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# Vertical transmission of Tetracapsuloides bryosalmonae (Myxozoa), the causative agent of salmonid proliferative kidney disease

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1 **Vertical transmission of *Tetracapsuloides bryosalmonae* (Myxozoa), the causative agent**
2 **of salmonid proliferative kidney disease**
3
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20 **Short running title:** Vertical transmission of *Tetracapsuloides bryosalmonae*
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26 **SUMMARY**

27 The freshwater bryozoan, *Fredericella sultana*, is the main primary host of the myxozoan
28 endoparasite, *Tetracapsuloides bryosalmonae* which causes proliferative kidney disease
29 (PKD) of salmonid fish. Because spores that develop in bryozoan colonies are infectious to
30 fish, bryozoans represent the ultimate source of PKD. Bryozoans produce numerous seed-like
31 dormant stages called statoblasts that enable persistence during unfavourable conditions and
32 achieve long distance dispersal. The possibility that *T. bryosalmonae* may undergo vertical
33 transmission via infection of statoblasts has been the subject of much speculation since this is
34 observed in close relatives. This study provides the first evidence that such vertical
35 transmission of *T. bryosalmonae* is extensive by examining the proportions of infected
36 statoblasts in populations of *F. sultana* on two different rivers systems and confirms its
37 effectiveness by demonstrating transmission from material derived from infected statoblasts
38 to fish hosts. Vertical transmission in statoblasts is likely to play an important role in the
39 infection dynamics of both bryozoan and fish hosts and may substantially contribute to the
40 widespread distribution of PKD.

41

42 Key words: Bryozoa, *Fredericella sultana*, statoblasts, maternal colonies, *Tetracapsuloides*
43 *bryosalmonae*, persistent infection, dispersal

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45 **KEY FINDINGS**

46 - Vertical transmission of *Tetracapsuloides bryosalmonae* is achieved via statoblasts
47 - Infected statoblasts show greater hatching success than uninfected statoblasts
48 - Infected statoblasts can be present in maternal colonies that have lost infection
49 - Dispersal of infected statoblasts likely contributes to the wide distribution of PKD
50 - Vertical transmission likely promotes persistent infection of bryozoan populations

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53 **INTRODUCTION**

54 The myxozoan parasite *Tetracapsuloides bryosalmonae,* is the causative agent of Proliferative
55 Kidney Disease (PKD) in salmonid fish (Anderson *et al*. 1999; Canning *et al*. 1999; Feist *et*
56 *al*. 2001). Mortality due to PKD in farmed fish ranges from 20% to 100%, leading to
57 increased costs of production (Ferguson and Ball, 1979; Clifton-Hadley *et al*. 1986; Feist and
58 Longshaw, 2006). The impacts of PKD on wild fish populations are generally poorly known
59 although a recent outbreak in Norway (Sterud *et al.* 2007) demonstrates the potential for mass
60 mortality and PKD is attributed to long term declines in Swiss brown trout populations
61 (Borsuk *et al*. 2006).

62 The life cycle of *T. bryosalmonae* involves exploitation of freshwater bryozoans (Phylum
63 Bryozoa, Class Phylactolaemata) as primary hosts (see Okamura *et al.* 2011, for review). The
64 parasite first develops as covert infections when single cells are associated with the bryozoan
65 body wall (Morris and Adams, 2006a). When conditions are favourable for host growth (e.g.
66 high temperatures and food levels for hosts; Tops *et al*. 2006; Hartikainen and Okamura,
67 2012) these cells detach and develop within the bryozoan body cavity to form sacs filled with
68 spores infectious to fish. Because these sacs can be observed with a stereomicroscope we refer
69 to such infections as overt (see Okamura *et al.* 2011, for review).

70 Freshwater bryozoans are sessile colonial invertebrates that occur in nearly all types of
71 freshwater environments (Massard and Geimer, 2008). They are suspension-feeders that
72 capture phytoplankton and other microorganisms by creating feeding currents with ciliated
73 tentacles (Massard and Geimer, 2008). Sexual reproduction is facultative, occuring briefly in
74 early summer and resulting in the release of short-lived swimming larvae (Wood and
75 Okamura, 2005). Much greater emphasis is on the asexual production of numerous dormant

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76 statoblasts, which are small (<1mm), multicellular seed-like stages comprised of germinal
77 tissue surrounded by resistant chitinous valves (Wood and Okamura, 2005). Statoblasts can
78 tolerate poor conditions such as desiccation, freezing, and lack of food (Hengherr and Schill,
79 2011). When favourable conditions return statoblasts hatch and form new colony (Wood and
80 Okamura, 2005). Because of their resistant nature, small size (0.5–1 mm), and high
81 production, statoblasts likely act as a means of dispersal within and between sites. Evidence in
82 support of this includes the presence of statoblasts in waterfowl guts (Figuerola *et al.* 2004;
83 Mouranval *et al.* 2007) and faecal material (Green *et al.* 2008), gene flow patterns associated
84 with migratory waterfowl routes (Freeland *et al*. 2000; Figuerola *et al.* 2005) and the ability of
85 statoblasts to hatch after passing through duck digestive tracts (Charalambidou *et al.* 2003).
86 Statoblasts have also been found in the gut of many fish species, including largemouth bass,
87 *Micropterus salmoides* (Lacepède), crappie, *Pomoxis annularis* (Rafinesque), and common
88 carp, *Cyprinus carpio* (Linneaus) (Marković *et al.* 2009). Statoblasts carried in ship ballast
89 tanks are likely to contribute to distributions of bryozoans worldwide (Kipp *et al*. 2010; Briski
90 *et al*. 2011).

91 Given the strong dispersal potential of statoblasts, it is of interest to know whether if *T.*
92 *bryosalmonae* can be vertically transmitted via statoblasts. In addition, vertical transmission
93 may explain the presence of infected bryozoans in sites free of salmonids (Tops *et al.* 2004) as
94 it may contribute to persistent infection in local bryozoan populations. Such vertical
95 transmission has been demonstrated for another bryozoan-malacosporean system
96 (*Buddenbrockia allmani* infecting the bryozoan, *Lophopus crystallinus*) whereby new
97 colonies developed from statoblasts were shown by PCR and sequencing to carry infection
98 (Hill and Okamura, 2007). The bizarre observation of the release of worm-like organisms
99 probably representative of *Buddenbrockia plumatellae* from statoblasts of *Plumatella fungosa*

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100 also provides evidence for vertical transmission (Taticchi *et al.* 2004). However, until very
101 recently investigations have failed to find evidence for infections of *T. bryosalmonae* in
102 bryozoan statoblasts (e.g. Grabner and El-Matbouli, 2008).

103 It is now clear that during overt infection *T. bryosalmonae* severely reduces statoblast
104 production, causes malformation of statoblasts and thus almost entirely castrates the infected
105 zooids of its main bryozoan host, *Fredericella sultana* (Hartikainen *et al.* 2013). *T.*
106 *bryosalmonae* infections have been detected by PCR in a fraction of the few statoblasts
107 produced in such overtly-infected bryozoans. However, these infections were not transmitted
108 successfully to the mature offpsring hatched from the same statoblast sources, suggesting
109 either selective mortality of infected offspring or that the infections were subsequently lost
110 (Hartikainen *et al.* 2013). Whether vertical transmission of infection is effective thus remains
111 an open question. The aims of the current study were to: 1) examine the occurrence and extent
112 of vertical transmission of *T. bryosalmonae* in *F. sultana* statoblasts, and; 2) determine the
113 effectiveness of vertical transmission by undertaking transmission studies across the *T.*
114 *bryosalmonae* life cycle.

115 **MATERIALS AND METHODS**

116 *The occurrence and extent of infection of statoblasts*

117 *F. sultana* colonies associated with submerged tree roots were collected from the River Lohr,
118 Germany (50°01’08’’ N; 09°32’43’’ E) on the 1$^{st}$ of August 2011 and from the Rivers Avon
119 (11°27’32’’ N; 41°46’29’’ E) and Dun (16°86’07’’ N; 43°42’63’’ E) in southern England on
120 29$^{th}$ August 2012 and 5$^{th}$ September 2012. Material from the Lohr was used in tranmission
121 experiments and material from the Rivers Avon and Dun were used to measure infection
122 prevalence and vertical transmission to statoblast offspring.

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123 Tree roots with attached colonies from the River Lohr were glued to Petri dishes, placed in an
124 aerated 10 L bucket filled with Bryozoan Medium C (BMC) (Kumar *et al.* 2013) and
125 maintained at 18 $^{0}$C ($\pm$2 $^{0}$C) by feeding with cultured algae as described in Grabner and El-
126 Matbouli (2008) . Briefly, 6 algal species (*Cryptomonas ovata*, *Cryptomonas*
127 spp.,*Chlamydomonas* spp., *Chlamydomonas reinhardii, Synechococcus* spp., *Synechococcus*
128 *leopoliensis*) were cultured in sterile Guillard's WC-Medium. *C. ovata* comprised 80% of
129 food provided to *F. sultana* colonies and the remaining 20% was comprised of the other algae.
130 Food was provided daily by adding 500 ml of the algal culture (concentration of 6.5 to 7.5 x
131 $10^6$ cells/ml) to the bucket and the water was changed every week.

132 After 49 days, microscopical examination of the bryozoans in BMC medium revealed cells
133 swirling in the host body cavity and the subsequent development of overt *T. bryosalmonae*
134 infections in 28% of colonies observed (n=100). Thirty statoblasts dissected from overtly-
135 infected laboratory-cultured colonies originally collected from the field were retained for PCR
136 assays. To minimise the possibility of contamination, statoblasts were incubated in distilled
137 water at 4 $^{0}$C for 24 h (spores degrade in < 24 h at 18 $^{0}$C; De Kinkelin *et al*. 2002) and were
138 then washed several times with distilled water prior to preserving in RNA Later until DNA
139 extraction.

140 Colonies from the Rivers Avon and Dun were collected by detaching several branches from
141 each colony with forceps and placing these branches together in a tube specific for that colony
142 and maintained at room temperature (20 $^{0}$C) for 24 h prior to collecting all the mature
143 statoblasts that were present in the material. Mature statoblasts were identified by the dark
144 brown colour of valves. Statoblasts were dissected from bryozoans using a stereomicroscope
145 (100 x magnification) which also enabled determining whether *T. bryosalmonae* sacs were
146 present within the bryozoan body cavity. Colonies lacking overt infections were subject to



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147 PCR to test for the presence of covert infections. Statoblasts from the same brood (i.e. from
148 the same maternal colony) from the Rivers Avon and Dun were placed in 1.5 ml tubes with
149 deionised water and stored at 4 $^0$C (Rivers Avon and Dun) for five months. After five months,
150 the statoblasts of the same brood (i.e. originating from a single maternal colony) were placed
151 in Petri dishes filled with artificial pond water and were allowed to hatch at 20 $^0$C over a
152 period of seven days. The hatched statoblasts were preserved in 100% ethanol for PCR assays
153 for detection of infection of individual statoblasts. The tissue of maternal colonies was also
154 preserved in ethanol to determine infection status. Associations between river, maternal
155 colony infection, statoblast hatching status and statoblast infection were tested using Chi-
156 square (for two-way analyses) and G tests (three-way analyses).

157 DNA extractions used either a QIAamp DNA mini Kit (QIAGEN) (Lohr samples) or a
158 modified hexadecyltrimethylammonium bromide (CTAB) protocol (Tops and Okamura,
159 2003) (Avon and Dun samples). Two *T. bryosalmoae*-specific PCR assays were used. 1)
160 Statoblasts from the River Lohr were diagnosed using a nested PCR with 5F (5'-
161 CCTATTCAATTGAGTAGGAGA-3') and 6R (5'-GGACCTTACTCGTTTCCGACC-3')
162 (Kent *et al*. 1998) in the first round, followed by a second round PCR using PKD-real F (5'-
163 TGTCGATTGGACACTGCATG-3') and PKD-real R (5'-ACGTCCGCAAACTTACAGCT-
164 3') (Grabner and El-Matbouli, 2009). The final 166 bp amplicon was verified to originate
165 from *T. bryosalmonae* via purification from 1.5% agarose gels, cloning and sequencing.
166 Briefly, the amplicon was purified using a MinElute gel extraction kit (QIAGEN), cloned into
167 the pCR4-TOPO vector (TOPO TA, Invitrogen) and the recombinant plasmids purified from
168 *Escherichia coli* using a QIAprep Miniprep (QIAGEN). Cloned PCR products were
169 sequenced in a commercial sequencing laboratory (LGC Genomics GmbH, Berlin, Germany).
170 2) Statoblasts from the Rivers Avon and Dun were subjected to PCR with the primers 514F

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171 new (5'-ATTCAGGTCCATTCGTGAGTAACAAGC-3') and 776R (5'-
172 CTGCCCTTAATTGGGTGTATCAGC-3') (diagnostic amplicon size 244 bp, PCR
173 conditions as in Hartikainen *et al.* 2013). The PCR products were visualised on a 1.5%
174 agarose electrophoretic gel and five positive samples were verified by direct sequencing on
175 ABI Prism 3700xl using BigDye v1.1 chemistry.

176 *Is vertical transmission in statoblasts effective?*

177 We conducted a laboratory-based transmission study using bryozoan colonies originated from
178 the River Lohr to determine whether infection of statoblasts results in effective vertical
179 transmission to colonies that subsequently develop and that these in turn can promote further
180 transmission of infection to fish. Material for this study was gained by cohabiting two-month
181 old specific pathogen free (SPF) colonies for 8 h per day for 14 days with 5 brown trout that
182 had been infected by exposure to naturally infected *F. sultana* colonies from the River Lohr
183 eight weeks earlier. The cohabitation experiment was conducted several times in order to
184 obtain as many statoblasts as possible, using different SPF *F. sultana* colonies. A total of 19
185 colonies (originating from the River Lohr) were present on the petri dishes and at the
186 beginning of exposure each had at least three zooids. In between bouts of cohabitation fish
187 were transferred back to their aquarium and fed with commercial fish feed. *F. sultana*
188 colonies were maintained in culture systems (buckets; see above) after the 8 hof cohabitation.
189 These colonies were then allowed to grow on following exposure to fish until they produced
190 statoblasts. Statoblasts were collected from these colonies, washed and placed in distilled
191 water for 24 h at 4 $^{0}$C.

192 Statoblasts produced by colonies exposed to infected fish were used in a study to address
193 whether infection was successfully vertically transmitted to daughter colonies that hatched
194 from these statoblasts and whether this infection could in turn be transmitted to fish. For this



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195 study we cohabited two colonies derived from these statoblasts and that were observed to
196 have spores and sacs of *T. bryosalmonae* with five SPF brown trout (4 cm in length) for eight
197 hours daily for two weeks. For the control, five SPF brown trout fish were cohabitated with
198 SPF *F. sultana* colonies (two colonies comprising 18 zooids) from long-term laboratory stock.
199 The aquaria were checked daily for fish mortality and extraordinary behaviour. After co-
200 habitation, fish were placed in 20 L aquaria with flow-through water. Eight weeks post-
201 infection, all five fish from each group were dissected and kidney, liver and spleen were
202 sampled for DNA extraction and *T. bryosalmonae*-specific PCR assays using 5F and 6R
203 primer sets.

204 Immunohistochemistry was performed on 30 statoblasts collected from the colonies exposed
205 to fish (described above) and from uninfected colonies deriving from our laboratory stock.
206 Statoblasts were washed with distilled water, incubated for 24 h at 4 $^{0}$C and fixed in 10%
207 neutral buffered formalin and paraffin embedded 4 µm sections were labelled with *T.*
208 *bryosalmonae* specific monoclonal antibody P01 (Aquatic Diagnostics LTD). Endogenous
209 peroxidase activity in deparaffinised sections was blocked with 3% $H_2O_2$ in phosphate
210 buffered saline (PBS) for 10 min. Following rinses with Tris-buffered saline (TBS), non-
211 specific protein binding sites were blocked with normal sheep serum (1:10 dilution in TBS)
212 for 30 min. The sections were then incubated for 60 min in a humid chamber at room
213 temperature with the reconstituted P01 antibody (20 µg/mL in PBS). After three washes with
214 TBS for 2 min each, the sections were incubated for 30 min with 100µL of goat anti-mouse
215 polyclonal antibody (IgG) conjugated with horseradish peroxidase (Dako EnVision+ System-
216 HRP AEC kit). After four washes with TBS for 2 min each, slides were incubated with the
217 AEC substrate for 4–5 min until a pink color appeared. The reaction was stopped by

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218 immersion of the slides in distilled water for 3–4 min. Sections were then mounted and
219 examined microscopically.

220 **RESULTS**

221 *The occurrence and extent of infection of statoblasts*

222 The *T. bryosalmonae*-specific PCR assays demonstrated parasite presence in statoblasts
223 produced by colonies collected from all three rivers as well as in statoblasts collected from
224 SPF *F. sultana* colonies exposed to infected brown trout (e.g. see Fig. 1). Sequencing of
225 positive samples showed 99% similarity to the 18S rRNA gene of *T. bryosalmonae* (GenBank
226 accession numbers, FJ981823 and AF190669).

227 We collected 181 statoblasts from 47 *F. sultana* colonies from the River Avon and 57
228 statoblasts from 27 colonies from the River Dun. Only one overtly-infected colony (with 11 *T.*
229 *bryosalmonae* sacs) was observed (River Avon). This colony had two mature uninfected
230 statoblasts. A high proportion of the remaining colonies were covertly infected (68% from
231 River Avon and 74% from River Dun) (see Fig. 2) but infection status (covertly infected vs.
232 uninfected) at the time of field collection was not dependent on river (Chi-Square Test:
233 $\chi^2$=0.30, df=1, p=0.59). Infected statoblasts were obtained from colonies that were both
234 covertly infected and uninfected (presumably having lost their infection recently) at the time
235 of collection in both sites (see Fig. 3). Higher proportions of infected statoblasts were
236 collected from the River Dun material (35% and 45% of statoblasts collected from covertly-
237 infected and uninfected maternal colonies, respectively) than from the River Avon material
238 (29% and 19% of statoblasts collected from covertly-infected and uninfected maternal
239 colonies, respectively) (Fig. 3) (statoblast infection status was not dependent on river: G=1.8,
240 df=1, p=0.18). Notably, the highest proportions of infected statoblasts were collected from

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241 uninfected maternal colonies from the River Dun (45%) but from covertly-infected maternal
242 colonies from the River Avon (29%) (Fig. 3) (statoblast infection status was not dependent on
243 maternal colony status: G=0.30, df=1, p=0.58). There was also no association between river,
244 maternal colony infection status and statoblast infection (2x2x2 G test: G=3.6, df=4,
245 p=0.463).

246 We assessed the frequency of vertical transmission by considering the infection status of
247 pooled statoblasts produced by all infected colonies. Here we included statoblasts produced
248 by uninfected maternal colonies that had produced at least one infected statoblast. Although
249 there was a slightly higher proportion of infected statoblasts (as revealed by PCR) in material
250 collected from the River Dun than in the River Avon (39% and 30%, respectively: see Fig. 4)
251 infection status was not dependent on river (Chi-Square Test: $\chi^2$=1.51, df=1, p=0.22). The
252 percentages of these infected statoblasts that hatched were 94% (n=46) and 100% (n=21) for
253 the Rivers Avon and Dun, respectively. The uninfected colonies with no infection in any of
254 the statoblast offspring produced 15 statoblasts in the Avon and 3 statoblasts in the Dun. The
255 percentage of statoblasts that hatched were 87% and 100%, respectively.

256 There were notably higher proportions of hatched statoblasts that were infected (50% on the
257 River Dun, 30% on the River Avon) relative to unhatched infected statoblasts (0% on the
258 River Dun, 21% on the River Avon) (Fig. 5) and the effect was greatest for material from the
259 River Dun. These patterns are reflected by the dependency of statoblast infection on hatching
260 status (2x2 G test: G=6.56, df=1, p=0.0104) (uninfected statoblasts did not hatch as well as
261 infected ones) but not on river (2x2 G test: G=1.6, df=1, p=0.2059), although statoblast
262 hatching status was dependent on river (2x2 G test: G=6.62, df=1, p=0.0101) (Fig. 5).
263 Overall, therefore, river, statoblast infection and hatching status were inter-dependent (2x2x2
264 G test: G=22.68, df=4, p<0.0001).



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265 *Is vertical transmission in statoblasts effective?*

266 Six of the 30 statoblasts collected for hatching from laboratory-infected *F. sultana* colonies
267 (20 %) successfully hatched. We confirmed that infection was successfully vertically
268 transmitted to two of the six hatched colonies (33%) by observing free spores and mature sacs
269 in two colonies. These infected colonies were each comprised of 2–3 zooids and were
270 subsequently used in exposures to fish. The SPF brown trout that were cohabitated for two
271 weeks with these two infected colonies didn't show any abnormal behaviour or mortalities
272 during the whole trial, and they were positive for *T. bryosalmonae* after eight weeks post-
273 infection (Fig. 6). Band intensities suggest the the *T. bryosalmonae* stages were most
274 abundant in the kidney but were also present in liver and spleen. No DNA was amplified from
275 control fish.

276 The use of *T. bryosalmonae* specific monoclonal antibodies revealed the presence of cryptic
277 stages in the infected statoblasts collected from laboratory infected *F. sultana* colonies (Fig.
278 7), which appeared as small red signals (arrows, Fig. 7). No such signal was observed in the
279 negative control (Fig. 8).

280

281 **DISCUSSION**

282 *Patterns of infection of maternal colonies and statoblasts*

283 We obtained evidence for surprisingly high levels of covert infection in the maternal colonies
284 that were collected from both the Rivers Avon and Dun (68% and 74%, respectively) relative
285 to levels that were monitored during a previous study that examined temporal prevalences of
286 infection in bryozoan populations in the River Cerne, also in southern England (the highest



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287 prevalence of infection being ~50%; Tops, 2004). There are several factors that may
288 contribute to explaining these high levels of covert infection including: the development of
289 new primers in this study, if bryozoans in these systems are more susceptible to infection, if a
290 greater number of infectious spores are present or if there is a greater degree of vertical
291 transmission of infection to new colonies produced from infected statoblasts or infected
292 colony fragments thus promoting the spread of infection through populations (Okamura *et al.*
293 2011).

294 We found that infected statoblasts were produced in maternal colonies that were both covertly
295 infected and uninfected during the time the statoblasts were collected. These results suggest
296 that some colonies lose infection but nevertheless pass infection to progeny produced during a
297 prior period of infection.This may have happened, for instance, if the colony had effectively
298 sealed off infected regions containing statoblasts by inward pinching of the peritoneum as was
299 observed to occur a colony of *Plumatella fungosa* infected with *Buddenbrockia plumatellae*
300 (Canning *et al.* 2002). An alternate explanation is failure in DNA extraction from the maternal
301 colony or that there was insufficient *T. bryosalmonae* DNA to form a clear band on the
302 agarose gel. A possible explanation for apparent rescue of some statoblats from infection is
303 the inclusion of infected and uninfected portions of the colony in the same DNA extraction,
304 giving a mixture of infected and uninfected statoblasts from an infected parent.

305 A substantial proportion of statoblasts produced by bryozoans from both the Rivers Avon and
306 Dun were infected (30% and 39%, respectively), a finding further corroborated by detection
307 of cryptic stages in infected statoblasts in our monoclonal antibody study. We also found that
308 most infected statoblasts hatched and that this effect was greatest for material from the River
309 Dun (94% and 100% in the Rivers Avon and Dun, respectively). Indeed, the hatching success
310 was significantly associated with infection, with more infected than uninfected statoblasts

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311 successfully hatching. This may indicate complex interactions between the colony ability to
312 support both infections and produce viable statoblasts or parasite mediated stimulation of
313 statoblast hatching. Our laboratory studies complemented our above findings by
314 demonstrating that the presence of *T. bryosalmonae* in statoblasts can indeed result in
315 subsequent overt infections in colonies that develop from statoblasts and that these infections,
316 in turn, are transmitted to fish.

317 *Implications of vertical transmission via statoblasts*

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318 Statoblasts function as dormant propagules that allow bryozoans to persist during
319 unfavourable periods. In addition, there is good evidence that buoyant statoblasts are likely to
320 be dispersed over great distances by waterfowl and ship ballast. Our demonstration that
321 vertical transmission via statoblasts is effective provides evidence that *T. bryosalmonae* will
322 be dispersed in space or time by hitchiking along with its host, thereby similarly evading
323 unfavourable conditions and achieving colonisation of new habitats. *F. sultana* statoblasts are
324 not buoyant, yet vertical transmission via statoblasts and dispersal with bryozoan hosts may
325 explain the widespread distribution of PKD in the northern hemisphere. Indeed, long distance
326 dispersal achieved by infected statoblasts may explain phylogeographic patterns that suggest
327 Europe may have been colonised by *T. bryosalmonae* from North America (Henderson and
328 Okamura, 2004).

329 The capacity for vertical transmission via statoblasts demonstrated here for *T. bryosalmonae*
330 appears to be a general characteristic of malacosporeans since it has been shown in
331 *Buddenbrockia allmani* (Hill and Okamura, 2007) and appears to be achieved by
332 *Buddenbrockia plumatellae* (Taticchi *et al.* 2004). This capacity should enable
333 malacosporeans to persist indefinitely in local populations of bryozoans (Hill and Okamura,

334 2007) and, in terms of PKD, is likely to contribute to the regular annual outbreaks observed in
335 many fish farms (El-Matbouli and Hoffmann, 2002; Okamura *et al*. 2011). Vertical
336 transmission via colony fragmentation (Morris and Adams, 2006; Hill and Okamura, 2007)
337 provides another mechanism by which malacosporean infections can be amplified within local
338 populations. Significantly, in these colonial hosts, the mechanisms of vertical transmission
339 documented to date are achieved by infection of clonal propagules (asexually-produced
340 statoblasts, fragments of maternal colonies). Thus, it will achieve persistent infection of clonal
341 genotypes over time and space which, in turn, will spread the risk of loss of infection from
342 clonal genotypes.

343 A body of work now demonstrates that *T. bryosalmonae* undergoes developmental cycling
344 between covert and overt infections dependent on host condition. The former cause no
345 detectable effect on bryozoan host fitness and are expressed when hosts are in poor condition
346 while overt infections temporarily effectively castrate bryozoan hosts and are expressed when
347 hosts are in good condition (Tops *et al.* 2006; Hartikainen and Okamura, 2012). The overall
348 driver of such developmental cycling appears to be a strategy for *T. bryosalmonae* to enable
349 long term persistence in bryozoan populations by minimising virulence while occasionally
350 undergoing horizontal transmission to fish. Such low virulence is anticipated when the
351 reproductive interests of both host and parasite are aligned (Bull *et al.* 1991) as is the case for
352 vertical transmission. Previous to this study it was predicted that vertical transmission of *T.*
353 *bryosalmonae* by statoblasts must occur (e.g. Tops *et al.* 2004; Grabner and El-Matbouli,
354 2008; Tops *et al.* 2009). By providing the first evidence that such vertical transmission is
355 apparently extensive and effective, our study significantly contributes to our understanding of
356 infection dynamics in both bryozoan and fish hosts.

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361

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515 **Figure captions**

516 **Figure 1** PCR amplification of *Tetracapsuloides bryosalmonae* DNA from statoblasts
517 collected from *T. bryosalmonae* infected *Fredericella sultana* colonies under laboratory
518 conditions: Lane 2 to 4 first round PCR amplified by 5F-6R primers and lane 7 to 9 nested
519 PCR amplified by PKD-real F-R primers. Lane 1 and 5: 100bp DNA ladder (Invitrogen); lane
520 2: infected statoblasts DNA; lane 3: positive control; lane 4: SPF statoblasts DNA; Lane 6 and
521 10: 50bp DNA ladder (Fermentas); Lane 7: infected statoblasts DNA; Lane 8: positive
522 control; Lane 9: SPF statoblasts DNA.

523 **Figure 2** The percentage of maternal colonies that were covertly-infected and uninfected at
524 the time of collection from Rivers Avon and Dun. One overtly-infected colony collected from
525 the River Avon is not included. N values above bars are the numbers of colonies in each
526 infection category per river.

527 **Figure 3** The percentage of statoblasts that were infected according to the infection status of
528 their maternal colony (covertly-infected or uninfected at the time of collection from the field)
529 from the Rivers Avon and Dun. Two uninfected statoblasts from an overtly-infected colony
530 collected from the River Avon are not included. N values above bars are the numbers of
531 statoblasts collected from uninfected and covertly infected maternal colonies per river.

532 **Figure 4** The percentage of infected and uninfected statoblasts collected from infected
533 colonies (including covertly-infected maternal colonies and maternal colonies with infected
534 statoblasts) from the Rivers Avon and Dun. Two uninfected statoblasts from an overtly-
535 infected colony collected from the River Avon are not included. N values above bars are the
536 numbers of statoblasts according to their infection status per river.

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537 **Figure 5** The percentages of hatched and unhatched infected statoblasts collected from
538 infected colonies (including covertly-infected maternal colonies and maternal colonies with
539 infected statoblasts) from the Rivers Avon and Dun. Numbers above bars are n-values.

540 **Figure 6** PCR amplification of *Tetracapsuloides bryosalmonae* DNA from brown trout
541 (*Salmo trutta*) samples cohabitated with *F. sultana* raised under laboratory conditions from
542 infected statoblasts: Lane 1: 100 bp DNA ladder (Invitrogen); Lane 2-4: infected kidney, liver
543 and spleen DNA samples of brown trout, respectively; Lane 5: negative control; Lane 6:
544 positive control.

545 **Figure 7** Horizontal section of infected statoblast of *Fredericella sultana* with
546 *Tetracapsuloides bryosalmonae* monoclonal antibody, revealed the presence of cryptic stages
547 inside the statoblast (arrows), enlarged section (lower left) in small windows focusing on
548 cryptic stages of *T. bryosalmonae* (arrows), and intact statoblast (upper left). The image
549 shows the dark brown chitinous valve of the statoblast containing vitellogenic material and
550 putative stained parasite stages.

551 **Figure 8** Specific pathogen free *Fredericella sultana* statoblast with *Tetracapsuloides*
552 *bryosalmonae* monoclonal antibody

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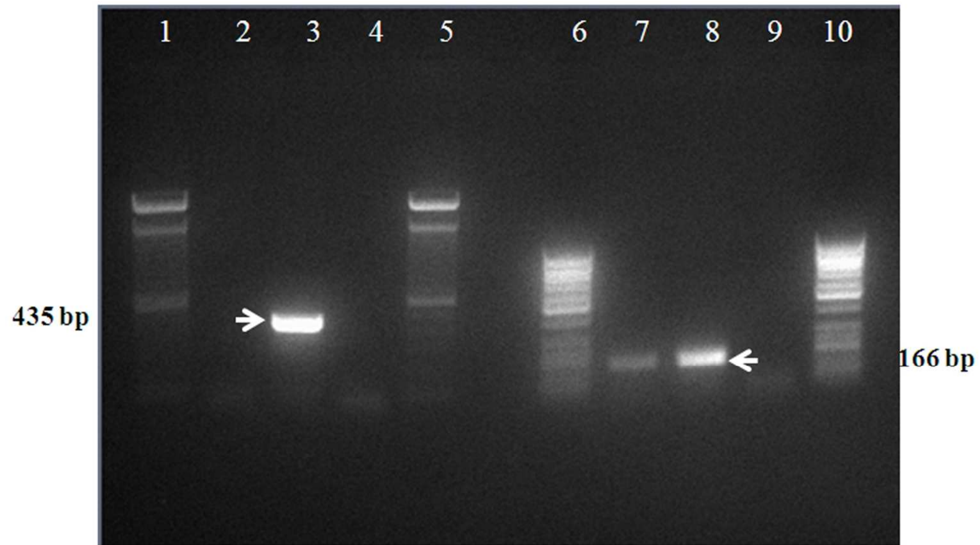


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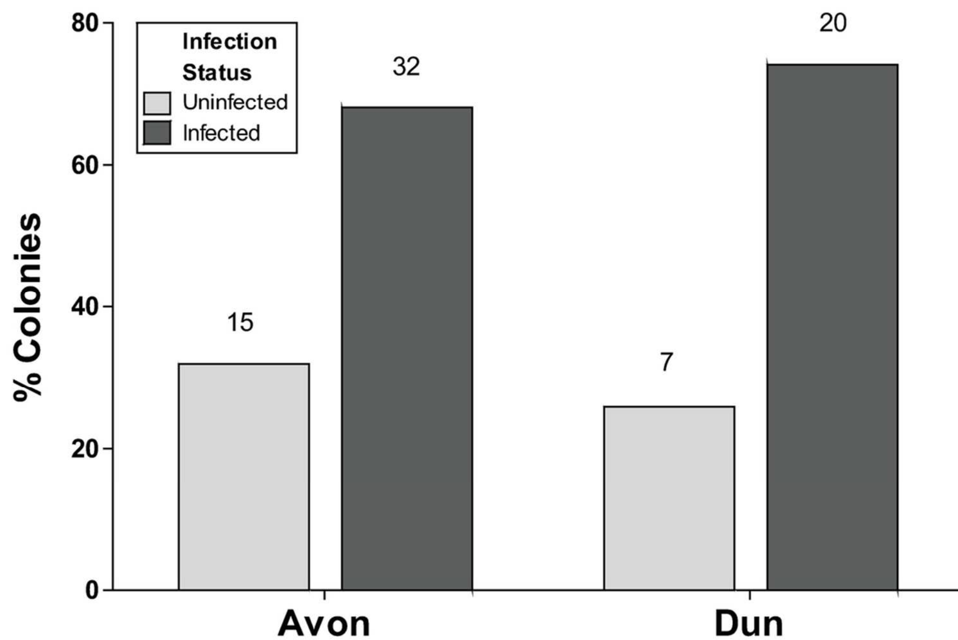


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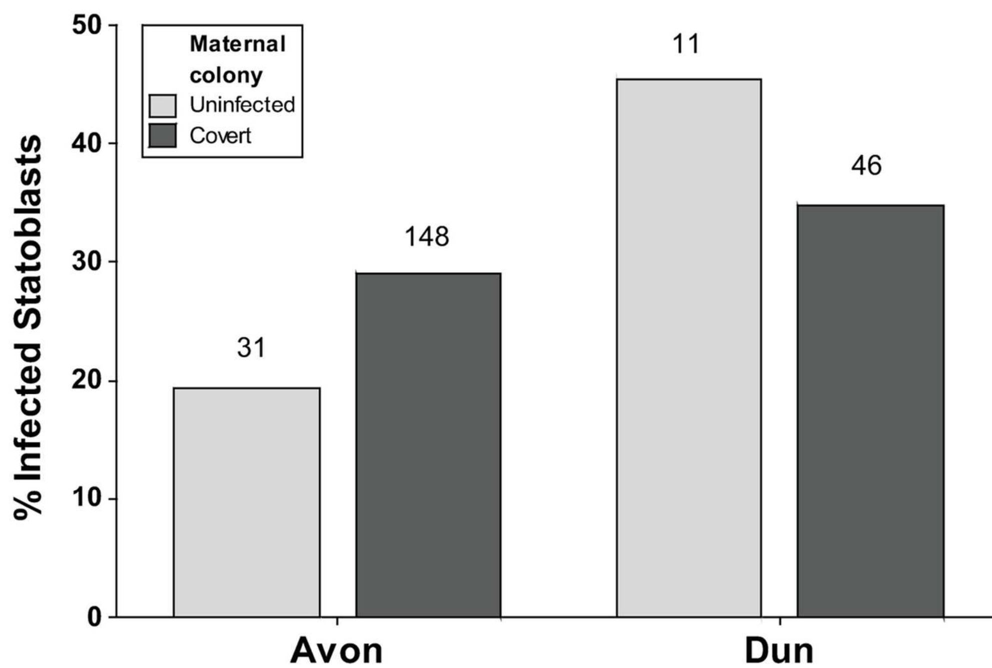


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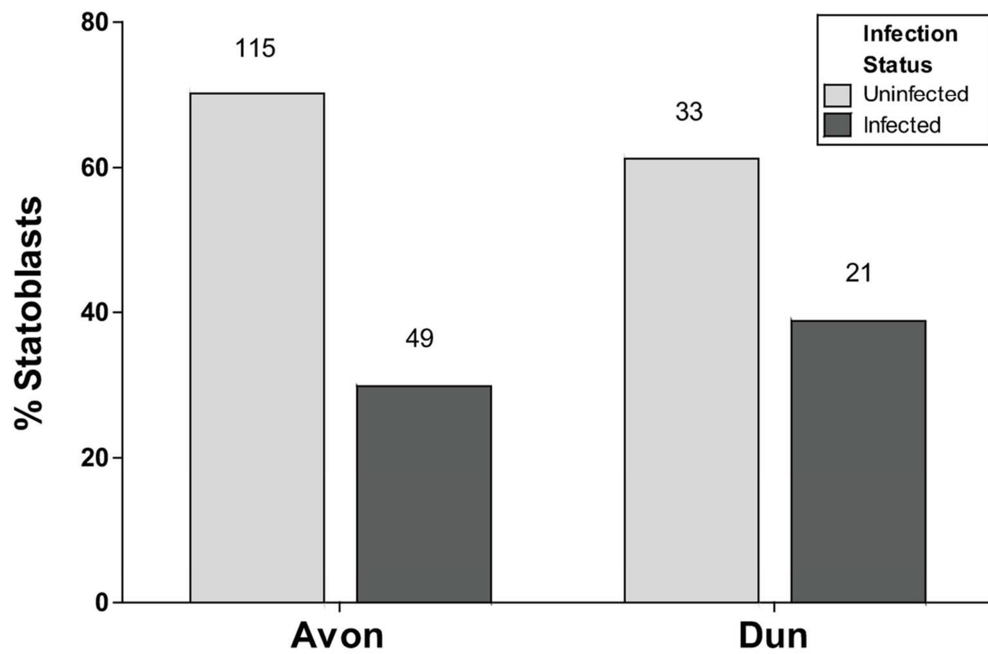


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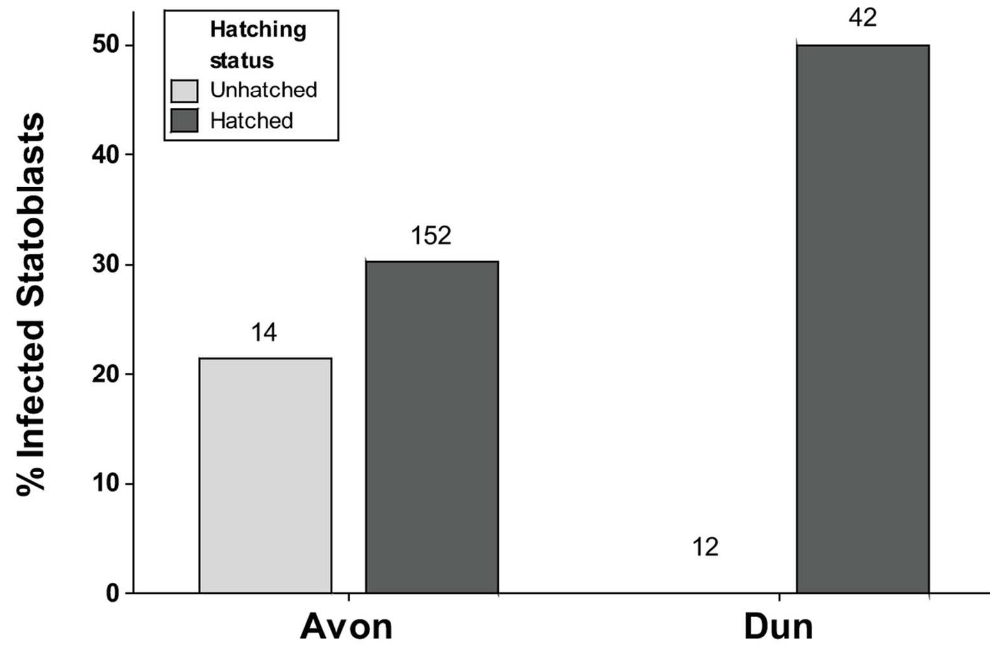


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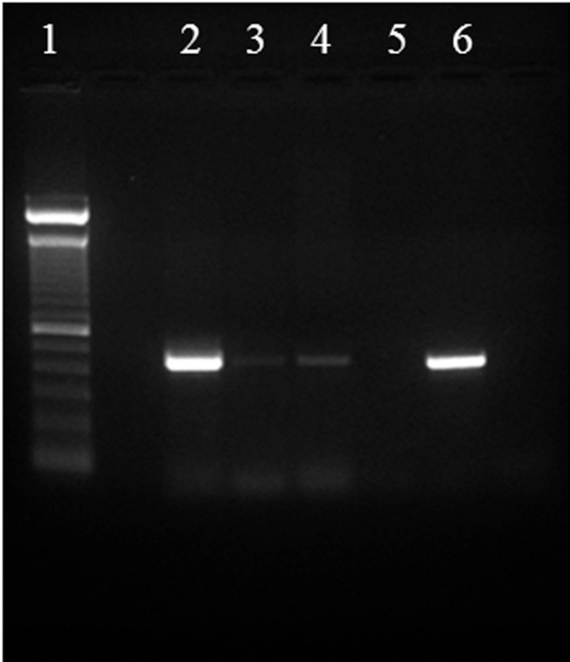


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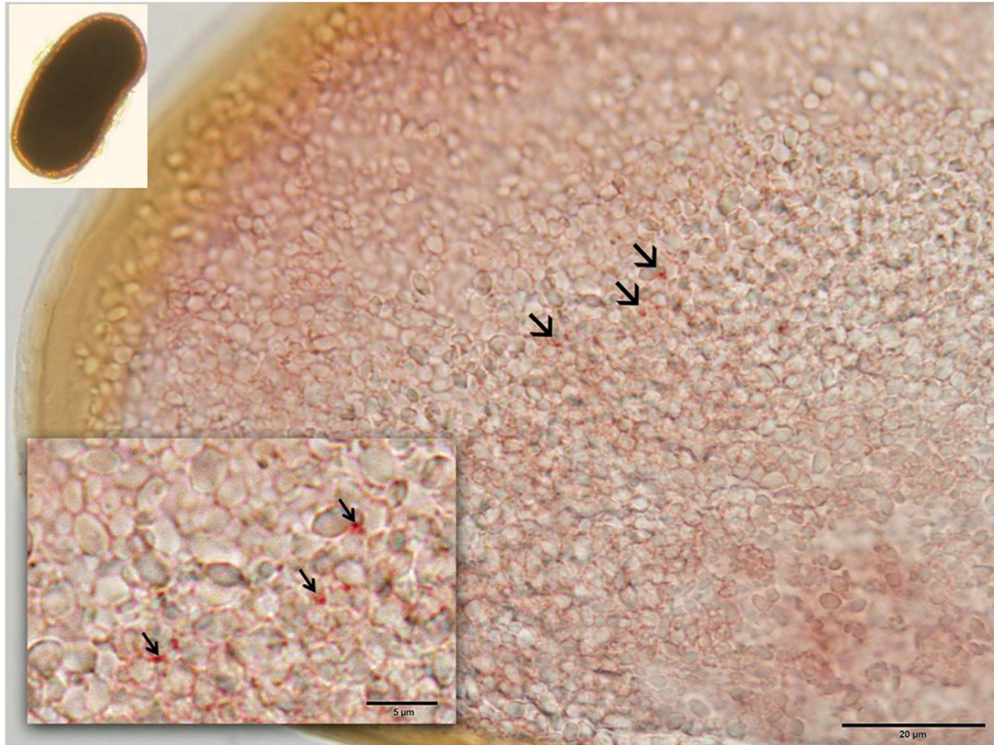


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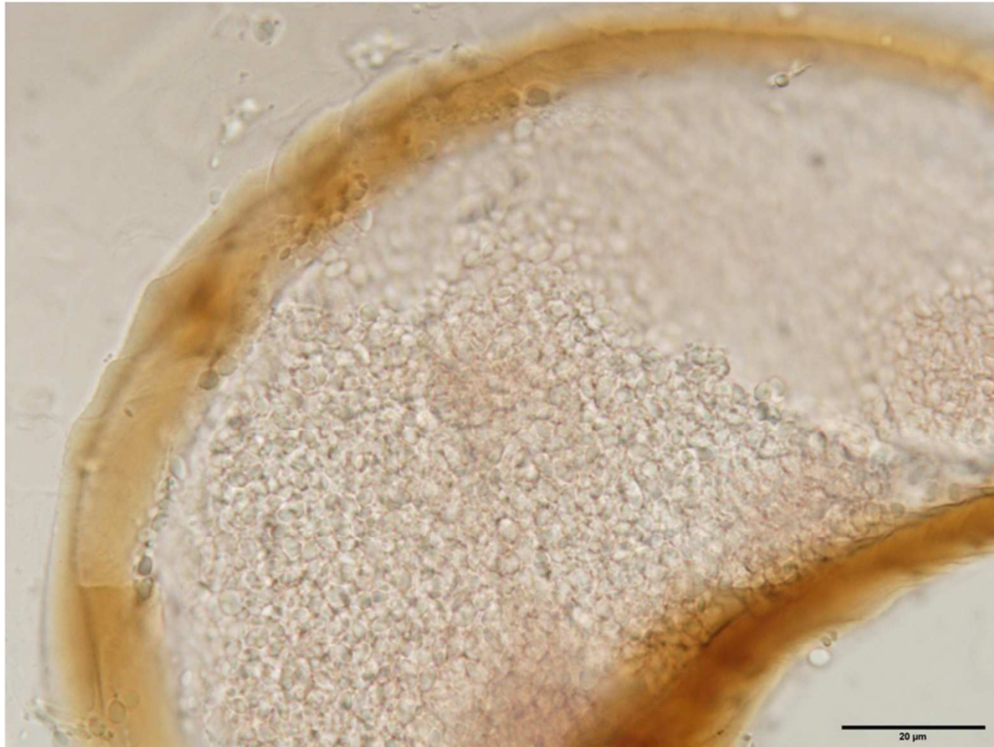


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