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Review

Can Myxosporean parasites compromise fish and amphibian reproduction?

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For Review Only

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26 **Abstract**

27

28 Research into fish and amphibian reproduction has increased exponentially in
29 recent years due to the expansion of the aquaculture industry, the need to recover
30 fishery populations, the impact of endocrine disruptors on the aquatic environment, and
31 the global decline of amphibian populations. This review focuses on a group of
32 parasites, the Myxozoa, which affect fish and amphibian reproduction. Lists of the
33 myxosporeans which specifically infect gonads are provided. Most of these are parasitic
34 of freshwater hosts, and most amphibian cases are reported from testes. Sex specificity
35 and sex reversal are discussed in relation to gonadal parasitism. The immune response
36 of the fish to the infection is described and the contribution of the immunoprivilege of
37 gonads to host invasion is emphasized. The pathological effect of these parasites can be
38 significant, especially in aquacultured broodstocks, on some occasions leading to
39 parasitic castration. Although myxosporean parasites are currently not very frequent in
40 gonads, their impact could increase in the future due to the transactions in the global
41 market. Their easy release into the aquatic environment with spawning could make their
42 spreading even more feasible. In the absence of commercial drugs or vaccines to treat
43 and prevent these infections, there is an urgent need to develop specific, rapid and
44 reliable diagnostic tools to control and manage animal movements. In addition, much
45 effort is still to be made on deciphering the life cycle of these organisms, their invasion
46 strategies and their immune evasion mechanisms.

47 1. WHY MYXOSPOREA AND REPRODUCTION?

48

49 Research into fish reproduction has increased exponentially in recent years due to the
50 parallel expansion of the aquaculture industry, the need to recover fishery populations
51 and the impact of endocrine disruptors on the aquatic environment (Mills & Chichester
52 2005; Powell *et al.* 2005). The interest in amphibian reproduction has also increased due
53 to the global decline of wild populations (Souder 2000; Pough 2007). Nevertheless the
54 knowledge of the effect of pathogens, and particularly that of parasites, on fish and
55 amphibian reproduction is scarce and scattered through different disciplines and fields
56 of expertise. In fish, most investigations have focused on the effect of parasites on
57 reproductive behaviour, sexual ornamentation and the relationship with
58 immunocompetence (Barber 2000 ; Skarstein *et al.* 2001; Ottová *et al.* 2005), and much
59 attention has been dedicated to the impact of some digeneans on the reproductive
60 success of declining frog populations (reviewed by Johnston *et al.* 2004). Collective
61 data suggests that Myxozoa could emerge as a serious pathological threat for the
62 reproduction of these aquatic organisms.

63 The Myxozoan clade comprises more than 2180 species, most of which are fish
64 parasites, and infect any tissue and host organ (Lom & Dyková 2006). In recent years,
65 Myxozoa have received extensive attention due to their pathological impact on both
66 fisheries and aquaculture (Alvarez-Pellitero & Sitjà-Bobadilla 1993; Rigos *et al.* 1999;
67 Moran *et al.* 1999; Palenzuela 2006), but also for the still intriguing aspects of their life
68 cycle and controversial taxonomy (Kent *et al.* 2001; Canning & Okamura 2004;
69 Jiménez-Guri *et al.* 2007). This review will focus on those myxosporeans which affect
70 fish and amphibian reproduction, due to the pathological effect that some of them have
71 on aquacultured broodstocks, and their detrimental effect on the reproductive success in

72 wild stocks. Some aspects related to their transmission and host-parasite relationship
73 will receive special attention.

74 Parasites can affect fish reproduction either directly or indirectly, depending on
75 the target organ. This review will concentrate on the first case, when the parasite is
76 present in the gonad. Nevertheless, worthy of mention among non-gonadal infections is
77 the case of the multivalvulid *Kudoa paniformis*. This parasite negatively affects the
78 reproductive effort of the Pacific hake (*Merluccius productus*), with the decrease of its
79 fecundity being proportional to the intensity of infection. Thus, a female with a muscle
80 parasite load of 340 pseudocysts/g has only 10% of the fecundity of non-infected fish
81 (Alderstein & Dorn 1998). As with this case, the exact mechanisms involved in the
82 alteration of fish reproduction by non-gonadal parasites have not yet been elucidated.
83 Several possibilities have been suggested: 1) The parasite extracts energy and nutrients
84 from the host, which are not destined to reproductive effort; and 2) The parasite induces
85 physiological, immunological or ethological changes in the host, which impair mating,
86 gonad maturation, or larval survival.

87 The incidence of parasites in fish and amphibian gonads is difficult to assess
88 since gonads are not always examined in parasite monitoring. Nevertheless, in recent
89 years, more attention has been paid to gonads due to the impact of endocrine disruptors
90 in aquatic animals (Blazer 2002), and to the world amphibian decline (Pasmans *et al.*
91 2006). Despite their huge diversity and vast host number, myxosporeans have not been
92 described in gonads as frequently as in other organs. A list of species specifically
93 affecting the gonads of fish and amphibians is presented in tables 1 and 2 (see the
94 electronic supplementary material), respectively. Most are parasites of freshwater hosts,
95 as only *Ceratomyxa auerbachii*, *Kudoa ovivora* and *Sphaerospora testicularis* have been
96 reported from marine fish. It is also noteworthy that most are not only specific of

97 gonads, but also of a particular sex (see section 5). For amphibians, all the known
98 records concern *Myxobolus* species and infect anurans, since there is only one
99 unpublished finding of a myxosporean in the testes of an imported salamander
100 (*Ambystoma maculatum*) kept in a German pet shop (F. Mutchsman, *pers. comm.*)

101 Tables 1 and 2 do not include species which have been reported in other organs
102 as well as in gonads, or others which are systemic and gonads are just another site. This
103 is the case of *Kudoa iwatai* found in the ovaric stroma, in addition to other organs of
104 gilthead sea bream (*Sparus aurata*) (Fig. 1A) (Diamant *et al.* 2005). *Myxobolus*
105 *diaphanus* in *Fundulus diaphanus* (Cone & Easy 2005) and *Sphaerospora lucioperca* in
106 *Stizostedion lucioperca* (Moshu 1992) are cases of ovaric infection in addition to other
107 fish organs. In European sea bass (*Dicentrarchus labrax*), *Sphaerospora dicentrarchi*, a
108 systemic histozoic parasite (Sitjà-Bobadilla & Alvarez-Pellitero 1992a), can be found in
109 the testicular stroma, within the connective tissue (Fig. 1B, C) and also in the
110 seminiferous lumen, when the normal architecture of the testicular cyst has been
111 disrupted by *S. testicularis* in mixed infections (Fig. 1D). Amongst amphibians, a
112 *Myxobolus* sp. was found to systemically infect 19 out of 25 *Bufo regularis* imported
113 from Egypt, including gonads (testes and ovaries), which were completely destroyed by
114 the parasite (Fig. 1 E-F) (F. Mutschmann, *pers. comm.*).

115

116 2. MACROSCOPIC AND ANATOMICAL LESIONS

117

118 Gonad infections do not generally produce external signs until the intensity of infection
119 is high. European sea bass infected with *Sphaerospora testicularis* during the spawning
120 season show abdominal distension due to the accumulation of ascites or gonad
121 hypertrophy. The genital pore can also appear enlarged and reddish. Testes can

122 sometimes be hyaline or yellowish with hemorrhagic foci (Fig. 2A-C). At the end of
123 this period, infected testes appear necrotic, hardened and much larger than non-infected
124 ones (Fig. 2D, E). Tilapia ovaries infected with *Myxobolus dahomeyensis* appear with
125 whitish nodules consisting of suppurating thick liquid visible through the membrane,
126 which contains large amounts of spores (Gbankoto *et al.* 2001). In some other cases, if
127 normal gonad development is impaired, part of the abdomen appears sunken. In
128 addition, *Clarias gariepinus* infected ovaries exhibited whitish macroscopic nodules
129 corresponding to *Myxobolus gariepinus* plasmodia (Reed *et al.* 2003), and ovaries
130 infected by *M. kainjiae* are enlarged, whitish, and with soft consistency (Obiekezie &
131 Okaeme 1990). In any case, the external appearance of the fish is altered and its
132 commercial value is decreased or it may even be rejected by consumers. Obviously, if
133 these fish are part of a broodstock, they are no longer useful as breeders.

134 In amphibians, no gross pathology was found associated with the myxosporean
135 testicular infection with *Myxobolus fallax* (Browne *et al.* 2002), *M. bufonis* (Upton *et al.*
136 1992) or *Myxobolus* sp. (Théodoridès *et al.* 1981). However, the testes infected with *M.*
137 *chimbuensis* may appear enlarged and slightly discoloured (Ewers 1973), and frogs
138 heavily parasitized with *M. hylae* appear sickly and emaciated, with their testes swollen
139 (Johnston & Bancroft 1918) or filling almost all the abdominal cavity (Browne *et al.*
140 2002). In addition, testes of *Bufo maculatum* heavily infected by *Myxobolus bufonis*
141 exhibit whitish irregular nodules of different sizes, which correspond to masses of the
142 parasite (F. Mutschmann, *pers. comm.*) (Fig. 2F).

143

144 3. PATHOLOGY

145

146 Gonad infection can lead to parasitic castration, depending on the exact location and
147 intensity of infection. Parasites located in the ovaric or testicular stroma are less
148 pathogenic than those located within oocytes or seminiferous tubules. Examples of the
149 first situation are *Henneguya testicularis* (Azevedo *et al.* 1997), *Myxobolus testicularis*
150 (Tajdari *et al.* 2005), *Myxobolus paranensis* (Bonetto & Pignalberi 1965), *Myxobolus*
151 *algonquinensis* or *Sphaerospora ovophila* (Xiao & Desser 1997). A mixed case is
152 *Myxobilatus* sp. in the three-spined stickleback (*Gasterosteus aculeatus*), which invades
153 both the ovaric stroma and the seminiferous tubules. In both sexes, heavy infections
154 may lead to parasitic castration, as infected tubules contained few or no spermatids or
155 spermatozoa and the parasite almost entirely replaced the contents of the ovaries. The
156 impact of this myxosporean on the host population dynamics is unknown, but an effect
157 on the host reproductive success is likely (Longshaw *et al.* 2007) (Fig. 3A, B).
158 *Henneguya amazonica* is an intermediate case. It massively infects the ovaric stroma,
159 but can also enter the oocyte, lying between the zona pellucida and the follicular
160 epithelium, which leaves the inner part of the oocyte free of the infection and may be
161 fertilised normally (Torres *et al.* 1994).

162 When parasites are located in the germinal tissue, both the destruction of the
163 already mature gonad and the inhibition of normal gonad development have been
164 reported. Some examples will be given below. The development of *M. dahomeyensis* in
165 tilapia ovaries leads to the formation of cavities and the destruction of the oocytes. The
166 ovary wall maintains its integrity, but the proliferation of the parasite inside the oocyte
167 leads to its exhaustion and the final disintegration of its membrane (Gbankoto *et al.*
168 2001) (Fig. 3 C). *M. kainjiae* also invades mature oocytes (Obiekezie & Okaeme 1990).
169 *Kudoa ovivora* infects the ovary of Caribbean fish belonging to Labridae (Fig. 3D, E).
170 Its plasmodia and spores develop inside oocytes, within the internal oocyte layer and the

171 vitelline granules, which are gradually reduced until oocytes are fully occupied by
172 parasitic stages, and attain even larger dimensions than healthy oocytes. The final result
173 is that the reproductive effort is largely reduced as infected oocytes, if fertilised, do not
174 divide, whereas the energy investment in reproduction is the same as in non-parasitized
175 fish (Swearer & Robertson 1999).

176 In European sea bass, *S. testicularis* invokes parasitic castration because of the
177 destruction of both testicular germinal cells and Sertoli cells which are essential for
178 normal spermatogenesis (Nóbrega *et al.* 2009). The infection starts with a few
179 trophozoites in the lumen of seminiferous tubules (Fig. 3G). At this stage, diagnosis of
180 the infection with a fresh drop of seminal fluid is fairly difficult (Fig. 3F). In the next
181 step, the parasite proliferates in the lumen, feeding on spermatozoa (Sitjà-Bobadilla &
182 Alvarez-Pellitero 1993a) and blocking spermatogenesis in the testicular cysts. TEM
183 observations have shown that Sertoli cells are damaged, and therefore their normal
184 phagocytic function, essential at the end of the reproductive season, is altered as well as
185 their function as nutritional and architectural support to spermatogonia in the testicular
186 cysts (Sitjà-Bobadilla & Alvarez-Pellitero 1993b). In a further step, the lumen is
187 completely occupied by parasitic stages, the germinal tissue is almost completely
188 destroyed, and only a few spermatogonia are left (Fig. 3I). The infection is thus easy to
189 detect in fresh smears of seminal fluid, which appears very fluid and yellowish (Fig.
190 3H). In the final stages of the invasion, testicular cysts are disrupted, the myxosporean
191 spreads beyond the testes and ascites appears in the abdominal cavity.

192 In amphibians, the information on the pathology is very scarce, but collective
193 data also point towards the damage of the reproductive capacity. *Litoria darlingtoni*
194 infected by *M. chimbuensis* exhibited very few sperm and little meiotic activity (Ewers
195 1973), and the plasmodia of *Myxobolus* sp. occupied almost all the testis of the

196 Egyptian toad *Bufo regularis*, causing atrophy of the testicular tissue, necrosis of the
197 tubular cells, destruction of the seminiferous tubules and extensive haemorrhages
198 (Mubarak & Abed 2001). Other myxosporeans not yet identified to the species level
199 caused destruction of the gonads in several amphibian species imported to Germany
200 from different tropical countries (F. Mutschmann, *pers. comm.*) (Fig. 4A-F).

201

202 **4. HOST IMMUNE REACTION**

203

204 Gonads are a special site when it comes to the immune response, as some parts are
205 isolated from the immune system. This means that they could become a paradise for
206 invaders and also the Achilles' heel of the immune system. The immunoprivilege of the
207 male gonad was thought to lie exclusively on the blood-testis barrier (BTB), maintained
208 by Sertoli cell functions. This physical barrier between the general circulation and
209 testicular tissue stops the pass of exogenous micro-organisms to the seminiferous
210 tubules, conceals testicular antigens from the immune systems and prevents effector cell
211 access. The main reason for this barrier is to avoid the reaction of the immune system
212 against gametes (haploid), which are considered allogenic for the host (diploid),
213 avoiding autoimmune reactions (Mochida & Takahashi 1993). Damage to reproductive
214 organs would pose a threat to the survival of the species. Nevertheless, recent research
215 in mammals has revealed that the immunotolerance of testis is established and
216 maintained not only by the BTB, but also by immunological factors (Fijak & Meinhard
217 2006).

218 This would explain the absence of cellular reaction against parasites whilst they
219 are located in the lumen, as happens with *S. testicularis*. However, when the testicular
220 cysts are disrupted, and the infection spreads beyond testes, leucocytic infiltration

221 consisting of granulocytes and macrophages is evident. At the end of the spawning
222 season, most infected tubular lumen are occupied by encapsulated parasites or parasitic
223 debris, with a strong fibrotic host reaction. These capsules can become highly
224 pigmented and granuloma can even remain until the next spawning season (Fig. 3J).
225 Granulocyte and pigmented cell accumulations are abundant close to the capsules, and
226 probably constitute accumulations of phagocytic cells, cell debris and oxidized lipids
227 (see Blazer 2002). In the three-spined stickleback, an increased thickening of the
228 interstitial tissue and a fibrotic reaction around some tubules was also apparent in testes
229 infected by *Myxobilatus* sp. However, in light infections there was a limited host
230 response (Longshaw *et al.* 2007). Parasite encapsulation is a host reaction which aims to
231 isolate the parasite and to prevent its dispersal, as described in several myxosporoses.
232 However, in *S. testicularis* infections, the efficacy of these fibrotic capsules is
233 questionable because they can prevent parasite spreading with seminal fluid, but they
234 also result in the accumulation of residual material in the germinal tissue, even up to the
235 next spawning season. Therefore, these “scars” of the past infections can reduce
236 spermatogenesis in the future.

237 In ovaries, parasites also evade host immune reaction while located inside
238 oocytes. They provide a very suitable place for the development of the parasite since
239 they are full of nutritive substances. Thus far, no host immune reaction against
240 myxosporean infecting oocytes has been reported.

241 No information is available on the immune response of amphibians against
242 myxosporeans.

243

244 **5. SEX SPECIFICITY AND SEX REVERSAL**

245

246 The number of myxosporean parasites reported in fish ovaries is higher than that in
247 testes, but most amphibians records are from testes (see Tables 1 and 2). Generally, the
248 reasons for this sex specificity are unknown, although several possibilities can be
249 hypothesised: 1) Specific nutritional requirements: oocytes and spermatozoa have clear
250 differences in composition and size, and therefore offer a different type of nutrients to
251 the parasites; 2) Particular ways of transmission and host-parasite co-evolution; and 3)
252 Immunological differences among host sexes. In birds and mammals, the highest
253 prevalence of infection of certain parasites in males has been attributed to the
254 immunosuppressive effect of testosterone (see Zuk 1996). In fact, sex ration and social
255 stressors such as courtship and fighting for females also can alter immune
256 responsiveness (Binuramesh *et al.* 2006). For *S. testicularis*, a trophic hypothesis could
257 be postulated, as trophozoites develop within the seminal fluid, feeding on spermatozoa,
258 and oocytes are obviously much larger, and the ovaric environment is clearly different.
259 The timing of the infection seems to be related to this fact, as immature sea bass were
260 never found infected, and the parasite proliferates in synchronization with spawning
261 (Sitjà-Bobadilla & Alvarez-Pellitero 1993c). Similarly, in infections by *M.*
262 *dahomeyensis*, immature oocytes are unaffected (Gbankoto *et al.* 2001).

263 In gonochoristic fish species, sex-reversal is an abnormal situation and has been
264 suggested as a marker of exposure to estrogenic substances in the aquatic environment
265 (Jobling *et al.* 1998). In sea bass, the presence of ovarian tissue in the testes of non-
266 parasitized males (ovo-testis) has been described (Blázquez *et al.* 1999). However, a
267 higher percentage of ovo-testis is found in males infected by *S. testicularis* than in
268 uninfected ones under the same conditions (author's unpublished observations). In these
269 cases, previtellogenic oocytes are scattered through the testicular tissue, even inside the
270 tubular lumen, and surrounded by spermatozoa. This phenomenon could be due to a

271 hormonal dysfunction invoked by the destruction of Sertoli cells, since these cells have
272 been described to have steroidogenic functions in several fish species (Nóbrega *et al.*
273 2009).

274 In sequential hermaphrodite fish a possible parasitic influence on the sex
275 allocation of its host has been postulated for the protogynous *Thalassoma bifasciatum*.
276 Thus, the data suggest that females infected by *Kudoa ovivora* not only have a lower
277 reproductive success, but also change sex earlier and at a smaller size than uninfected
278 ones (Schärer & Vizoso 2003). This host-parasite system has implications for the field
279 of evolutionary ecology. Sex change can be viewed as “host death” from the point of
280 view of the parasite, as the host tissue in which the parasite sporulates disappears,
281 potentially leading to conflicts of interest between host and parasite over sex change
282 (Schärer & Vizoso 2003).

283

284 **6. TRANSMISSION**

285

286 The life cycle pattern of *Myxoporeia* entails the alternation of a vertebrate host and
287 definitive one (an invertebrate). Thus, spores released from fish are infective for the
288 involved invertebrate, but the generality of this life cycle has not yet been established.
289 In fact, fish-to-fish transmission has been demonstrated for members of the genus
290 *Enteromyxum* (Diamant 1997; Redondo *et al.* 2005). The final consequence of gonad
291 infection is the release of the parasite with gametes during spawning, which favours its
292 transmission to the environment and therefore to the next putative host. If invertebrate
293 hosts were not involved, then horizontal or vertical transmission could happen. The first
294 has been described for some parasites with holoxene cycles, such as microsporeans and
295 coccidians. Amongst fish myxosporeans, it has been proposed for *Kudoa ovivora* since

296 infected oocytes have distinctive features which favour their ingestion by hosts
297 (Swearer & Robertson 1999), and the epidemiological data of *S. testicularis* suggest that
298 fish-to-fish transmission might take place (Sitjà-Bobadilla & Alvarez-Pellitero 2003c),
299 but this has not been demonstrated experimentally. In amphibians, testicular infection
300 by *Myxobolus* spp. may favour sexual transmission of spores during spawning, allowing
301 spore deposition in aquatic environments, where invertebrate stages may be
302 encountered, and perhaps subsequent infection of tadpoles may occur (Browne *et al.*
303 2002). The shedding of *M. fallax* spores has been demonstrated to be sexually mediated
304 through reproductive hormones without affecting fertility (Browne *et al.* 2006).

305 Vertical transmission (to the next generation) can happen when gametes are
306 intracellularly infected (uniparental, transovarian), or when the parasites accompany
307 gametes during fertilisation, and enter the embryo rapidly (biparental, perinatal) (Fig.
308 5). This has been described for microsporean parasites of invertebrates and vertebrates,
309 including fish (Summerfelt & Warner 1970). It is suspected to be the case of *H.*
310 *amazonica*, as infected oocytes seem to be viable, and there is a possibility that they
311 may proceed with normal development, fertilization and survive hatching (Torres *et al.*
312 1994).

313

314 **7. CONCLUSIONS AND FUTURE PERSPECTIVES FOR RESEARCH**

315

316 The effect of some myxosporean parasites on the reproduction of their hosts is
317 considerable, leading to parasitic castration on some occasions. In testicular infections,
318 the immuneprivilege of gonads contributes to the invasion of the host. Although
319 myxosporean parasites are not currently very frequent in gonads, their impact could
320 increase in the future due the transactions in the global market. Thus, the trade of fish,

321 their gametes and embryos as a result of fish farming and the need for re-stocking wild
322 populations and the market of ornamental fish, together with the movements of
323 amphibians for food consumption, pet shops and biomedical research exert a high risk
324 for the transmission and spreading of gonadal myxosporeans. The likely fish-to-fish
325 transmission of some of these, and their easy release into the aquatic environment with
326 spawning could make their spreading even more feasible. As for Myxozoa in general,
327 there is an urgent need to find effective treatments of these gonadal myxosporoses, since
328 only experimental trials have been performed for *S. testicularis* (Sitjà-Bobadilla &
329 Alvarez-Pellitero 1992b). In the absence of useful parasiticides and vaccines, control
330 measures can only be based on preventive and good management measures of the
331 broodstocks. Thus, the development of specific, rapid and reliable diagnostic tools to
332 control and manage animal movements is crucial. In addition, much effort is still to be
333 made on deciphering the life cycle of these organisms, their invasion strategies and
334 immune evasion mechanisms. Furthermore, we are still largely in the dark concerning
335 the endocrine disruption made by gonadal parasites and the evolution of host-parasite
336 systems to balance the host (to reproduce) and parasite (to grow on reproductive organs)
337 interests.

338

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340

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348

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556 **Figure captions**

557

558 Fig. 1. Non specific infections of fish and amphibian gonads. (A) *Kudoa iwatai* in
559 gilthead sea bream ovary. Masses of spores (*). (B-D) *Sphaerospora*
560 *dicentrarchi* in European sea bass testis: (B) Typical bag-like group of spores in
561 the connective tissue; (C) Mature spores (arrows) embedded in a pigmented cell
562 accumulation in the interstitial tissue during the spawning season; (D) Spore
563 (arrow) in a fish also infected with *Sphaerospora testicularis* (arrowheads). (E-
564 F) *Myxobolus* sp. in *Bufo regularis* testis. Stainings of histological sections:
565 Hematoxylin-eosin (A); toluidine blue (B-D); Ziehl-Nielsen (E); fresh smear (F).
566 Bar scales = 20 µm (A, C, E); = 10 µm (B, D, F). Histological material of (A)
567 provided by Dr. A. Diamant (NCM, Israel); (E-F) illustrations provided by Dr.
568 F. Mutschmann (Exomed, Germany).

569 Fig. 2. Gross lesions produced by *Sphaerospora testicularis* in European sea bass
570 during (A-C) and after (D, E) the spawning season. (A) Slight infection with
571 haemorrhagic foci (arrowhead); (B) Strong infection with hardened yellowish
572 appearance and visible nodules (arrowheads); (C) Hyalinized margins
573 (arrowhead) in a strongly infected testis; (D) Massive necrosis in sea bass testis
574 (arrow) (Picture courtesy of Carlos Zarza, Skreting, Spain); (E) Detail of the
575 necrosis. (F) Massive infection of *Bufo maculatum* testis with *Myxobolus bufonis*
576 (Picture by F. Mutschmann, Exomed, Germany).

577 Fig. 3. Pathological effects of myxosporeans in fish gonads. (A-B) *Myxobilatus* sp. in
578 *Gasterosteus aculeatus*, notice the scarce oocytes left in the ovary (A, arrows)
579 and a detail of spores in the ovaric stroma (B, arrow). (C) Scanning electronic
580 microscopic image of a tilapia ovary with oocytes destroyed by *Myxobolus*

581 *dahomeyensis*. (D, E) *Kudoa ovivora* from the ovary of *Thalasoma bifasciatum*:
 582 (D) detail of a spore with scanning electron microscopy; (E) fresh smear, phase
 583 contrast. (F-J) *Sphaerospora testicularis* from European sea bass: fresh smears
 584 of seminal fluid with slight (F, Nomarski) and heavy (H, phase contrast)
 585 infections; sections of testis with slight (G, trophozoites (arrows) are visible in
 586 the seminiferous lumen) and massive infections (I) during the spawning period
 587 and after spawning (J). Notice the seminiferous tubuli completely occupied by
 588 parasitic stages (* in I), the thickened interstitial tissue (I) and the granulomata
 589 with parasite debris (* in J). Histological sections stained with hematoxylin-
 590 eosin (A-B) or toluidine blue (G, I, J). Bar scales = 1 mm (A, C); 10 μm (B, F,
 591 G, H); 20 μm (J); 25 μm (E); 125 μm (I). Illustrations courtesy of Dr. M.
 592 Longshaw (CEFAS, U.K.) (A-B), Dr. A. Marques (University of Montpellier II,
 593 France) (C) and Dr. S. E. Swearer (University of Melbourne, Australia) (D, E).

594 Fig. 4. Pathological effects of myxosporeans in amphibian gonads. *Myxobolus* sp. in
 595 *Hyperolius concolour* testis (A, B). *Myxobolus* sp. in *Litoria caerulea* ovary (C,
 596 D). *Myxobolus* sp. in *Litoria infrafrenata* (E, F). Masses of parasite stages are
 597 indicated with *. Histological sections stained with hematoxylin-eosin (A, C, E,
 598 F). Bar scales = 1 mm (A, C); 200 μm (E); 10 μm (B, D, F). Pictures courtesy of
 599 Dr. F. Mutschmann (Exomed, Germany) (A-D) and Dr. P. Zwart (Utrecht
 600 University, The Netherlands) (E-F).

601 Fig. 5. Diagrammatic representation of the vertical transmission strategies of parasites.
 602 A: Viable non-infected oocyte. Intracellularly and massively infected oocytes
 603 (B) are not viable, and cannot vertically transmit the parasites. Parasites are
 604 transmitted transovarically to the offspring when are located either in the inner
 605 part or the outer layers of oocytes, in slight infections (C). When the parasite is

606 located close to the oocyte (D) it is transmitted perinatally. In both cases, the
607 parasite migrates to the gonadal primordium in larvae (E), and later on
608 proliferates when gonad matures (F). Vertical transmission has not been
609 demonstrated yet for myxosporeans.

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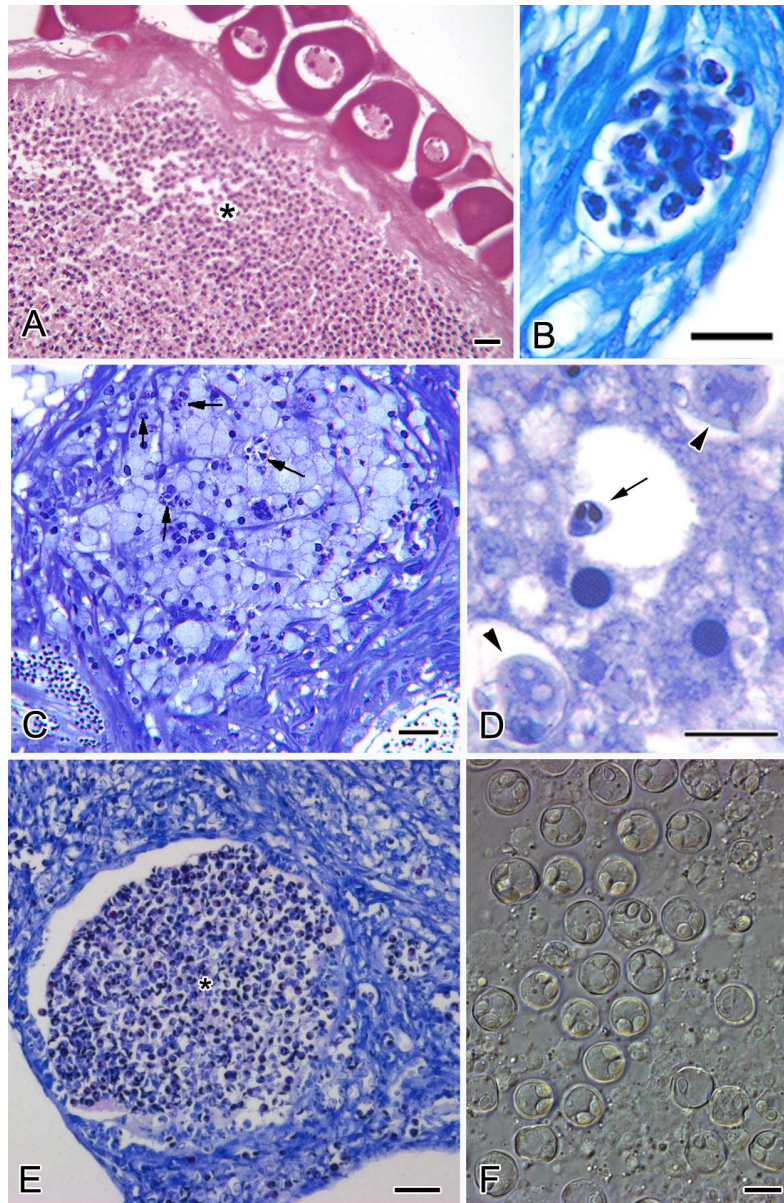


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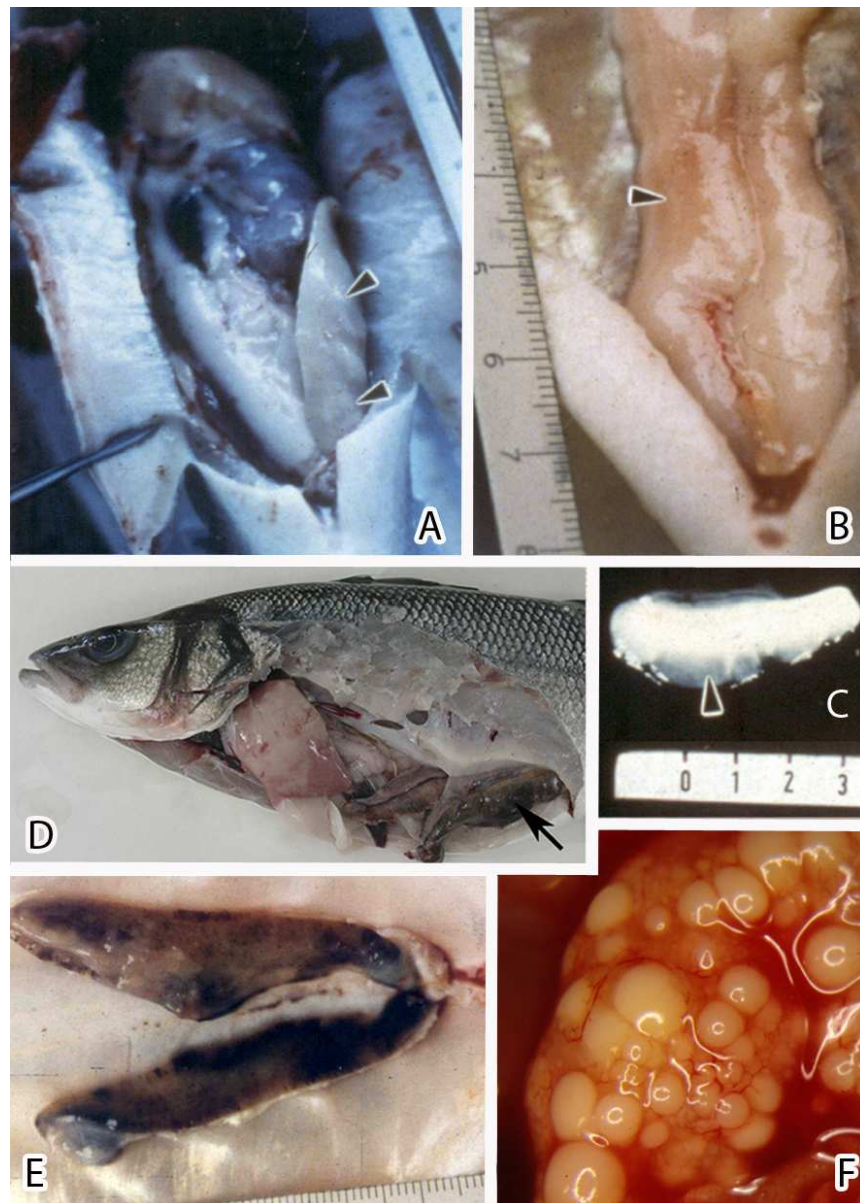


Fig. 2. Gross lesions produced by *Sphaerospora testicularis* in European sea bass during (A-C) and after (D, E) the spawning season. (A) Slight infection with haemorrhagic foci (arrowhead); (B) Strong infection with hardened yellowish appearance and visible nodules (arrowheads); (C) Hyalinized margins (arrowhead) in a strongly infected testis; (D) Massive necrosis in sea bass testis (arrow) (Picture courtesy of Carlos Zarza, Skreting, Spain); (E) Detail of the necrosis. (F) Massive infection of *Bufo maculatum* testis with *Myxobolus bufonis* (Picture by. F. Mutschmann, Exomed, Germany).

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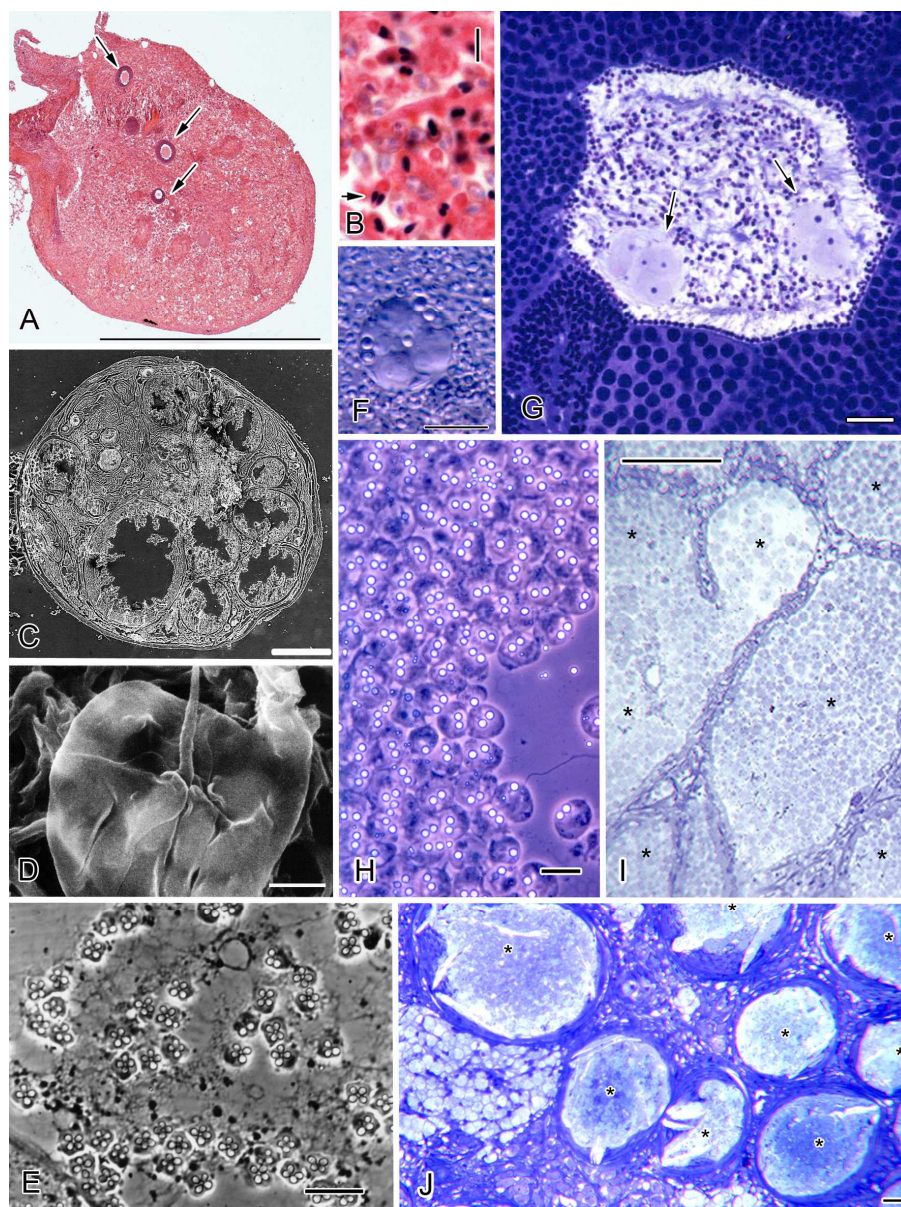


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courtesy of Dr. M. Longshaw (CEFAS, U.K.) (A-B), Dr. A. Marques (University of Montpellier II, France) (C) and Dr. S. E. Swearer (University of Melbourne, Australia) (D, E).
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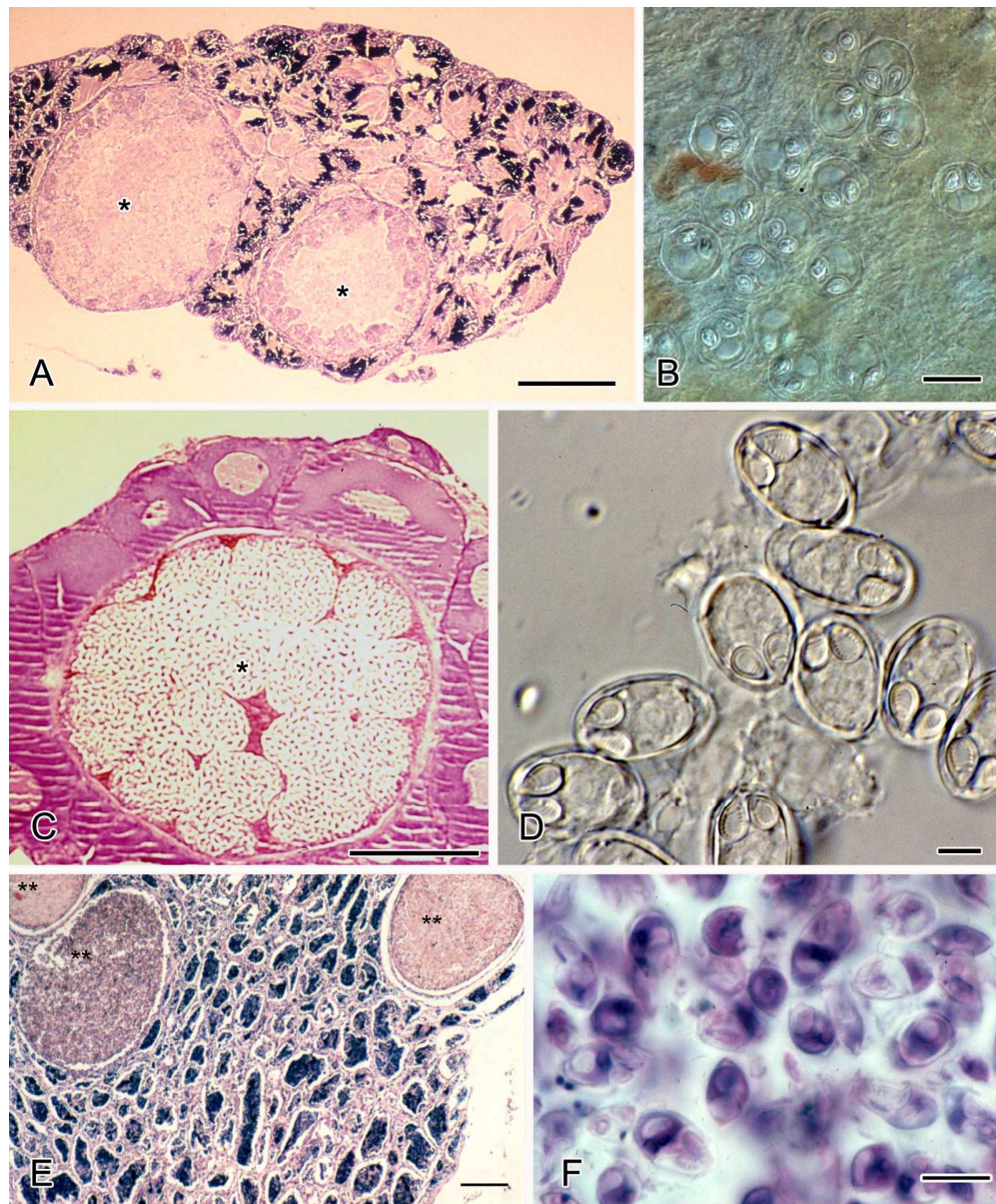


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Fig. 5 Sitjà-Bobadilla

