporting kinetic spermatozoa

629 parameters have contributed to explore numerous ecology issues of different fish species
630 belonging to different taxa (Figure 2). During this section, a wide range of topics such as
631 breeder ageing, seasonal changes, characterization of populations, etc. are going to be
632 addressed through the sperm quality perspective.

633 In fish species with an annual reproductive cycle, the quality of sperm usually oscillates
634 across the spawning season both in the wild and in captivity individuals. Most of these
635 differences may be due to physiological changes and environmental cues related to fish
636 reproduction, and sperm motility assessment will reveal the optimal period in which
637 sperm should be collected in the hatchery (captive fish), or will simply supply information
638 about the breeding cycle of the species (wild fish). Table 6 summarizes the seasonal
639 changes in sperm motility on several fish, and over the data different sequential patterns
640 (depending on the timing of sperm quality peak) can be found among species. Type I
641 pattern includes species whose sperm production (quality and quantity) is higher at the
642 beginning of the spawning season, to subsequently decrease. Species such as Russian
643 sturgeon (*A. gueldenstaedtii*), halibut (*H. hippoglossus*), brook trout (*S. trutta*) or turbot
644 (*S. maximus*) show this breeding pattern. Type II species show the highest sperm motility
645 peak right in the middle of the breeding season, so sperm motion performance is
646 maximum during the central months. Many marine species such as European sea bass (*D.*
647 *labrax*), cod (*G. morhua*), red porgy (*P. pagrus*) or the ocean pout (*Macrozoarces*
648 *americanus*) present this sperm quality pattern. Finally, type III pattern includes species
649 whose sperm quality is higher at the end of the spawning season, achieving the peak
650 values of sperm motility at the final months of the breeding season. Key species in
651 freshwater aquaculture such as common carp (*C. carpio*) or the European perch (*P.*
652 *fluviatilis*) show this motility pattern. Lastly, there are species such as Persian sturgeon
653 (*A. persicus*), common barbel (*B. barbatus*) or South American catfish (*Rhamdia quelen*)
654 that not present significant differences in the percentage of motile spermatozoa
655 throughout the breeding season, so sperm motility usually remains high and constant
656 during this period.

657 Moreover, sperm motility can be also applied to inquire inter-populations differences or
658 even in the kinetic characteristics between related species belonging to the same genus.
659 Concerning inter-populations studies, some authors have reported notably differences
660 studying the same species between different populations and environments. In lake
661 minnow (*Eupallasella percunurus*), the two populations analyzed were markedly different
662 in several sperm quality biomarkers such as milt volume, sperm concentration, and sperm

663 motility (Dietrich *et al.* 2014). As a result, authors estimated 134 million motile
664 spermatozoa per ejaculate (volume × concentration × percentage of motility) in the
665 “Siedliszcze” population compared to 480 million motile sperm in males from “Guzy”
666 population.

667 In Atlantic salmon (*S. salar*), two wild populations (belonging from Wiezpra and Vistula
668 river) were compared in the terms of sperm motility and motility time (Biernaczyk *et al.*
669 2012). Authors reported that both parameters were dependent on the origin of fish:
670 salmon ascending the River Wieprza showed the highest sperm quality values (both in
671 sperm motility and motility time), while animals caught in the Vistula river showed
672 lowest values. In this case, sperm quality was largely dependent on environmental
673 conditions, which were able to explain the 75% of sperm variability. Other authors have
674 studied in depth the sperm trait differences between wild and farmed animals, pondering
675 the possible impacts of escaped farmed fish on wild fish populations. In this sense,
676 Lehnert *et al.* (2012) reported that Chinook salmon (*O. tshawytscha*) farmed males had
677 significantly greater percentage of motile spermatozoa compared to wild males,
678 suggesting that farming practices may lead to increased sperm performance from
679 selective pressure on the aquaculture environment. Authors also reported that these results
680 do highlight the potential for substantial introgression resulting from male-male
681 competition between farmed and wild Chinook salmon in the wild. However, other
682 studies focused on this topic showed different outcomes: Skjæraasen *et al.* (2010) and
683 Butts *et al.* (2011) reported that wild male cod (*G. morhua*) had greater sperm
684 performance compared to farmed cod; whereas Rideout *et al.*, (2004) observed no
685 difference in sperm traits between wild and farmed haddock (*H. hippoglossus*).

686 Concerning inter-species studies, interesting approaches can be done through sperm
687 motility data. Gallego *et al.* (2014) analyzed the sperm motion parameters of swimmer
688 (pufferfish and European eel) and sessile (sea urchin and ascidian) species, reporting
689 sperm motion patterns totally opposite. In this context, sessile species displayed notably
690 higher values than swimmer species in terms of motility time,, keeping high motility
691 values during a longer time. Authors linked the sperm motion patterns to the species-
692 specific lifestyles, postulating that sessile organisms (which show limited or no
693 movement) need sperm with a capacity to swim for long distances to find the oocytes,
694 while swimmer male organisms can move toward the female and release gametes near it,
695 and therefore the spermatozoa does not need to swim for such a long time.

696

697 **3.5 Ecotoxicology**

698 Aquatic ecosystems are repositories of substantial quantities of natural and man-made
699 environmental contaminants (EC), and fish sperm motility has become a valuable tool for
700 assessing the EC toxicity (Hatef *et al.* 2013). At present, around 100 scientific
701 publications reporting the impact of ECs on sperm motion performance have contributed
702 to understand the toxicity mechanisms and action sites of ECs, and this knowledge can
703 be nowadays applied for wider range of topics.

704 ECs are diverse along the natural environment and include a heterogeneous group formed
705 by heavy metals, pesticides, biocides or pharmaceuticals, which can usually lead to
706 diminished reproductive parameters, including sperm production and sperm motility
707 parameters (Segner 2011). It is important to take into account that EC effects are
708 extremely variable, and several factors such as the target species, the EC concentrations
709 and the duration of exposure will be key elements on the impact on the sperm motion
710 performance. In this context, Table 6 summarizes the main ECs affecting sperm motility
711 of fish species, indicating the minimum EC dose at which sperm motion performance was
712 significantly affected.

713 Within the main ECs, xenoestrogens are part of a group of synthetic and naturally
714 endocrine disruptors that specifically have estrogen-like effects. Bisphenol-A (BPA), a
715 synthetic chemical used in the production of epoxy resins and polycarbonate plastics, has
716 estrogenic potency and several studies have evidenced its effect on fish sperm motility.
717 For example, brown trout (*S. trutta*) males exposed to BPA concentrations of 1.75 and
718 2.40 µg/L showed low sperm quality (sperm density, motility rate, and swimming
719 velocity) than control males at the beginning of the spawning season (Lahnsteiner *et al.*
720 2005). In fact, production of high quality sperm was restricted to the end of the spawning
721 season, and delayed for approximately 4 weeks in comparison to the control. In goldfish
722 males (*Carassius auratus*), sperm motility was significantly decreased in the BPA-treated
723 groups after 20 or 30 days of exposure, and significant decrease in sperm velocity was
724 observed at 30, 60 and 90 s post-activation in the BPA-treated groups at all exposure
725 times (10, 20 and 30 days) (Hatef *et al.* 2012).

726 Estradiol (E2) or ethynylestradiol (EE2) are another xenoestrogens easy to find, which
727 usually comes from oral contraceptives, and its occurrence in surface waters is the result
728 of local sewage discharges (Arcand-Hoy & Benson 1998). Recently, both xenoestrogens
729 have been implicated as the primary contaminants contributing to the estrogenic activity
730 in surface waters, and negative effects about reproduction issues have been reported in

731 both freshwater and seawater species. In fighting fish (*Betta splendens*), an exposure for
732 4 weeks of 100 ng/L reduced significantly sperm swimming velocity and, in rainbow trout
733 (*O. mykiss*), exposures for 12 weeks of 10 ng/L also decreased sperm motility parameters.
734 In pejerrey fish (*Odontesthes bonariensis*), although no significant differences in motility
735 parameters were observed between the control group and E2 and EE2 acting separately,
736 a significant decrease in sperm motility was recorded for combined effect of estrogenic
737 agents (E2 + EE2) (Gárriz *et al.* 2015).

738 Furthermore, heavy metals are considered as the most dangerous pollutants around the
739 world, and the toxicity of accumulated metals is determined not only by the type of metal,
740 but also by the physical and chemical properties of water and the protective mechanisms
741 of fish species (Hatef *et al.* 2013). In this sense, and regarding species-specific effect,
742 Lahnsteiner *et al.* (2004) studied the impact of different heavy metals (zinc, mercury and
743 cadmium) on sperm motility parameters in four teleosts belonging to the most
744 representative freshwater families (Salmonidae, Cyprinidae, Gadidae and Clariidae), and
745 they concluded that toxic concentrations of all pollutants differed markedly for each
746 species. In this sense, African catfish (*C. gariepinus*) spermatozoa were the most resistant,
747 European chub (*L. cephalus*) and burbot (*Lota lota*) spermatozoa showed medium
748 resistance and brown trout (*S. trutta*) spermatozoa were the most sensitive to the heavy
749 metals used. The impact of heavy metals has been also reported in other freshwater
750 species such as common carp (*C. carpio*), in which zinc and cadmium affected
751 significantly sperm motility at 50 mg/L after 24 h of incubation (Chyb & Kime 2000;
752 Dietrich *et al.* 2011); or in rainbow trout (*O. mykiss*), where cadmium and mercury
753 decreased the percentage of motile spermatozoa after 4 h of incubation at 10 mg/L
754 (Dietrich *et al.* 2010). By contrast, scarce studies have been carried out in marine species.
755 In this context, some data in European sea bass indicate that copper ($\text{CuCl}_2 \cdot 2\text{H}_2\text{O}$) and
756 lead (PbCl_2) did not affect sperm motility when the activating media contained up to 100
757 ppm of these metal salts. In contrast, mercury (HgCl_2) was able to completely arrest the
758 spermatozoa motion at concentrations as low as 0.1 mg/L (Abascal *et al.* 2007).

759 Finally, some pesticides and biocides can also interfere with normal biological processes
760 causing deleterious effects on the reproductive axes of species from aquatic ecosystems.
761 Within this class of chemicals, dichlorodiphenyltrichloroethane (DDT) and tributyltin
762 (TBT) has been the most studied biocides. Regarding DDT, although banned from use
763 many years ago, it is still widely used in some developing countries. Some studies have
764 reported that large exposures (~40 days) of DDT in stinging catfish (*H. fossilis*) can affect

765 negatively sperm motility (Singh *et al.* 2008). In this sense, non-exposed animals showed
766 80-100% of total motility, whereas DDT-exposed fish showed only 20-40% of motile
767 sperm. In African catfish (*C. gariepinus*) and Mozambique tilapia (*O. mossambicus*),
768 CASA results showed a decrease in motility parameters from the control fish values
769 (Marchand *et al.* 2008, 2010). Specifically, the decrease in motility for *O. mossambicus*
770 was statistically significant (80% against 54%, DDT-area against non-polluted area,
771 respectively). Regarding TBT, widely used as antifouling agent, it is considered another
772 common contaminant that has been shown to be capable of embryotoxicity, genotoxicity
773 and endocrine disruption. Studies in African catfish (*C. gariepinus*) showed that TBT had
774 a significant effect on motility at 0.27 µg/L after 24 h exposure in catfish (Rurangwa *et al.*
775 *al.* 2002). However, same trials carried out in common carp (*C. carpio*) showed that
776 sperm motility was only significantly affected by TBT exposure at only 2.7 µg/L
777 (Rurangwa *et al.* 2002).

778 To sum up, sperm motility assessment became a valuable tool to check and understand
779 toxicity mechanisms and sites of action of different ECs through *in vitro* and *in vivo*
780 techniques. Although EC effects are extremely variable depending on the target species
781 or on the concentration and the duration of exposure, changes in the sperm motion
782 performance can serve as a useful biomarker for biomonitoring these agents and their
783 potential effects on reproductive function.

784

785 **3.6 Sperm competition**

786 Sperm competition, which occurs when the sperm from two or more males compete for
787 the fertilization of eggs, is a widespread process that occurs in a wide range of animal
788 taxa, including fish (Stockley *et al.* 1997; Stoltz & Neff 2006). This process usually lead
789 to behavioural, morphological and physiological adaptations both for males
790 characteristics (breeder size, body colour, etc.) and sperm traits (head or flagellum size,
791 total motility, swimming velocity, etc.), so sperm motion assessment become a useful tool
792 for studying this phenomenon (Reinhardt & Otti 2012). In this sense, and despite being a
793 topic of recent interest, about a hundred scientific reports have been published during the
794 last two decades (Figure 2).

795 Dominance hierarchies are common among males living in groups during the mating
796 season, and they have been one of the most studied aspects over sperm competition.
797 Within these hierarchies, males can adopt different reproductive strategies according to
798 their social position (dominant or subordinate), and sperm performance will be often

799 linked to the chosen strategy (Serrano *et al.* 2006). Theoretical models predict that
800 dominant males invest more in “attractiveness” (size, colour, behaviour, etc.) than in
801 gametes (spermatozoa), so sperm quality should be *a priori* lower in dominant than in
802 subordinate males (Ball & Parker 1996). On their hand, subordinate males are not going
803 to invest in “attractiveness”, so they will have more resources for enhancing their
804 gametes. This trade-off between social investment and sperm performance has been
805 studied in several fish species, overall salmonids. For example, in Arctic char (*S. alpinus*),
806 sperm velocity was significantly higher in subordinate than in dominant males, suggesting
807 that males with social dominance are unable to maintain high sperm velocity (Serrano *et*
808 *al.* 2006). In Atlantic salmon (*S. salar*), in which there are two alternative reproductive
809 tactics (ARTs; small sneaking “parr” males *versus* large dominant “anadromous” males),
810 parr males compensate their behavioural subordination by producing physiologically
811 superior spermatozoa. In this sense, the proportions of motile spermatozoa were notably
812 greater in the parr males (Vladić & Järvi 2001). In Chinook salmon, *O. tshawytscha*, parr
813 (jacks) males invested significantly more of their somatic tissue into gonads compared
814 with anadromous (hooknoses) males, and parr males showed higher motility and velocity
815 values (90% and 70 $\mu\text{m/s}$, respectively) than dominant males (85% and 55 $\mu\text{m/s}$,
816 respectively). In masu salmon (*Oncorhynchus masou*), sperm velocity and motility were
817 also significantly higher in parr males compared to anadromous males activated by river
818 water (Makiguchi *et al.* 2016). However, in that study, sperm velocity and motility did
819 not differ between the ARTs in the presence of ovarian fluid (OF, produced by females
820 and released with the eggs) on the activation media, so these results could suggest that
821 OF biases paternity in competitive fertilization contexts playing a role in cryptic female
822 choice. In this sense, it is important to note that OF usually plays an important role during
823 fertilization process, and numerous manuscripts have shown an increase of sperm motility
824 and velocity in species such as lake trout, *Salvelinus namaycush* (Butts *et al.* 2012), Arctic
825 char, *Salvelinus alpinus* (Turner & Montgomerie 2002; Urbach *et al.* 2005), Alpine
826 whitefish, *Coregonus sp.* (Urbach *et al.* 2007), three-spined stickleback, *Gasterosteus*
827 *aculeatus* (Elofsson *et al.* 2006), rainbow trout (Wojtczak *et al.* 2007) or Caspian brown
828 trout, *Salmo trutta caspius* (Hatef *et al.* 2009). Therefore, OF effects should be taken into
829 account during the sperm competition studies with the aim of not masking the “true”
830 sperm kinetic parameters obtained by the dominant or non-dominant males.
831 On the contrary, even when theory predicts that dominant males should have lower
832 quality sperm, some studies have shown no effects, or even the opposite situation. In

833 rainbow trout (*O. mykiss*), no significant differences in sperm quality (motility) or
834 quantity (weight of expressible milt) were evident between fish with different social status
835 (Cardwell *et al.* 1996). In bluegill (*Lepomis macrochirus*), in which males can use three
836 mating tactics (“sneakers”, which streak spawn; “satellites”, which mimic females; and
837 “parentals”, which are territorial), there was no difference in sperm flagellum length,
838 curvilinear swim speed or path linearity among the different mating types (Stoltz & Neff
839 2006). Regarding the opposite effect, male breeding coloration was positively correlated
840 with sperm velocity in three-spined sticklebacks (*G. aculeatus*), and “attractive”
841 (colourful) males showed the fast spermatozoa (Mehlis *et al.* 2013).

842 Cichlids represent another interesting group to study adaptations resulting from sperm
843 competition, because there is a tremendous diversity in their mating behaviours (Morita
844 *et al.* 2014). Fitzpatrick *et al.* (2007) tested in *Telmatochromis vittatus*, a small shell-
845 brooding cichlid, the evolution of sperm parameters across four different reproductive
846 tactics present in this species (pirate, territorial, satellite, and sneaker). Because sneakers
847 usually spawn in the presence of another male, sneakers face the highest levels of sperm
848 competition and pirates the lowest, whereas satellites and territorials experience
849 intermediate levels. In accordance with sperm competition theory, sneakers’ spermatozoa
850 swam faster ($>40 \mu\text{m/s}$) than sperm from males adopting the other reproductive tactics
851 (territorial and satellite), whereas sperm from pirates was slowest ($<30 \mu\text{m/s}$) (Fitzpatrick
852 *et al.* 2007). Fitzpatrick *et al.* (2009) also provided, after examine sperm characteristics
853 in 29 cichlid species, an evidence that species experiencing greater levels of sperm
854 competition have faster-swimming sperm. In this sense, authors reported that species
855 subject to a high level of competition (polygynous) had relatively larger and longer
856 gonads able to provide faster-swimming and longer-lived spermatozoa compared with
857 species experiencing lower sperm competition (monogamous). However, other study
858 carried out by Morita *et al.* (2014) among 28 cichlid species showed that sperm velocity
859 was not correlated with sperm competition rank, whereas motility time was considerably
860 longer in bower-building species (high competition rank) compared with species that do
861 not build bowers (low competition rank).

862 In this context, spermatozoa motion assessment can serve as a useful tool for studying the
863 evolution of alternative reproductive strategies and mating systems in different fish taxa,
864 and several kinetic parameters such as total motility, swimming velocity and/or motility
865 time will provide data to carry out sperm competition studies.

866

867 **4. Standardization of the procedures for assessing sperm motility**

868 The evaluation of sperm motility and other kinetic parameters have become an essential
869 tool for investigating a wide range of topics in numerous fish species (see section 3), and
870 more than 1500 manuscripts have been already published applying fish sperm motility as
871 a tool research. However, an evident lack of standardization assessing the sperm motion
872 has often provided a low reproducibility between trials, making difficult to interpret and
873 compare the results intra- and inter-labs (Boryshpolets *et al.* 2013; Gallego *et al.* 2013c).
874 Therefore, a serial of biological and technical settings both for subjective (made by a
875 technician) and objective (made by CASA system) assessments should be taken into
876 account before sperm kinetic evaluation.

877 Biological settings such as sperm collection (see section 3.3.4), the initial dilution in a
878 species-specific extender, the storage temperature before analysis (see section 3.2.1), the
879 sperm:activation medium ratio, or the timing after sperm motility triggering can notably
880 influence the sperm kinetic evaluation (Fauvel *et al.* 2010). In this sense, Billard and
881 Cosson (1992) reported that a relatively high dilution ratio (close to 1/1000) is necessary
882 to initiate simultaneous motility of spermatozoa in cyprinids. At low dilutions, only some
883 of the spermatozoa were activated after mixing with diluent, whereas others became
884 progressively activated afterwards, so sperm dilution became a key issue for assessing
885 sperm motility. On their hand, kinetic characteristics of fish sperm are often species-
886 specific, and the timing in which the technician (both during subjective or objective)
887 assess sperm motility plays a crucial role regarding the species evaluated. For example,
888 spermatozoa from freshwater species usually present a shorter longevity (1-2 min) than
889 marine species, thus freshwater sperm must be evaluated during the first seconds after
890 activation (Billard & Cosson 1992). However, because there is a lot of variability on
891 sperm longevity among marine species, early sperm evaluations (first 15-30 seconds) are
892 often recommended for all teleost fish (Gallego *et al.* 2014).

893 Technical settings for assessing sperm motility can involve a wide range of factors such
894 as optical contrast, lens magnification, type and depth of the chamber used, etc. (Amann
895 & Waberski 2014). For example, studies carried out in European eel (*A. anguilla*) showed
896 that different magnification lens (10x vs 20x) affected significantly the measurement of
897 sperm kinetic parameters (Gallego *et al.* 2013c). In this case, the number of spermatozoa
898 captured by the 20x magnification lens was much less than those assessed with the 10x
899 lens, therefore the variability coefficients obtained by the wider lens (20x) were much

900 higher than those obtained with the 10x lens. Thus, the results obtained using the 10x lens
901 should be, *a priori*, more accurate and precise than the results obtained using the 20x lens.
902 CASA settings also play a key role for estimating sperm kinetic parameters, and several
903 factors such as the frame rate of recording (or frames per second; fps), the track sampling
904 time, the focal position of swimming sperm cells inside the open drop, the field of
905 observation location, or even the type of CASA used can notably affect sperm kinetic
906 results (Amann & Waberski 2014; Lu *et al.* 2014). In this sense, Gallego *et al.* (2013)
907 reported for a teleost fish that the number of fps influenced the sperm quality parameters
908 provided by a CASA system. Even the frame rate setting had no effect either on motility
909 or on progressive motility, parameters such as curvilinear velocity, straightness, and beat
910 cross frequency were significantly affected. Authors then demonstrated that low frame
911 rates underestimated the real value of kinetic traits, while a higher fps setting provided a
912 more accurate reconstruction of the sperm trajectories, closely resembling the real
913 trajectory.

914 In other study, Boryshpolets *et al.*, 2013 examined different CASA systems (CRISMAS;
915 Hobson Sperm Tracker; and Image J) on the same video recordings using three
916 taxonomically different fish species (sterlet: *A. ruthenus*; common carp, *C. carpio*; and
917 rainbow trout, *O. mykiss*). Authors reported that motility parameters were highly affected
918 by the species and the CASA used for analyses, so special care should be taken with
919 regard to CASA settings, recording conditions, and quality of video recordings
920 (Boryshpolets *et al.* 2013).

921 To sum up, to make it possible to compare the results obtained by different laboratories,
922 all studies using sperm motility assessment must describe its methodology in detail,
923 particularly concerning biological and technical settings. Unfortunately, in most
924 publications, details of these parameters are not provided, thus reducing the possibility of
925 comparing the results intra- and inter-labs. Therefore, it becomes imperative to harmonize
926 common procedures and established protocols to be used in many research groups
927 assessing fish sperm motility for enhancing the reliability, comparability, and
928 applicability of data produced by different laboratories (Rosenthal *et al.* 2010).

929

930 **5. New emerging tools for sperm quality analysis**

931 Although a relatively high number of sperm quality biomarkers have been successfully
932 applied in several fish species, the new demands on basic research imply the arrival of
933 new techniques for sperm analysis, which in a not so far future will be used by fish

934 farming companies (Cabrita *et al.* 2014). This new generation of tools, reviewed by
935 Cabrita *et al.* (2014), will improve the knowledge on sperm quality assessment,
936 complementing the information provided by sperm motion assessment.

937 Genome analysis represent the first emerging tool being a candidate to become a great
938 sperm quality biomarker. In this context, although the evaluation of chromatin damage
939 has been poorly considered in the assessment of sperm quality, some studies have recently
940 related with the fertilization success in fish (Pérez-Cerezales *et al.* 2010a). In addition, it
941 is important to take into account that chromatin modifications could happen even in the
942 absence of measurable effects on other sperm characteristics (like sperm motility), so
943 damage spermatozoa would be able to reach and fertilize the ova, being able to cause
944 harmful effects on the offspring (Pérez-Cerezales *et al.* 2010b). Different methods can be
945 applied to the evaluation of chromatin damage, and most of them related to the detection
946 of fragments or packaging failures. Comet assay or SCGE (single cell gel electrophoresis)
947 is the technique most commonly used and has been validated in numerous fish species
948 (Cabrita *et al.* 2005a; Beirão *et al.* 2008; Pérez-Cerezales *et al.* 2009; Nathanailides *et al.*
949 2011). This method is based on the different electrophoretic migration patterns of DNA
950 fragments, where cells (spermatozoa) with damaged DNA usually present a comet-like
951 tail structure, being longer is the DNA structure damage is bigger.

952 Another techniques based on the differential migration of chromatin fragments, such as
953 the SCD (sperm chromatin dispersion test), are nowadays emerging in fish and they need
954 to be set up for different fish species. In fact, this method have been only used in tench
955 (*T. tinca*) sperm, reporting a good correlation with the results obtained simultaneously
956 using the comet assay (López-Fernández *et al.* 2009). Finally, DNA fragmentation can
957 also be assessed more specifically using the TUNEL (terminal deoxynucleotidyl
958 transferase dUTP nick end labelling) assay method, based on the addition of a fluorescent
959 nucleotide to the 3'OH end of the strand. So, as more fragmented is the spermatozoa
960 DNA, higher the fluorescence emit by the nucleus. This technique has been successfully
961 applied in European sea bass (*D. labrax*) and gilthead seabream (*S. aurata*) (Cabrita *et al.*
962 2011).

963 Moreover, transcriptomic analysis can represent another tool for predicting gamete
964 quality. Although traditionally the use of microarray had not focused on the evaluation of
965 the RNA profile in breeding males, studies in mammals reporting key roles of residual
966 mRNAs from spermatogenesis (Lalancette *et al.* 2008) have allowed the beginning of the
967 research in this topic in fish species. Guerra *et al.* (2013), for example, reported a different

968 approach to investigate on the role of mRNAs as quality markers in fish spermatozoa. In
969 this sense, authors were able to define a set of transcripts that had a different profile in
970 testicular cells from good and bad zebrafish breeders, reporting then a correlation between
971 specific transcripts and sperm quality. Although these results were provided using model
972 species such as zebrafish (*D. rerio*), it opened up the possibility of exploring these
973 findings to key species of aquaculture sector (Guerra *et al.* 2013).

974 To sum up, the great potential of emerging technologies such as genomic, transcriptomic
975 and/or proteomic could establish the first step towards the possibility of selecting fish
976 breeder performance from a molecular point of view (Cabrita *et al.* 2014; Labbé *et al.*
977 2017; Robles *et al.* 2017). The identification of predictive estimators or markers of sperm
978 quality would have major applications in research, fish farms and biotechnological
979 industries.

980

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985

986 **References**

987 Aas GH, Refstie T, Gjerde B (1991) Evaluation of milt quality of Atlantic salmon.
988 *Aquaculture* **95**: 125–132.

989 Abascal FJ, Cosson J, Fauvel C (2007) Characterization of sperm motility in sea bass:
990 The effect of heavy metals and physicochemical variables on sperm motility.
991 *Journal of Fish Biology* **70**: 509–522.

992 Ajala OO, Owoyemi AO (2016) Effect of dietary vernonia amygdalina del.
993 Supplementation on some biological parameters of milt of male African catfish
994 (*Clarias gariepinus*). *Bulgarian Journal of Veterinary Medicine* **19**: 30–39.

995 Al-Lawati H, Kamp G, Bienefeld K (2009) Characteristics of the spermathecal contents
996 of old and young honeybee queens. *Journal of Insect Physiology* **55**: 116–121.

997 Alavi SMH, Cosson J, Karami M, Abdolhay H, Amiri BM (2004) Chemical composition
998 and osmolality of seminal fluid of *Acipenser persicus*; their physiological
999 relationship with sperm motility. *Aquaculture Research* **35**: 1238–1243.

1000 Alavi SMH, Rodina M, Hatef A, Stejskal V, Policar T, Hamáčková J *et al.* (2010) Sperm

- 1001 motility and monthly variations of semen characteristics in *Perca fluviatilis*
1002 (Teleostei: Percidae). *Czech Journal of Animal Science* **55**: 174–182.
- 1003 Allison LNN (1961) The effect of tricaine methanesulfonate (M.S. 222) on the motility
1004 of brook trout sperm. *Progressive Fish-Culturist* **23**: 46–47.
- 1005 Amann RP, Waberski D (2014) Computer-assisted sperm analysis (CASA): Capabilities
1006 and potential developments. *Theriogenology* **81**: 5–17.
- 1007 Aoki K, Okamoto M, Tatsumi K, Ishikawa Y (1997) Cryopreservation of medaka
1008 spermatozoa. *Zoological Science* **14**: 641–644.
- 1009 Aramli MS, Kalbassi MR, Nazari RM (2014) Monthly fluctuations during the breeding
1010 season of sperm density, volume, motility, and composition of seminal and coelomic
1011 fluid in broodfish of Persian sturgeon, *Acipenser persicus* Borodin, 1897. *Journal of*
1012 *Applied Ichthyology* **30**: 261–266.
- 1013 Aramli MS, Golshahi K, Nazari RM, Aramli S, Banan A (2015) Effectiveness of glucose-
1014 methanol extender for cryopreservation of *Huso huso* spermatozoa. *Animal*
1015 *Reproduction Science* **162**: 37–42.
- 1016 Aramli MS, Golshahi K, Nazari RM, Golpour A, Aramli S (2016a) Influence of
1017 Glutamine Supplementation on Motility and Fertilization Success of Frozen–
1018 Thawed Persian Sturgeon (*Acipenser persicus*) Sperm. *Reproduction in Domestic*
1019 *Animals* **51**: 474–477.
- 1020 Aramli MS, Golshahi K, Banan A, Sotoudeh E (2016b) Reliable collection of Caspian
1021 brown trout (*Salmo trutta caspius*) sperm using a catheter. *Reproduction in Domestic*
1022 *Animals* **51**: 831–834.
- 1023 Araújo JÊXS, Streit DP, Ribeiro JS de A, Martins E de FF, Souza FN, de Oliveira CAL
1024 *et al.* (2014) Ovopel and carp pituitary extract as spawning inducers in males of the
1025 Amazon catfish *Leiarius marmoratus* (Gill, 1970). *Brazilian Archives of Biology*
1026 *and Technology* **57**: 882–886.
- 1027 Arcand-Hoy LD, Benson WH (1998) Fish reproduction: An ecologically relevant
1028 indicator of endocrine disruption. *Environmental Toxicology and Chemistry* **17**: 49–
1029 57.
- 1030 Asturiano JF, Sorbera LA, Carrillo M, Zanuy S, Ramos J, Navarro JC *et al.* (2001)
1031 Reproductive performance in male European sea bass (*Dicentrarchus labrax*, L.) fed
1032 two PUFA-enriched experimental diets: A comparison with males fed a wet diet.
1033 *Aquaculture* **194**: 173–190.
- 1034 Asturiano JF, Pérez L, Garzón DL, Marco-Jiménez F, Peñaranda DS, Vicente JS *et al.*

- 1035 (2004) Physio-chemical characteristics of seminal plasma and development of media
1036 and methods for the cryopreservation of European eel sperm. *Fish Physiology and*
1037 *Biochemistry* **30**: 283–293.
- 1038 Asturiano JF, Cabrita E, Horváth (2017) Progress, challenges and perspectives on fish
1039 gamete cryopreservation: A mini-review. *General and Comparative Endocrinology*
1040 **245**: 69–76.
- 1041 Atasever M, Bozkurt Y (2015) Effect of different photoperiod regimes on sperm quality,
1042 fecundity and fertilization in rainbow trout (*Oncorhynchus mykiss*). *Turkish Journal*
1043 *of Fisheries and Aquatic Sciences* **15**: 517–523.
- 1044 Babiak I, Ottesen O, Rudolfson G, Johnsen S (2006a) Chilled storage of semen from
1045 Atlantic halibut, *Hippoglossus hippoglossus* L. I: Optimizing the protocol.
1046 *Theriogenology* **66**: 2025–2035.
- 1047 Babiak I, Ottesen O, Rudolfson G, Johnsen S (2006b) Quantitative characteristics of
1048 Atlantic halibut, *Hippoglossus hippoglossus* L., semen throughout the reproductive
1049 season. *Theriogenology* **65**: 1587–1604.
- 1050 Babiak I, Marschhäuser V, Ottesen O, Rudolfson G, Eggen B, Babiak J (2012) Effects of
1051 extender, storage and sperm-to-egg ratio on cryopreservation success of Atlantic cod
1052 (*Gadus morhua* L.) sperm. *Journal of Applied Ichthyology* **28**: 941–947.
- 1053 Baeza R, Mazzeo I, Vílchez MC, Gallego V, Peñaranda DS, Pérez L *et al.* (2015)
1054 Relationship between sperm quality parameters and the fatty acid composition of the
1055 muscle, liver and testis of European eel. *Comparative Biochemistry and Physiology,*
1056 *Part A* **181**: 79–86.
- 1057 Bagheri T, Imanpoor MR, Jafari V, Bennetau-Pelissero C (2013) Reproductive
1058 impairment and endocrine disruption in goldfish by feeding diets containing soybean
1059 meal. *Animal Reproduction Science* **139**: 136–144.
- 1060 Bai C, Wang X, Lu G, Wei L, Liu K, Gao H *et al.* (2013) Cooling rate optimization for
1061 zebrafish sperm cryopreservation using a cryomicroscope coupled with SYBR14/PI
1062 dual staining. *Cryobiology* **67**: 117–123.
- 1063 Ball MA, Parker GA (1996) Sperm Competition Games: External Fertilization and
1064 Adaptive'' Infertility. *Journal of Theoretical Biology* **180**: 141–150.
- 1065 Bani A, Haghi Vayghan A, NaserAlavi MG (2016) The effects of salinity on reproductive
1066 performance and plasma levels of sex steroids in Caspian kutum *Rutilus frisii kutum*.
1067 *Aquaculture Research* **47**: 3119–3126.
- 1068 Beirão J, Cabrita E, Soares F, Herráez MP, Dinis MT (2008) Cellular damage in

1069 spermatozoa from wild-captured *Solea senegalensis* as detected by two different
1070 assays: Comet analysis and Annexin V-Fluorescein staining. *Journal of Applied*
1071 *Ichthyology* **24**: 508–513.

1072 Beirão J, Soares F, Herráez MP, Dinis MT, Cabrita E (2011) Changes in *Solea*
1073 *senegalensis* sperm quality throughout the year. *Animal Reproduction Science* **126**:
1074 122–129.

1075 Beirão J, Soares F, Pousão-Ferreira P, Diogo P, Dias J, Dinis MT *et al.* (2015) The effect
1076 of enriched diets on *Solea senegalensis* sperm quality. *Aquaculture* **435**: 187–194.

1077 Bencic DC, Krisfalusi M, Cloud JG, Ingermann RL (1999) ATP levels of chinook salmon
1078 (*Oncorhynchus tshawytscha*) sperm following in vitro exposure to various oxygen
1079 tensions. *Fish Physiology and Biochemistry* **20**: 389–397.

1080 Bencic DC, Ingermann RL, Cloud JG (2001) Does CO₂ enhance short-term storage
1081 success of chinook salmon (*Oncorhynchus tshawytscha*) milt? *Theriogenology* **56**:
1082 157–166.

1083 Bennetau-Pelissero C, Breton B, Bennetau B, Le Menn F, Kaushik S (2002) Effect of
1084 genistein enriched diet on the sex steroid endocrinology and the reproductive
1085 efficiency of the rainbow trout *Oncorhynchus mykiss*. *Revue de Médecine*
1086 *Vétérinaire* **153**: 513–516.

1087 Bernáth G, Zarski D, Krejszef S, Palińska-Zarska K, Bokor Z, Król J *et al.* (2015)
1088 Optimization of conditions for the cryopreservation of Eurasian perch (*Perca*
1089 *fluviatilis* Linnaeus, 1758) sperm. *Journal of Applied Ichthyology* **31**: 94–98.

1090 Biernaczyk M, Formicki K, Bartel R, Mongialo Z (2012) Characteristics of gametes of
1091 the Atlantic salmon (*Salmo salar* L.) restored in northern Poland. *Journal of Applied*
1092 *Ichthyology* **28**: 66–74.

1093 Billard R, Cosson MP (1992) Some problems related to the assessment of sperm motility
1094 in freshwater fish. *Journal of Experimental Zoology* **261**: 122–131.

1095 Billard R, Cosson J, Noveiri SB, Pourkazemi M (2004) Cryopreservation and short-term
1096 storage of sturgeon sperm, a review. *Aquaculture* **236**: 1–9.

1097 Blancas-Arroyo GA, Figueroa-Lucero G, Barriga-Sosa IDLA, Arredondo-Figueroa JL
1098 (2004) Effects of an artificial photothermal cycle on the reproduction of the shortfin
1099 silverside, *Chirostoma humboldtianum*, Valenciennes, 1835 (Pisces:
1100 Atherinopsidae). *Aquaculture* **241**: 575–585.

1101 Bobe J, Labbe C (2009) Chilled storage of sperm and eggs. In: *Methods in Reproductive*
1102 *Aquaculture, Marine and Freshwater Species. Section IV. Marine Biology*, pp. 219–

1103 235. CRC Press.

1104 Bone Q, Marshall NB, Blaxter JHS (1995) *Diversity of Fishes*. Wiley-Blackwell.

1105 Borges A, Rodrigues Siqueira D, Follmann Jurinitz D, Zanini R, do Amaral F, Lacerda
1106 Grillo M *et al.* (2005) Biochemical composition of seminal plasma and annual
1107 variations in semen characteristics of jundiá *Rhamdia quelen* (Quoy and Gaimard,
1108 Pimelodidae). *Fish Physiology and Biochemistry* **31**: 45–53.

1109 Boryshpolets S, Kowalski RK, Dietrich GJ, Dzyuba B, Ciereszko A (2013) Different
1110 computer-assisted sperm analysis (CASA) systems highly influence sperm motility
1111 parameters. *Theriogenology* **80**: 758–765.

1112 Bozkurt Y, Ögrent F, Secer FS (2009) Effect of different extenders and storage periods
1113 on motility and fertilization success of grass carp (*Ctenopharyngodon idella*) sperm
1114 during spawning season. *Tarim Bilimleri Dergisi* **15**: 277–284.

1115 Bromage N, Randall C, Davies B, Thrush M, Duston J, Carillo M *et al.* (1993)
1116 Photoperiodism and the control of reproduction and development in farmed fish. In:
1117 *Aquaculture: Fundamental and Applied Research*. pp. 81–102. American
1118 Geophysical Union.

1119 Burness G, Moyes CD, Montgomerie R (2005) Motility, ATP levels and metabolic
1120 enzyme activity of sperm from bluegill (*Lepomis macrochirus*). *Comparative*
1121 *Biochemistry and Physiology - A Molecular and Integrative Physiology* **140**: 11–17.

1122 Butts IAE, Rideout RM, Burt K, Samuelson S, Lush L, Litvak MK *et al.* (2010)
1123 Quantitative semen parameters of Atlantic cod (*Gadus morhua*) and their
1124 physiological relationships with sperm activity and morphology. *Journal of Applied*
1125 *Ichthyology* **26**: 756–762.

1126 Butts IAE, Trippel EA, Ciereszko A, Soler C, Słowińska M, Alavi SMH *et al.* (2011)
1127 Seminal plasma biochemistry and spermatozoa characteristics of Atlantic cod
1128 (*Gadus morhua* L.) of wild and cultivated origin. *Comparative Biochemistry and*
1129 *Physiology - A Molecular and Integrative Physiology* **159**: 16–24.

1130 Butts IAE, Johnson K, Wilson CC, Pitcher TE (2012) Ovarian fluid enhances sperm
1131 velocity based on relatedness in lake trout, *Salvelinus namaycush*. *Theriogenology*
1132 **78**: 2105–2109.

1133 Butts IAE, Baeza R, Strotrup JG, Krüger-Johnsen M, Jacobsen C, Pérez L *et al.* (2015)
1134 Impact of dietary fatty acids on muscle composition, liver lipids, milt composition
1135 and sperm performance in European eel. *Comparative Biochemistry and Physiology*
1136 *-Part A : Molecular and Integrative Physiology* **183**: 87–96.

- 1137 Cabas I, Chaves-Pozo E, García-Alcazar A, Meseguer J, Mulero V, Garcia-Ayala A
1138 (2013) The effect of 17-ethynylestradiol on steroidogenesis and gonadal cytokine
1139 gene expression is related to the reproductive stage in marine hermaphrodite fish.
1140 *Marine Drugs* **11**: 4973–4992.
- 1141 Cabrita E, Robles V, Rebordinos L, Sarasquete C, Herráez MP (2005a) Evaluation of
1142 DNA damage in rainbow trout (*Oncorhynchus mykiss*) and gilthead sea bream
1143 (*Sparus aurata*) cryopreserved sperm. *Cryobiology* **50**: 144–153.
- 1144 Cabrita E, Robles V, Cuñado S, Wallace JC, Sarasquete C, Herráez MP (2005b) Effect
1145 of dominance status on sex hormone levels in laboratory and wild-spawning male
1146 trout. *Cryobiology* **50**: 273–284.
- 1147 Cabrita E, Sarasquete C, Martínez-Páramo S, Robles V, Beirão J, Pérez-Cerezales S *et*
1148 *al.* (2010) Cryopreservation of fish sperm: Applications and perspectives. *Journal*
1149 *of Applied Ichthyology* **26**: 623–635.
- 1150 Cabrita E, Ma S, Diogo P, Martínez-Páramo S, Sarasquete C, Dinis MT (2011) The
1151 influence of certain aminoacids and vitamins on post-thaw fish sperm motility,
1152 viability and DNA fragmentation. *Animal Reproduction Science* **125**: 189–195.
- 1153 Cabrita E, Martínez-Páramo S, Gavaia PJ, Riesco MF, Valcarce DG, Sarasquete C *et al.*
1154 (2014) Factors enhancing fish sperm quality and emerging tools for sperm analysis.
1155 *Aquaculture* **432**: 389–401.
- 1156 Canyurt MA, Akhan S (2008) Effect of dietary vitamin E on the sperm quality of rainbow
1157 trout (*Onchorynchus mykiss*). *Aquaculture Research* **39**: 1014–1018.
- 1158 Cardwell JR, Sorensen PW, Van Der Kraak GJ, Liley NRR (1996) Effect of Dominance
1159 Status on Sex Hormone Levels in Laboratory and Wild-Spawning Male Trout.
1160 *General and Comparative Endocrinology* **101**: 333–341.
- 1161 Carneiro PCF, Segui MS, Ióris Filho CR, Mikos JD (2006) Viabilidade do sêmen do
1162 jundiá, *Rhamdia quelen*, armazenado sob refrigeração. *Revista Academica* **4**: 11–16.
- 1163 Cejko BI, Targońska K, Kowalski RK, Zarski D, Sarosiek B, Kucharczyk D *et al.* (2012)
1164 The effectiveness of hormonal preparations (Ovopel, Ovaprim, LHRHa, hCG and
1165 CPE) in stimulating spermiation in dace *Leuciscus leuciscus* (L.). *Journal of Applied*
1166 *Ichthyology* **28**: 873–877.
- 1167 Cejko BI, Zarski D, Krejszef S, Kucharczyk D, Kowalski RK (2013) Effect of hormonal
1168 stimulation on milt volume, number of sperm, and sperm motility in the crucian carp,
1169 *Carassius carassius* (L.). *Israeli Journal of Aquaculture - Bamidgeh* **65**: 912–918.
- 1170 Cejko BI, Zarski D, Judycka S, Kucharczyk D, Sarosiek B, Kowalski RK (2014) Effect

1171 of two commercial preparations containing different GnRH analogues with
 1172 dopamine antagonists on barbel *Barbus barbus* (L.) sperm quantity and quality.
 1173 *Aquaculture International* **22**: 97–109.

1174 Cejko BI, Krejszef S, Sarosiek B, Zarski D, Judycka S, Kowalski RK (2015)
 1175 Biochemical factors of common carp *Cyprinus carpio* L. 1758, seminal plasma and
 1176 its relationship with sperm motility parameters. *Journal of Applied Ichthyology* **31**:
 1177 10–17.

1178 Cejko BI, Kucharczyk D (2015) Application of dopaminergic antagonist:
 1179 Metoclopramide, in reproduction of crucian carp *Carassius carassius* (L.) under
 1180 controlled conditions. *Animal Reproduction Science* **160**: 74–81.

1181 Cejko BI, Judycka S, Kujawa R (2016) The effect of different ambient temperatures on
 1182 river lamprey (*Lampetra fluviatilis*) egg and sperm production under controlled
 1183 conditions. *Journal of Thermal Biology* **62**: 70–75.

1184 Chalde T, Gárriz Á, Sanches EA, Miranda LA (2016) Influence of pejerrey *Odontesthes*
 1185 *bonariensis* (Valenciennes, 1835) broodstock age on gamete quality, reproductive
 1186 performance and plasma sex steroid levels during the spawning season. *Aquaculture*
 1187 *Research* **47**: 969–982.

1188 Chen J, Saili KS, Liu Y, Li L, Zhao Y, Jia Y *et al.* (2017) Developmental bisphenol A
 1189 exposure impairs sperm function and reproduction in zebrafish. *Chemosphere* **169**:
 1190 262–270.

1191 Chen SL, Ji XS, Yu GC, Tian YS, Sha ZX (2004) Cryopreservation of sperm from turbot
 1192 (*Scophthalmus maximus*) and application to large-scale fertilization. *Aquaculture*
 1193 **236**: 547–556.

1194 Christ S, Toth G, McCarthy H, Torsella J, Smith M (1996) Monthly variation in sperm
 1195 motility in common carp assessed using computer-assisted sperm analysis (CASA).
 1196 *Journal of Fish Biology* **48**: 1210–1222.

1197 Christensen JM, Tiersch TR (2005) Cryopreservation of channel catfish sperm: Effects
 1198 of cryoprotectant exposure time, cooling rate, thawing conditions, and male-to-male
 1199 variation. *Theriogenology* **63**: 2103–2112.

1200 Chyb J, Kime D (2000) The influence of zinc on sperm motility of common carp—a
 1201 computer assisted studies. *Archiwum Rybactwa* **8**: 5–14.

1202 Chyb J, Kime DE, Szczerbik P, Mikoajczyk T, Epler P (2001) Computer-assisted analysis
 1203 (CASA) of common carp *Cyprinus carpio* L. spermatozoa motility in the presence
 1204 of cadmium. *Archives of Polish Fisheries* **9**: 173–182.

- 1205 Ciereszko A, Dabrowski K (2000) Effect of ascorbic acid supplement in vitro on rainbow
1206 trout sperm viability. *Aquaculture International* **8**: 1–8.
- 1207 Ciereszko A, Hatef A, Křišann J, Dzyuba B, Boryshpolets S, Rodina M *et al.* (2015)
1208 Sperm morphology, physiology, motility, and cryopreservation in percidae. In:
1209 *Biology and Culture of Percid Fishes: Principles and Practices*. (Eds P. Kestemont,
1210 K. Dabrowski & R.C. Summerfelt), pp. 163–191. Springer Netherlands, Dordrecht.
- 1211 Ciereszko a, Dabrowski K (1995) Sperm quality and ascorbic acid concentration in
1212 rainbow trout semen are affected by dietary vitamin C: an across-season study.
1213 *Biology of Reproduction* **52**: 982–988.
- 1214 Crowe TP, Russell R (2009) Functional and Taxonomic Perspectives of Marine
1215 Biodiversity. In Marine Hard Bottom Communities. In: *Marine Hard Bottom*
1216 *Communities: Patterns, Dynamics, Diversity, and Change*. (Ed. M. Wahl), pp. 375–
1217 390. Springer Berlin Heidelberg, Berlin, Heidelberg.
- 1218 Cruz-Casallas PE, Medina-Robles VM, Velasco-Santamaría YM (2007) Seasonal
1219 variation of sperm quality and the relationship between spermatocrit and sperm
1220 concentration in Yamú *Brycon amazonicus*. *North American Journal of Aquaculture*
1221 **69**: 159–165.
- 1222 Dabrowski K, Rinchar J, Lee K-J, Blom JH, Ciereszko A, Ottobre J (2000) Effects of
1223 diets containing gossypol on reproductive capacity of rainbow trout (*Oncorhynchus*
1224 *mykiss*). *Biology of Reproduction* **62**: 227–234.
- 1225 Dada AA (2012) Effect of ascorbic acid supplementation in broodstock feed on sperm
1226 quality of african sharptooth catfish (*Clarias gariepinus*). *Indian Journal of Animal*
1227 *Research* **46**: 213–218.
- 1228 DeGraaf JD, Berlinsky DL (2004) Cryogenic and refrigerated storage of Atlantic cod
1229 (*Gadus morhua*) and haddock (*Melanogrammus aeglefinus*) spermatozoa.
1230 *Aquaculture* **234**: 527–540.
- 1231 DeGraaf JD, King V W, Benton C, Berlinsky DL (2004) Production and storage of sperm
1232 from the black sea bass *Centropristis striata* L. *Aquaculture Research* **35**: 1457–
1233 1465.
- 1234 Dietrich GJ, Kowalski R, Wojtczak M, Dobosz S, Goryczko K, Ciereszko A (2005)
1235 Motility parameters of rainbow trout (*Oncorhynchus mykiss*) spermatozoa in relation
1236 to sequential collection of milt, time of post-mortem storage and anesthesia. *Fish*
1237 *Physiology and Biochemistry* **31**: 1–9.
- 1238 Dietrich GJ, Wojtczak M, Dobosz S, Kuźmiński H, Kowalski R, Kotłowska M *et al.*

- 1239 (2007) *Characterization of whitefish (Coregonus lavaretus) sperm motility: Effects*
1240 *of pH, cations and ovarian fluid.*
- 1241 Dietrich GJ, Dietrich M, Kowalski RK, Dobosz S, Karol H, Demianowicz W *et al.* (2010)
1242 Exposure of rainbow trout milt to mercury and cadmium alters sperm motility
1243 parameters and reproductive success. *Aquatic Toxicology* **97**: 277–284.
- 1244 Dietrich GJ, Pecio A, Sikorska J, Wolnicki J, Słowińska M, Hliwa P *et al.* (2014)
1245 Characterization of lake minnow *Eupallasella percnurus* semen in relation to sperm
1246 morphology, regulation of sperm motility and interpopulation diversity. *Journal of*
1247 *Fish Biology* **85**: 446–455.
- 1248 Dietrich GJ, Nynca J, Szczepkowski M, Dobosz S, Szczepkowska B, Ciereszko A (2016)
1249 The effect of cryopreservation of semen from whitefish (*Coregonus lavaretus*) and
1250 northern pike (*Esox lucius*) using a glucose-methanol extender on sperm motility
1251 parameters and fertilizing ability. *Aquaculture* **464**: 60–64.
- 1252 Dietrich MA, Dietrich GJ, Hliwa P, Ciereszko A (2011) Carp transferrin can protect
1253 spermatozoa against toxic effects of cadmium ions. *Comparative Biochemistry and*
1254 *Physiology - C Toxicology and Pharmacology* **153**: 422–429.
- 1255 Ding F, Lall SP, Li J, Lei J, Rommens M, Milley JE (2011) Cryopreservation of sperm
1256 from Atlantic halibut (*Hippoglossus hippoglossus*, L.) for commercial application.
1257 *Cryobiology* **63**: 56–60.
- 1258 Diogo P, Martins G, Gavaia P, Pinto W, Dias J, Cancela L *et al.* (2015) Assessment of
1259 nutritional supplementation in phospholipids on the reproductive performance of
1260 zebrafish, *Danio rerio* (Hamilton, 1822). *Journal of Applied Ichthyology* **31**: 3–9.
- 1261 Dzyuba B, Boryshpolets S, Cosson J, Dzyuba V, Fedorov P, Saito T *et al.* (2014) Motility
1262 and fertilization ability of sterlet *Acipenser ruthenus* testicular sperm after
1263 cryopreservation. *Cryobiology* **69**: 339–341.
- 1264 Dzyuba B, Bondarenko O, Fedorov P, Gazo I, Prokopchuk G, Cosson J (2017) Energetics
1265 of fish spermatozoa: The proven and the possible. *Aquaculture* **472**: 60–72.
- 1266 Dzyuba V, Cosson J (2014) Motility of fish spermatozoa: From external signaling to
1267 flagella response. *Reproductive Biology* **14**: 165–175.
- 1268 El-Ebiary EH, Wahbi OM, El-Greisy ZA (2013) Influence of dietary Cadmium on sexual
1269 maturity and reproduction of Red Tilapia. *Egyptian Journal of Aquatic Research* **39**:
1270 313–317.
- 1271 Elofsson H, Van Look KJW, Sundell K, Sundh H, Borg B (2006) Stickleback sperm
1272 saved by salt in ovarian fluid. *The Journal of Experimental Biology* **209**: 4230–4237.

- 1273 Falahatkar B, Poursaeid S (2014) Effects of hormonal manipulation on stress responses
1274 in male and female broodstocks of pikeperch *Sander lucioperca*. *Aquaculture*
1275 *International* **22**: 235–244.
- 1276 Fauvel C, Suquet M, Cosson J (2010) Evaluation of fish sperm quality. *Journal of Applied*
1277 *Ichthyology* **26**: 636–643.
- 1278 Feindel NJ, Benfey TJ, Trippel EA (2010) Competitive spawning success and fertility of
1279 triploid male Atlantic cod *Gadus morhua*. *Aquaculture Environment Interactions* **1**:
1280 47–55.
- 1281 Felip A, Piferrer F, Carrillo M, Zanuy S (1999) The relationship between the effects of
1282 UV light and thermal shock on gametes and the viability of early developmental
1283 stages in a marine teleost fish, the sea bass (*Dicentrarchus labrax* L.). *Heredity* **83**:
1284 387–397.
- 1285 Fitzpatrick JL, Desjardins JK, Milligan N, Montgomerie R, Balshine S (2007)
1286 Reproductive-tactic-specific variation in sperm swimming speeds in a shell-
1287 brooding cichlid. *Biology of Reproduction* **77**: 280–284.
- 1288 Flajšhans M, Rodina M, Halačka K, Vetešník L, Gela D, Lusková V *et al.* (2008)
1289 Characteristics of sperm of polyploid Prussian carp *Carassius gibelio*. *Journal of*
1290 *Fish Biology* **73**: 323–328.
- 1291 Foresti F (2000) Biotechnology and fish culture. *Hydrobiologia* **420**: 45–47.
- 1292 Fujimoto T, Yasui GS, Yoshikawa H, Yamaha E, Arai K (2008) Genetic and reproductive
1293 potential of spermatozoa of diploid and triploid males obtained from interspecific
1294 hybridization of *Misgurnus anguillicaudatus* female with *M. mizolepis* male.
1295 *Journal of Applied Ichthyology* **24**: 430–437.
- 1296 Gage MJG, Macfarlane CP, Yeates S, Ward RG, Searle JB, Parker GA (2004)
1297 Spermatozoal traits and sperm competition in Atlantic salmon: relative sperm
1298 velocity is the primary determinant of fertilization success. *Current Biology* **14**: 44–
1299 47.
- 1300 Gallego V, Mazzeo I, Vílchez MC, Peñaranda DS, Carneiro PCF, Pérez L *et al.* (2012)
1301 Study of the effects of thermal regime and alternative hormonal treatments on the
1302 reproductive performance of European eel males (*Anguilla anguilla*) during induced
1303 sexual maturation. *Aquaculture* **354–355**: 7–16.
- 1304 Gallego V, Pérez L, Asturiano JF, Yoshida M (2013a) Relationship between spermatozoa
1305 motility parameters, sperm/egg ratio, and fertilization and hatching rates in
1306 pufferfish (*Takifugu niphobles*). *Aquaculture* **416–417**: 238–243.

- 1307 Gallego V, Pérez L, Asturiano JF, Yoshida M (2013b) Study of pufferfish (Takifugu
1308 niphobles) sperm: Development of methods for short-term storage, effects of
1309 different activation media and role of intracellular changes in Ca²⁺ and K⁺ in the
1310 initiation of sperm motility. *Aquaculture* **414–415**: 82–91.
- 1311 Gallego V, Carneiro PCF, Mazzeo I, Vílchez MC, Peñaranda DS, Soler C *et al.* (2013c)
1312 Standardization of European eel (*Anguilla anguilla*) sperm motility evaluation by
1313 CASA software. *Theriogenology* **79**: 1034–1040.
- 1314 Gallego V, Pérez L, Asturiano JF, Yoshida M (2014) Sperm motility parameters and
1315 spermatozoa morphometric characterization in marine species: A study of swimmer
1316 and sessile species. *Theriogenology* **82**: 668–676.
- 1317 Gallego V, Cavalcante SS, Fujimoto RY, Carneiro PCF, Azevedo HC, Maria AN (2017)
1318 Fish sperm subpopulations: Changes after cryopreservation process and relationship
1319 with fertilization success in tambaqui (*Colossoma macropomum*). *Theriogenology*
1320 **87**: 16–24.
- 1321 Garcia RRF, Vasconcelos ACN, Povh JA, Oberst ER, Eloy LR, Streit DP (2016)
1322 Different extenders solutions for tambaqui semen cooling. *Pesquisa Agropecuaria*
1323 *Brasileira* **51**: 780–784.
- 1324 Gárriz Á, Menéndez-Helman RJ, Miranda LA (2015) Effects of estradiol and
1325 ethinylestradiol on sperm quality, fertilization, and embryo-larval survival of
1326 pejerrey fish (*Odontesthes bonariensis*). *Aquatic Toxicology* **167**: 191–199.
- 1327 Gasparini C, Simmons LW, Beveridge M, Evans JP (2010) Sperm swimming velocity
1328 predicts competitive fertilization success in the green swordtail *Xiphophorus helleri*.
1329 *PLoS ONE* **5**: e12146.
- 1330 Gee W (1916) Effects of acute alcoholization on the germ cells of *Fundulus heteroclitus*.
1331 *The Biological Bulletin* **31**: 379–406.
- 1332 Giardina A, Larson SF, Wisner B, Wheeler J, Chao M (2009) Long-term and acute effects
1333 of zinc contamination of a stream on fish mortality and physiology. *Environmental*
1334 *Toxicology and Chemistry* **28**: 287.
- 1335 Golpour A, Akhoundian M, Khara H, Rahbar M, Dadras H (2013) Changes of sperm
1336 quality parameters in Caspian roach (*Rutilus rutilus caspicus*) during spawning
1337 migration. *Czech Journal of Animal Science* **58**: 117–124.
- 1338 Goryczko K, Dososz S, Mäkinen T, Tomasik L (1991) UV-irradiation of rainbow trout
1339 sperm as a practical method for induced gynogenesis. *Journal of Applied*
1340 *Ichthyology* **7**: 136–146.

- 1341 Goudie CA, Simco BA, Davis KB, Liu Q (1995) Production of gynogenetic and polyploid
1342 catfish by pressure-induced chromosome set manipulation. *Aquaculture* **133**: 185–
1343 198.
- 1344 Guerra SM, Valcarce DG, Cabrita E, Robles V (2013) Analysis of transcripts in gilthead
1345 seabream sperm and zebrafish testicular cells: MRNA profile as a predictor of
1346 gamete quality. *Aquaculture* **406–407**: 28–33.
- 1347 Hachero-Cruzado I, Forniés A, Herrera M, Mancera JM, Martínez-Rodríguez G (2013)
1348 Sperm production and quality in brill *Scophthalmus rhombus* L.: Relation to
1349 circulating sex steroid levels. *Fish Physiology and Biochemistry* **39**: 215–220.
- 1350 Hadi-Alavi S, Psenicka M, Rodina M, Policar T, Linhart O (2008) Changes of sperm
1351 morphology, volume, density and motility and seminal plasma composition in
1352 *Barbus barbus* (Teleostei: Cyprinidae) during the reproductive season. *Aquatic*
1353 *Living Resources* **21**: 75–80.
- 1354 Hajiahmadian M, Sarvi Moghanlou K, Agh N, Farrokhi Ardabili F (2016) Semen
1355 characteristics of rainbow trout (*Oncorhynchus mykiss*) following diets containing
1356 different vegetable fatty acid levels. *Reproduction in Domestic Animals* **51**: 979–
1357 984.
- 1358 Hajirezaee S, Amiri BMM, Mirvaghefi ARR (2010) Changes in sperm production, sperm
1359 motility, and composition of seminal fluid in caspian brown trout, *salmo trutta*
1360 *caspicus*, over the course of a spawning season. *Journal of Applied Aquaculture* **22**:
1361 157–170.
- 1362 Halimi M, Mohammadi A, Norousta R, Khara H, Karimi MR (2015) Spermiation time
1363 affect the milt quality indices of the Russian sturgeon, *Acipenser gueldenstaedtii*,
1364 Brandt & Ratzeburg, 1833. *Aquaculture Research* **46**: 2426–2430.
- 1365 Hatéf A, Niksirat H, Alavi SMH (2009) Composition of ovarian fluid in endangered
1366 Caspian brown trout, *Salmo trutta caspius*, and its effects on spermatozoa motility
1367 and fertilizing ability compared to freshwater and a saline medium. *Fish Physiology*
1368 *and Biochemistry* **35**: 695–700.
- 1369 Hatéf A, Alavi SMH, Linhartova Z, Rodina M, Policar T, Linhart O (2010) In vitro effects
1370 of Bisphenol A on sperm motility characteristics in *Perca fluviatilis* L. (Percidae;
1371 Teleostei). *Journal of Applied Ichthyology* **26**: 696–701.
- 1372 Hatéf A, Alavi SMH, Butts IAE, Policar T, Linhart O (2011) Mechanism of action of
1373 mercury on sperm morphology, adenosine triphosphate content, and motility in
1374 *Perca fluviatilis* (Percidae; Teleostei). *Environmental Toxicology and Chemistry* **30**:

1375 905–914.

1376 Hatef A, Alavi SMH, Abdulfatah A, Fontaine P, Rodina M, Linhart O (2012) Adverse
1377 effects of bisphenol A on reproductive physiology in male goldfish at
1378 environmentally relevant concentrations. *Ecotoxicology and Environmental Safety*
1379 **76**: 56–62.

1380 Hatef A, Alavi SMH, Golshan M, Linhart O (2013) Toxicity of environmental
1381 contaminants to fish spermatozoa function in vitro-A review. *Aquatic Toxicology*
1382 **140–141**: 134–144.

1383 Havelka M, Hulák M, Ráb P, Rábová M, Lieckfeldt D, Ludwig A *et al.* (2014) Fertility
1384 of a spontaneous hexaploid male Siberian sturgeon, *Acipenser baerii*. *BMC genetics*
1385 **15**: 5.

1386 He Q, Zhao E, Lu Y, Yan M, Huang C, Dong Q (2012) Evaluation of activation and
1387 storage conditions for sperm of yellow drum *Nibea albiflora*. *Aquaculture* **324–325**:
1388 319–322.

1389 He S, Woods LC (2004) Changes in motility, ultrastructure, and fertilization capacity of
1390 striped bass *Morone saxatilis* spermatozoa following cryopreservation. *Aquaculture*
1391 **236**: 677–686.

1392 Henrotte E, Kaspar V, Rodina M, Psenicka M, Linhart O, Kestemont P (2010) Dietary n-
1393 3/n-6 ratio affects the biochemical composition of Eurasian perch (*Perca fluviatilis*)
1394 semen but not indicators of sperm quality. *Aquaculture Research* **41**: 31–38.

1395 Horváth A, Labbé C, Jesenšek D, Hoitsy G, Bernáth G, Kaczkó D *et al.* (2015) Post-thaw
1396 storage of sperm from various salmonid species. *Journal of Applied Ichthyology* **31**:
1397 119–124.

1398 Horváth Á, Urbányi B, Mims SD, Bean WB, Gomelsky B, Tiersch TR (2006) Improved
1399 cryopreservation of sperm of paddlefish (*Polyodon spathula*). *Journal of the World*
1400 *Aquaculture Society* **37**: 356–362.

1401 Hossain MS, Sarder MRI (2013) Cryogenic freezing of silver carp spermatozoa for
1402 conservation of gene pool. *Progressive Agriculture* **20**: 99–106.

1403 Huang C, Dong Q, Walter RB, Tiersch TR (2004) Sperm cryopreservation of green
1404 swordtail *Xiphophorus helleri*, a fish with internal fertilization. *Cryobiology* **48**:
1405 295–308.

1406 Huang C, Sun C, Su X, Zhao X, Miao M, Liu Y *et al.* (2009) Sperm cryopreservation in
1407 guppies and black mollies. A generalized freezing protocol for livebearers in
1408 Poeciliidae. *Cryobiology* **59**: 351–356.

- 1409 Hulak M, Kaspar V, Psenicka M, Gela D, Li P, Linhart O (2010) Does triploidization
1410 produce functional sterility of triploid males of tench *Tinca tinca* (L.). *Reviews in*
1411 *Fish Biology and Fisheries* **20**: 307–315.
- 1412 Izquierdo MS, Fernández-Palacios H, Tacon AGJ (2001) Effect of broodstock nutrition
1413 on reproductive performance of fish. *Aquaculture* **197**: 25–42.
- 1414 Jenkins-Keeran K, Woods LC (2002) An evaluation of extenders for the short-term
1415 storage of striped bass milt. *North American Journal of Aquaculture* **64**: 248–256.
- 1416 Jenkins JA, Tiersch TR (1997) A preliminary bacteriological study of refrigerated
1417 channel catfish sperm. *Journal of the World Aquaculture Society* **28**: 282–288.
- 1418 Jiang M, Wu F, Huang F, Wen H, Liu W, Tian J *et al.* (2016) Effects of dietary Zn on
1419 growth performance, antioxidant responses, and sperm motility of adult blunt snout
1420 bream, *Megalobrama amblycephala*. *Aquaculture* **464**: 121–128.
- 1421 Judycka S, Szczepkowski M, Ciereszko A, Dietrich GJ (2015) New extender for
1422 cryopreservation of Siberian sturgeon (*Acipenser baerii*) semen. *Cryobiology* **70**:
1423 184–189.
- 1424 Kashani ZH, Imanpoor MR, Shabani A, Gorgin S (2011) The effect of vitamin E and
1425 highly unsaturated fatty acid on growth, survival and haematocrit of goldfish
1426 (*Carassius auratus gibelio*). *International Journal of Bioflux Society* **4**: 334–338.
- 1427 Kashani ZH, Imanpoor MRI (2012) Long-Term Effects and Interactions of Different
1428 Levels of Dietary Vitamin C and E and Highly Unsaturated Fatty Acid on Sperm
1429 Parameters in Goldfish (*Carassius auratus gibelio*). *Journal of Aquaculture*
1430 *Research & Development* **3**: 3–6.
- 1431 Kawamura K, Ueda T, Aoki K, Hosoya K (1999) Spermatozoa in triploids of the rosy
1432 bitterling *Rhodeus ocellatus ocellatus*. *Journal of Fish Biology* **55**: 420–432.
- 1433 Kestemont P, Henrotte E (2015) Nutritional requirements and feeding of broodstock and
1434 early life stages of eurasian perch and pikeperch. *Biology and Culture of Percid*
1435 *Fishes: Principles and Practices* 539–564.
- 1436 Kime DE, Ebrahimi M, Nysten K, Roelants I, Rurangwa E, Moore HDM *et al.* (1996)
1437 Use of computer assisted sperm analysis (CASA) for monitoring the effects of
1438 pollution on sperm quality of fish; application to the effects of heavy metals. *Aquatic*
1439 *Toxicology* **36**: 223–237.
- 1440 Kime DE, Van Look KJW, McAllister BG, Huyskens G, Rurangwa E, Ollevier F (2001)
1441 Computer-assisted sperm analysis (CASA) as a tool for monitoring sperm quality in
1442 fish. *Comparative Biochemistry and Physiology - C Toxicology and Pharmacology*

- 1443 **130**: 425–433.
- 1444 Kopeika E, Kopeika J, Zhang T (2007) Cryopreservation of fish sperm. *Methods in*
1445 *Molecular Biology* **368**: 203–217.
- 1446 Koteeswaran R, Pandian TJ (2002) Live sperm from post-mortem preserved Indian
1447 catfish. *Current Science* **82**: 447–450.
- 1448 Kowalska A, Siwicki AK, Kowalski RK (2017) Dietary resveratrol improves immunity
1449 but reduces reproduction of broodstock medaka *Oryzias latipes* (Temminck &
1450 Schlegel). *Fish Physiology and Biochemistry* **43**: 27–37.
- 1451 Kowalski RK, Cejko BI, Sarosiek B, Kucharczyk D, Targońska K, Glogowski J (2012)
1452 Temporal changes in motility parameters of dace *Leuciscus leuciscus* (L.) sperm
1453 obtained from spermatid ducts and directly from testicles. *Polish Journal of Natural*
1454 *Sciences* **27**: 193–201.
- 1455 Kucharczyk D, Kujawa R, Luczynski M, Glogowski J, Babiak I, Wyszomirska E (1997)
1456 Induced spawning in bream, *Abramis brama* (L.), using carp and bream pituitary
1457 extract and hCG. *Aquaculture Research* **28**: 139–144.
- 1458 Kutluyer F, Kayim M, Öğretmen F, Büyükleblebici S, Tuncer PB (2014)
1459 Cryopreservation of rainbow trout *Oncorhynchus mykiss* spermatozoa: Effects of
1460 extender supplemented with different antioxidants on sperm motility, velocity and
1461 fertility. *Cryobiology* **69**: 462–466.
- 1462 Labbé C, Robles V, Herraez MP (2017) Epigenetics in fish gametes and early embryo.
1463 *Aquaculture* **472**: 93–106.
- 1464 Lahnsteiner F, Berger B, Weismann T, Patzner R (1996) Motility of spermatozoa of
1465 *Alburnus alburnus* (Cyprinidae) and its relationship to seminal plasma composition
1466 and sperm metabolism. *Fish Physiology and Biochemistry* **15**: 167–179.
- 1467 Lahnsteiner F, Berger B, Weismann T, Patzner RA (1998) Determination of semen
1468 quality of the rainbow trout, *Oncorhynchus mykiss*, by sperm motility, seminal
1469 plasma parameters, and spermatozoal metabolism. *Aquaculture* **163**: 163–181.
- 1470 Lahnsteiner F, Mansour N, Berger B (2004) The effect of inorganic and organic pollutants
1471 on sperm motility of some freshwater teleosts. *Journal of Fish Biology* **65**: 1283–
1472 1297.
- 1473 Lahnsteiner F, Berger B, Kletzl M, Weismann T (2005) Effect of bisphenol A on
1474 maturation and quality of semen and eggs in the brown trout, *Salmo trutta fario*.
1475 *Aquatic Toxicology* **75**: 213–224.
- 1476 Lalancette C, Miller D, Li Y, Krawetz SA (2008) Paternal contributions: New functional

1477 insights for spermatozoal RNA. *Journal of Cellular Biochemistry* **104**: 1570–1579.

1478 Lanes CFC, Okamoto MH, Bianchini A, Marins LF, Sampaio LA (2010) Sperm quality
1479 of Brazilian flounder *Paralichthys orbignyanus* throughout the reproductive season.
1480 *Aquaculture Research* **41**: e199–e207.

1481 Lavens P, Sorgeloos P, Dhert P, Devresse B (1996) *Broodstock management and egg and*
1482 *larval quality*. Oxford ; Boston : Blackwell Science.

1483 Lebeda I, Dzyuba B, Rodina M, Flajshans M (2014) Optimization of sperm irradiation
1484 protocol for induced gynogenesis in Siberian sturgeon, *Acipenser baerii*.
1485 *Aquaculture International* **22**: 485–495.

1486 Lee CS, Tamaru CS, Kelley CD, Moriwake A, Miyamoto GT (1992) The effect of salinity
1487 on the induction of spawning and fertilization in the striped mullet, *Mugil cephalus*.
1488 *Aquaculture* **102**: 289–296.

1489 Legendre M, Alavi SMH, Dzyuba B, Linhart O, Prokopchuk G, Cochet C *et al.* (2016)
1490 Adaptations of semen characteristics and sperm motility to harsh salinity: Extreme
1491 situations encountered by the euryhaline tilapia *Sarotherodon melanotheron*
1492 *heudelotii* (Dumeril, 1859). *Theriogenology* **86**: 1251–1267.

1493 Lehnert SJ, Heath DD, Pitcher TE (2012) Sperm trait differences between wild and
1494 farmed Chinook salmon (*Oncorhynchus tshawytscha*). *Aquaculture* **344–349**: 242–
1495 247.

1496 Linhart O, Gela D, Rodina M, Kocour M (2004) Optimization of artificial propagation in
1497 European catfish, *Silurus glanis* L. *Aquaculture* **235**: 619–632.

1498 Linhart O, Rodina M, Flajshans M, Mavrodiev N, Nebesarova J, Gela D *et al.* (2006)
1499 Studies on sperm of diploid and triploid tench, *Tinca tinca* (L.). *Aquaculture*
1500 *International* **14**: 9–25.

1501 Liu Q, Wang X, Wang W, Zhang X, Xu S, Ma D *et al.* (2015) Effect of the addition of
1502 six antioxidants on sperm motility, membrane integrity and mitochondrial function
1503 in red seabream (*Pagrus major*) sperm cryopreservation. *Fish Physiology and*
1504 *Biochemistry* **41**: 413–422.

1505 Van Look KJW, Kime DE (2003) Automated sperm morphology analysis in fishes: The
1506 effect of mercury on goldfish sperm. *Journal of Fish Biology* **63**: 1020–1033.

1507 López-Fernández C, Gage MJG, Arroyo F, Gosálbez A, Larrán AM, Fernández JL *et al.*
1508 (2009) Rapid rates of sperm DNA damage after activation in tench (*Tinca tinca*:
1509 Teleostei, Cyprinidae) measured using a sperm chromatin dispersion test.
1510 *Reproduction* **138**: 257–266.

- 1511 Lu JC, Huang YF, Lü NQ (2014) Computer-aided sperm analysis: Past, present and
1512 future. *Andrologia* **46**: 329–338.
- 1513 Luckenbach JA, Godwin J, Daniels H V., Beasley JM, Sullivan C V., Borski RJ (2004)
1514 Induction of diploid gynogenesis in southern flounder (*Paralichthys lethostigma*)
1515 with homologous and heterologous sperm. *Aquaculture* **237**: 499–516.
- 1516 Lujčić J, Bernath G, Marinovic Z, Radojkovic N, Simic V, Ćirković M *et al.* (2017)
1517 Fertilizing capacity and motility of tench *Tinca tinca* (L., 1758) sperm following
1518 cryopreservation. *Aquaculture Research* **48**: 102–110.
- 1519 Lüpold S, Calhim S, Immler S, Birkhead TR (2009) Sperm morphology and sperm
1520 velocity in passerine birds. *Proceedings. Biological Sciences* **276**: 1175–1181.
- 1521 Makiguchi Y, Torao M, Kojima T, Pitcher TE (2016) Reproductive investment patterns
1522 and comparison of sperm quality in the presence and absence of ovarian fluid in
1523 alternative reproductive tactics of masu salmon, *Oncorhynchus masou*.
1524 *Theriogenology* **86**: 2189–2193.
- 1525 Manning A, Burton M, Crim L (2004) Reproductive evaluation of triploid yellowtail
1526 flounder, *Limanda ferruginea* (Storer). *Aquaculture* **242**: 625–640.
- 1527 Marchand MJ, Pieterse GM, Barnhoorn IEJ (2008) Preliminary results on sperm motility
1528 and testicular histology of two feral fish species, *Oreochromis mossambicus* and
1529 *Clarias gariepinus*, from a currently DDT-sprayed area, South Africa. *Journal of*
1530 *Applied Ichthyology* **24**: 423–429.
- 1531 Marchand MJ, Pieterse GM, Barnhoorn IEJ (2010) Sperm motility and testicular
1532 histology as reproductive indicators of fish health of two feral fish species from a
1533 currently DDT sprayed area, South Africa. *Journal of Applied Ichthyology* **26**: 707–
1534 714.
- 1535 Martínez-Páramo S, Horváth Á, Labbé C, Zhang T, Robles V, Herráez P *et al.* (2017)
1536 Cryobanking of aquatic species. *Aquaculture* **472**: 156–177.
- 1537 Mehlis M, Hilke LK, Bakker TCM (2013) Attractive males have faster sperm in three-
1538 spined sticklebacks *Gasterosteus aculeatus*. *Current Zoology* **59**: 761–768.
- 1539 Mewes JK, Meurer F, Tessaro L, Buzzi AH, Sypereck MA, Bombardelli RA (2016)
1540 Diets containing crude glycerin damage the sperm characteristics and modify the
1541 testis histology of Nile tilapia broodstock. *Aquaculture* **465**: 164–171.
- 1542 Migaud H, Bell G, Cabrita E, McAndrew B, Davie A, Bobe J *et al.* (2013) Gamete quality
1543 and broodstock management in temperate fish. *Reviews in Aquaculture* **5**: S194–
1544 S223.

- 1545 Montano GA, Kraemer DC, Love CC, Robeck TR, O'Brien JK (2012) Evaluation of
1546 motility, membrane status and DNA integrity of frozen-thawed bottlenose dolphin
1547 (*Tursiops truncatus*) spermatozoa after sex-sorting and recryopreservation.
1548 *Reproduction* **143**: 799–813.
- 1549 Montgomery TM, Brown AC, Gendelman HK, Ota M, Clotfelter ED (2014) Exposure to
1550 17 α -ethinylestradiol decreases motility and ATP in sperm of male fighting fish *Betta*
1551 *splendens*. *Environmental Toxicology* **29**: 243–252.
- 1552 Morisawa M (2008) Adaptation and strategy for fertilization in the sperm of teleost fish.
1553 *Journal of Applied Ichthyology* **24**: 362–370.
- 1554 Morita M, Takemura A, Okuno M (2004) Acclimation of sperm motility apparatus in
1555 seawater-acclimated euryhaline tilapia *Oreochromis mossambicus*. *Journal of*
1556 *Experimental Biology* **207**: 337–345.
- 1557 Morita M, Awata S, Yorifuji M, Ota K, Kohda M, Ochi H (2014) Bower-building
1558 behaviour is associated with increased sperm longevity in Tanganyikan cichlids.
1559 *Journal of Evolutionary Biology* **27**: 2629–2643.
- 1560 Müller T, Baska F, Niklesz C, Horn P, Varadi B, Bercsenyi M (2005) The testis histology
1561 of artificially matured European eel (*Anguilla anguilla* L.) at the end of sexual
1562 maturation, and spermatozoa ultrastructure in freshwater rearing. *Acta Biologica*
1563 *Hungarica* **56**: 169–172.
- 1564 Munkittrick KR, Moccia RD (1987) Seasonal changes in the quality of rainbow trout
1565 (*Salmo gairdneri*) semen: Effect of a delay in stripping on spermatocrit, motility,
1566 volume and seminal plasma constituents. *Aquaculture* **64**: 147–156.
- 1567 Murgas LDS, Miliorini AB, Franciscatto RT, Maria AN (2004) Spermatic viability of
1568 piracanjuba (*Brycon orbignyanus*) semen cooled at 4°C. *Revista Brasileira de*
1569 *Zootecnia* **33**: 1361–1365.
- 1570 Mylonas CC, Papadaki M, Divanach P (2003) Seasonal changes in sperm production and
1571 quality in the red porgy *Pagrus pagrus* (L.). *Aquaculture Research* **34**: 1161–1170.
- 1572 Mylonas CC, Fostier A, Zanuy S (2010) Broodstock management and hormonal
1573 manipulations of fish reproduction. *General and Comparative Endocrinology* **165**:
1574 516–534.
- 1575 Mylonas CC, Duncan NJ, Asturiano JF (2016) Hormonal manipulations for the
1576 enhancement of sperm production in cultured fish and evaluation of sperm quality.
1577 *Aquaculture* **472**: 21–44.
- 1578 Nandi S, Routray P, Gupta SD, Rath SC, Dasgupta S, Meher PK *et al.* (2007)

1579 Reproductive performance of carp, *Catla catla* (Ham.), reared on a formulated diet
1580 with PUFA supplementation. *Journal of Applied Ichthyology* **23**: 684–691.

1581 Nathanailides C, Chanzaropoulos T, Barbouti A, Perdikaris C, Zhang T (2011) DNA
1582 fragmentation, linear velocity and fertilising ability of reactivated cryopreserved
1583 goldfish sperm using different cryoprotectants. *Biotechnology* **10**: 514–520.

1584 Navarro RD, Keley F, Pereira S, Felizardo VO, Murgas DS, Pereira MM (2014)
1585 Cryopreservation of semen of Thailand tilapia (*Oreochromis spp.*) fed diet with
1586 different oil sources. *Maringá* **36**: 399–404.

1587 Nowosad J, Kucharczyk D, Liszewski T, Targońska K, Kujawa R (2014) Comparison of
1588 temperature shock timing to induced artificial mitotic gynogenesis and androgenesis
1589 in common tench. *Aquaculture International* **23**: 45–53.

1590 Nyina-Wamwiza L, Milla S, Pierrard MA, Rurangwa E, Mandiki SNM, Van Look KJW
1591 *et al.* (2012) Partial and total fish meal replacement by agricultural products in the
1592 diets improve sperm quality in African catfish (*Clarias gariepinus*). *Theriogenology*
1593 **77**: 184–194.

1594 Nynca J, Dietrich GJ, Dobosz S, Grudniewska J, Ciereszko A (2014) Effect of
1595 cryopreservation on sperm motility parameters and fertilizing ability of brown trout
1596 semen. *Aquaculture* **433**: 62–65.

1597 Nynca J, Judycka S, Liszewska E, Dobosz S, Grudniewska J, Arai K *et al.* (2016) Utility
1598 of different sugar extenders for cryopreservation and post-thaw storage of sperm
1599 from Salmonidae species. *Aquaculture* **464**: 340–348.

1600 Ohta H, Kagawa H, Tanaka H, Okuzawa K, Hirose K (1996) Milt production in the
1601 Japanese eel *Anguilla japonica* induced by repeated injections of human chorionic
1602 gonadotropin. *Fisheries Science* **62**: 44–49.

1603 Ohta H, Izawa T (1996) Diluent for cool storage of the Japanese eel (*Anguilla japonica*)
1604 spermatozoa. *Aquaculture* **142**: 107–118.

1605 Oropesa AL, Martín-Hidalgo D, Fallola C, Gil MC (2015) Effects of exposure to 17-
1606 alpha-ethynylestradiol on sperm quality of tench (*Tinca tinca*). *Ecotoxicology and*
1607 *Environmental Safety* **120**: 318–325.

1608 Ottesen OH, Babiak I, Dahle G (2009) Sperm competition and fertilization success of
1609 Atlantic halibut (*Hippoglossus hippoglossus* L.). *Aquaculture* **286**: 240–245.

1610 Pandian TJ, Koteeswaran R (1998) Ploidy induction and sex control in fish.
1611 *Hydrobiologia* **384**: 167–243.

1612 Park C, Chapman F (2005) An extender solution for the short-term storage of sturgeon

- 1613 semen. *North American Journal of Aquaculture* **67**: 52–57.
- 1614 Parodi J, Guerra G, Cuevas M, Ramírez-Reveco A, Romero F (2017) Effects of storage
1615 time on the motility, mortality and calcium levels of Atlantic salmon *Salmo salar*
1616 spermatozoa. *Journal of Fish Biology* **90**: 1506–1516.
- 1617 Patterson JT, Green CC (2015) Physiological and reproductive response to varying
1618 quantitative lipid inclusion in diets for Gulf killifish *Fundulus grandis* Baird and
1619 Girard. *Aquaculture Research* **46**: 2236–2247.
- 1620 Peñaranda DS, Pérez L, Gallego V, Barrera R, Jover M, Asturiano JF (2010) European
1621 eel sperm diluent for short-term storage. *Reproduction in Domestic Animals* **45**:
1622 407–415.
- 1623 Perchec G, Jeulin C, Cosson J, André F, Billard R (1995) Relationship between sperm
1624 ATP content and motility of carp spermatozoa. *Journal of Cell Science* **108**: 747–
1625 753.
- 1626 Pérez-Cerezales S, Martínez-Páramo S, Cabrita E, Martínez-Pastor F, de Paz P, Herráez
1627 MP (2009) Evaluation of oxidative DNA damage promoted by storage in sperm from
1628 sex-reversed rainbow trout. *Theriogenology* **71**: 605–613.
- 1629 Pérez-Cerezales S, Martínez-Páramo S, Beirão J, Herráez MP (2010a) Fertilization
1630 capacity with rainbow trout DNA-damaged sperm and embryo developmental
1631 success. *Reproduction* **139**: 989–997.
- 1632 Pérez-Cerezales S, Martínez-Páramo S, Beirão J, Herráez MP (2010b) Evaluation of
1633 DNA damage as a quality marker for rainbow trout sperm cryopreservation and use
1634 of LDL as cryoprotectant. *Theriogenology* **74**: 282–289.
- 1635 Pérez L, Vílchez MC, Gallego VV, Morini M, Peñaranda DS, Asturiano JF (2016) Role
1636 of calcium on the initiation of sperm motility in the European eel. *Comparative*
1637 *Biochemistry and Physiology -Part A: Molecular and Integrative Physiology* **191**:
1638 98–106.
- 1639 Peruzzi S, Rudolfson G, Primicerio R, Frantzen M, Kaurić G (2009) Milt characteristics
1640 of diploid and triploid Atlantic cod (*Gadus morhua* L.). *Aquaculture Research* **40**:
1641 1160–1169.
- 1642 Piferrer F, Cal RM, Gómez C, Álvarez-Blázquez B, Castro J, Martínez P (2004) Induction
1643 of gynogenesis in the turbot (*Scophthalmus maximus*): Effects of UV irradiation on
1644 sperm motility, the Hertwig effect and viability during the first 6 months of age.
1645 *Aquaculture* **238**: 403–419.
- 1646 Piferrer F, Beaumont A, Falguière JC, Flajšhans M, Haffray P, Colombo L (2009)

1647 Polyploid fish and shellfish: Production, biology and applications to aquaculture for
1648 performance improvement and genetic containment. *Aquaculture* **293**: 125–156.

1649 Pongthana N, Penman DJ, Karnasuta J, McAndrew BJ (1995) Induced gynogenesis in the
1650 silver barb (*Puntius gonionotus*) and evidence for female homogamety. *Aquaculture*
1651 **135**: 267–276.

1652 Pšenička M, Flajšhans M, Hulák M, Kašpar V, Rodina M, Borishpolets S *et al.* (2010)
1653 The influence of ploidy level on ultrastructure and motility of tench *Tinca tinca* (L.)
1654 spermatozoa. *Reviews in Fish Biology and Fisheries* **20**: 331–338.

1655 Pšenička M, Kašpar V, Rodina M, Gela D, Hulák M, Flajšhans M (2011) Comparative
1656 study on ultrastructure and motility parameters of spermatozoa of tetraploid and
1657 hexaploid Siberian sturgeon *Acipenser baerii*. *Journal of Applied Ichthyology* **27**:
1658 683–686.

1659 Rahman MM, Gasparini C, Turchini GM, Evans JP (2015) Testing the interactive effects
1660 of carotenoids and polyunsaturated fatty acids on ejaculate traits in the guppy
1661 *Poecilia reticulata* (Pisces: Poeciliidae). *Journal of Fish Biology* **86**: 1638–1643.

1662 Rainis S, Mylonas CC, Kyriakou Y, Divanach P (2003) Enhancement of spermiation in
1663 European sea bass (*Dicentrarchus labrax*) at the end of the reproductive season
1664 using GnRHa implants. *Aquaculture* **219**: 873–890.

1665 Ramirez-Merlano JA, Medina-Robles V, Cruz-Casallas P (2011) Stational variation on
1666 seminal characteristics in bagre rayado *Pseudoplatystoma metaense* (Telostei,
1667 pimelodidae). *Revista MVZ Córdoba* **16**: 2336–2348.

1668 Reinhardt K, Otti O (2012) Comparing sperm swimming speed. *Evolutionary Ecology*
1669 *Research* **14**: 1039–1056.

1670 Richardson GF, Mcniven MA, Mansour N (2011) Effect of methanol concentration and
1671 thaw rate on the viability and fertility of cryopreserved Arctic char, *Salvelinus*
1672 *alpinus* (L.), spermatozoa. *Aquaculture Research* **42**: 1096–1100.

1673 Rideout RM, Trippel EA, Litvak MK (2004) Relationship between sperm density,
1674 spermatocrit, sperm motility and spawning date in wild and cultured haddock.
1675 *Journal of Fish Biology* **65**: 319–332.

1676 Riesco MF, Félix F, Matias D, Joaquim S, Suquet M, Cabrita E (2017) First study in
1677 cryopreserved *Crassostrea angulata* sperm. *General and Comparative*
1678 *Endocrinology* **245**: 108–115.

1679 Rinchard J, Lee KJ, Dabrowski K, Ciereszko A, Blom JH, Ottobre JS (2003) Influence
1680 of gossypol from dietary cottonseed meal on haematology, reproductive steroids and

1681 tissue gossypol enantiomer concentrations in male rainbow trout (*Oncorhynchus*
1682 *mykiss*). *Aquaculture Nutrition* **9**: 275–282.

1683 Robinson EH, Tiersch TR (1995) Effects of long-term feeding of cottonseed meal on
1684 growth, testis development, and sperm motility of male channel catfish *Ictalurus*
1685 *punctatus* broodfish. *Journal of the World Aquaculture Society* **26**: 426–431.

1686 Robles V, Riesco MF, Psenicka M, Saito T, Valcarce DG, Cabrita E *et al.* (2017) Biology
1687 of teleost primordial germ cells (PGCs) and spermatogonia: Biotechnological
1688 applications. *Aquaculture* **472**: 4–20.

1689 Rosenberg DL (1983) Fertilization success of coho salmon gametes: effects of storage
1690 under various atmospheric conditions, temperature acclimation, and temperature
1691 variations. *The Progressive Fish-Culturist* **45**: 84–87.

1692 Rosenthal H, Asturiano JF, Linhart O, Horvath A (2010) On the biology of fish gametes:
1693 Summary and recommendations of the Second International Workshop, Valencia,
1694 Spain, 2009. *Journal of Applied Ichthyology* **26**: 621.

1695 Rouxel C, Suquet M, Cosson J, Severe A, Quemener L, Fauvel C (2008) Changes in
1696 Atlantic cod (*Gadus morhua* L.) sperm quality during the spawning season.
1697 *Aquaculture Research* **39**: 434–440.

1698 Rurangwa E, Roelants I, Huyskens G, Ebrahimi M, Kime DE, Ollevier F (1998) The
1699 minimum effective spermatozoa:egg ratio for artificial insemination and the effects
1700 of mercury on sperm motility and fertilization ability in *Clarias gariepinus*. *Journal*
1701 *of Fish Biology* **53**: 402–413.

1702 Rurangwa E, Volckaert FAM, Huyskens G, Kime DE, Ollevier F (2001) Quality control
1703 of refrigerated and cryopreserved semen using computer-assisted sperm analysis
1704 (CASA), viable staining and standardized fertilization in African catfish (*Clarias*
1705 *gariepinus*). *Theriogenology* **55**: 751–769.

1706 Rurangwa E, Biegniewska A, Slominska E, Skorkowski EF, Ollevier F (2002) Effect of
1707 tributyltin on adenylate content and enzyme activities of teleost sperm: A
1708 biochemical approach to study the mechanisms of toxicant reduced spermatozoa
1709 motility. *Comparative Biochemistry and Physiology - C Toxicology and*
1710 *Pharmacology* **131**: 335–344.

1711 Rurangwa E, Kime DE, Ollevier F, Nash JP (2004) The measurement of sperm motility
1712 and factors affecting sperm quality in cultured fish. *Aquaculture* **234**: 1–28.

1713 Saad A, Billard R, Theron MC, Hollebecq MG (1988) Short-term preservation of carp
1714 (*Cyprinus carpio*) semen. *Aquaculture* **71**: 133–150.

- 1715 Sahinöz E, Aral F, Doğu Z (2007) Changes in Mesopotamian spiny eel, *Mastacembelus*
1716 *mastacembelus* (Bank & Solender in Russell, 1794) (Mastacembelidae) milt quality
1717 during a spawning period. *Theriogenology* **67**: 848–854.
- 1718 Sansone G, Fabbrocini A, Ieropoli S, Langellotti AL, Occidente M, Matassino D (2002)
1719 Effects of extender composition, cooling rate, and freezing on the motility of sea
1720 bass (*Dicentrarchus labrax*, L.) spermatozoa after thawing. *Cryobiology* **44**: 229–
1721 239.
- 1722 Sarmiento NLA, Martins EFF, Costa DC, Silva WS, Mattioli CC, Luz MR *et al.* (2017)
1723 Effects of supplemental dietary vitamin C on quality of semen from Nile tilapia
1724 (*Oreochromis niloticus*) breeders. *Reproduction in Domestic Animals* **52**: 144–152.
- 1725 Sarosiek B, Judycka S, Kowalski RK (2013) Influence of antioxidants on spermatozoa in
1726 the short-term storage of salmonidae milt. *Polish Journal of Natural Sciences* **28**:
1727 379–384.
- 1728 Sarosiek B, Judycka S, Kucharczyk D, Zarski D, Kowalski RK (2014) Motility
1729 parameters of perch spermatozoa (*Perca fluviatilis* L.) with cryoprotectors addition.
1730 *Aquaculture International* **22**: 167–172.
- 1731 Sarosiek B, Dryl K, Krejszeff S, Zarski D (2016) Characterization of pikeperch (*Sander*
1732 *luciperca*) milt collected with a syringe and a catheter. *Aquaculture* **450**: 14–16.
- 1733 Schultz IR, Battelle PNNL, Richland WA, Drum AS, Battelle MSL, Sequim WA *et al.*
1734 (2000) Environmental estrogens: Dose-response relationships for vitellogenin
1735 formation and reproductive toxicity in male rainbow trout. *Marine Environmental*
1736 *Research* **50**: 192–193.
- 1737 Segner H (2011) Reproductive and Developmental Toxicity in Fishes. In: *Reproductive*
1738 *and Developmental Toxicology*. pp. 1145–1166. Centre for Fish and Wildlife Health,
1739 University of Berne, Berne, Switzerland.
- 1740 Serrano J V, Folstad I, Rudolfson G, Figenschou L (2006) Do the fastest sperm within an
1741 ejaculate swim faster in subordinate than in dominant males of Arctic char?
1742 *Canadian Journal of Zoology* **84**: 1019–1024.
- 1743 Shaliutina A, Hulak M, Gazo I, Linhartova P, Linhart O (2013) Effect of short-term
1744 storage on quality parameters, DNA integrity, and oxidative stress in Russian
1745 (*Acipenser gueldenstaedtii*) and Siberian (*Acipenser baerii*) sturgeon sperm. *Animal*
1746 *Reproduction Science* **139**: 127–135.
- 1747 Sheikhzadeh N, Reza A, Razi Allah JJ, Hossein TN (2010) Effect of Ergosan on semen
1748 quality of male rainbow trout (*Oncorhynchus mykiss*) broodstock. *Animal*

- 1749 *Reproduction Science* **122**: 183–188.
- 1750 Singh PB, Sahu V, Singh V, Nigam SK, Singh HK (2008) Sperm motility in the fishes of
1751 pesticide exposed and from polluted rivers of Gomti and Ganga of North India. *Food*
1752 *and Chemical Toxicology* **46**: 3764–3769.
- 1753 Skjæraasen JE, Meager JJ, Karlsen, Mayer I, Dahle G, Rudolfson G *et al.* (2010) Mating
1754 competition between farmed and wild cod *Gadus morhua*. *Marine Ecology Progress*
1755 *Series* **412**: 247–258.
- 1756 Sørensen SR, Gallego V, Pérez L, Butts IAE, Tomkiewicz J, Asturiano JF (2013)
1757 Evaluation of methods to determine sperm density for the European eel, *Anguilla*
1758 *anguilla*. *Reproduction in Domestic Animals* **48**: 936–944.
- 1759 Stockley P, Gage MJ, Parker G, Møller P (1997) Sperm competition in fishes: the
1760 evolution of testis size and ejaculate characteristics. *The American Naturalist* **149**:
1761 933–954.
- 1762 Stoltz JA, Neff BD (2006) Sperm competition in a fish with external fertilization: The
1763 contribution of sperm number, speed and length. *Journal of Evolutionary Biology*
1764 **19**: 1873–1881.
- 1765 Stoss J, Refstie T (1983) Short-term storage and cryopreservation of milt from Atlantic
1766 salmon and sea trout. *Aquaculture* **30**: 229–236.
- 1767 Sullivan M, Brown AC, Clotfelter ED (2014) Dietary carotenoids do not improve motility
1768 or antioxidant capacity in cichlid fish sperm. *Fish Physiology and Biochemistry* **40**:
1769 1399–1405.
- 1770 Sun C, Huang C, Su X, Zhao X, Dong Q (2010) Optimization of handling and refrigerated
1771 storage of guppy *Poecilia reticulata* sperm. *Journal of Fish Biology* **77**: 54–66.
- 1772 Suquet M, Omnes MH, Normant Y, Fauvel C (1992) Influence of photoperiod, frequency
1773 of stripping and presence of females on sperm output in turbot, *Scophthalmus*
1774 *maximus* (L.). *Aquaculture Research* **23**: 217–225.
- 1775 Suquet M, Dreanno C, Dorange G, Normant Y, Quemener L, Gaignon JL *et al.* (1998)
1776 The ageing phenomenon of turbot spermatozoa: effects on morphology, motility and
1777 concentration, intracellular ATP content, fertilization, and storage capacities.
1778 *Journal of Fish Biology* **52**: 31–41.
- 1779 Suquet M, Dreanno C, Fauvel C, Cosson J, Billard R (2000) Cryopreservation of sperm
1780 in marine fish. *Aquaculture Research* **31**: 231–243.
- 1781 Suquet M, Cosson J, de la Gándara F, Mylonas CC, Papadaki M, Lallemand S *et al.* (2010)
1782 Sperm features of captive Atlantic bluefin tuna (*Thunnus thynnus*). *Journal of*

- 1783 *Applied Ichthyology* **26**: 775–778.
- 1784 Tanaka S (2002) Long-term cryopreservation of sperm of Japanese eel. *Journal of Fish*
1785 *Biology* **60**: 139–146.
- 1786 Targońska K, Kucharczyk D (2011) The application of hCG, CPH and Ovopel in
1787 successful artificial reproduction of goldfish (*Carassius auratus auratus*) under
1788 controlled conditions. *Reproduction in Domestic Animals* **46**: 651–655.
- 1789 Taylor P, Brown GG, Mims SD (1995) Storage, transportation, and fertility of undiluted
1790 and diluted paddlefish milt. *The Progressive Fish-Culturist* **57**: 64–69.
- 1791 Tessaro L, Toledo CPR, Neumann G, Krause RA, Meurer F, Natali MRM *et al.* (2012)
1792 Growth and reproductive characteristics of *Rhamdia quelen* males fed on different
1793 digestible energy levels in the reproductive phase. *Aquaculture* **326–329**: 74–80.
- 1794 Tizkar B, Kazemi R, Alipour A, Seidavi A, Naseralavi G, Ponce-Palafox JT (2015)
1795 Effects of dietary supplementation with astaxanthin and β -carotene on the semen
1796 quality of goldfish (*Carassius auratus*). *Theriogenology* **84**: 1111–1117.
- 1797 Toth GP, Christ SA, McCarthy HW, Torsella JA, Smith MK (1995) Computer-assisted
1798 motion analysis of sperm from the common carp. *Journal of Fish Biology* **47**: 986–
1799 1003.
- 1800 Tourmente M, Giojalas LC, Chiaraviglio M (2011) Sperm parameters associated with
1801 reproductive ecology in two snake species. *Herpetologica* **67**: 58–70.
- 1802 Truscott B, Idler D (1968) Sub-zero preservation of Atlantic salmon sperm. *Journal of*
1803 *the Fisheries Board of Canada* **25**: 363–372.
- 1804 Tsai S, Lin C (2012) Advantages and applications of cryopreservation in fisheries science.
1805 *Brazilian Archives of Biology and Technology* **55**: 425–434.
- 1806 Turner E, Montgomerie R (2002) Ovarian fluid enhances sperm movement in Arctic
1807 charr. *Journal of Fish Biology* **60**: 1570–1579.
- 1808 Tuset VM, Dietrich GJ, Wojtczak M, Słowińska M, De Monserrat J, Ciereszko A (2008)
1809 Relationships between morphology, motility and fertilization capacity in rainbow
1810 trout (*Oncorhynchus mykiss*) spermatozoa. *Journal of Applied Ichthyology* **24**: 393–
1811 397.
- 1812 Ubilla A, Fornari D, Figueroa E, Effer B, Valdebenito I (2015) Short-term cold storage
1813 of the semen of rainbow trout *Oncorhynchus mykiss* (Walbaum, 1792) incorporating
1814 DMSO in the sperm diluent. Effects on motility and fertilizing capacity. *Aquaculture*
1815 *Research* **46**: 37–44.
- 1816 Urbach D, Folstad I, Rudolfson G (2005) Effects of ovarian fluid on sperm velocity in

- 1817 Arctic charr (*Salvelinus alpinus*). *Behavioral Ecology and Sociobiology* **57**: 438–
1818 444.
- 1819 Urbach D, Bittner D, Lenz TL, Bernet D, Wahli T, Wedekind C (2007) Sperm velocity
1820 in an Alpine whitefish: Effects of age, size, condition, fluctuating asymmetry and
1821 gonad abnormalities. *Journal of Fish Biology* **71**: 672–683.
- 1822 Uthayakumar V, Sreedevi PR, Senthilkumar D, Munirasu S, Kiruba A,
1823 Ramasubramanian V (2013) Impact of seasonal variation and feeding on
1824 reproductive behavior of freshwater spiny eel *Mastacembelus armatus* from
1825 Cauvery River. *Asian Pacific Journal of Reproduction* **2**: 189–195.
- 1826 Vassallo-Agius R, Watanabe T, Yoshizaki G, Satoh S, Takeuchi Y (2001) Quality of eggs
1827 and spermatozoa of rainbow trout fed an n-3 essential fatty acid-deficient diet and
1828 its effects on the lipid and fatty acid components of eggs, semen and livers. *Fisheries
1829 Science* **67**: 818–827.
- 1830 Vazirzadeh A, Farhadi A, Naseri M, Jeffs A (2016) Comparison of methods to improve
1831 induction of spermiation in wild-caught carp (*Cyprinus carpio*), a threatened species
1832 from the Caspian Sea basin. *Animal Reproduction Science* **170**: 100–107.
- 1833 Vermeirssen ELM, Mazorra De Quero C, Shields RJ, Norberg B, Kime DE, Scott AP
1834 (2004) Fertility and motility of sperm from Atlantic halibut (*Hippoglossus
1835 hippoglossus*) in relation to dose and timing of gonadotrophin-releasing hormone
1836 agonist implant. *Aquaculture* **230**: 547–567.
- 1837 Verstegen J, Iguer-Ouada M, Onclin K (2002) Computer assisted semen analyzers in
1838 andrology research and veterinary practice. *Theriogenology* **57**: 149–179.
- 1839 Vílchez MC, Santangeli S, Maradonna F, Gioacchini G, Verdenelli C, Gallego V *et al.*
1840 (2015) Effect of the probiotic *Lactobacillus rhamnosus* on the expression of genes
1841 involved in European eel spermatogenesis. *Theriogenology* **84**: 1321–1331.
- 1842 Vílchez MC, Morini M, Peñaranda DS, Gallego V, Asturiano JF, Pérez L (2016) Sodium
1843 affects the sperm motility in the European eel. *Comparative Biochemistry and
1844 Physiology -Part A : Molecular and Integrative Physiology* **198**: 51–58.
- 1845 Vílchez MC, Morini M, Peñaranda DS, Gallego V, Asturiano JF, Pérez L (2017) Role of
1846 potassium and pH on the initiation of sperm motility in the European eel.
1847 *Comparative Biochemistry and Physiology -Part A : Molecular and Integrative
1848 Physiology* **203**: 210–219.
- 1849 Viveiros ATM, Godinho HP (2009) Sperm quality and cryopreservation of Brazilian
1850 freshwater fish species: A review. *Fish Physiology and Biochemistry* **35**: 137–150.

- 1851 Viveiros ATM, Orfão LH, Maria AN, Allaman IB (2009) A simple, inexpensive and
1852 successful freezing method for curimba *Prochilodus lineatus* (Characiformes)
1853 semen. *Animal Reproduction Science* **112**: 293–300.
- 1854 Viveiros ATM, Amaral TB, Orfão LH, Isaú ZA, Caneppele D, Leal MC (2011) Sperm
1855 cryopreservation of tiete tetra *Brycon insignis* (Characiformes): Effects of
1856 cryoprotectants, extenders, thawing temperatures and activating agents on motility
1857 features. *Aquaculture Research* **42**: 858–865.
- 1858 Viveiros ATM, Taffarel TR, Leal MC (2014) Osmolality and composition of the extender
1859 during the cold storage of *Prochilodus lineatus* (Characiformes: Prochilodontidae)
1860 sperm. *Neotropical Ichthyology* **12**: 643–648.
- 1861 Vladić T, Järvi T (2001) Sperm quality in the alternative reproductive tactics of Atlantic
1862 salmon: the importance of the loaded raffle mechanism. *Proceedings. Biological*
1863 *Sciences* **268**: 2375–2381.
- 1864 Vuthiphandchai V, Thadsri I, Nimrat S (2009) Chilled storage of walking catfish (*Clarias*
1865 *macrocephalus*) semen. *Aquaculture* **296**: 58–64.
- 1866 Vuthiphandchai V, Wilairattanadilok K, Chomphuthawach S, Sooksawat T, Nimrat S
1867 (2015) Sperm cryopreservation of silver barb (*Barbodes gonionotus*):
1868 Cryoprotectants, cooling rate and storage time on sperm quality. *Aquaculture*
1869 *Research* **46**: 2443–2451.
- 1870 Wagner E, Arndt R, Hilton B (2002) Physiological stress responses, egg survival and
1871 sperm motility for rainbow trout broodstock anesthetized with clove oil, tricaine
1872 methanesulfonate or carbon dioxide. *Aquaculture* **211**: 353–366.
- 1873 Wang Z, Crim L (1997) Seasonal changes in the biochemistry of seminal plasma and
1874 sperm motility in the ocean pout, *Macrozoarces americanus*. *Fish Physiology and*
1875 *Biochemistry* **16**: 77–83.
- 1876 Wayman WR, Tiersch TR, Thomas RG (1998) Refrigerated storage and cryopreservation
1877 of sperm of red drum, *Sciaenops ocellatus* L. *Aquaculture Research* **29**: 267–273.
- 1878 Williot P, Kopeika EF, Goncharov BF (2000) Influence of testis state, temperature and
1879 delay in semen collection on spermatozoa motility in the cultured Siberian sturgeon
1880 (*Acipenser baeri* Brandt). *Aquaculture* **189**: 53–61.
- 1881 Wojtczak M, Dietrich GJ, Słowińska M, Dobosz S, Kuźmiński H, Ciereszko A (2007)
1882 Ovarian fluid pH enhances motility parameters of rainbow trout (*Oncorhynchus*
1883 *mykiss*) spermatozoa. *Aquaculture* **270**: 259–264.
- 1884 Xu H, Huang L, Liang M, Zheng K, Wang X (2015) Effect of dietary vitamin E on the

- 1885 sperm quality of turbot (*Scophthalmus maximus*). *Journal of Ocean University of*
1886 *China* **14**: 695–702.
- 1887 Yang H, Hazlewood L, Walter RB, Tiersch TR (2009) Sperm cryopreservation of a live-
1888 bearing fish, *Xiphophorus couchianus*: Male-to-male variation in post-thaw motility
1889 and production of F1 hybrid offspring. *Comparative Biochemistry and Physiology -*
1890 *C Toxicology and Pharmacology* **149**: 233–239.
- 1891 Yang H, Norris M, Winn R, Tiersch TR (2010) Evaluation of cryoprotectant and cooling
1892 rate for sperm cryopreservation in the euryhaline fish medaka *Oryzias latipes*.
1893 *Cryobiology* **61**: 211–219.
- 1894 Yang H, Cuevas-Uribe R, Savage MG, Walter RB, Tiersch TR (2012) Sperm
1895 cryopreservation in live-bearing *Xiphophorus* fishes: offspring production from
1896 *Xiphophorus variatus* and strategies for establishment of sperm repositories.
1897 *Zebrafish* **9**: 126–134.
- 1898 Yavas I, Bozkurt Y (2011) Effect of different thawing rates on motility and fertilizing
1899 capacity of cryopreserved grass carp (*Ctenopharyngodon idella*) sperm.
1900 *Biotechnology and Biotechnological Equipment* **25**: 2254–2257.
- 1901 Yavas I, Bozkurt Y, Yildiz C (2014) Cryopreservation of scaly carp (*Cyprinus carpio*)
1902 sperm: Effect of different cryoprotectant concentrations on post-thaw motility,
1903 fertilization and hatching success of embryos. *Aquaculture International* **22**: 141–
1904 148.
- 1905 Yoshikawa H, Morishima K, Kusuda S, Yamaha E, Arai K (2007) Diploid sperm
1906 produced by artificially sex-reversed clone loaches. *Journal of Experimental*
1907 *Zoology Part A: Ecological Genetics and Physiology* **307**: 75–83.
- 1908 Yossa R, Sarker PK, Proulx M, Vandenberg GW (2015) The effects of the dietary biotin
1909 on zebrafish *Danio rerio* reproduction. *Aquaculture Research* **46**: 117–130.
- 1910 You F, Xu J, Zhu X, Xu Y, Zhang P (2008) Effect of ultraviolet irradiation on sperm of
1911 the left-eyed flounder, *Paralichthys olivaceus*. *Journal of the World Aquaculture*
1912 *Society* **39**: 414–422.
- 1913 Zhao Y, Psenicka M, Fujimoto T, Saito T, Yasui GS, Yamaha E *et al.* (2012) Motility,
1914 morphology, mitochondria and ATP content of diploid spermatozoa from sex-
1915 reversed clonal diploid and neo-tetraploid loach, *Misgurnus anguillicaudatus*.
1916 *Journal of Applied Ichthyology* **28**: 1006–1012.
- 1917 Zhao Y, Fujimoto T, Pšenička M, Saito T, Arai K (2016) Non-motile tetraploid
1918 spermatozoa of *Misgurnus* loach hybrids. *Fisheries Science* **82**: 127–135.

1919 Zilli L, Schiavone R, Zonno V, Storelli C, Vilella S (2004) Adenosine triphosphate
1920 concentration and beta-D-glucuronidase activity as indicators of sea bass semen
1921 quality. *Biology of Reproduction* **70**: 1679–1684.
1922

1923 **Figure legends**

1924

1925 **Figure 1.** Evolution of number of manuscripts published from 1975 to 2016 in SCI
1926 journals using fish sperm motility as a research tool (assessed both by subjective or
1927 objective method).

1928

1929 **Figure 2.** Number of manuscripts published by research area (sperm storage, sperm
1930 physiology, broodstock management, breeding cycle, ecotoxicology, and sperm
1931 competition) in SCI journals using fish sperm motility as a research tool (assessed both
1932 by subjective or objective method).

1933

1934 **Table legends**

1935

1936 **Table 1.** Studies on short- and medium-term sperm storage protocols applied to several
1937 fish species indicating the temperature (°C) and ratio (sperm:extender) used. Table gives
1938 the best results reached for each species in terms on sperm motility (percentage of motile
1939 cells). Numbers in brackets indicate the storage days and the absence of brackets means
1940 1-week storage.

1941

1942 **Table 2.** Studies on sperm cryopreservation of fish species belonging from different
1943 groups. Table gives the best results reached for each species in terms on post-thaw
1944 motility (percentage of motile cells), indicating the cryo-medium used for each specie.

1945

1946 **Table 3.** Effect on sperm motility (percentage of motile cells) of different dietary
1947 components (carotenoids, lipids, proteins, vitamins, and others) applied on different fish
1948 species. Positive effect on sperm motility respect to the control group are represented by
1949 “+”; negative effect are represented by “-“; and non-effect are represented by “=”.

1950

1951 **Table 4.** Sperm motility (percentage of motile cells) and velocity ($\mu\text{m/s}$) of different
1952 polyploid fish (2n, 3n, 4n, and 6n) on several teleost species.

1953

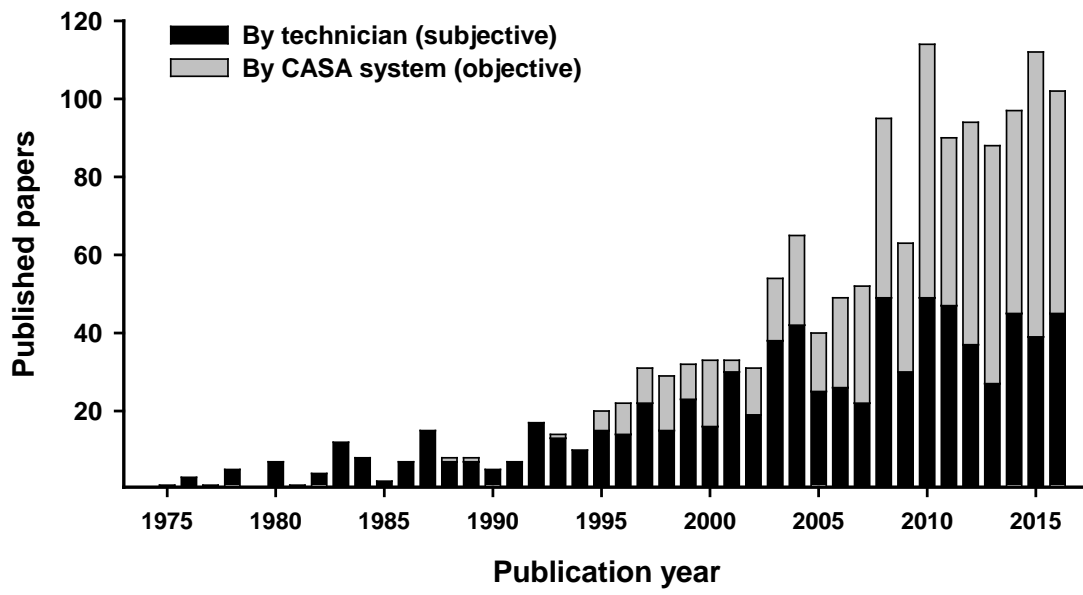
1954 **Table 5.** Seasonal changes in sperm motility (percentage of motile cells) throughout the
1955 breeding season on several fish species. Data are structured regarding sequential patterns

1956 of motility: Type I, species whose sperm motility is higher at the beginning of the
1957 spawning season; Type II, species whose sperm motility is higher at the middle of the
1958 spawning season; Type III, species whose sperm motility is higher at the end of the
1959 spawning season. Table also shows if fish belong from fish farms (captive) or in the wild.
1960 **Solea senegalensis* spawn naturally in two periods (late spring to the beginning of summer and
1961 early autumn, when temperatures are similar)

1962

1963 **Table 6.** Main environmental contaminants (ECs) affecting sperm motility (percentage
1964 of motile cells) on different fish species, indicating the minimum EC dose at which sperm
1965 motion performance was significantly affected. Values in brackets indicate the time
1966 exposure, *in vivo* or *in vitro*.

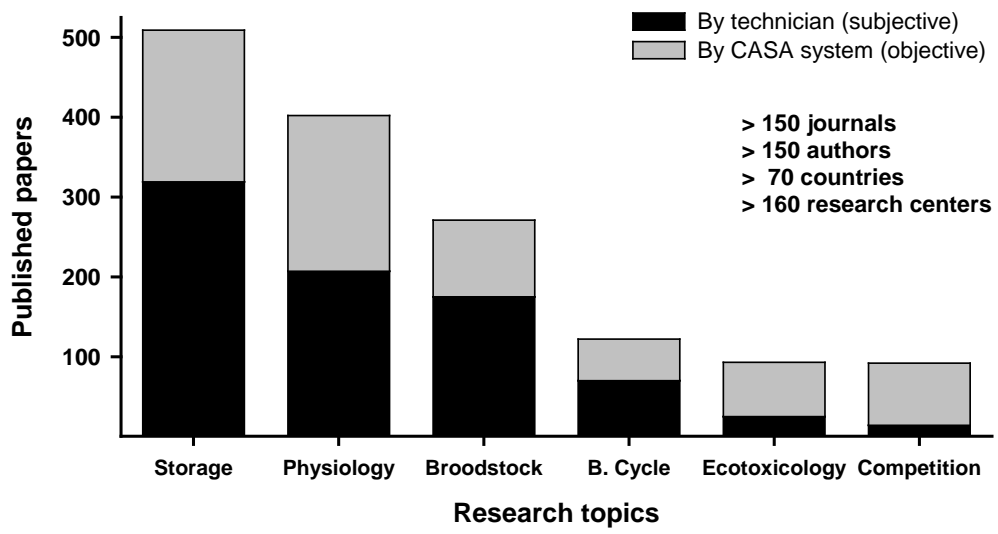
1967 **Figure 1**



1968
1969

1970 **Figure 2**

1971



1972

1973

Table 1

Species	Motility (%)		T°	Ratio	Reference
	Short-term	Medium-term			
<i>Acipenser baerii</i>	50-55 (6)	-	4	1:100	Shaliutina <i>et al.</i> (2013)
<i>Acipenser gueldenstaedtii</i>	50-55 (6)	-	4	1:100	Shaliutina <i>et al.</i> (2013)
<i>Acipenser oxyrinchus</i>	70-75	40-65 (28)	4	1:3	Park and Chapman (2005)
<i>Anguilla anguilla</i>	25-30	-	4	1:50	Peñaranda <i>et al.</i> (2010)
<i>Anguilla japonica</i>	80-85	55-60 (21)	3	1:50	Ohta and Izawa (1996)
<i>Brycon orbignyianus</i>	55-60	-	4	1:3	Murgas <i>et al.</i> (2004)
<i>Clarias macrocephalus</i>	30-35	15-20 (10)	4	1:4	Vuthiphandchai <i>et al.</i> (2009)
<i>Colossoma macropomum</i>	20-25 (5)	-	6	1:4	Garcia <i>et al.</i> (2016)
<i>Cyprinus carpio</i>	90-95	25-30 (17)	4	1:100	Saad <i>et al.</i> (1988)
<i>Gadus morhua</i>	50-55	15-20 (30)	3	1:3	DeGraaf and Berlinsky (2004)
<i>Hippoglossus hippoglossus</i>	85-90	50-55 (50)	4	1:5	Babiak <i>et al.</i> (2006a)
<i>Ictalurus punctatus</i>	25-30	-	4	-	Jenkins and Tiersch (1997)
<i>Melanogrammus aeglefinus</i>	60-65	20-25 (30)	3	1:3	DeGraaf and Berlinsky (2004)
<i>Morone saxatilis</i>	5-10	-	3	1:3	Jenkins-Keeran and Woods (2002)
<i>Nibea albiflora</i>	20-25 (1)	-	4	1:2	He <i>et al.</i> (2012)
<i>Oncorhynchus mykiss</i>	70-75	50-55 (14)	4	1:2	Ubilla <i>et al.</i> (2015)
<i>Oncorhynchus tshawytscha</i>	40-45	40-45 (14)	4	-	Bencic <i>et al.</i> (2001)
<i>Perca fluviatilis</i>	70-75	55-60 (17)	4	1:9	Sarosiek <i>et al.</i> (2014)
<i>Poecilia reticulata</i>	20-25	-	4	1:50	Sun <i>et al.</i> (2010)
<i>Polyodon spatula</i>	75-100	5-25 (56)	2	1:1	Taylor <i>et al.</i> (1995)
<i>Prochilodus lineatus</i>	15-20	-	6-8	1:9	Viveiros <i>et al.</i> (2014)
<i>Takifugu niphobles</i>	75-80	15-20 (15)	4	1:50	Gallego <i>et al.</i> (2013b)
<i>Rhamdia quelen</i>	45-50	25-30 (12)	6	-	Carneiro <i>et al.</i> (2006)
<i>Salmo salar</i>	45-50	35-40	4	1:2	Parodi <i>et al.</i> (2017)
<i>Salvelinus alpinus</i>	60-65	10-15 (15)	4	1:10	Sarosiek <i>et al.</i> (2013)
<i>Sciaenops ocellatus</i>	35-40	10-15 (10)	4	1:10	Wayman <i>et al.</i> (1998)

1976 **Table 2**

Group	Species	Motility (%)		Medium	Reference
		Fresh	Post-thaw		
Salmonids	<i>Coregonus clupeaformis</i>	87	52	9% methanol + 0.18 M	Nynca <i>et al.</i> (2016)
	<i>Coregonus lavaretus</i>	80	50	7.5% methanol + 0.15 m glucose	Dietrich <i>et al.</i> (2016)
	<i>Oncorhynchus mykiss</i>	≥90	≥60	10% DMSO + 7,5% egg yolk	Kutluyer <i>et al.</i> (2014)
	<i>Salmo salar</i>	93	62	9% methanol + 0.18 M glucose	Nynca <i>et al.</i> (2016)
	<i>Salmo trutta</i>	90	74	7.5% methanol + 0.15 m glucose	Nynca <i>et al.</i> (2014)
	<i>Salvelinus alpinus</i>	>70	28	15% methanol	Richardson <i>et al.</i> (2011)
	<i>Salvelinus fortinalis</i>	83	57	9% methanol + 0.18 M sucrose	Nynca <i>et al.</i> (2016)
Cyprinids	<i>Barbodes gonionoutus</i>	91	83	10% DMSO	Vuthiphandchai <i>et al.</i> (2015)
	<i>Ctenopharyngodon idella</i>	91	83	5% glycerol + 0.35 M glucose	Yavas and Bozkurt (2011)
	<i>Cyprinus carpio</i>	95	93	15% egg yolk	Yavas <i>et al.</i> (2014)
	<i>Hypophthalmichthys molitrix</i>	91	81	10% DMSO	Hossain & Sarder (2013)
	<i>Perca fluviatilis</i>	82	54	10% methanol	Bernáth <i>et al.</i> (2015)
	<i>Tinca tinca</i>	>80	45	5% DMSO	Lujčić <i>et al.</i> (2017)
Sturgeons	<i>Acipenser baerii</i>	80	50	10% methanol + glucose	Judycka <i>et al.</i> (2015)
	<i>Acipenser persicus</i>	95	80	10% methanol + 10 mM glutamine	Aramli <i>et al.</i> (2016a)
	<i>Acipenser ruthenus</i>	92	57	10% methanol	Dzyuba <i>et al.</i> (2014)
	<i>Huso huso</i>	82	50	10% methanol + 0.2 M glucose	Aramli <i>et al.</i> (2015)
	<i>Polyodon spathula</i>	90	85	10% methanol	Horváth <i>et al.</i> (2006)
Characiformes	<i>Brycon insignis</i>	98	82	5% BTS + 5% glucose	Viveiros <i>et al.</i> (2011)
	<i>Colossoma macropomum</i>	>90	>70	10% methyl-glycol + 5% Egg yolk	Gallego <i>et al.</i> (2017)
	<i>Esox lucius</i>	80	60	7.5% methanol + 0.15 M glucose	Dietrich <i>et al.</i> (2016)
	<i>Oreochromis niloticus</i>	70	65	10% methanol	Navarro <i>et al.</i> (2014)
	<i>Prochilodus lineatus</i>	100	88	10% methyl-glycol + 5% glucose	Viveiros <i>et al.</i> (2009)
Model species	<i>Danio rerio</i>	84	46	8% DMSO or 4% methanol	Bai <i>et al.</i> (2013)
	<i>Oryzias latipes</i>	85	52	10% methanol	Yang <i>et al.</i> (2010)

	<i>Poecilia latipinna</i>	80	50	20% glycerol	Huang <i>et al.</i> (2009)
	<i>Poecilia reticulata</i>	75	60	20% glycerol	Huang <i>et al.</i> (2009)
Catfishes	<i>Clarias gariepinus</i>	95	71	8% DMSO + 10% egg yolk	Rurangwa <i>et al.</i> (2001)
	<i>Ictalurus punctatus</i>	87	48	5% methanol	Christensen and Tiersch (2005)
Marine fish	<i>Anguilla anguilla</i>	75	47	10% methanol + 5% egg yolk	Herranz-Jusdado, pers. comm., 2017)
	<i>Anguilla japonica</i>	60	46	10% DMSO	Tanaka (2002)
	<i>Dicentrarchus labrax</i>	>90	>50	10% egg yolk + Na-pyruvate	Sansone <i>et al.</i> (2002)
	<i>Gadus morhua</i>	85	70	10% glycerol + 10% egg yolk	Babiak <i>et al.</i> (2012)
	<i>Hippoglossus hippoglossus</i>	80	75	10% DMSO	Ding <i>et al.</i> (2011)
	<i>Morone saxatilis</i>	88	53	5% DMSO+50 mM glycine	He and Woods (2004)
	<i>Pagrus major</i>	88	78	15% methanol + 100mM threalose	Liu <i>et al.</i> (2015)
	<i>Scophthalmus maximus</i>	>80	77	10% DMSO	Chen <i>et al.</i> (2004)
	<i>Sparus aurata</i>	95	70	5% DMSO	Cabrita <i>et al.</i> (2005b)
Int. fertilization	<i>Xiphophorus helleri</i>	95	77	14% glycerol	Huang <i>et al.</i> (2004)
	<i>Xiphophorus couchianus</i>	95-90	35-40	14% glycerol	Yang <i>et al.</i> (2009)
	<i>Xiphophorus variatus</i>	57	37	10% glycerol	Yang <i>et al.</i> (2012)

Table 3

Diet component	Type	Species	Effect on motility	Reference
Carotenoids	Astaxanthin β -carotene	<i>Amatitlania nigrofasciata</i>	=	Sullivan <i>et al.</i> (2014)
	Astaxanthin β -carotene	<i>Carassius auratus</i>	+	Tizkar <i>et al.</i> (2015)
	Zeaxanthin, Astaxanthin, β -carotene	<i>Poecilia reticulata</i>	=	Rahman <i>et al.</i> (2015)
Lipids	Fats	<i>Rhamdia quelen</i>	+	Tessaro <i>et al.</i> (2012)
	DHA	<i>Solea senegalensis</i>	+	Beirão <i>et al.</i> (2015)
	EFA	<i>Oncorhynchus mykiss</i>	+	Vassallo-Agius <i>et al.</i> (2001)
	EPA, DHA, ARA	<i>Anguilla anguilla</i>	+	Butts <i>et al.</i> (2015)
	HUFAs	<i>Carassius auratus</i>	=	Kashani and Imanpoor (2012)
	PUFAs	<i>Catla catla</i>	=	Nandi <i>et al.</i> (2007)
	PUFAs	<i>Poecilia reticulata</i>	+	Rahman <i>et al.</i> (2015)
	Phospholipids	<i>Danio rerio</i>	+	Diogo <i>et al.</i> (2015)
	PUFAs	<i>Dicentrarchus labrax</i>	=	Asturiano <i>et al.</i> (2001)
	Fish oil	<i>Fundulus grandis</i>	=	Patterson and Green (2015)
	HUFAs:SFA	<i>Oncorhynchus mykiss</i>	+	Hajjahmadian <i>et al.</i> (2016)
	HUFAs ratio	<i>Perca fluviatilis</i>	=	Kestemont and Henrotte (2015)
	n-3/n-6 ratio	<i>Perca fluviatilis</i>	=	Henrotte <i>et al.</i> (2010)
Proteins	Soybean meal (replacement)	<i>Carassius auratus</i>	-	Bagheri <i>et al.</i> (2013)
	Vegetable meal (replacement)	<i>Clarias gariepinus</i>	=	Ajala and Owoyemi (2016)
	Agricultural meal (replacement)	<i>Clarias gariepinus</i>	+	Nyina-Wamwiza <i>et al.</i> (2012)
	Cottonseed meal (replacement)	<i>Ictalurus punctatus</i>	=	Robinson and Tiersch (1995)
	Cottonseed meal (replacement)	<i>Oncorhynchus mykiss</i>	=	Rinchard <i>et al.</i> (2003)
	Cottonseed meal (replacement)	<i>Oncorhynchus mykiss</i>	=	Dabrowski <i>et al.</i> (2000)
Vitamins	Biotin	<i>Danio rerio</i>	+	Yossa <i>et al.</i> (2015)
	Vitamin C	<i>Oreochromis niloticus</i>	+	Sarmiento <i>et al.</i> (2017)
	Vitamin C	<i>Clarias gariepinus</i>	+	Dada (2012)
	Vitamin C	<i>Oncorhynchus mykiss</i>	+	Ciereszko and Dabrowski (1995, 2000)

	Vitamin C and E	<i>Carassius auratus</i>	+	Kashani <i>et al.</i> (2011)
	Vitamin E	<i>Scophthalmus maximus</i>	+	Xu <i>et al.</i> (2015)
	Vitamin E	<i>Solea senegalensis</i>	+	Beirão <i>et al.</i> (2015)
	Vitamin E	<i>Oncorhynchus mykiss</i>	+	Canyurt and Akhan (2008)
Others	Zinc	<i>Megalobrama amblycephala</i>	+	Jiang <i>et al.</i> (2016)
	Cadmium	<i>Oreochromis mossambicus</i>	-	El-Ebiary <i>et al.</i> (2013)
	Genistein	<i>Oncorhynchus mykiss</i>	-	Bennetau-Pelissero <i>et al.</i> (2002)
	Glycerin	<i>Oreochromis niloticus</i>	-	Mewes <i>et al.</i> (2016)
	Reservatrol	<i>Oryzias latipes</i>	+	Kowalska <i>et al.</i> (2017)
	<i>Lactobacillus rhamnosus</i>	<i>Anguilla anguilla</i>	+	Vílchez <i>et al.</i> (2015)
	Ergosan	<i>Oncorhynchus mykiss</i>	+	Sheikhzadeh <i>et al.</i> (2010)

1979
1980

Table 4

Species	Type	Motility (%)	Velocity ($\mu\text{m/s}$)	Reference
<i>Acipenser baerii</i>	4n	95	155	Pšenička <i>et al.</i> (2011)
	6n	100	181	
<i>Acipenser baerii cierto</i>	2n	95	170	Havelka <i>et al.</i> (2014)
	6n	100	152	
<i>Carassius gibelio</i>	2n	69	-	Flajšhans <i>et al.</i> (2008)
	3n	23	-	
	4n	45	-	
<i>Gadus morhua</i>	2n	90	12	Peruzzi <i>et al.</i> (2009)
	3n	84	11	
	2n	-	55	Feindel <i>et al.</i> (2010)
	3n	-	56	
<i>Limanda ferruginea</i>	2n	90-100	-	Manning <i>et al.</i> (2004)
	3n	>10	-	
<i>Misgurnus anguillicaudatus</i>	2n	91	-	Zhao <i>et al.</i> (2012)
	4n	90	-	
	2n	>80	-	Yoshikawa <i>et al.</i> (2007)
	3n	10	-	
	2n	90	-	Fujimoto <i>et al.</i> (2008)
	3n	1.5	-	
	2n	91.7	-	Zhao <i>et al.</i> (2016)
<i>Rhodeus ocellatus</i>	2n	98	-	Kawamura <i>et al.</i> (1999)
	3n	87	-	
<i>Tinca tinca</i>	2n	92-100	90-100	Hulak <i>et al.</i> (2010)
	3n	87-96	90-100	

2n	98	99	Pšenička <i>et al.</i> (2010)
3n	94	91	
2n	93-100	82-110	Linhart <i>et al.</i> (2006)
3n	37-77	~90	

1982

1983

1984 **Table 5**

	Species	Condition	Motility			Reference
			Early	Middle	Last	
Type I	<i>Acipenser gueldenstaedtii</i>	Captive	65-70	65-70	40-45	Halimi <i>et al.</i> (2015)
	<i>Hippoglossus hippoglossus</i>	Captive	80-85	80-85	20-30	Babiak <i>et al.</i> (2006b)
	<i>Salmo trutta</i>	Captive	65-70	55-60	50-55	Hajirezaee <i>et al.</i> (2010)
	<i>Scophthalmus maximus</i>	Captive	80-100		60-80	Suquet <i>et al.</i> (1998)
	<i>Solea senegalensis</i> *	Captive	55-60		35-40	Beirão <i>et al.</i> (2011)
Type II	<i>Centropristis striata</i>	Captive		80-85	40-45	DeGraaf <i>et al.</i> 2004)
	<i>Dicentrarchus labrax</i>	Captive		90-100	35-50	Rainis <i>et al.</i> (2003)
	<i>Gadus morhua</i>	Captive	40-45	50-55	35-40	Rouxel <i>et al.</i> (2008)
	<i>Macrozoarces americanus</i>	Captive	<25	>75	40-50	Wang and Crim (1997)
	<i>Mastacembelus mastacembelus</i>	Wild	45-50	80-85	65-70	Sahinöz E <i>et al.</i> (2007)
	<i>Oncorhynchus mykiss</i>	Captive	75-80	80-85	55-60	Munkittrick and Moccia (1987)
	<i>Pagrus pagrus</i>	Captive	45-50	90-95	85-90	Mylonas <i>et al.</i> (2003)
	<i>Rutilus rutilus</i>	Wild	60-65	80-85	70-75	Golpour <i>et al.</i> (2013)
Type III	<i>Cyprinus carpio</i>	Captive	60-60	80-90	80-90	Christ <i>et al.</i> (1996)
	<i>Mastacembelus armatus</i>	Captive	0-10	10-20	30-40	Uthayakumar <i>et al.</i> (2013)
	<i>Odontesthes bonariensis</i>	Captive	40-45	20-25	60-65	Chalde <i>et al.</i> (2016)
	<i>Perca fluviatilis</i>	Captive	80-85	90-95	90-95	Alavi <i>et al.</i> (2010)
	<i>Scophthalmus rhombus</i>	Captive	20-40	40-60	60-80	Hachero-Cruzado <i>et al.</i> (2013)
	<i>Solea senegalensis</i> *	Captive	55-60		60-65	Beirão <i>et al.</i> (2011)
No diff.	<i>Acipenser persicus</i>	Captive	90	80-85	80-85	Aramli <i>et al.</i> (2014)
	<i>Barbus barbus</i>	Captive	60-65	60-65	60-65	Hadi-Alavi <i>et al.</i> (2008)
	<i>Brycon amazonicus</i>	Wild	100	95		Cruz-Casallas <i>et al.</i> (2007)
	<i>Paralichthys orbignyanus</i>	Wild	50-75	50-75	50-75	Lanes <i>et al.</i> (2010)
	<i>Pseudoplatystoma metaense</i>	Captive	>90	>95	>90	Ramirez-Merlano <i>et al.</i> (2011)
	<i>Rhamdia quelen</i>	Captive	>90		>90	Borges <i>et al.</i> (2005)

1985 **Table 6**

ECs	Species	Mode	Dosis (Time exposure)	Reference
Bisphenol A	<i>Danio rerio</i>	<i>In vivo</i>	0.1 µM (2 months)	Chen <i>et al.</i> (2017)
	<i>Perca fluviatilis</i>	<i>In vitro</i>	1.5 mM	Hatef <i>et al.</i> (2010)
	<i>Carassius auratus</i>	<i>In vivo</i>	4.5 µg/L (20 days)	Hatef <i>et al.</i> (2012)
	<i>Salmo trutta</i>	<i>In vivo</i>	1.75 µg/L (spawning period)	Lahnsteiner <i>et al.</i> (2005)
EE2	<i>Tinca tinca</i>	<i>In vivo</i>	50 µg/kg (30 days, injected)	Oropesa <i>et al.</i> (2015)
	<i>Oncorhynchus mykiss</i>	<i>In vivo</i>	10 ng/L (12 weeks)	Schultz <i>et al.</i> (2000)
	<i>Odontesthes bonariensis</i>	<i>In vitro</i>	45 ng/L + 350 ng/L E ₂	Gárriz <i>et al.</i> (2015)
	<i>Betta splendens</i>	<i>In vivo</i>	100 ng/L (4 weeks)	Montgomery <i>et al.</i> (2014)
	<i>Sparus aurata</i>	<i>In vivo</i>	5 µg/L (28 days, by feeding)	Cabas <i>et al.</i> (2013)
Zinc	<i>Salmo trutta</i>	<i>In vitro</i>	5.9 mg/L	Giardina <i>et al.</i> (2009)
	<i>Clarias gariepinus</i>	<i>In vitro</i>	2000 mg/L (24h)	Kime <i>et al.</i> (1996)
	<i>Cyprinus carpio</i>	<i>In vitro</i>	50 mg/L (24h)	Chyb and Kime (2000)
	<i>Lota lota</i>	<i>In vitro</i>	75 mg/L	Lahnsteiner <i>et al.</i> (2004)
	<i>Leuciscus cephalus</i>	<i>In vitro</i>	7.5 mg/L	Lahnsteiner <i>et al.</i> (2004)
Cadmium	<i>Clarias gariepinus</i>	<i>In vitro</i>	100 mg/L (24h)	Kime <i>et al.</i> (1996)
	<i>Cyprinus carpio</i>	<i>In vitro</i>	10 mg/L (2h)	Chyb <i>et al.</i> (2001)
	<i>Oncorhynchus mykiss</i>	<i>In vitro</i>	10 mg/L (4h)	Dietrich <i>et al.</i> (2010)
	<i>Cyprinus carpio</i>	<i>In vitro</i>	50 mg/L (24h)	Dietrich <i>et al.</i> (2011)
	<i>Lota lota</i>	<i>In vitro</i>	25 mg/L	Lahnsteiner <i>et al.</i> (2004)
Mercury	<i>Oncorhynchus mykiss</i>	<i>In vitro</i>	10 mg/L (4h)	Dietrich <i>et al.</i> (2010)
	<i>Perca fluviatilis</i>	<i>In vitro</i>	62 µM	Hatef <i>et al.</i> (2011)
	<i>Perca fluviatilis</i>	<i>In vitro</i>	31 µM (3h)	Hatef <i>et al.</i> (2011)
	<i>Clarias gariepinus</i>	<i>In vitro</i>	0.001 mg/L	Rurangwa <i>et al.</i> (1998)
	<i>Dicentrarchus labrax</i>	<i>In vitro</i>	0.1 mg/L (5 min)	Abascal <i>et al.</i> (2007)
	<i>Carassius auratus</i>	<i>In vitro</i>	>1 mg/L (24 h)	Van Look and Kime (2003)

DDT	<i>Oreochromis mossambicus</i>	<i>In vivo</i>	>0.01 µg L*	Marchand <i>et al.</i> (2008)
	<i>Clarias gariepinus</i>	<i>In vivo</i>	>0.01 µg L*	Marchand <i>et al.</i> (2008)
	<i>Heteropneustes fossilis</i>	<i>In vivo</i>	0.1 mg/L (40 days)	Singh <i>et al.</i> (2008)
TBT	<i>Cyprinus carpio</i>	<i>In vitro</i>	2.7 µg/L	Rurangwa <i>et al.</i> (2002)
	<i>Cyprinus carpio</i>	<i>In vitro</i>	0.27 µg/L (24h)	Rurangwa <i>et al.</i> (2002)
	<i>Clarias gariepinus</i>	<i>In vitro</i>	0.27 µg/L (24h)	Rurangwa <i>et al.</i> (2002)

1986

1987