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7 **Hidden infections and changing environments**

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11 Beth Okamura

12 Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD,
13 United Kingdom14 b.okamura@nhm.ac.uk

15 +44 (0)2079 426631

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18 Abstract

19 It is increasingly evident that cryptic stages of many parasites cause asymptomatic infections
20 in a diversity of hosts. This review examines what may cause these infectious agents to
21 persist as asymptomatic infections in invertebrates and how environmental change is linked
22 with the subsequent development of overt infection and disease. In many systems disease
23 dynamics are closely associated with host condition which, in turn, is linked with
24 environmental change. Symbionts (commensals and mutualists) display similar dynamics
25 when environmental change causes them to exert negative effects on their hosts. Although
26 such asymptomatic infections are demonstrated in a range of invertebrate hosts they are
27 greatly undersampled because most invertebrate diseases are uninvestigated, infections are
28 difficult to detect, and many parasite groups are poorly characterised. A better understanding
29 of the diversity and distribution of parasites that cause asymptomatic infections and of their
30 complex relationships with invertebrate hosts will enable a fuller appreciation of context-
31 dependent host-parasite interactions and will address the biased focus on diseases of
32 invertebrates of practical importance. The existence of such infections could underlie novel
33 disease outbreaks that might otherwise be attributed to invasives while altered disease
34 dynamics may provide an additional and complementary indicator of ecosystem change.

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40 **Introduction**

41 Many parasites and pathogens can persist as cryptic stages that exert little to no effects on
42 their hosts. When conditions are appropriate, such hidden enemies proliferate and may
43 transform into distinct stages that result in disease and obvious infection. Well-known
44 examples of disease agents associated with asymptomatic infections are *Mycobacterium*
45 *tuberculosis* and species of *Plasmodium*, causing tuberculosis and malaria, respectively.
46 Disease ensues when parasite proliferation follows immunosuppression (tuberculosis) or
47 when dormant stages are reactivated and develop into new forms (malaria). In keeping with
48 the real world continuum between parasitism and mutualism (Combes 2001) similar
49 dynamics are revealed when, under certain conditions, symbionts proliferate and exert
50 negative effects on their hosts. For example, enhanced nitrate levels can increase the
51 growth of 'mutualistic' zooxanthellae thereby causing a decrease in calcification and, by
52 extension, coral growth (Marubini and Davies 1996).

53 This review describes how such hidden infectious agents persist as asymptomatic infections
54 in invertebrates and the implications of environmental change for infection dynamics. I
55 largely focus on a system that provides an unusually comprehensive understanding of these
56 issues – myxozoans that cycle between covert and overt infections in bryozoan hosts.
57 Similarities with other systems are also evaluated in order to explore common context-
58 dependent aspects of disease dynamics, to demonstrate how poorly we understand the
59 diversity of agents causing hidden infections and to highlight how changing disease profiles
60 may act as indicators of ecosystem change. I begin by reviewing different types of
61 asymptomatic infections and describing their detection.

62 **Characterising asymptomatic infections**

63 Asymptomatic infections that cause little or no disease but are nevertheless transmissible
64 are often referred to as 'silent' or 'latent' in the medical literature. For example, during the

65 'clinical latency phase' HIV infection can still be transmitted to new hosts even though
66 infected individuals may show no symptoms. Persistent asymptomatic infections that are not
67 horizontally transmitted have been defined as covert infections (Sorrell et al. 2009). Covert
68 infections may be caused by particular developmental stages of parasites and pathogens
69 (henceforth referred to collectively as parasites) that impose little costs on host fitness during
70 extended periods of persistence - for example, specialised bacterial cells with arrested or
71 reduced growth (Balaban et al. 2004). In other cases, early developmental stages may be
72 sustained, an example being single cell stages of some myxozoans located beneath the
73 basal lamina (Fig. 1A) (Canning et al. 2008). Covert infection stages may be dormant or they
74 may slowly replicate as indicated, for instance, by the continuous low-level expression of
75 viral proteins in e.g. insect hosts (Hughes et al. 1997; Vilaplana et al. 2010).

76 Cryptic stages causing asymptomatic infections may be located in immunoprivileged sites.
77 Alternatively, they may be tolerated to avoid the damaging effects of an inflammatory
78 immune response or they may be undetected. For example, slowly replicating hypnozoites
79 of *Plasmodium vivax* in the liver (Wells et al. 2010) may not be apparent to immune cells
80 because of low signal production (e.g. peptides) (Janeway et al. 2001).

81 Asymptomatic infections are inherently difficult to assess because disease is not evident.
82 Their detection thus requires molecular, histological or other approaches. For wild animals
83 this is typically done using PCR and RT-PCR. They may also be inferred, for example, by
84 emergence of disease in insects held under parasite-free laboratory conditions (Bonsall et al.
85 2005) or when asymptomatic individuals are challenged with other infections (e.g. Hughes et
86 al. 1993).

87 **Dynamics of asymptomatic covert infections: insights from** 88 **myxozoans**

89 Myxozoans are a radiation of endoparasitic cnidarians that exploit invertebrates and
90 vertebrates as primary and secondary hosts, respectively, and cause several devastating
91 fish diseases, including whirling disease, proliferative kidney disease (PKD) and
92 enteronecrosis (Jones et al. 2015). PKD causes substantial economic loss to rainbow trout
93 aquaculture in Europe, has contributed to declines of brown trout populations in Swiss rivers
94 and impacts North American hatcheries (Hedrick et al. 1986; Okamura et al. 2011). The
95 disease is caused by the malacosporean myxozoan, *Tetracapsuloides bryosalmonae*, which
96 uses freshwater bryozoans as primary hosts. Interactions between *Fredericella sultana* (the
97 most common bryozoan host) and *T. bryosalmonae* have therefore been extensively
98 investigated in order to understand patterns of PKD outbreaks and spread because spores
99 released from bryozoans infect fish. As described below, this body of work demonstrates
100 that covert infections enable persistent infection of highly clonal invertebrate hosts, creating
101 a substantial disease reservoir for fish. Insights on covert infections in another myxozoan-
102 bryozoan system are included to illustrate common patterns.

103 **Developmental cycling in freshwater bryozoan hosts**

104 Early single cell stages of malacosporeans (myxozoans that exploit freshwater bryozoan
105 hosts) are associated with the body wall (Fig. 1A) and cause covert infection. These cryptic
106 stages develop into multicellular, spore-filled sacs (or worms, in the case of some species of
107 *Buddenbrockia*; Hartikainen et al. 2014a) that proliferate in the body cavity during overt
108 infection (Fig. 1B). Spores are infectious to fish. Covert infections of *T. bryosalmonae* are
109 generally retained after overt infections disappear (e.g. in some 63% of *F. sultana* colonies;
110 Tops et al. 2009).

111 Tops et al. (2006) showed that as temperatures increase a greater proportion of *F. sultana*
112 colonies sustain overt infections of *T. bryosalmonae* and that the time for overt infection to
113 develop (latency) decreases. Monitoring of host responses demonstrated that overt infection
114 development occurs when warmer temperatures promote bryozoan growth (Tops et al.

115 2009). Overt infections reduce host growth at higher temperatures whereas the growth of
116 covertly infected and uninfected bryozoans is similar regardless of temperature (unless
117 bryozoans invest in statoblast production). Greater nutrients and hence food levels for
118 bryozoan hosts similarly promote the development of overt infections of *T. bryosalmonae*
119 and the growth of *F. sultana* (Hartikainen et al. 2009; Hartikainen and Okamura 2012). In this
120 case, overt infections reduce bryozoan growth as nutrient levels decrease. These studies
121 demonstrate that *T. bryosalmonae* exhibits host condition-dependent developmental cycling
122 (Fig. 2A). When *F. sultana* is in good condition and growing rapidly (high temperatures and
123 food levels) overt infections are triggered to develop from cryptic stages; however, when the
124 host environment is sub-optimal and host condition is depressed covert infections are
125 maintained. The retention of covert infections enables developmental cycling of parasites
126 between covert and overt infections and explains the waxing and waning of overt infections
127 that are observed in laboratory-maintained bryozoan hosts, including infections of both
128 *Buddenbrockia allmani* in *Lophopus crystallinus* (Hill and Okamura 2007) and of *T.*
129 *bryosalmonae* in *F. sultana* (Fig. 2B) (Tops 2004; Tops et al. 2009).

130 **Covert and overt infections over space and time**

131 The ability to regulate demands on bryozoan hosts contingent on their condition would
132 suggest that infections could be highly persistent over space and time. Indeed, covert
133 infection prevalences of *T. bryosalmonae* characterised by PCR every 45 days over a 12
134 month period in *F. sultana* populations in each of three rivers in southern England ranged
135 from 35-92% (mean = 65.6%; SD = 22.8%; n = 8) in one river, 0-76% (mean = 42.7%; SD =
136 24.1%; n = 8) in a second river, and 27-72% (mean = 45.6%; SD = 14.2%; n = 8) in a third
137 river (pooled data for bryozoan populations established on three tree root systems per river
138 on eight sampling dates; Fontes 2015; Fontes et al. submitted). Such persistent covert
139 infections represent a reservoir of disease for fish and contribute to annual outbreaks of PKD
140 on fish farms (see below) (Fig. 2B). Overt infections can develop rapidly (e.g. within three
141 days of laboratory culture; Canning and Okamura 2004) and are expressed in the field at

142 high prevalences over a relatively brief window of time in the UK (for weeks; Tops 2004).
143 Sampling every three weeks from spring to autumn revealed mean overt infection
144 prevalences that ranged from 0.2 to 22% (peak infection in early June) (n = 150 per
145 sampling date) in a fourth river system in southern England (Tops 2004).

146 Covert infections of *B. allmani* in *L. crystallinus* (Fig. 1A) appear to be common, having been
147 detected in all bryozoan populations sampled in the UK (n=3), Switzerland (n=1) and Italy
148 (n=1) (Hill and Okamura 2007). Prevalences of covert infection ranged from 9-59% and of
149 overt infections from 0-13% (sampling a UK population every 90 days from October 2003 to
150 January 2005, n \geq 29 per sampling date; Hill and Okamura 2007).

151 **Transmission of infections in clonal hosts**

152 Freshwater bryozoans undergo extensive clonal reproduction via: the iteration of zooids
153 which increases colony size, colony fission or fragmentation, and the production of seed-like
154 propagules (statoblasts) that typically serve as dormant stages. Infection transmission during
155 colony replication is effectively vertical as it generates new colonies that carry infection. As
156 outlined below, such transmission can be substantial and is thus likely to amplify covert
157 infection prevalence. It also enables parasites to exploit the same host genotypes over time
158 and space.

159 Infection of statoblasts has been demonstrated by PCR of colonies newly hatched from
160 statoblasts. Thus, *T. bryosalmonae* infections were detected in 39% (n=54) and 30%
161 (n=164) of young colonies hatched from statoblasts deriving from parental colonies of *F.*
162 *sultana* in two river systems (Abd-Elfattah et al. 2013). Similarly, infections of *B. allmani* were
163 inferred for nine out of 10 *L. crystallinus* statoblasts assayed from bryozoans in a single site
164 (Hill and Okamura 2007). Infection of statoblasts must generally be achieved by cryptic
165 stages during covert infection because overt infections inhibit statoblast production (Tops
166 2004; Hill and Okamura 2007; Hartikainen and Okamura 2012). In addition, the few

167 malformed statoblasts that are produced during overt infection exhibit greatly reduced
168 hatching success (Hartikainen et al. 2013).

169 Infections are also passed to colonies created by fission and fragmentation. Currents and
170 other forms of disturbance cause branches of *F. sultana* to detach and these can reattach in
171 new microhabitats (Wood 1973). Detached fragments of *F. sultana* were sampled from
172 bryozoan populations on three submerged tree root systems in three rivers and at two time
173 periods (Fontes 2015; Fontes et al. submitted). Overall some 49% of fragments (n=414)
174 carried covert infections and infection status varied across roots, rivers and times. Covert
175 infection prevalences of fragments collected in June and September were, respectively,
176 25.3% (n=95) and 79.5% (n=44) in one river, 42.1% (n=95) and 39.4% (n=66) in a second
177 river, and 38.5% (n=13) and 73.3% (n=101) in the third river (pooled data for fragments
178 detached from three root systems per river). In *L. crystallinus*, overt infections (sacs of *B.*
179 *allmani*; Fig. 1b) were observed in both daughter colonies produced by 64 of 65 colony
180 fission events (Hill and Okamura 2007) implying very high levels of infection transmission.

181 **Hidden infections in invertebrates: the great unknown**

182 There is growing evidence that a range of aquatic and terrestrial invertebrates are infected
183 by parasites capable of persisting as covert infections and associated research identifies
184 some common features. Covert viral infections of insect pests have long been recognised
185 and have been referred to as 'latent', 'occult' or 'non-apparent' infections (see Anderson and
186 May 1981 and references therein). More recent studies provide further evidence for covert
187 infections in wild populations of insects, crustaceans, and bivalves as well as freshwater
188 bryozoans, with molecular diagnostics (PCR, qPCR) demonstrating covert infection
189 prevalences that are frequently > 50% (Table 1). Vertical transmission characterises many of
190 these invertebrate host-parasite systems, although it has not been explicitly examined in
191 some (Table 1). Persistent infections within populations, high infection prevalences and
192 dispersal of infectious agents are all likely to be facilitated by such vertical transmission.

193 Collectively, this evidence suggests that substantial proportions of susceptible invertebrates
194 may sustain asymptomatic infections that are likely to be widespread and to persist over
195 time.

196 The diversity of agents causing such hidden infections remains poorly known. This is partly
197 because many invertebrates are small and overlooked, there are relatively few invertebrate
198 pathologists, and infections may be highly patchy and exhibit no external gross pathology
199 until late in infection or host death. Detection thus requires appropriate expertise and
200 destructive host sampling to characterise disease agents. Furthermore, there is a general
201 bias for detection of parasites in invertebrates that are of particular significance to
202 agriculture, forestry and fisheries or to ecosystem function (e.g. grazing by *Daphnia*,
203 pollination by bees). Finally, the potential for symbionts (commensals and mutualists) to
204 cause negative effects on hosts is generally disregarded. However, with the advent of the
205 Anthropocene, overlooking cryptic stages and asymptomatic infections poses particular risks
206 in view of growing evidence that disease dynamics may be influenced by environmental
207 change.

208 Molecular detection is revealing substantial novel diversity in various endoparasitic taxa that
209 exploit invertebrate hosts, including haplosporidians (Hartikainen et al. 2014b), mikrocytids
210 (Hartikainen et al. 2014c) and microsporidians (Stentiford et al. 2013). For example,
211 amplicon sequencing of environmental samples has increased the number of microsporidian
212 lineages by >100% and has revealed several highly distinct novel lineages (Hartikainen et al.
213 2014b). This approach has also provided substantial insights into radiations of unique
214 mikrocytid lineages while targeted screening provides evidence that *Paramikrocytis canceri*
215 exploits an exceptionally wide range of invertebrates, including molluscs, decapods and
216 annelids (Hartikainen et al. 2014c). Finally, many parasite taxa once regarded as single
217 species based on morphological criteria, are being shown to comprise multiple species
218 according to sequence data (e.g. in myxozoans; Atkinson et al. 2015). These discoveries of
219 novel and hidden diversity suggest a huge and largely unknown diversity of endoparasites

220 that exploit invertebrate hosts. Many of these may persist causing asymptomatic infections
221 for long periods of time.

222 **Persistence and dispersal of infectious agents**

223 Covert infections are particularly linked with traits enabling persistence of infectious agents
224 within hosts. Low demands exerted by dormant or slowly replicating cryptic stages effect low
225 virulence and should promote persistence. Covert infections also commonly undergo vertical
226 transmission (Table 1) that will promote persistence and may amplify infection prevalences
227 locally. Such persistent infections may be actively maintained by parasites or result from
228 host suppression of overt infection. Host suppression is unlikely to explain patterns of host
229 condition-dependent development in bryozoans because this would require hosts in poor
230 condition to suppress overt infection – an unlikely scenario in view of the costs of
231 continuously mounting an immune response during long periods of sub-optimal conditions.
232 Other evidence that persistent infections are controlled by parasites includes demonstration
233 of specialised bacterial persistor cells (Balaban et al. 2004) and of transcription factors (e.g.
234 DosR in *Mycobacterium tuberculosis*; reviewed by Boon and Dick 2012) that regulate
235 development – both suggesting specific parasite adaptations for arrested or reduced growth
236 during covert infection. The retention of covert infection stages following bouts of overt
237 infection may represent a form of bet hedging to cope with environmental stochasticity, for
238 example in herpes viruses (Stumpf et al. 2002) and in myxozoans in bryozoans.

239 Dispersal of infected hosts may enable infection persistence over time and space. For
240 example, dispersive statoblasts carry cryptic stages (see above) and there is substantial
241 evidence that waterfowl act as vectors of statoblast dispersal (Freeland et al. 2000a,
242 Figerola et al. 2004, 2005), including from North America to Europe (Freeland et al. 2000b,
243 Henderson and Okamura 2004). The relationship between vertical transmission, dispersal
244 and persistence of infectious agents is worthy of further investigation in view of range
245 extensions in response to climate change.

246 **Environmental change and disease dynamics**

247 **Context-dependent disease dynamics and their implications**

248 Activation of microscopic, persistent quiescent stages to cause disease in invertebrates is
249 generally associated with changing environments and often these are linked to changes in
250 host condition. For example, overt viral infections of insects develop when hosts experience
251 stressful conditions, including infections of other parasites, high temperatures, and
252 overcrowding (Table 1; see also Anderson and May 1981 for review of early studies). Viral
253 diseases of oysters and shrimp are similarly noted to develop when hosts are exposed to
254 stressors (Table 1). In contrast, overt infections of *T. bryosalmonae* develop when hosts are
255 in peak form as a result of sustained warm temperatures and high food levels that promote
256 host growth.

257 Context-dependent dynamics may also be expressed if changing conditions alter host-
258 symbiont interactions as exemplified in the earlier cited example of nitrate levels increasing
259 the growth of zooxanthellae to the detriment of coral host growth (Marubini and Davies
260 1996). If such symbionts are only detected when disease is observed they are likely to be
261 regarded as parasites even though negative effects on hosts may be confined to unusual
262 circumstances. Increased understanding of the complexities of host-symbiont interactions in
263 their broadest sense (e.g. those involving organisms traditionally regarded as parasites,
264 commensals and mutualists) will enable better prediction of how environmental change may
265 influence invertebrate health and disease development.

266 The association of disease development with environmental change has implications in view
267 of accelerated changes that now characterise environments globally. In particular, the
268 dynamics of invertebrate diseases may be altered as changing environments influence host
269 and parasite responses. Such altered dynamics may include:

- 270 • shifts in the timing of disease outbreaks (due to e.g. warming temperatures)

- 271 • prolonged or diminished periods of disease (as a consequence of parasite- and host-
272 specific responses to environmental variation)
- 273 • the apparent disappearance of disease (e.g. if conditions are unsuitable for overt
274 infection development)
- 275 • the appearance of a previously undetected disease (if asymptomatic infections are
276 widespread)

277 **Warning of ecosystem change**

278 The strong link between disease and environmental change suggests that changes in
279 invertebrate disease dynamics or distributions may provide generically useful indicators of
280 ecosystem change. Thus, indicators based on altered disease profiles could complement
281 indicators arising from special understanding of specific systems (Pace et al. 2015) or from
282 long term monitoring to identify the behaviour of state variables (Batt et al. 2013).

283 Furthermore, because disease is contingent on the intimate interactions of at least two
284 organisms, disease indicators will reflect integrated responses and may provide insights on
285 the underlying mechanisms of change as outlined below in the case of PKD.

286 Outbreaks of PKD in sites in Norway (Sterud et al. 2007) and Iceland (Kristmundsson et al.
287 2010) in regions where previously the disease was undetected are suggestive of
288 environmental change and associated altered dynamics of covert infections. Unusually warm
289 conditions at these sites may have caused PKD outbreaks by instigating overt infection and
290 prolonged spore production in bryozoan hosts (Tops et al. 2006). Alternatively or
291 additionally, warmer conditions may have stressed fish harbouring previously chronic
292 undetected infections, causing clinical PKD and mortality. These northerly PKD outbreaks
293 thus demonstrate that novel diseases need not be attributed to invasive parasites but can
294 result from endoparasites that remain hidden within ecosystems as endemic asymptomatic
295 infections. The possibility that emerging diseases may be explained by endemic but

296 inapparent causative agents is rarely considered because research tends to focus on
297 invasive disease agents and ecosystem change (e.g. Crowl et al. 2008).

298 **Conclusion**

299 There are many unknowns regarding the diversity and dynamics of asymptomatic, hidden
300 infections in invertebrates. An important question is whether and when environmental
301 change may, for example, constrain host resistance evolution because resistance-conferring
302 traits are costly and require trading off against other aspects of fitness (e.g. fecundity,
303 growth). Consideration of such issues is beyond the scope of this paper but see Altizer and
304 Pederson (2008) for review of how environmental change may influence the evolutionary
305 dynamics of hosts and parasites and the consequent disease risks for wild populations.
306 What does appear to be predictable is that environmental change will exacerbate some
307 invertebrate diseases. Such diseases may be caused by endemic endoparasites that have
308 been overlooked and lurk as covert infections. In other cases, infectious agents may be
309 released from inhibition by host immune responses or competitive interactions with other
310 parasites or symbionts. Invertebrate diseases may also develop when parasites are
311 introduced to new environments by human activities or when vertical transmission
312 establishes infection in dispersal stages that colonise new habitats. Similarly, environmental
313 change may cause disease or impact the health of invertebrates hosting commensal or
314 mutualistic symbionts if compromised host defences enable opportunistic exploitation of host
315 resources. These changing disease dynamics may provide warning of further biotic change
316 that doesn't require extensive knowledge and long term monitoring of ecosystems. A better
317 understanding of the diversity and complex relationships of endoparasites with their
318 invertebrate hosts will enable a fuller appreciation of parasite biodiversity and of how our
319 changing world may impact organismal interactions.

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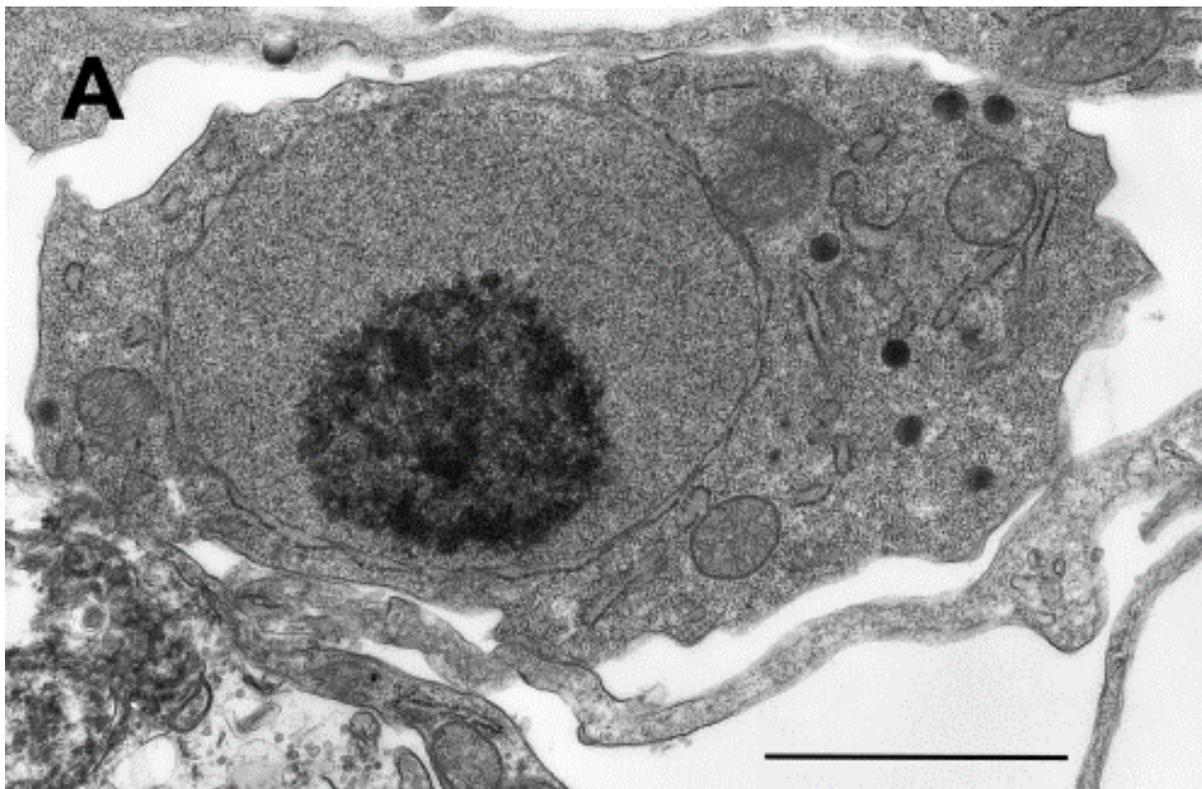
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Fig. 1 Infection stages of the sac-forming malacosporean myxozoan, *Buddenbrockia allmani*, in the freshwater bryozoan host, *Lophopus crystallinus*. **(A)** Single cells of *B. allmani* beneath the peritoneum associated with covert infection. Scale bar = 3 μm . **(B)** Spore-filled sacs of *B. allmani* are readily observed by stereomicroscopy in the bryozoan body cavity causing overt infections. Scale bar = 500 μm .

Fig. 2 Contingent dynamics of myxozoan parasite, *Tetracapsuloides bryosalmonae*, and its freshwater bryozoan host, *Fredericella sultana*. **(A)** Host-condition dependent cycling between avirulent covert (single cells associated with the body wall) and virulent overt infection (numerous spore-filled sacs in the body cavity) resulting in the release of spores infectious to fish. **(B)** The effects of host-condition dependent cycling through time, illustrating initial infection of *F. sultana* colony and the subsequent iterated impacts of covert and overt infection dynamics on propagule production and periodic castration (during overt infection) as mediated by the persistence of cryptic stages of *T. bryosalmonae*. Note that infections may eventually be lost (as illustrated) but reinfection may occur.



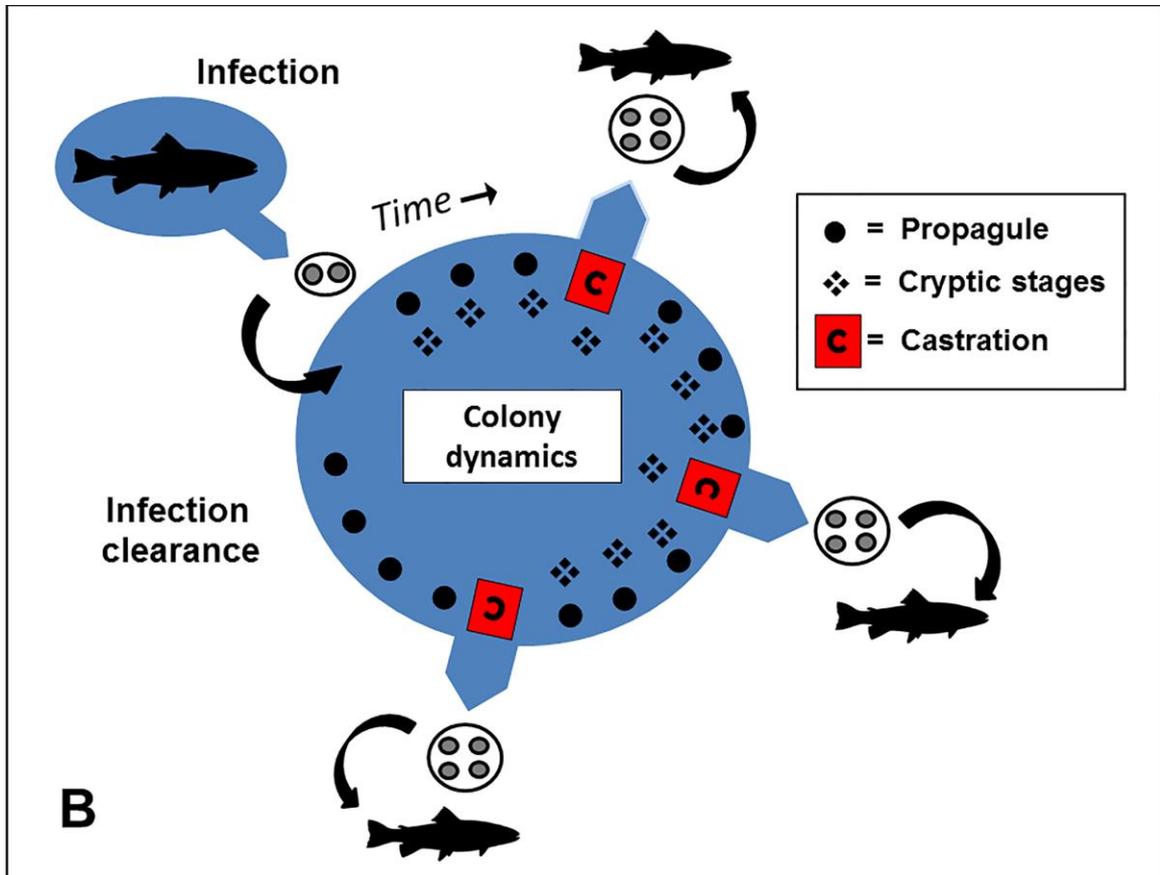
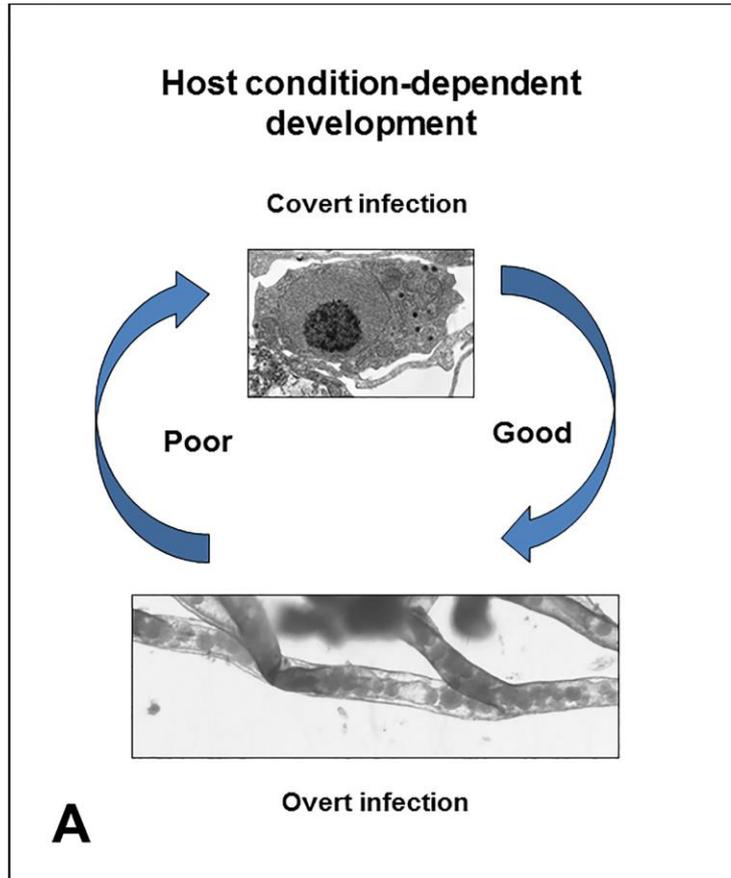


Table 1. Asymptomatic covert infections in insects and aquatic invertebrates studied in recent years, including insights on prevalences, drivers of overt infection and capacity for vertical transmission. Numerical superscripts link results with specific references when required. Covert infections of myxozoans in bryozoan hosts are elaborated more fully in the text but relevant data are included for comparative purposes.

Parasite	Host	Covert infection prevalence	Overt infection drivers	Vertical transmission	References
<i>MbNPV</i> (nucleopolyhedro virus in Baculoviridae)	<i>Mamestra brassicae</i> larvae (cabbage moth)	50-100% (PCR: 10 populations collected variously over 2 yrs; n<10 in 7 populations)	Challenge with heterologous baculoviruses	PCR shows infection in 75% and 80% of 1 st generation larvae and in 100% of 5 th generation larvae deriving from mated adults from 2 populations [n _≤ 5]	Burden et al. 2003
<i>PiGV</i> (granulovirus in Baculoviridae)	<i>Plodia interpunctella</i> larvae (Indian meal moth)	100% larvae, 100% pupae, 30% adults after challenge with PiGV at 5 th instar (PCR: 10 individuals per life history stage)	unknown	PCR shows infection passed by both sexes to progeny and 80-90%, [n=10] of 2 nd generation progeny are infected	Burden et al. 2002
<i>SpexNPV</i> (nucleopolyhedro virus in Baculoviridae)	<i>Spodoptera exempta</i> larvae (African armyworm)	97% (PCR: 33 adult moths derived from field-collected larvae and pupae); 60% (RT-PCR: 10 adult moths derived from field-collected material)	Long term persistence in relatively stress-free laboratory conditions implies stressful host conditions trigger overt infection	78-100% of 2 nd and 7 th generation larvae and adults by PCR; 25-50% by RT-PCR [relatively low n-values]	Vilaplana et al. 2010
Iridoviruses (possibly 3 species ²)	<i>Simulium</i> larvae (blackfly)	17%, 30% and 23% ¹ at a single time; 17-37% in spring, 0% in summer, 0-20% in autumn ² (PCR on	Host stress is suggested in keeping with baculoviruses ¹ ; Supported by elevated	unknown	¹ Williams 1993; ² Williams 1995

		3 populations [n=30 or 50 per population per time] in 1 river in both studies)	covert infection levels at low host densities, low temperatures and [presumably] slower host growth ²		
DWV (deformed wing virus, a positive strand RNA virus)	<i>Apis mellifera</i> (honeybee)	100% (RT-PCR of 4 German hives [n≥40 bees/hive]); 40% (24 bees from 3 Swedish hives) ¹ ; 95% of pupae, 80% of larvae, 79% of adults (RT-PCR of 2 hives in USA; n=24 for each stage) ²	Infestations of ectoparasitic mite, <i>Varroa destructor</i> , that causes varroosis in honeybees ³	Via infected sperm causing 100% infection of eggs in 6 out of 8 cases; Via gonad causing 100% infection of unfertilised eggs in 2 of 8 cases and 9% in 1 of 8 cases (n=24 eggs sampled) ³	¹ Yue and Genersch 2005; ² Chen et al. 2005; ³ Yue et al. 2007
OsHV-1 (Ostreid herpesvirus 1)	Many bivalves, most studies on <i>Crassostrea gigas</i> ; <i>Ostrea edulis</i> (oysters)	46% (qPCR: n= 54) & 47% (qPCR: n=46) in <i>C. gigas</i> populations in 2 sites in northern California ¹ ; 79% in <i>O. edulis</i> population (qPCR: n=14) in northern California ¹	Rapid increase in water temperature ² ; Adverse conditions for host ³	Viral DNA in the gonad suggests potential or vertical transmission ⁴	¹ Burge et al. 2011; ² Renault et al. 2014; ³ See references in Burge et al. 2007; ⁴ Arzul et al. 2002
White spot syndrome virus (WSSD)	Wide range of crustaceans (devastating disease in shrimp aquaculture); other invertebrates may	e.g. 6-77% & 40-88% of wild-caught asymptomatic shrimp and crabs, respectively (PCR: sample sizes≥5) ²	Stressors to hosts (e.g.rapid change in salinity, drop in temperature) ³	Suspected ³	¹ Stentiford et al. 2009; ² Lo & Kou 1998; ³ See references in Stentiford et al. 2009

	act as vectors ¹				
<i>Tetracapsuloides bryosalmonae</i> (myxozoan)	<i>Fredericella sultana</i> (freshwater bryozoan)	0-92% ¹ (PCR: populations in 3 rivers sampled every 45 days over 2 yrs; n _≥ 25 with one exception)	Good host condition ^{2,3}	39% [n=54] and 30% [n=164] of statoblasts from 2 populations by PCR ⁴ ; 25-80% of colony fragments sampled over 2 time periods in 3 rivers, [n _≥ 44 with one exception] ⁵	¹ Fontes 2015; ² Tops et al. 2006; ³ Hartikainen & Okamura 2012; ⁴ Abd-Elfattah et al. 2013; ⁵ Fontes 2015; ⁵ Fontes et al. submitted
<i>Buddenbrockia allmani</i> (myxozoan)	<i>Lophopus crystallinus</i> (freshwater bryozoan)	9-59% (PCR: in 5 populations in the UK, Switzerland & Ireland n _≥ 29)	Unknown	98% of daughter colonies produced by fission (n=65)	Hill & Okamura 2007