NATURAL
HISTORY
MUSEUM

Title	Hidden Infections and Changing Environments
Authors	Okamura, B
Description	The file attached is the Accepted/final draft post-refereeing version of the article.

1

2

3

4

5

6

Hidden infections and changing environments

8

9

10

Beth Okamura

Department of Life Sciences, Natural History Museum, Cromwell Road, London, SW7 5BD, United Kingdom

b.okamura@nhm.ac.uk

+44 (0)2079 426631

16

17

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.

35

36

37 Total number words: 5166

38

39

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.

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 hypnozooites

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.

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.

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.

Environmental change and disease dynamics

Context-dependent disease dynamics and their implications

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

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

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

- 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).

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.

Acknowledgements

331   **References**

332   Abd-Elfattah A, Fontes I, Kumar G, Soliman H, Hartikainen H, Okamura B, El-Matbouli M.

333   2014. Vertical transmission of *Tetracapsuloides bryosalmonae* (Myxozoa), the

334   causative agent of salmonid proliferative kidney disease. Parasitol 141:482–490.

335   Altizer S, Harvell D, Friedle E. 2003. Rapid evolutionary dynamics and disease threats to

336   biodiversity. Trends Ecol Evol 18:589–96.

337   Altizer S, Pedersen A. 2008. Host-pathogen evolution, biodiversity and disease risk for

338   natural populations. In: Carroll S, Fox C, editors. Conservation biology: evolution in

339   action. New York, USA: Oxford University Press. p. 259-77.

340   Anderson RM, May RM. 1981. The population dynamics of microparasites and their

341   invertebrate hosts.. Phil Trans R Soc B 291:451-524.

342   Arzul I, Renault T, Thebault A, Gerard A. 2002. Detection of oyster herpesvirus DNA and

343   proteins in asymptomatic *Crassostrea gigas* adults. Virus Res 84:151–60.

344   Atkinson SD, Bartošová-Sojková P, Whipps CM, Bartholomew JL. 2015. Approaches for

345   characterising myxozoan species. In: Okamura B, Gruhl A, Bartholomew JL, editors.

346   Myxozoan evolution, ecology and development, 1 ed. Cham, Switzerland: Springer

347   International Publishing. p.111-23.

348 Balaban NQ, Merrin J, Chait R, Kowalik L, Leibler S. 2004. Bacterial persistence as a

349   phenotypic switch. Science 305:1622-25.

350 Batt RD, Carpenter SR, Cole JJ, Pace ML, Johnson RA. 2013. Changes in ecosystem

351   resilience detected in automated measures of ecosystem metabolism during a whole-

352   lake manipulation. Proc Natl Acad Sci USA 110:17398–403.

353 Bonsall MB, Sait SM, Hails RS. 2005. Invasion and dynamics of covert infection strategies

354   in structured insect–pathogen populations. J Anim Ecol 74: 464-474.

355 Boon C, Dick T. 2012. How *Mycobacterium tuberculosis* goes to sleep: the dormancy

356   survival regulator DosR a decade later. Future Microbiol. 7:513-18.

357 Burden JP, Griffiths CM, Cory JS, Smith P, Sait SM. 2002 Vertical transmission of sublethal

358   granulovirus infection in the Indian meal moth, *Plodia interpunctella*. Mol Ecol 11:547-

359   55.

360 Burden JP, Nixon CP, Hodgkinson AE, Possee RD, Sait SM, King LA, Hails RS. 2003.

361   Covert infections as a mechanism for long-term persistence of baculoviruses. Ecol Lett

362   6:524-31.

363 Burge CA, Strenge RE, Friedman CS. 2011. Detection of the oyster herpesvirus in

364   commercial bivalve in northern California, USA: conventional and quantitative PCR.

365   Dis Aquat Org 94:107-16.

366 Burge CA, Judah LR, Conquest LL, Griffin FJ, Cheney DP, Suhrbier A, Vadopalas B, Olin

367   PG, Renault T, Friedman CS. 2007. Summer seed mortality of the Pacific oyster,

368   Crassostrea gigas Thunberg grown in Tomales Bay, California, USA: The influence of

369   oyster stock, planting time, papthogens, and environmental stressors. J Shellfish Res

370   26: 163–172.

371 Canning EU, Okamura B. 2004. Biodiversity and evolution of the Myxozoa. Adv Parasitol

372   56:43-131.

373 Canning EU, Curry A, Okamura B. 2008. Early development of the myxozoan

374   *Buddenbrockia plumatellae* in the bryozoans *Hyalinella punctata* and *Plumatella*

375   *fungosa*, with comments on taxonomy and systematics of the Myxozoa. Fol Parasitol

376   55:241-255.

377 Chen YP, Higgins JA, Feldlaufer MF. 2005. Quantitative real-time teverse transcription-PCR

378   analysis of deformed wing virus infection in the honeybee (*Apis mellifera* L.). Appl Env

379   Microbiol 71:436-41.

380 Combes C. 2001. Parasitism: the ecology and evolution of intimate interactions. Chicago,

381   USA: The University of Chicago Press.

382 Crowl TA, Crist TO, Parmenter RR, Belovsky G, Lugo AE. 2008. The spread of invasive

383   species and infectious disease as drivers of ecosystem change. Front Ecol Environ

384   6:238–46.

385 Figuerola J, Green AJ, Black K, Okamura B. 2004. The influence of gut morphology on

386   passive transport of freshwater bryozoans by waterfowl in Doñana (southwest Spain).

387   Can J Zool 82:835-40.

388 Figuerola J, Green AJ, Michot TC. 2005. Invertebrate eggs can fly: evidence of waterfowl-

389   mediated gene flow in aquatic invertebrates. Am Nat 165:274-80.

390 Fontes I. 2015. Life history, distribution and invertebrate host-parasite interactions of the

391   causative agent of proliferative kidney disease (PKD), *Tetracapsuloides*

392   *bryosalmonae*. Dissertation. University of Aberdeen.

393 Ford SE, Bushek D. 2012. Development of resistance to an introduced marine pathogen by
394   a native host. J Mar Res 70:205–23.

395 Freeland JR, Noble LR, Okamura B. 2000a. Genetic consequences of the metapopulation
396   biology of a facultatively sexual freshwater invertebrate. J Evol Biol 13:383-95.

397 Freeland JR, Romualdi C, Okamura B. 2000b. Gene flow and genetic diversity: a
398   comparison of freshwater bryozoan populations in Europe and North America.
399   Heredity 85:498–508.

400 Hartikainen H, Ashford OS, Berney C, Okamura B, Feist SW, Baker-Austin C, Stentiford GD,
401   Bass D. 2014b. Lineage-specific molecular probing reveals novel diversity and
402   ecological partitioning of haplosporidians. ISME 8:177-186.

403 Hartikainen H, Fontes I, Okamura B. 2013. Parasitism and phenotypic change in colonial
404   hosts. Parasitol 140:1403–12.

405 Hartikainen H, Gruhl A, Okamura B. 2014a. Diversification and repeated morphological
406   transitions in endoparasitic cnidarians (Myxozoa: Malacosporea). Mol Phylogenet Evol
407   76:261–69.

408 Hartikainen H, Johnes P, Moncrieff C, Okamura B. 2009. Bryozoan populations reflect
409   nutrient enrichment and productivity gradients in rivers. Freshw Biol 54:2320–34

410 Hartikainen H, Okamura B. 2012. Castrating parasites and colonial hosts. Parasitol 139:547-
411   56.

412 Hartikainen H, Stentiford G, Bateman KS, Berney C, Feist SW, Longshaw M, Okamura B,
413   Stone D, Ward G, Wood C, Bass D. 2014c. Mikrocytids: a novel radiation of parasitic
414   protists with a broad invertebrate host range and distribution. Curr Biol 24:807-12.

415 Hedrick RP, Kent ML, Smith CE. 1986. Proliferative kidney disease of salmonid fishes. U.S.
416   Dept. of Interior, Fish and Wildlife Service, Fish Dis. Leaflet 74:9.

417   Henderson MW, Okamura B. 2004. The phylogeography of salmonid proliferative kidney

418   disease in Europe and North America. Proc R Soc B 1549:1729-36.

419   Hill SLL, Okamura B. 2007. Endoparasitism in colonial hosts: patterns and processes.

420   Parasitol 134:841-852.

421   Hughes DS, Possee RD, King LA. 1993. Activation and detection of a latent baculovirus

422   resembling Mamestra brassicae nuclear polyhedrosis virus in *M. brassicae* insects.

423   Virology 194:608-15.

424   Hughes DS, Possee RD, King LA. 1997. Evidence for the presence of a low-level, persistent

425   baculovirus infection of *Mamestra brassicae* insects. J Gen Virol 78:1801-5.

426   Janeway CA Jr, Travers P, Walport M, Shlomchik M. 2001. Immunobiology: The Immune

427   System in Health and Disease. 5th edition. New York: Garland Science.

428   Jones SRM, Bartholomew JL, Zhang JY. 2015. Mitigating myxozoan disease impacts on wild

429   fish populations. In: Okamura B, Gruhl A, Bartholomew JL, editors. Myxozoan

430   evolution, ecology and development, 1 ed. Cham, Switzerland: Springer International

431   Publishing. p. 397-413.

432   Kim, K, Harvell CD. 2004. The rise and fall of a six-year coral epizootic. Am. Nat. 164, S52–

433   S53.

434   Kristmundsson Á, Antonsson T, Árnason F. 2010. First record of Proliferative Kidney

435   Disease in Iceland. Bul Eur Ass Fish Path 30:35-40.

436   Lo C-F, Kou G-H. 1998. Virus-associated white spot syndrome of shrimp in Taiwan: a

437   review. Fish Pathol 33:365-71.

438   Marubini F, Davies PS. 1996. Nitrate increases zooxanthellae population density and

439   reduces skeletogenesis in corals. Mar Biol 127:319-28.

440   Okamura B, Hartikainen H, Schmidt-Posthaus H, Wahli T. 2011. Proliferative kidney disease

441   as an emerging disease: the importance of life cycle complexity and environmental

442   change. Freshw Biol 56:735-53.

443   Pace ML, Carpenter SR, Cole JJ. 2015. With and without warning: managing ecosystems in

444   a changing world. Front Ecol Env 13:460-7.

445   Renault T, Bouquet AL, Maurice J-T, Lupo C, Blachier P. 2014. Ostreid herpesvirus 1

446   infection among Pacific Oyster (*Crassostrea gigas*) spat: relevance of water

447   temperature to virus replication and circulation prior to the onset of mortality. Appl

448   Environ Microbiol 80:5419–26.

449   Sorrell I, White A, Pedersen AB, Hails RS, Boots M. 2009. The evolution of covert, silent

450   infection as a parasite strategy. Proceedings of the Royal Society B 276: 2217-2226.

451   Stentiford GD, Bonami J-R, Alday-Sanz V. 2009. A critical review of susceptibility of

452   crustaceans to Taura syndrome, Yellowhead disease and White Spot Disease and

453   implications of inclusion of these diseases in European legislation. Aquaculture 291: 1-

454   17.

455   Stentiford GD, Feist SW, Stone DM, Bateman KS, Dunn AM. 2013. Microsporidia: diverse,

456   dynamic, and emergent pathogens in aquatic systems. Trends Parasitol 29:567-78.

457   Sterud E, Forseth T, Ugedal O, Poppe TT, Jørgensen A, Bruheim T, Fjeldstad H-P, Mo TA.

458   2007. Severe mortality in wild Atlantic salmon *Salmo salar* due to proliferative kidney

459   disease (PKD) caused by *Tetracapsuloides bryosalmonae* (Myxozoa). Dis Aquat Org

460   77:191-8.

461   Stumpf MP, Laidlaw Z, Jansen VA. 2002. Herpes viruses hedge their bets. Proc Natl Acad

462   Sci USA. 99:15234-7.

463   Tops S. 2004. Ecology, life history and diversity of malacosporeans. Dissertation, University

464   of Reading.

465 Tops S, Hartikainen H-L, Okamura B. 2009 The effects of infection by *Tetracapsuloides*

466   *bryosalmonae* (Myxozoa) and temperature on *Fredericella sultana* (Bryozoa). Int J

467   Parasitol 39:1003-10.

468 Tops S, Lockwood W, Okamura B. 2006. Temperature-driven proliferation of

469   *Tetracapsuloides bryosalmonae* in bryozoan hosts portends salmonid declines. Dis

470   Aquat Org 70:227-236.

471 Vilaplana L, Wilson, K, Redman EM, Cory JS. 2010. Pathogen persistence in migratory

472   insects: high levels of vertically-transmitted virus infection in field populations of the

473   African armyworm. Evol Ecol 24:147-160.

474 Wells TN, Burrows JN, Baird JK. 2010. Targeting the hypnozoite reservoir of *Plasmodium*

475   *vivax*: the hidden obstacle to malaria elimination. Trends Parasitol 26:145-151.

476 Williams T. 1993. Covert iridovirus infection of blackfly larvae. Proc R Soc Lond B 251: 225-

477   230.

478 Williams T. 1995. Patterns of covert infection by invertebrate pathogens: iridescent viruses of

479   blackflies. Mol Ecol 4:447-57.

480 Wood TS. 1973 Colony development in species of *Plumatella* and *Fredericella* (Ectoprocta:

481   Phylactolaemata). In Animal Colonies, development and function through time.

482   Boardman RS, Cheetham, AH, Oliver WAJ, editors. Stroudsburg, USA: Hutchinson

483   and Ross. p. 395–432.

484 Yue C, Genersch E. 2005. RT-PCR analysis of Deformed wing virus in honeybees (*Apis*

485   *mellifera*) and mites (*Varroa destructor*). J Gen Virol. 86:3419-24.

486 Yue C, Schröder M, Gisder S, Genersch E. 2007. Vertical-transmission routes for deformed

487   wing virus of honeybees (*Apis mellifera*). J Gen Virol. 88:2329-36.

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.
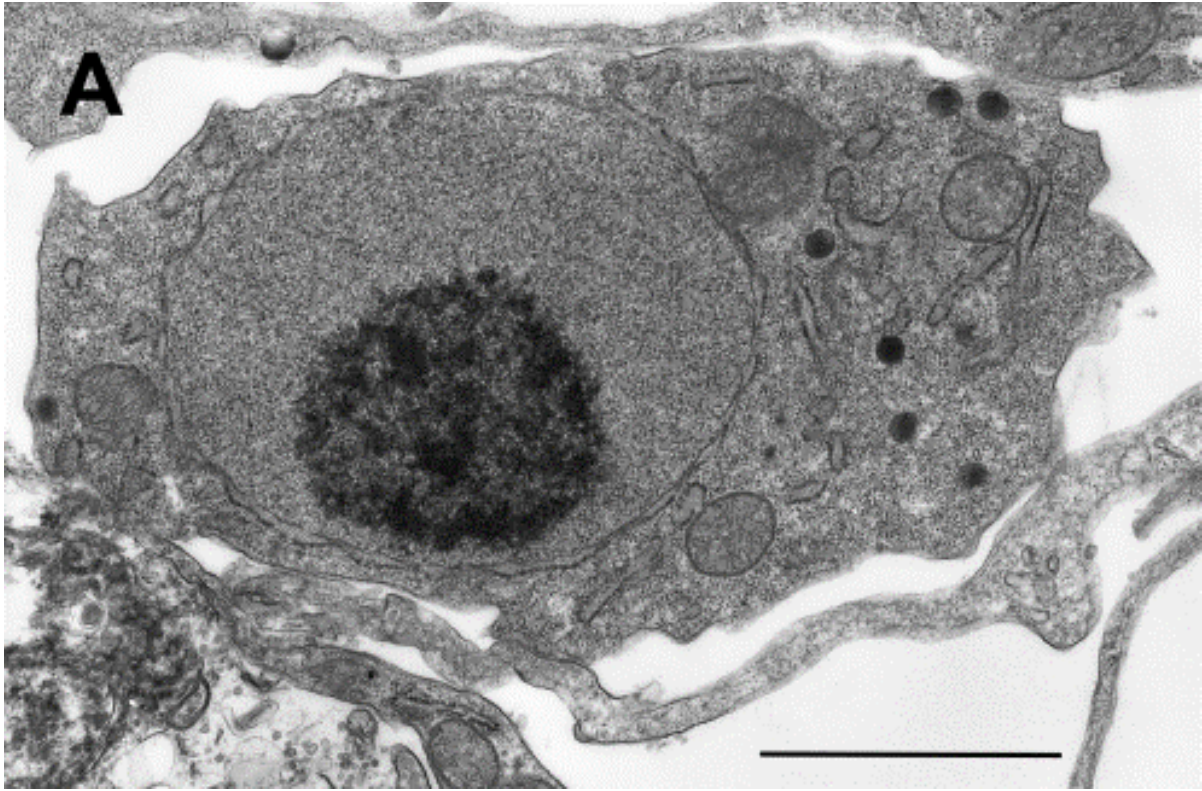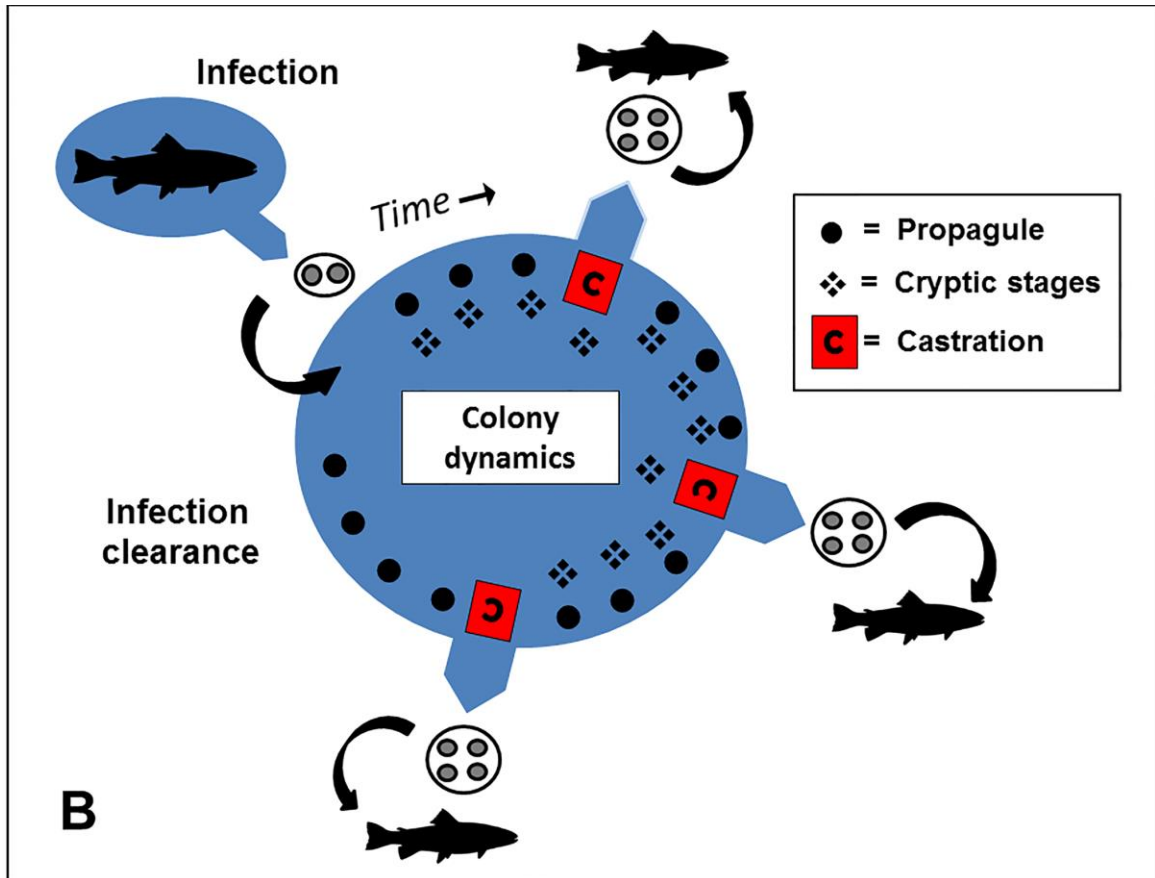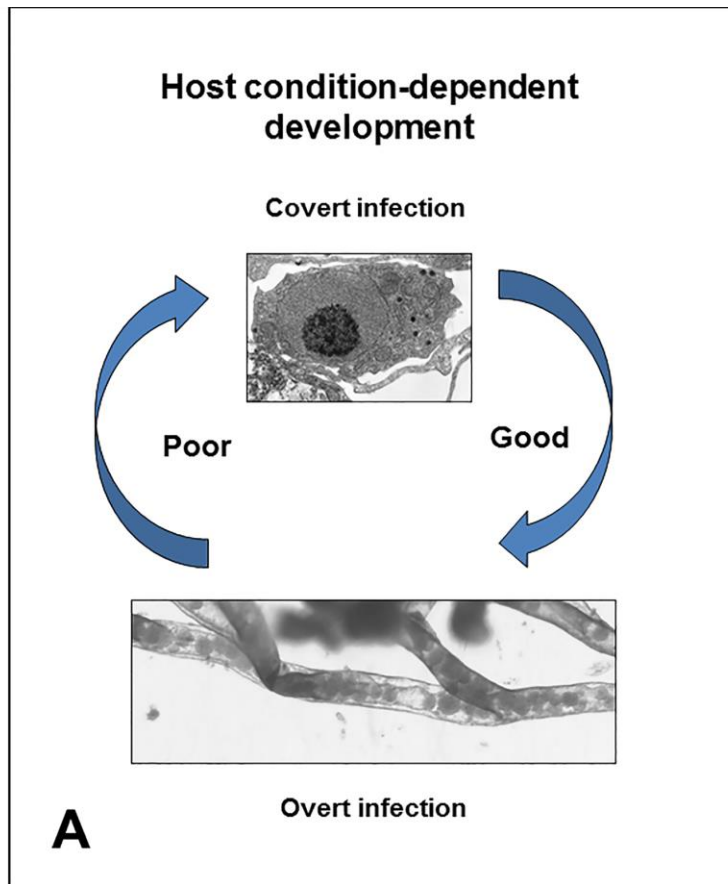
Table 1. Asymptomatic covert infections in insects and aquatic invertebrates studied in recent years, including insights on prevalences, drivers of overt infection and capacity for vertical transmission. Numerical superscripts link results with specific references when required. Covert infections of 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
*Mb*NPV (nucleopolyhedrovirus in Baculoviridae)	*Mamestra brassicae* 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 1st generation larvae and in 100% of 5th generation larvae deriving from mated adults from 2 populations [n$\leq$5]	Burden et al. 2003
*Pi*GV (granulovirus in Baculoviridae)	*Plodia interpunctella* larvae (Indian meal moth)	100% larvae, 100% pupae, 30% adults after challenge with PiGV at 5th instar (PCR: 10 individuals per life history stage)	unknown	PCR shows infection passed by both sexes to progeny and 80-90%, [n=10] of 2nd generation progeny are infected	Burden et al. 2002
*Spex*NPV (nucleopolyhedrovirus in Baculoviridae)	*Spodoptera exempta* 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 2nd and 7th generation larvae and adults by PCR; 25-50% by RT-PCR [relatively low n-values]	Vilaplana et al. 2010
Iridoviruses (possibly 3 species[2])	*Simulium* 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)	*Apis mellifera* (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, *Varroa destructor*, 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 *Crassostrea gigas; Ostrea edulis* (oysters)	46% (qPCR: n= 54) & 47% (qPCR: n=46) in *C. gigas* populations in 2 sites in northern California[1]; 79% in *O. edulis* 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]				
Tetracapsuloides bryosalmonae (myxozoan)	*Fredericella sultana* (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
Buddenbrockia allmani (myxozoan)	*Lophopus crystallinus* (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