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Life cycle complexity, environmental change and the emerging status of salmonid proliferative kidney disease

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SUMMARY

1. Proliferative kidney disease (PKD) is a disease of salmonid fish caused by the endoparasitic myxozoan, *Tetracapsuloides bryosalmonae*, which uses freshwater bryozoans as primary hosts. Clinical PKD is characterised by a temperature-dependent proliferative and inflammatory response to parasite stages in the kidney.

2. Evidence that PKD is an emerging disease includes outbreaks in new regions, declines in Swiss brown trout populations and the adoption of expensive practices by fish farms to reduce heavy losses. Disease-related mortality in wild fish populations is almost certainly underestimated because of e.g. oversight, scavenging by wild animals, misdiagnosis and fish stocking.

3. PKD prevalences are spatially and temporally variable, range from 0 to 90–100% and are typically highest in juvenile fish.

4. Laboratory and field studies demonstrate that (i) increasing temperatures enhance disease prevalence, severity and distribution and PKD-related mortality; (ii) eutrophication may promote outbreaks. Both bryozoans and *T. bryosalmonae* stages in bryozoans undergo temperature- and nutrient-driven proliferation.

5. *Tetracapsuloides bryosalmonae* is likely to achieve persistent infection of highly clonal bryozoan hosts through vertical transmission, low virulence and host condition-dependent cycling between covert and overt infections. Exploitation of fish hosts entails massive proliferation and spore production by stages that escape the immune response. Many aspects of the parasite's life cycle remain obscure. If infectious stages are produced in all hosts then the complex life cycle includes multiple transmission routes.

6. Patterns of disease outbreaks suggest that background, subclinical infections exist under normal environmental conditions. When conditions change, outbreaks may then occur in regions where infection was hitherto unsuspected.

7. Environmental change is likely to cause PKD outbreaks in more northerly regions as warmer temperatures promote disease development, enhance bryozoan biomass and increase spore production, but may also reduce the geographical range of this unique multihost-parasite system. Coevolutionary dynamics resulting from host–parasite interactions that maximise fitness in previous environments may pose problems for sustainability, particularly in view of extensive declines in salmonid populations and degradation of many freshwater habitats.

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Introduction

Proliferative kidney disease (PKD) is a widespread disease of wild and farmed salmonid fish in the Northern Hemisphere (for review see Hedrick, MacConnell & de Kinkelin, 1993; El-Matbouli & Hoffman, 1994; Canning & Okamura, 2004). All salmonids appear to be susceptible but our understanding of disease development and pathology is based largely on studies of rainbow trout, *Oncorhynchus mykiss* (Walbaum). Clinical PKD is characterised by a massive inflammatory response caused by cells proliferating in the kidney and spleen in salmonid fish infected with the causative agent, the myxozoan *Tetracapsuloides bryosalmonae* (Canning *et al*., 2002). The disease is highly problematic for fish farms and hatcheries, where up to 100% of stock can be infected and mortalities can reach 95% (Hedrick *et al*., 1984; Feist & Longshaw, 2006). Fish with PKD often die following secondary infections (Feist & Bucke, 1993). In contrast, the impact of PKD on wild fish populations is poorly understood for reasons we later explore.

It has long been known that the development and pathology of PKD are influenced by temperature with recent studies highlighting temperature-dependent mortality (Bettge *et al*., 2009a,b). These links between disease progression, mortality and temperature suggest that PKD outbreaks may be exacerbated by climate change, and, indeed evidence implies that this is already happening. Evaluation of this evidence along with other data indicates that PKD is an emerging disease that poses a significant and growing risk to salmonid health. The aims of this paper are therefore to: (i) review the evidence that PKD is an emerging disease; (ii) evaluate the environmental and biological drivers of disease emergence, and; (iii) enable understanding and prediction of disease dynamics. As host–parasite interactions are fundamental to disease development, we begin by describing the biology and life cycle of *T. bryosalmonae*.

Tetracapsuloides bryosalmonae: the causative agent of PKD

Although PKD has been recognised since the early 1900s and was shown to be caused by a myxozoan 25 years ago (Kent & Hedrick, 1985), the disease source was a mystery until 1999 when freshwater bryozoans were revealed as invertebrate hosts (Anderson, Canning & Okamura, 1999). This discovery allowed the causative agent to be described, and the subsequent erection of a new myxozoan class (the Malacosporea) (Canning *et al*., 2000) to accommodate myxozoans parasitic in freshwater bryozoans.

Myxozoans are endoparasites of vertebrates and invertebrates. Some are highly pathogenic in fish, developing as stages in organ cavities or tissues and causing many diseases, including whirling disease, ceratomyxosis and swim bladder inflammation (El-Matbouli, Fischer-Scherl & Hoffman, 1992; Feist & Longshaw, 2006). Their phylogenetic affinities have long been controversial (see Canning & Okamura, 2004 for review); however, a recent study provides strong evidence that myxozoans are a clade of highly derived and morphologically degenerate cnidarians (Jiménez-Guri *et al*., 2007). These results support earlier suggestions of a cnidarian affinity based on the presence of intracellular organelles with extrusible filaments (polar capsules) that are used to attach to hosts and resemble cnidarian nematocysts (Weill, 1938; see also Siddall *et al*., 1995).

The identification of bryozoans as hosts has enabled rapid progress in understanding the ecology and life cycle of *T. bryosalmonae*, although much yet remains unclear. Freshwater bryozoans (Phylum Bryozoa, Class Phylactolaemata) are benthic, colonial suspension-feeding animals. Colonies can grow to indefinite size by budding of individual zooids that share a common body cavity (the coelom) (Wood & Okamura, 2005). The parasite develops first as covert infections when single-cell stages are associated with the bryozoan body wall (Morris & Adams, 2006a). During overt infection, multicellular sacs (up to 350 μm in diameter) develop from single-cell stages and proliferate in the body cavity of bryozoan hosts (Canning *et al*., 1999, 2000; Morris & Adams, 2007) (Fig. 1). Spores (*c*. 20 μm in diameter) are produced within mature sacs (Canning *et al*., 1999, 2000), each possessing two internal amoeboid cells and four polar capsules. A single sac of 350 μm can be estimated to contain between 2800 and 4000 spores depending on spore packing density and calculating as aggregations

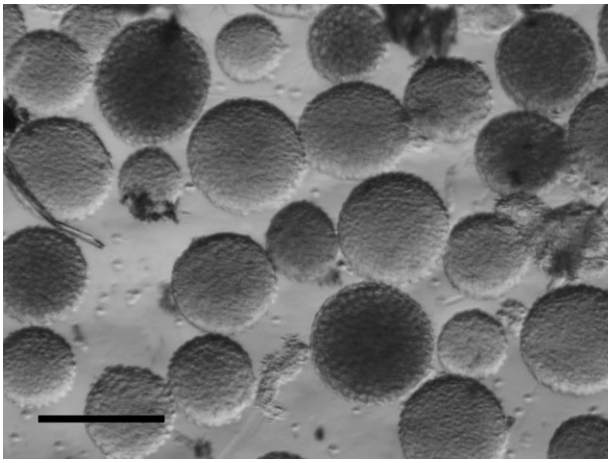

Fig. 1 Sacs of *Tetracapsuloides bryosalmonae* expelled from the body cavity of bryozoan hosts. Scale bar = 350 μm.

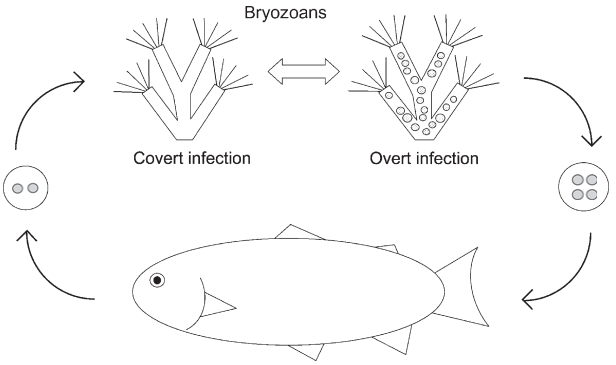

Fig. 2 The life cycle of *Tetracapsuloides bryosalmonae*. Spores (with two polar capsules) released from fish infect bryozoans to cause covert infections of single cell stages. Overt infection entails the development of sacs within bryozoan hosts that can be observed via stereomicroscopy. Spores (with four polar capsules) that develop in sacs infect fish. Note cycling can occur between covert and overt infection.

of spherical spores that fill the volume of a spherical sac (Martin *et al*., 1997). Meiosis during spore development (Canning *et al*., 2000) identifies bryozoans as primary hosts. Spores released from bryozoans into the water attach to fish via the eversible filament contained within polar capsules. Spore development and release does not guarantee loss of infection in bryozoans, and the parasite can cycle between covert and overt infection (Fig. 2). Infections of *T. bryosalmonae* have been detected in bryozoans ranging from putatively primitive to derived species (Anderson *et al*., 1999).

Infection of fish is achieved by amoeboid cells in spores that invade via the skin or gills (Morris, Adams & Richards, 2000b; Longshaw *et al*., 2002) and enter the vascular system. These unicellular extrasporogonic stages multiply in the blood prior to reaching the kidney as the main site of further development, but parasites can also invade and proliferate in other organs. The extrasporogonic stages undergo further proliferation in the interstitium of the kidney causing an inflammatory response and damage to kidney tissues. These stages may eventually be eliminated and the damage they caused repaired (Feist & Longshaw, 2006). Some of these extrasporogonic stages migrate into the lumen of kidney tubules, where they differentiate into attached pseudoplasmodia. Single spores develop within the pseudoplasmodia, each containing a single amoeboid cell and two polar capsules (Kent & Hedrick, 1985, 1986; Morris & Adams, 2008). The spores are excreted in urine (Hedrick *et al*., 2004) and, in the case of at least brown (*Salmo trutta* Linnaeus) and brook (*Salvelinus fontinalis* Mitchill) trout (Morris & Adams, 2006b; Grabner & El-Matbouli, 2008), are infective to bryozoans (see Fig. 2). The site of invasion of bryozoan hosts is unknown.

External clinical signs of PKD include pale, anaemic gills and abdominal swelling. Internally, kidney and spleen are hyperplastic because of a proliferative and inflammatory response to the presence of parasitic stages (Hedrick *et al*., 1993; El-Matbouli & Hoffman, 1994) (Fig. 3). As the specific immune responses of fish are usually suppressed at low temperatures (Le Morvan, Troutaud & Deschaux, 1998), PKD development is temperature-dependent. During the onset of PKD, key immune regulatory cytokines are downregulated and severe kidney pathology ensues, including abnormal increases in lymphocytes, granulomatous lesions and renal atrophy (Chilmonczyk, Monge & de Kinkelin, 2002). Additionally, the activity of granulocytes (components of the immune system) is depressed, increasing the risk of contracting bacterial diseases (Chilmonczyk *et al*., 2002). Thus, increasing temperatures act as promoters for other diseases that may exacerbate the mortality rates in fish immunocompromised by PKD infection. However, laboratory experiments on rainbow trout clearly indicate that mortality can result in the absence of secondary infections demonstrating that infection by *T. bryosalmonae* alone leads to the death of fish (Bettge *et al*., 2009a,b).

The apparent lack of development of spores in many salmonid species had been interpreted to

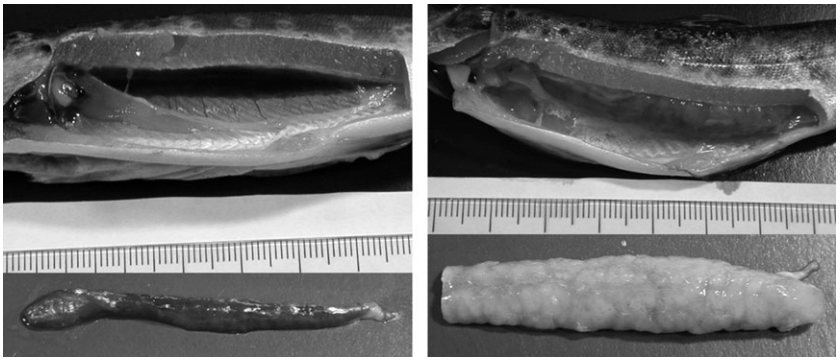

Fig. 3 Brown trout without proliferative kidney disease (PKD) (left) and with PKD (right) with their kidneys removed. Note hyperplastic kidney on right resulting from proliferative and inflammatory response of the fish to the presence of *Tetracapsuloides bryosalmonae* in kidney tissue.

signify that these were accidental hosts (Hedrick *et al*., 1993), but this requires re-evaluation in light of what is now known of spore maturation in malacosporeans (Canning *et al*., 2002). In addition, identification of the disease may be hindered by absence of clinical signs, misdiagnosis or not accounting for temporal variation in development (Hedrick *et al*., 2004; Grabner & El-Matbouli, 2008). Most reports of PKD are for rainbow and brown trout but outbreaks have been described in Atlantic salmon (*Salmo salar* Linnaeus) in Europe and chinook, [*Oncorhynchus tshawytscha* (Walbaum)] and coho [*Oncorhynchus kisutch* (Walbaum)] salmon in North America (Hedrick *et al*., 1993; Wahli *et al*., 2002, 2007; Sterud *et al*., 2007). In Europe, grayling [*Thymallus thymallus* (Linnaeus)], Arctic charr [*Salvelinus alpinus* (Linnaeus)] and pike (*Esox lucius* Linnaeus) can show signs of the disease but infected brook trout exhibit no clinical signs (Bucke, Feist & Clifton-Hadley, 1991; Feist & Bucke, 1993).

PKD as an emerging disease

Evidence that PKD is an emerging disease in wild salmonid populations derives mainly from Norway, the U.S.A. and Switzerland. This evidence indicates that PKD is spreading, causing major mortality events, and playing a significant role in fish declines. As will be discussed in our concluding section, there is also an indication that the disease is manifesting itself in a new way, perhaps as a result of environmental change.

In 2006, populations of Atlantic salmon and brown trout experienced a severe outbreak of PKD that caused an estimated 85% reduction in Atlantic salmon parr densities in the River Åelva in Central Norway (Sterud *et al*., 2007). This represented a notable northward extension for PKD outbreaks. Similarly, PKD outbreaks in 1990 and 1991 in a remote population of feral cutthroat trout represented the first case of PKD for the state of Montana (MacConnell & Peterson, 1992).

The programme 'Fischnetz' was established in 1998 to investigate the causes of declines in brown trout in Swiss lowland rivers. A major driver for the investigation was the reduction in brown trout catches over time in cantonal fishing records. These records, which date back to the 1970s, indicate declines in catches from 1980 onwards (Frick *et al*., 1998). The Fishnetz programme measured factors that might explain catch declines in rivers throughout Switzerland along with assessments of fish health. Bayesian modelling incorporating all variables identified PKD as an important factor in explaining low fish population densities (Borsuk *et al*., 2006) and causing an inferred mortality of >25% in some rivers.

The economically driven and widespread adoption of expensive farming methods by commercial fish farms provides further compelling evidence for the emergence of PKD in recent years Although there are no published data, many fish farmers describe the disease as once unproblematic but now a major issue. As a result, many fish farms (e.g. in the U.K.: O. Robinson, pers. comm.; in Italy: M. Fioravanti, pers. comm.; C. Ghittino, pers. comm.) have adopted specific management practices to avoid excessive mortality because of PKD. These entail exposing fish fry or fingerlings to *T. bryosalmonae* infections in late summer or autumn when temperatures start to decrease. Fish thus exposed either do not develop or demonstrate reduced clinical signs of PKD in the following year when temperatures increase. The practice of stocking fish in late summer or autumn involves substantial time and effort. When possible, well water is used for holding fish during risky periods prior to later exposure to infection. The fish

may thus require transport from well water to exposure sites and then must be cared for and fed through the winter. Such management practice substantially reduces production by extending the time that fish stocks must be maintained, often in a limited set of holding areas prior to ongrowth. Since wild salmonid populations are typically associated with waterbodies linked to fish farms they may also be experiencing the increased disease prevalence and severity that drove fish farmers to adopt expensive management practices. However, ironically, these practices are also likely to obscure the situation because catastrophic mortality is now avoided on farms and other diseases are therefore generally of primary concern.

Constraints on demonstrating disease emergence in wild fish populations

While there are some records of PKD in wild fish populations and evidence that it is increasing in range or prevalence (percentage of population with infection), knowledge of PKD and other diseases in wild fish is generally extremely poor (Feist *et al*., 2002; Wahli *et al*., 2007). Disease-related mortality of wild fish is almost certainly underestimated, since it has to be on a large enough scale to be noticeable (Wootten & McVicar, 1982). Understanding loss at the population level is further hampered when predators or scavengers are present, as will almost invariably be the case.

Sampling protocols can also compromise disease detection. For instance, fry may be undersampled by use of seine nets and traditional fishing techniques such as trolled lures (Feist *et al*., 2002). However, a major impediment is that detecting PKD requires killing salmonids to obtain kidney tissue, a practice likely to be frowned upon by authorities, owners of waterbodies and biologists themselves. The reluctance to kill many fish no doubt explains the relatively small sample sizes in studies of wild fish that lead to wide confidence intervals for estimates of prevalence (Feist *et al*., 2002; Wahli *et al*., 2007; Peeler *et al*., 2008). Data on PKD prevalences over time in wild populations are therefore rare and highly compromised by sample size.

Fish stocking may additionally complicate the assessment of disease dynamics in wild populations. Stocking practices will mask mortality, while variability in stocking will further obscure disease prevalence over time. Of course, stocking in itself can be a potential indicator of declines in wild fish populations that may, in part, be caused by disease.

Finally, when mortality is observed, the underlying cause may be misdiagnosed. For instance, death may be attributed solely to secondary infections. In other cases, lack of expertise or equipment may preclude diagnosis, since this is based on histological or molecular analysis.

Epidemiology of PKD

Prevalences of PKD in wild fish are spatially and temporally variable. Wahli *et al.* (2002) detected *T. bryosalmonae* infection in brown and rainbow trout in 40% of sites sampled in Switzerland in 2000–01 (n = 139). In most sites prevalence was <40%, but in two sites 90–100% of fish were infected. The study also obtained evidence for higher infection prevalences in wild than in farmed fish. A later study focusing on young-of-year brown trout found infections in 56% of sites in Swiss rivers in 2004 (n = 91) and again demonstrated site variation in prevalences (Wahli *et al*., 2007). The majority of sites had infection prevalences >50%, with 10 and six sites having infection prevalences of 81–90 and 91–100%, respectively. Surveys conducted in 1997 in England and Wales characterised prevalences in brown trout populations ranging from 11 to 43% in PKD-positive rivers with no infection detected in 9 of 14 rivers. A later survey in 2003 found *T. bryosalmonae* infections in brown trout in six of seven rivers in southwest England, with prevalences ranging from 2.5 to 35.7% in the six PKD-positive rivers (Peeler *et al*., 2008). As previously noted, the small sample sizes used in these surveys result in large confidence intervals for prevalence estimates within sites and also the possibility that low prevalence of infection existed at sites where PKD was not recorded. Nevertheless, the greater variance in the prevalence of PKD between sites within a river compared with that between rivers suggests that within-site features are most important in determining prevalence. These could include water quality (see later discussion of eutrophication) and the distribution of bryozoans (Peeler *et al*., 2008).

Juvenile fish are regarded as most at risk from PKD but any fish that have not previously been exposed to *T. bryosalmonae* are susceptible (Feist & Longshaw, 2006). Wootten & McVicar (1982) obtained evidence

for higher prevalences of *T. bryosalmonae* infection in wild brown trout fry than in 1+ or older fish in a Scottish river. However, Wahli *et al.* (2002) found infection prevalence peaked in two size classes of brown trout in Swiss rivers and farms. One peak was for fish <1 year. The second peak for older fish was probably abnormal, resulting from disproportionate collection of more evidently diseased fish and inclusion of farmed fish that were kept in tanks exposed to water sources not inhabited by bryozoans (well or ground water) when young. Schager, Peter & Burkhardt-Holm (2007) demonstrated a negative relationship between the density of young-of-year brown trout and infection by *T. bryosalmonae*, with the highest densities and lowest infection prevalences occurring in narrow streams with a steep slope in low-altitude Swiss rivers. This result suggests that age-class/prevalence relationships may be highly dynamic as a result of fish movements or differential mortality.

A further complication to interpreting age-class/prevalence relationships is uncertainty over infection status. Young fish that survive exposure to *T. bryosalmonae* generally do not develop clinical PKD in the following year when temperatures rise (Ferguson & Ball, 1979; Foott & Hedrick, 1987; de Kinkelin & Loriot, 2001), thus apparently acquiring immunity following infection. However, there is also evidence that spore-forming stages within kidney tubules remain after the disappearance of stages in kidney interstitium and the decline of clinical PKD (Kent & Hedrick, 1986; Morris *et al.*, 2000a). Foott & Hedrick (1987) showed that these stages can persist for at least 1 year in rainbow trout. Whether persistence is achieved throughout the lifetime of fish hosts is unknown, but several observations are relevant to this question. First, low numbers of spore-forming stages in kidney tubules have been observed in grayling, brown trout and Atlantic salmon outside the PKD season (Morris *et al.*, 2000a). These stages are not associated with pathology (Morris *et al.*, 2000a). This evidence suggests that low-grade subclinical kidney tubule infections may persist indefinitely at least in some fish hosts (e.g. brown trout, grayling or Atlantic salmon) allowing the continuous production of infectious spores. Furthermore, since fish health is apparently unimpaired, such subclinical infections are likely to be overlooked unless targeted by specifically designed longitudinal studies to follow infections over the lifetime of fish hosts. Whether subclinical infections in wild fish derive from new infections or from persistence of a number of parasites has still to be elucidated. Preliminary results indicate a full elimination of parasites in rainbow trout under laboratory conditions where new infections are not possible (K. Bettge, pers. comm.). However, parasites have been recorded all year long in brown trout kept in river water (C. Schubiger, pers. comm.). It is possible that warm, laboratory conditions (18 °C) are conducive to elimination. Further studies are clearly required to examine the presence and significance of persistent, low-grade kidney tubule infections in salmonid species.

Drivers of disease

In this section, we explore what factors may enhance the prevalence, severity and distribution of PKD. In particular, we consider how increasing temperatures and eutrophication influence the development and effects of *T. bryosalmonae* in fish and bryozoan hosts. We also consider how parasite strategies to exploit hosts may contribute to disease emergence.

Disease development in fish

Both laboratory and field studies demonstrate the role of temperature in disease development. For instance, laboratory studies demonstrate that fish will develop clinical PKD at 12–18 °C but not at 9 °C (Clifton-Hadley, Richards & Bucke, 1986) and that reduced water temperature suppresses the effects of the disease (Clifton-Hadley *et al.*, 1986; de Kinkelin & Loriot, 2001; Bettge *et al.*, 2009b). Peak prevalences in wild and farmed fish typically occur during the summer (Hedrick *et al.*, 1993; Wahli *et al.*, 2002) and the disease is generally not manifested in the autumn or winter despite fish being infected (Hedrick *et al.*, 1993). However, PKD can develop during winter on farms where water temperatures are abnormally warm (Schlotfeldt, 1983; Smith *et al.*, 1984). In addition, drought conditions that result in elevated water temperatures can be particularly devastating on fish farms (T. Wahli, pers. obs.). Examples of PKD emergence in wild and feral fish populations are also linked to temperature. Thus, the recent severe outbreak in Norwegian Atlantic salmon and brown trout was associated with warmer waters as a result of water abstraction (Sterud *et al.*, 2007), while the

outbreak in feral cutthroat trout in Montana was linked with drought (MacConnell & Peterson, 1992). In addition, declines in brown trout populations attributed to PKD have occurred in Swiss rivers that are undergoing warming (Borsuk *et al.*, 2006). Conversely, recent surveys of *T. bryosalmonae* infections in brown trout populations in 287 sites throughout Switzerland (Wahli *et al.*, 2008) revealed no relationship between altitude (used as a proxy for temperature) and infection prevalence (or infection intensity). However, the altitude–temperature relationship was complicated by unusually cold or warm water sources affecting river temperatures in some sites, including warmer water in the two high-altitude sites with high prevalences. In addition, factors other than temperature may influence infection development and severity, including oxygen levels, fish movements, normal altitudinal limits to bryozoan or parasite distributions, the effect of host condition on the development of *T. bryosalmonae* in bryozoans (see later discussion) and concentrations of infectious spores.

Temperature also influences incubation time and severity of infection. Field transmission trials demonstrate that clinical signs of the disease (e.g. kidney swelling) take about 8 weeks to develop in early and late summer in rainbow trout fingerlings but only about 4–5 weeks in July (El-Matbouli & Hoffman, 1994). Laboratory studies have shown that infected rainbow trout held at 15 and 19 °C had fully swollen kidneys, while those held at 9 and 12 °C showed little renal swelling (Clifton-Hadley *et al.*, 1986). Recent histological studies specifically examining the effects of temperature on the course of infection in rainbow trout have demonstrated that higher temperatures accelerate lesion severity and parasite numbers (Bettge *et al.*, 2009b). In addition, analysis by RT-PCR has provided evidence that parasite load during the initial phase of infection was reduced in fish kept at 12 °C relative to fish maintained in higher temperatures (Bettge *et al.*, 2009a). Both the histological and RT-PCR-based studies demonstrated striking variation in cumulative mortality among groups, with 5–10% of fish dying at 12 °C but around 80–90% at 18 °C (Bettge *et al.*, 2009a,b). The pathological effects observed led the researchers to propose that kidney dysfunction that compromises osmoregulation and haematopoiesis, combined with the higher demand for oxygen, may prove fatal. Subsequent investigations of the further course of the disease have indicated that the main effect of temperature is temporal variation in disease progression rather than differences in severity of lesions or numbers of parasites *per se* (Bettge, 2008; Schmidt-Posthaus *et al.*, submitted). In general, these recent studies suggest that multiple factors may contribute to temperature-driven mortality in diseased fish (Bettge, 2008; Schmidt-Posthaus *et al.*, submitted).

Another environmental factor suggested to influence PKD outbreaks is eutrophication. This issue was specifically studied in Germany where PKD was endemic in a trout hatchery on the Singold Brook from 1979 to 1998 (El-Matbouli & Hoffman, 2002). During most of this period, effluent from a sewage treatment plant (STP) was introduced upstream from the hatchery; however, in 1998 the treated water was diverted. Hatchery prevalences of PKD dropped to 15% in 1998 and to 5% in 2000, while previous levels ranged from 40 to 80%. Prevalences of PKD in the wild fish population showed a similar pattern. Prior to diversion, the prevalence was significantly lower in a site upstream (14%) from the STP than in a site downstream (56%). In 1998, prevalences dropped to 8% upstream and 17% downstream, and in 2000 no infected fish were sampled. There was no evidence for changing temperature regimes during the period of study.

An association of PKD and eutrophication has also been suggested in some Swiss lowland rivers, but there are confounding factors (e.g. Burkhardt-Holm & Scheurer, 2007). For instance, PKD prevalence and intensity can show downstream increases in parallel with increases in both nutrients and temperature (Zimmerli *et al.*, 2007). In addition, water quality in most Swiss rivers has steadily been improving because of sewage treatment (Binderheim-Bankay, Jakob & Liechti, 2000). However, sewage treatment does not eliminate all pollutants, particularly not micropollutants. Furthermore, an improvement in water quality may not be accompanied by biotic responses because of slow recovery or alternative stable states (Scheffer *et al.*, 1993; Carpenter *et al.*, 1998). For instance, eutrophic conditions may have favoured the establishment of dense bryozoan populations, which once developed may be highly persistent (see next section). In addition, sources of non-point pollution (e.g. fertilizers from agriculture, pesticides, run-off from areas supporting livestock; atmospheric deposition) may also be important

(Carpenter *et al.*, 1998; Burkhardt-Holm & Scheurer, 2007; Coors *et al.*, 2008).

Disease development in ryozoans

The effects of temperatures on the development of *T. bryosalmonae* in its most common freshwater bryozoan host, *Fredericella sultana* (Blumenbach), have been investigated by following the development of infection in field-collected colonies with unknown infection status in controlled laboratory conditions (10, 14 and 20 °C) over periods of 4 weeks (Tops, Lockwood & Okamura, 2006). The basic responses of *T. bryosalmonae* are depicted in Fig. 4. *Tetracapsuloides bryosalmonae* consistently reacted to increasing temperatures at three different times of year (summer, autumn, winter) by developing into overt infections (spore-producing sacs) in a greater proportion of bryozoan hosts. As temperatures decreased, covert infections were maintained in a larger proportion of colonies. Higher temperatures generally reduced the time for overt infections to develop (decreased latency), and the duration of overt infection was longest at 14 °C suggesting a unimodal relationship to temperature. A second set of trials provided evidence that a change in temperature triggers overt infection, these developing only after bryozoans were transferred to 20 °C following maintenance at 10 °C for either 3 or 6 weeks in both summer and autumn. Temperatures for the site from which the bryozoans were collected range from 6 to 16 °C (Tops, 2004; Tops *et al.*, 2006). This study therefore shows that increases in temperature and prolonged periods of warmer temperatures promote the development of overt infections.

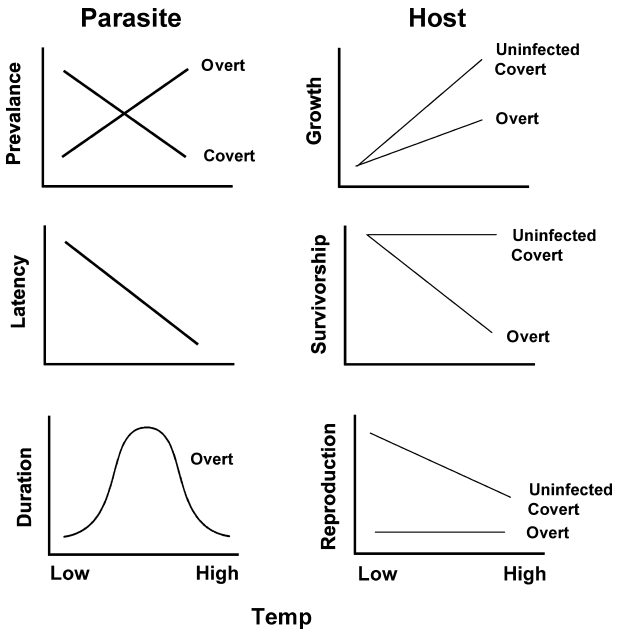

Fig. 4 Schematic responses of *Tetracapsuloides bryosalmonae* (parasite) and bryozoan host to increasing temperatures.

The effects of increasing temperatures and parasitism on bryozoan hosts are of course also important to consider. Tops, Hartikainen & Okamura (2009) characterised host responses (summarised in Fig. 4) in the same set of trials described earlier. Increasing temperatures generally increased growth and caused some mortality. The effects of infection depended on both temperature and infection status. Overtly infected bryozoans sustained lower growth rates than both covertly infected and uninfected bryozoans at 14 and 20 °C and also experienced greater mortality at 20 °C. Nevertheless, the higher growth rate of overtly infected bryozoans at 20 °C than at 14 or 10 °C implies that increasing temperatures will be associated with a higher biomass of infected bryozoans. The growth rates of covertly infected and uninfected bryozoans were generally similar regardless of temperature, although covertly infected bryozoans that produced statoblasts (asexual, dormant stages) exhibited lower growth. The propensity to produce statoblasts was similar in covertly infected and uninfected bryozoans, while statoblast production almost ceased in overtly infected bryozoans. Overt infection thus reduces reproduction by asexual propagules, although we know nothing about the effects of infection on sexual reproduction.

An important conclusion from the aforementioned studies is that increasing temperatures can be expected to result in the release of greater numbers of spores infective to fish hosts. This is because increasing temperatures: (i) directly promote the proliferation of *T. bryosalmonae* in bryozoan hosts; (ii) enhance the growth of bryozoan hosts, thereby producing a greater biomass for parasites to exploit; (iii) are associated with low virulence except in, at present, extreme conditions (a constant temperature of 20 °C for 4 weeks). Increases in spore numbers may be of little significance to disease dynamics, since it has been shown that clinical PKD can result following infection by one or only a few spores (McGurk *et al.*, 2006). However, caution must be adopted when extrapolating results of simple, controlled laboratory

studies to the field where infection dynamics may be influenced by many factors, such as rapid dilution of spore concentrations, effects of turbulence on transmission, variation in infectivity of parasite strains or in host susceptibility (Grabner & El-Matbouli, 2009) and escalation in virulence as a result of multiple infections (Galvani, 2003).

The potential link between PKD and eutrophication may be explained by responses of bryozoan hosts, since increasing nutrient concentrations should promote primary production, thereby enhancing food resources for bryozoans. Hartikainen *et al.* (2009) obtained strong evidence from combined laboratory and field studies that nutrient concentrations influence bryozoan abundance. Field studies determined the concentrations of statoblasts retained in traps deployed on rivers characterised by low, intermediate and high nutrient concentrations. This approach to estimating abundance is possible because most species release floating statoblasts, and the number of statoblasts produced is a good proxy of bryozoan biomass. The study found that the concentrations of statoblasts of all bryozoan species increased significantly with nutrient concentration. The latter may also promote greater concentrations of *T. bryosalmonae*-infected statoblasts, since malacosporean infections of statoblasts have been documented (Taticchi *et al.*, 2004; Hill & Okamura, 2007).

The effects of eutrophication on parasite development and host–parasite interactions have also been assessed in a series of microcosm-based studies (Hartikainen, 2007; Hartikainen & Okamura, submitted). These studies revealed that greater nutrient/food levels promote both parasite and host growth and provide further evidence that low virulence characterises infections. As was the case with increasing temperature, eutrophication can therefore also be expected to cause the release of greater numbers of spores infective to fish hosts as a result of the effect of increasing food levels supporting greater bryozoan and parasite biomass. Of course in both cases, there will be limits to this effect via direct or indirect negative feedback loops, such as increased competition, predation or eventual harm caused by changing temperatures and nutrient levels.

Parasite strategies to exploit hosts

The proliferation of single cells during covert infection of bryozoan hosts (Morris & Adams, 2006a) should enable the spread of infection along with the growth of the colonial host and thus contribute to persistent infection, particularly since covert infections exert little to no effect on bryozoan hosts (Tops *et al.*, 2009) and because clonal reproduction makes bryozoans potentially immortal hosts. Such proliferation and spread will also maximise the chances of achieving vertical transmission by colony fragmentation (Morris & Adams, 2006c; Hill & Okamura, 2007) and possibly via statoblasts. The transmission of infection via statoblasts was shown in a closely related bryozoan-malacosporean system when new colonies developed from statoblasts that were stored in dark, cold conditions for 5–17 months were shown by PCR and sequencing to carry infection (Hill & Okamura, 2007; see also Taticchi *et al.*, 2004). Such vertical transmission should be highly significant as it will promote persistent infection of clonal genotypes over time and space by spreading the risk of loss of infection from clonal genotypes as well as facilitating location of particularly favourable microhabitats for host growth. It may also contribute to the low virulence demonstrated in microcosm studies, since the reproductive interests of both host and parasite are aligned (Bull, Molinieux & Rice, 1991; Tops *et al.*, 2009). Indeed, selection for low virulence may be particularly likely for parasites that exploit hosts whose highly clonal life cycles offer a long-lived, genetically homogeneous resource and ample opportunities for vertical transmission, such as those characterising freshwater bryozoans (Hill & Okamura, 2007). These issues relate to theoretical predictions for how virulence vs. transmission trade-offs are influenced by infection duration which, in turn, is related to host longevity (e.g. see review by Frank, 1996). Infections of clonal genotypes should be lost eventually because of stochastic events and the negative effects of overt infection. Nevertheless, the enhanced opportunities for prolonged infection afforded by clonal replication and low virulence will greatly delay this loss.

In common with a number of parasites, such as *Plasmodium* species that cause malaria, or certain viruses, *T. bryosalmonae* can cycle between different stages of infection in bryozoan hosts. Thus, the infection can either become overt, causing disease symptoms and leading to horizontal transmission, or it may remain covert, or 'silent' (Sorrell *et al.*, 2009). This is observed in *T. bryosalmonae* when overt infection disappears and reappears in study material

and during routine monitoring of bryozoans in microcosms (Tops *et al.*, 2009), indicating an interim period of covert infection. PCR has shown that in 63% of cases (n = 40), covert infection is retained after the disappearance of sacs in bryozoans maintained in microcosms at 20 °C (Tops *et al.*, 2009). The high prevalences of overt infections in late spring and autumn (Tops, 2004; Tops *et al.*, 2006; Hartikainen, 2007; Hartikainen & Okamura, unpubl. data) may also be explained by cycling between covert and overt infections in field populations. The life cycle of freshwater bryozoans is likely to contribute to such cycling, since extensive clonal reproduction provides a continuous host resource for exploitation. Furthermore, parasite development appears to be condition-dependent, with overt infections developing particularly when hosts are undergoing enhanced growth as a result of higher temperatures or food levels in laboratory microcosms (Hartikainen, 2007; Tops *et al.*, 2009; Hartikainen & Okamura, submitted). The high field prevalences of overt infection in late spring and autumn are consistent with this scenario, since these high prevalences coincide with periods of enhanced bryozoan growth that is probably supported by spring and autumn peaks in primary production. Moreover, *T. bryosalmonae* exhibits flexibility in its response to host condition. Thus, overt infections have been observed continuously for months in favourable laboratory conditions but show high prevalences for only weeks in field populations (Tops *et al.*, 2009). This disparity in infection duration in laboratory and field situations and the year-round availability of fish hosts provides evidence against the alternative hypothesis that developmental timing is driven by opportunities to achieve horizontal transmission.

These general patterns of development suggest that *T. bryosalmonae* may be a prudent parasite, using the condition of bryozoan hosts to determine the development of different stages. Thus, overt infection proceeds when hosts are in good condition (exemplified by rapid growth) and can sustain the costs of sac proliferation. However, when host condition is poor, the parasite persists as single-cell stages that pose little cost. Such host-condition dependent cycling of developmental stages should promote infection of clonal hosts over long periods, particularly since winter persistence of colonies of the most common host, *F. sultana*, means that even individual colonies are potentially immortal. Field sampling indicates that *T. bryosalmonae* infections are characterised by persistence within bryozoan populations with infection observed routinely within some years and also across multiple years in bryozoan populations sampled variously in southern England, Germany, France and Switzerland (Tops, 2004; Tops *et al.*, 2006; Hartikainen, unpubl. data; Okamura, unpubl. data). Annual outbreaks of PKD in farmed fish also suggest a persistent infection source.

In some cases, however, the development of overt infection occurs when hosts are likely to be in poor condition. Thus, overt infections are detected in a small proportion of bryozoans experiencing suboptimal conditions in both field (Tops, 2004) and laboratory studies (Tops *et al.*, 2006; Hartikainen, 2007). Such infections are presumably responsible for infection of fish during winter periods (Gay, Okamura & de Kinkelin, 2001). These observations suggest that development of overt infection may occasionally be influenced by factors unrelated to host condition. Alternatively, *T. bryosalmonae* may respond to gradients in host condition in more subtle ways. For instance, it may effect escape from hosts in such poor condition that the host may die. In such circumstances, the parasite is likely to be highly virulent.

The possibility that the host controls parasite development by mounting an immune response to prevent overt infection is of course another explanation. However, this explanation would imply that suppression of overt infection is achieved by an immune response during unfavourable periods for the host. This seems unlikely since mounting an immune response is energetically costly and immunocompromised hosts are typically those experiencing stress (Moret & Schmid-Hempel, 2000).

The ability to follow the ongoing development of *T. bryosalmonae* in bryozoan hosts has enabled insights that are more difficult to obtain from fish hosts, since characterising development over time requires sacrifice of fish. There are also numerous confounding factors in the interpretation of parasite development that obscure the identification of potential parasite strategies for exploiting fish hosts. For instance, only some permissive hosts (brown and brook trout) have been identified (Morris & Adams, 2006b; Grabner & El-Matbouli, 2008), and most work has been conducted on infection in rainbow trout. In addition, focus has largely been on the development of parasite stages in the kidney interstitium because it is this

stage that induces the host inflammatory response. Also, for practical reasons, many studies have subjected fish to infection by exotic parasite strains that may provoke unusual reactions (e.g. rainbow trout exposed to infection in European waters). Furthermore, many studies are conducted at abnormally high temperatures with the aim of characterising clinical disease progression. Finally, little is known about the persistence of the spore-forming stages in kidney tubules in salmonids. In short, to characterise the normal behaviour of *T. bryosalmonae* in fish hosts, it is necessary as far as possible to consider development at temperatures to which the host and parasite are adapted, to focus on known permissive hosts (brown or brook trout), to study native host–parasite combinations and to undertake longitudinal studies to characterise the development and persistence of all parasite stages in fish over time.

Given the above caveats, what seems clear is a parasite strategy of exploiting fish hosts by extensive proliferation as extrasporogonic single-cell stages, initially in the blood and subsequently in the kidney. If the host survives infection of the kidney interstitium, then the evidence to hand suggests a strategy of persistence of spore-producing stages in kidney tubules after the disease-causing stages in the kidney interstitium have disappeared. We suggest that, under normal conditions, fish hosts generally survive infection of the kidney interstitium, since death prior to spore production in kidney tubules will make them dead-end hosts. The presence of spores in old fish and also during the winter suggests that spore production may persist for long periods, perhaps even over the lifetime of the fish host. We therefore propose a parasite strategy that involves bombardment of the fish kidney by replication of extrasporogonic stages some of which will escape the immune response to gain access to the kidney tubules where spore production may then proceed continuously at low levels over a prolonged period of time.

The scenario proposed earlier may be more complicated if there are undetected, facultative hosts in the life cycle of *T. bryosalmonae* or if immature extrasporogonic stages in dead fish can cause infection. So far there is no evidence for infection of hosts other than fish and bryozoans by *T. bryosalmonae*, despite extensive search for the causative agent of PKD prior to the discovery of bryozoan hosts. However, the possible infection of bryozoans by exposure to macerated infected kidney (Morris, Morris & Adams, 2002) suggests that pre-spore stages in fish tissues that escape scavengers might also be transmitted to bryozoans (but see Tops *et al*., 2004). If so, infection by *T. bryosalmonae* of a broad range of salmonid hosts (and pike; Morris *et al*., 2000a) that vary in their response to infection is of potential significance as a parasite strategy. It is clear that some species develop severe clinical disease, while others (e.g. brook trout) lack disease symptoms (Feist & Bucke, 1993). There also appears to be considerable variation in the numbers of spores produced in different salmonid hosts (Feist & Bucke, 1993; Feist & Longshaw, 2006), and it remains unclear whether spores develop in certain hosts (Morris & Adams, 2006b). *Tetracapsuloides bryosalmonae* may therefore exploit a broad range of fish hosts to gain multiple transmission routes via spores excreted in fish urine and via extrasporogonic stages in fish tissues. The functional consequences of infecting a range of both fish and bryozoan hosts are entirely unknown and require elucidation if we are to gain a comprehensive view of the strategies employed across the complex life cycle of *T. bryosalmonae*.

Despite the constraints that preclude such a comprehensive view, we believe there is sufficient evidence to propose how *T. bryosalmonae* is adapted to exploit bryozoan and fish hosts (Table 1). Thus, *T. bryosalmonae* infects a broad range of bryozoan and fish hosts and, in many, causes low virulence under normal conditions. Cycling between overt and covert infections maximises the exploitation of highly clonal bryozoans and can achieve unlimited persistence in potentially immortal clones – a possibility precluded in fish by their eventual death. The sporadic development of overt infections, modulated by host condition, will effect horizontal transmission and contribute to persistent infection in bryozoans, but whether spores are periodically produced in fish remains to be investigated. The clonal life cycle of bryozoans entails substantial opportunities for vertical transmission that is almost certainly absent in fish.

Table 1 also summarises the various dispersal opportunities afforded by bryozoan and fish hosts. This will be achieved within water bodies by fish movements, colony fragmentation and rafting and by short-lived (<24 h; de Kinkelin, Gay & Forman, 2002), non-buoyant spores. Dispersal between water bodies

Table 1 Summary of how *Tetracapsuloides bryosalmonae* exploits bryozoan and fish hosts under normal environmental conditions according to parasite traits

Parasite traits	Bryozoan hosts	Fish hosts
Host specificity	Broad: infection of many species	Broad: infection of multiple species
Virulence	Low under normal conditions Exacerbated by high temperatures	Low under normal conditions in hosts that support spore production, high in hosts that do not Exacerbated by high temperatures
Persistence	Theoretically unlimited: Infection of potentially immortal clonal genotypes over space and time via multiplication, vertical transmission, and developmental cycling	Limited: Lifetime of individual fish
Horizontal transmission	Via cyclical spore production dependent on host condition Possibly via single cells in covert infections	Via spore production (cyclicity and effects of host condition unknown) Possibly via extrasporogonic stages
Dispersal within water bodies	Drift of colony fragments Rafting of surfaces with attached colonies Probably via statoblasts Drift of spores released from bryozoans	Fish movements Drift of spores released from fish
Dispersal between water bodies	Probably via waterfowl-mediated transport of statoblasts Possibly by occasional flooding Possibly via man (e.g. introduction of macrophytes with attached bryozoans; statoblasts transported in ballast water or attached to waders, fishing gear, boats, etc.)	Possibly via anadramous fish movements Possibly via occasional flooding Fish culture and stocking

may occasionally be achieved by anadromous fish, but most between-site dispersal is likely to result from waterfowl-mediated transport of infected statoblasts. A variety of evidence provides support for waterfowl acting as vectors of dispersal, including studies of gene flow among bryozoan populations associated with waterfowl movements (Freeland, Noble & Okamura, 2000; Figuerola, Green & Michot, 2005) and the presence and viability of statoblasts in waterfowl guts and faeces (Charalambidou, Santamaría & Figuerola, 2003; Figuerola *et al.*, 2004; Mouranval *et al.*, 2007). As mentioned earlier, it remains to be demonstrated that statoblasts carry infections of *T. bryosalmonae*, but we predict this to be the case in view of the minimal effects of covert infection on bryozoan hosts and the equal propensity of covertly and uninfected colonies to produce statoblasts. Indeed it is difficult to reconcile the broad distribution of PKD across the northern hemisphere without invoking such dispersal, particularly since there is little genetic variation in ITS-1 sequences of *T. bryosalmonae* among many sites sampled across Europe (Henderson & Okamura, 2004). The presence of infected bryozoan populations in sites lacking salmonids is also best explained by such dispersal (Okamura *et al.*, 2001; Canning & Okamura, 2004); note that clonal reproduction would promote the indefinite persistence of infection in such sites until an opportunity for horizontal transmission arises either by fish introduction or dispersal to a new site. Flooding may also occasionally effect between-site dispersal, while fish stocking represents a potential human-mediated route of dispersal as discussed further in the next section.

Human-mediated movements of hosts and parasites

Humans have effected large movements of fish and probably of bryozoan hosts. Such movements may extend the geographical range of the disease and be associated with changing patterns of virulence because of novel combinations of fish, bryozoan and parasite genotypes.

Salmonids, and most particularly rainbow trout, have been introduced extensively around the world as a result of aquaculture and fisheries. Such introductions may transport disease via infected stock and may also promote increases in the incidence or severity of previously existing diseases (see Poulin *et al.*, this issue). Diseases may also be introduced through food used in aquaculture or gear associated with fishing. An example of the former is salmonid

whirling disease which, like PKD, is caused by a myxozoan and is likely to have been introduced to North America via frozen fish food (Hoffman, 1970). In addition, there is evidence for human-mediated transport of bryozoan statoblasts via shipping. Thus, Wood & Okamura (1999) found that *Asajirella gelatinosa*, a species native to eastern Asia, was present in a water body connected to the Panama Canal in 1992. More recently, statoblasts of exotic species have been sampled from ballast sediments of ships in the Great Lakes (Kipp *et al*., 2010). The highly resistant nature of statoblasts and their ability to undergo prolonged periods of dormancy should facilitate introduction to distant localities.

Perhaps surprisingly, so far there is little evidence that human-mediated host movements have effected introductions of *T. bryosalmonae*. Thus, although salmonids have been introduced to many countries in the southern hemisphere, as far as we are aware, PKD is restricted to the northern hemisphere. In addition, a phylogeographical study based on ITS-1 sequence analysis of *T. bryosalmonae* infections in a range of salmonid species (Henderson & Okamura, 2004) provided no evidence that recent human activities had introduced southern European strains to other European regions nor North American strains to Europe, although rainbow trout originated in North America. At the time, these results were interpreted as evidence that fish are accidental hosts. The subsequent demonstration that brown and brook trout are permissive hosts and release spores infective to bryozoans (Morris & Adams, 2006b; Grabner & El-Matbouli, 2008) somewhat challenges this interpretation, but the host status of other salmonids, and especially rainbow trout, requires demonstration. Note, however, that host status may be highly complicated and context-dependent. For instance, introduced fish might be inappropriate hosts for local parasite strains but hybridisation with local salmonids (e.g. Allendorf & Leary, 1988) may alter their suitability to local parasites. Further complications may be expected if fish become locally adapted following past introductions (e.g. Hendry *et al*., 2000; Miller, Close & Kapuscinski, 2004). In general, the issue of human-mediated transport of parasite strains merits further scrutiny with increased sampling over sites linked by the potential for frequent introductions and using genes that offer greater phylogeographical resolution than ITS-1.

Understanding and predicting disease dynamics

Based on our current understanding of disease dynamics, we predict that PKD outbreaks will increase in frequency and severity in more northerly regions. We also propose that PKD is and will continue to be an emerging disease because it is being manifested in new ways. Both the temporal and spatial patterns of disease outbreaks support this view. The existence of background, subclinical infections under normal environmental conditions across a broad geographical range would explain the outbreaks of PKD in Norway and Montana with increasing temperatures provoking the development of clinical PKD in both sites. This of course implies a broad distribution for infected bryozoan populations as indeed is indicated by the presence of *T. bryosalmonae* infections in brown trout over a wide range of elevations throughout Switzerland (Wahli *et al*., 2008). It may also be significant that *T. bryosalmonae* is characterised by broad host specificity and that freshwater bryozoans tolerate a wide range of environmental conditions, occurring in both lotic and lentic sites ranging from cool, clear, oligotrophic waters to warm, turbid, eutrophic environments (Okamura, pers. obs.; Wood, 1991; Hartikainen *et al*., 2009). This broad host specificity together with the presence of *T. bryosalmonae*-infected fish throughout Switzerland (Wahli *et al*., 2008) suggest that subclinical infections may be undetected across much of the geographical range of brown and brook trout if not of other salmonids. It is also possible that at range extremes infections in bryozoans normally remain covert as a result of low temperatures or suboptimal host condition. Such covert infections could represent an immediate source of future PKD outbreaks if the environment changes. The alternative explanation, that range expansion by *T. bryosalmonae* resulted in PKD outbreaks in Norway and Montana, is less plausible as this would entail: (i) establishment of infection in bryozoan populations within a short time, and; (ii) coincidental introduction just prior to temperature change in both sites.

The discovery of bryozoans as hosts of *T. bryosalmonae* has enabled recent and rapid progress in understanding the life cycle of *T. bryosalmonae* and the potential effects of environmental change. In particular, it can be predicted that extensive clonal reproduction in bryozoan hosts and low virulence of

infection will jointly play an especially important role in this complex system, contributing to indefinite persistence in bryozoan populations and to increases in prevalence and severity of disease in fish with environmental change, at least in the short term. In addition, the apparently widespread distribution of infected bryozoans, including at high elevations and latitudes, implies that environmental change could have devastating consequences for salmonid health. Furthermore, dispersal of bryozoans by waterfowl may promote rapid range shifts as birds respond to changing habitats. Evaluation of all evidence at hand regarding infections in both bryozoan and fish hosts indicates that increasing temperatures are likely to contribute to disease emergence. Eutrophication may have similar effects.

However, to better understand and predict current and future disease dynamics further insights into the ecology and life cycle of *T. bryosalmonae* are necessary. Indeed, the development of models to identify effective control policies for parasites with complex life cycles, such as *T. bryosalmonae*, is highly dependent on characterising the complex dynamics resulting from a multihost ecology (Lloyd-Smith *et al.*, 2009). Table 2 summarises a series of questions that represent gaps in our knowledge and whose answers are critical to enable more fully informed prediction and appropriate model development. Many of these questions have been alluded to in the foregoing discussion. However, an over-riding issue not previously developed relates to recognising that this system is already impacted by change. Freshwater habitats in general are widely accepted to represent the most degraded of ecosystems as a result of physical alteration, water abstraction, habitat loss, pollution, overexploitation, invasive species and high extinction rates (e.g. Ricciardi & Rasmussen, 1999; Revenga *et al.*, 2005). It is also clear that salmonid populations have undergone extensive declines and extinctions throughout their historical range (e.g. Lichatowich, 1999), and these continue in the present-day (Krkošek *et al.*, 2009). In many regions, such declines have been lost from memory, but archaeological, historical, fisheries and ecological records indicate dwindling salmonid stocks in inland lakes and rivers of Europe and England some 1000 years ago because of overfishing and habitat degradation (Barrett, Locker & Roberts, 2004; Lotze *et al.*, 2006). Yet, host–parasite interactions evolve as a consequence of coevolutionary dynamics in previous populations. Whether host–parasite interactions that maximise fitness through complex and interdependent outcomes in previous environments can be sustained in the highly non-equilibrium conditions of the present day remains a huge question.

Table 2 Critical questions that represent knowledge gaps and whose answers will enable future prediction of disease dynamics

What is the adaptive significance of broad host specificity?
Are some fish or bryozoans resistant to infection?
What is the geographical distribution of *Tetracapsuloides bryosalmonae* in bryozoan and fish hosts?
Are covert infections in bryozoan hosts suppressed at range extremes?
What are the methods of dispersal by *T. bryosalmonae* (and can it infect statoblasts)?
How persistent are infections in bryozoan populations?
Are spores produced throughout the life time of fish?
What are the consequences of non-equilibrium conditions for the sustainability of host–parasite interactions?

Rapid evolution in organisms with short life cycles (e.g. Lenski *et al.*, 1991; Yoshida *et al.*, 2003) and also in host–parasite systems involving simple parasite life cycles (e.g. Buckling & Rainey, 2002; Decaestecker *et al.*, 2007; Duffy *et al.*, 2009; Penczykowski *et al.*, this volume) suggests that some systems may be sustainable in the face of environmental change. Whether this will be the case for this highly aberrant, endoparasitic cnidarian with a complex life cycle remains very unclear in view of the combined effects of environmental degradation and declining populations of fish hosts in many regions. These considerations lead us to predict that at best this unique host–parasite system will be maintained over a reduced geographical range. This will arise because of disease emergence in fish populations that cannot sustain increases in prevalence and severity and that face other challenges related to environmental change. Indeed, there is every reason to suspect that extinctions and declines in salmonid populations have already resulted in range retractions of *T. bryosalmonae* unless persistence in clonal bryozoan populations or exploitation of diverse fish hosts has so far precluded this. The rich complexity of this host–parasite system may confer many routes for persistence, but a combination of man-made challenges, including overfishing and environmental change, poses novel challenges and increases the potential for synergistic effects of multiple

stressors (e.g. Coors & De Meester, 2008). Further research is required to characterise the complex, multihost ecology of *T. bryosalmonae* and to assess the interactions between this parasite and its hosts in our changing world.

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