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REVIEW

Disease ecology of wild fish: opportunities and challenges for linking infection metrics with behaviour, condition, and survival

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Abstract: Surmounting evidence supports that infectious agents play a critical role in shaping fish physiology, behaviour, and survival. The exclusion of disease-causing agents from fisheries research has resulted in major knowledge gaps that may limit the predictive capacity of ecological models. A major barrier in wild fisheries epidemiology is the logistical constraints associated with observing disease and obtaining samples from free-ranging fish, restricting the vast majority of research to laboratory studies or aquaculture facilities. For fisheries ecologists, including infectious agents can provide greater insight into observed phenomena, particularly with respect to fish physiology (e.g., metabolism), movement (e.g., migration rates), behaviour (e.g., habitat selection), personality (e.g., bold versus shy), and survival. Here we provide a brief introduction to the current understanding of disease ecology in wild fish and describe technological advances in both epidemiology and fisheries and aquatic sciences that can be used in tandem to create comprehensive studies of disease ecology in wild fishes. Combining nonlethal sampling and molecular genetic-based identification methods with field studies creates vast opportunities for innovative study designs that have the potential to address the true complexity of aquatic ecosystems.

Résumé : Des preuves de plus en plus nombreuses montrent que les agents infectieux jouent un rôle dans la modulation de la physiologie, du comportement et de la survie des poissons. L'exclusion des agents pathogènes de la recherche sur les pêches s'est traduite en d'importantes lacunes sur le plan des connaissances qui pourraient limiter la capacité prédictive des modèles écologiques. Les contraintes logistiques associées à l'observation de maladies et à l'obtention d'échantillons de poissons en liberté constituent un important obstacle en épidémiologie des ressources halieutiques sauvages, qui limite la vaste majorité des travaux de recherche à des études en laboratoire ou en pisciculture. Pour les écologistes des pêches, l'inclusion d'agents infectieux peut faciliter grandement la compréhension de phénomènes observés, particulièrement en ce qui concerne la physiologie (p. ex. métabolisme), les déplacements (p. ex. vitesses de migration), le comportement (p. ex. sélection de l'habitat), la personnalité (p.ex. téméraire ou timide) et la survie des poissons. Nous présentons une brève introduction à la compréhension actuelle de l'écologie des maladies chez les poissons sauvages et décrivons des avancées technologiques dans les domaines de l'épidémiologie et des sciences halieutiques et aquatiques qui peuvent être combinées pour concevoir des études intégrées de l'écologie des maladies chez les poissons sauvages. La combinaison de méthodes d'échantillonnage non létal et d'identification basée sur la génétique moléculaire à des études de terrain offre la possibilité de concevoir des schémas d'étude novateurs pouvant permettre d'aborder efficacement la complexité réelle des écosystèmes aquatiques. [Traduit par la Rédaction]

Introduction

Ectotherms such as fishes are vulnerable to infectious agents; environmental factors such as suboptimal temperatures can reduce host resistance (Jeffries et al. 2012) and increase infectious agent abundance (Paull and Johnson 2014) or virulence (Thomas and Blanford 2003; Teffer and Miller 2019), enhancing the likelihood of disease development (Snieszko 1974). Infectious diseases have already been implicated in the decline of some wild fish populations (Steinbach Elwell et al. 2009; Gibson-Reinemer et al. 2017), but the mechanisms and scales by which they reduce these populations remain dubious (Hellard et al. 2015). Compared with terrestrial disease ecology, where concepts rooted in evolutionary ecology often form the theoretical foundation of research, studies in aquatic disease ecology have somewhat lagged behind (see McCallum et al. 2004 for detailed discussion on the topic). This is perhaps because a great deal of the current understanding is garnered from experimental studies (Hellard et al. 2015) or observations of disease from aquaculture, particularly for microparasite species (Austin and Austin 2016). Fisheries epidemiology is unlike that of terrestrial systems due to its greater host species diversity, spatial and hydrological complexity of the aquatic environment, highly variable pathways of transmission, and the lack of observational data (McCallum et al. 2004; Miller et al. 2014). It is extremely challenging to collect individuals that are experiencing disease in aquatic ecosystems; moribund fish are likely vulnerable to predation, while mortalities are scavenged, sink to depths challenging to sample, or decompose quickly and thus of little use for examination (Herman 1990). These challenges have led fish disease ecologists to rely heavily on knowledge of infectious agents and host response garnered from cultured fish, where mortality, morbidity, and disease states are more easily observable, isolated, and examined. Application of these findings to wild populations, however, is precarious given that pathways

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of transmission do not reflect the natural environment and human intervention has caused substantial geno- and phenotypic divergence of cultured fish from their wild ancestors. Indeed many artificial breeding programs have created phenotypes that are completely absent from wild populations (Blanchet et al. 2008; Christie et al. 2012), which greatly reduces the relevance of these data in wild contexts.

One of the major knowledge gaps present in research on disease in wild fish is the incidence of co-infection (Sofonea et al. 2015; Kotob et al. 2017). In the wild, fish are chronically exposed to a heterogenous composition of infectious agents and are rarely burdened with a single pathogen at a given time, which can directly mediate host–pathogen dynamics (Hellard et al. 2015). In experimental work with wild fishes, presence of infectious agents that are not the focus of study may be viewed as inconsequential and thus not incorporated as factors to consider (Kotob et al. 2017). However, co-infection is a natural characteristic of wild animals, and incidence of co-infection has been identified in virtually all host taxa comprehensively examined (e.g., Pacific salmon (*Oncorhynchus* spp.; Miller et al. 2014; Bass et al. 2017; Thakur et al. 2018), Atlantic salmon (*Salmo salar*; Laurin et al. 2019), European grayling (*Thymallus thymallus*; Pylkkö et al. 2006), and rainbow trout (*Oncorhynchus mykiss*; Bandilla et al. 2005)). Bacterial species have been shown to opportunistically infect fish after the epithelial layer is compromised by the entry of other pathogens (Kanno et al. 1990), often termed "secondary infection" (Pylkkö et al. 2006). Theoretical modelling suggests within-host pathogen interactions determine pathogenicity, recovery, and transmission rates for individual agents (Sofonea et al. 2015), yet few empirical studies have demonstrated such phenomena (reviewed in Kotob et al. 2017).

The aforementioned constraints have contributed to major gaps in the research body addressing disease ecology in wild fish. However, advances in research technologies in both fisheries science and wildlife epidemiology have opened the door for comprehensive studies to investigate multiple scales of fisheries epidemiology in the wild. By strategically combining research methods and fostering collaboration among disciplines, we argue that infectious agent dynamics should be incorporated into investigations of fish ecology and behaviour. In the present article, we briefly outline the current application of several novel research methods in fish epidemiology and behavioural ecology, present areas of uncertainty and knowledge gaps that may be addressed through interdisciplinary collaboration, and highlight research opportunities in the context of free-ranging wild fishes. Parasites are defined here as species that have the capacity to negatively impact host fitness. The terms parasites and pathogens are used interchangeably throughout to refer to any disease-causing infectious agent (though some parasites may be commensal, leading to discussions on the merit of the term pathogen; see Méthot and Alizon 2014). The focus of this review is endoparasitic infections by both micro- and macroparasites; however, concepts within can be applied to an array of species in both aquatic and terrestrial environments.

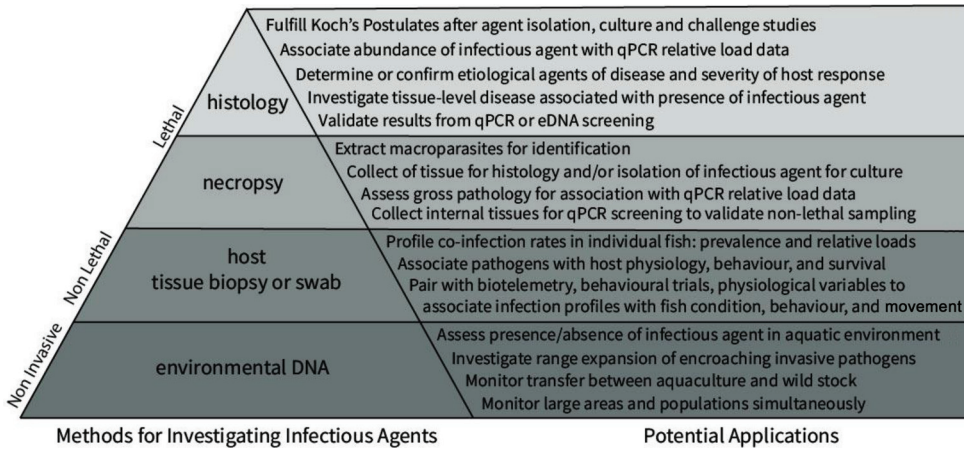
Context of wild fish epidemiology

Wildlife disease research is often focused on pathogens that hold high risk factors for human infection or negatively impact species important for aquaculture, rather than being focused on ecological phenomena. Helminth parasites — trematodes, cestodes, and nematodes — are the most common etiological agents of human infection from fish-borne pathogens (Sangaran and Sundar 2016). Humans act as either the definitive host, where sexual reproduction of the parasite occurs, or more often as incidental hosts that are not typical for the parasite's life cycle. Tapeworms of the genus *Diphyllobothrium* are transferred to their definitive host, including humans, through consumption of larval stages found in numerous species of freshwater fish (Kuchta et al. 2013). The nematode *Anisakis simplex*, found in marine and diadromous species, including salmonids, infect humans as an incidental host, causing gastrointestinal disturbance through questing behaviour in the gastrointestinal lamina (Audicana and Kennedy 2008). Both *Diphyllobothrium* spp. and *A. simplex* cause substantial pathology in humans and continue to be studied extensively. Comparatively, microbial agents (viruses, bacteria, fungi, and protozoans) of fishes are generally less likely to spill over to human populations. Low contact rates between humans and fish reduce exposure, and host specificity prevents transmission from fish to human. While 75% of emerging human disease are zoonotic in origin (Slingenbergh et al. 2004), the vast majority of such transfers are from terrestrial species. Most human microbial infection traced back to aquatic organisms are associated with harvest from water bodies contaminated with human sewage (Iwamoto et al. 2010). In such cases, infectious agents are those for which humans are the main reservoir (e.g., *Salmonella* and *Shigella* spp.), and aquatic organisms are involved as incidental vectors, typically filter feeders that are not negatively impacted by pathogen presence (Iwamoto et al. 2010).

Parasitic helminths rarely cause mortality in their hosts and are much less likely to result in population-scale mortality in the wild, so while they may be of interest from a human health perspective, they are typically not of great concern for wildlife conservation (Lafferty and Gerber 2002). From a conservation perspective, microbial pathogens (viruses, bacteria, protozoans, and fungi) are far more likely to negatively impact fish populations at a large scale (Bakke and Harris 1998). Yet because humans are generally not directly impacted by aquatic microbial pathogens that commonly infect fish, the limited knowledge of microbial pathogens in wild fish (specifically excluding aquaculture, where research funding is more available and industry-driven) is relatively constrained to research on acute agents that cause large-scale die-offs of cultured fish or those that may transfer to wild fish from aquaculture (Lafferty and Hofmann 2016). Given the cryptic nature of mortalities in aquatic environments, fisheries scientists may become aware of ecologically important infectious agents in the wild only after observable "fish kills", when mortality rates exceed consumption by predators and scavengers, sometimes resulting in high numbers of observed carcasses in waterways and on shorelines. Such events tend to be easily identifiable, can be catastrophic for isolated or small populations, and are of great public concern due to their shocking appearance. Unlike studying wild fish when pathogens are more typically distributed within the population (i.e., a negative binomial distribution), fish kills allow researchers to collect large numbers of mortalities to identify the causative agent (e.g., epizootic haematopoietic necrosis virus in Australian redfin perch (*Perca fluviatilis*); Langdon and Humphrey 1987; *Ichthyophthirius multifiliis* in freshwater species in the southern US; Allison and Kelly 1963; infectious hematopoietic necrosis virus in sockeye salmon (*Oncorhynchus nerka*) in western Canada; Williams and Amend 1976; *Ichthyophonus hoferi* in herring (*Clupea harengus*); Mellergaard and Spanggaard 1997; herpes virus in Australian pilchard (*Sardinops sagax*); Murray et al. 2003). Factors that cause large-scale outbreaks (e.g., thermal stress, introduced pathogens), which push disease rates over a threshold of visibility, are complex and difficult to identify or predict in many cases (Langdon and Humphrey 1987; Herman 1990; Adlard et al. 2015). This is particularly true when previous abundance and prevalence rates are not known for the affected population.

Viral haemorrhagic septicaemia virus (VHSV), a pathogen that is responsible for large-scale die-offs in over 30 species of fish from both freshwater and marine ecosystems globally (Escobar et al. 2017), exemplifies the complexities surrounding aquatic animal epidemiology. VHSV is arguably one of the best-studied wild infectious agents and may be present in as many as 140 species based on ecological niche modeling (Escobar et al. 2018). Research has identified susceptible species (Gadd et al. 2011; Moreno et al. 2014; Escobar et al. 2018), diversity in host specificity from VHSV

Fig. 1. Methods and potential applications for studies investigating wild fish pathogens. Nonlethal methods are genomics-based techniques that can be paired with other methods in fisheries science such as biotelemetry to study the consequences of infection in wild fish.



genotypes (Ogut and Altuntas 2014), and risk areas based on geographical characteristics (Escobar et al. 2018). However, despite such an extensive body of knowledge, little is known about VHSV in the context of wild fish ecology, including consequences on behavior, phenology, and fitness. Furthermore, co-infection with other pathogens may modulate host susceptibility to and transmission of VSHV; co-infection was therefore recently highlighted as another major knowledge gap in the understanding of the role VHSV plays in aquatic ecology (Escobar et al. 2018).

Methods for identifying pathogens

Prior to molecular genetic-based techniques, all screening for infectious agents in fish was conducted using lethal sampling to assess clinical signs of disease, conduct histopathological assessment of diseased tissues, and identify macroparasites and microbial agents using microscopy and (or) culture (Austin and Austin 2016). Histopathology — the study of visual abnormalities in cellular processes in tissues — remains the gold standard of diagnostics and remains necessary to characterize host response to pathogen presence (e.g., severity of disease). However, properly conducted histopathological analysis is labour-intensive, lacks sensitivity during early infection stages, and requires extensive specialized expertise that is typically reserved for specialized veterinary experts (Fig. 1). To ensure high-quality, accurate histopathology for disease research, fisheries researchers must therefore seek out such advanced training, engage in collaborations, or pay for diagnostic services. This is may be extremely prohibitive for many fisheries scientists based on availability of training, access to willing collaborators, and budget constraints.

Though not a replacement for histological examinations, molecular-based examination of tissues has vastly increased the speed and specificity of infectious agent detection in wildlife (Mendonca and Arkush 2004; Miller et al. 2016; Kralik and Ricchi 2017; Sana et al. 2018) and can be employed once aetiological agents of disease have been determined using traditional methods. Since the introduction of polymerase chain reaction (PCR) in 1985 (Saiki et al. 1989), DNA amplification and analysis has revolutionized methods in microbiology and is the foundation of a myriad of rapidly developing genomic technologies. To apply genomic techniques to research on infectious agents, one must reduce host tissue samples to only the genetic material present (i.e., RNA or DNA). The result is a mix of host and pathogen DNA and RNA that is used in analysis. From here, several different molecular techniques can be applied independently or complementarily, depending on the research question.

Metagenomic sequencing

Rapid advances in gene sequencing technology facilitated a cascade of molecular techniques and platforms that have greatly reduced time and cost of data acquisition. Next-generation sequencing (NGS) is now the most common high-throughput sequencing method that sequences many samples independently and simultaneously (Mardis 2008). It can be applied to any organism containing genetic material and is therefore a useful investigatory tool when investigating microbial pathogens (e.g., RNA viruses; Benton et al. 2015). Traditional NGS platforms produce millions of short sequences with read lengths between 50 and 300 base pairs (bp) (Mardis 2008); however, NGS technologies producing long reads (10 to >100 kb) such as MinION and PacBio have more recently been developed. There are many commercial NGS platforms available (e.g., Illumina, Life Technologies/Ion Torrent, PacBio, etc.); however, for simplicity, the Illumina Whole Genome Shotgun sequencing (WGS) and 16 S ribosomal RNA (rRNA) gene sequencing workflows will be referenced here. Both methods are useful for the identification and characterization of pathogenic agents and can be used independently or in tandem. The WGS approach typically works by sequencing sample isolate (genetic material), and sequencing libraries are constructed from sheared DNA that is bound to sequencing adapters that interact with complementary regions on the surface of the Illumina flow cell. Illumina uses cluster generation to amplify the library, which is then sequenced in both directions (termed paired-end sequencing; Head et al. 2014). 16S rRNA gene sequencing differs in that sequencing libraries are not constructed from sheared DNA; rather, they use a targeted approach using primers specific to the 16S rRNA gene that is then amplified within a sample to create the sequencing library and sequenced as described for WGS. The 16S rRNA gene is used here as a genetic marker, as it is a highly conserved hypervariable region within the bacterial ribosome, and so present in all bacterial species, and can therefore be used for bacterial taxonomy-related studies (Woese 1987). Following sequencing and quality filtering, sequence data are then aligned to a reference genome, if available, or assembled de novo using contig construction for WGS sequencing (Mardis 2008). Contigs can then be searched through public genetic sequence databases, such as GenBank, using the Blast Local Alignment Search Tool (BLAST; McGinnis and Madden 2004). 16S rRNA sequence data are typically assigned to an operational taxonomic unit based off a 97%–99% similarity of sequences and then assigned taxonomy using reference databases (e.g., SILVA). Consequently, 16S rRNA may have a more difficult time differentiating closely related bacterial species (Fox et al. 1992; Acinas et al. 2004).

The molecular resolution of 16 S rRNA sequencing is lower than that for WGS; therefore, it is not always useful in detecting pathogenic species or strains that have little differentiation (Grützke et al. 2019). It is, however, a useful tool for characterizing complex microbial communities and can offer insights into how communities shift in response to disease expression in an organism (Bartram et al. 2011; Li et al. 2016) or for determining phylogenetic relationships between bacteria models (Joung and Côté 2002). WGS is a useful investigatory tool in pathogen outbreaks, as the whole genome of the microbial pathogen is characterized and therefore can provide in-depth information on virulence factors, resistance genes, strain characterization, and other features related to pathogenesis (Sridhar et al. 2012; Tyagi et al. 2019). Duchaud et al. (2007) published the first complete genome of a fish pathogen, *Flavobacterium psychrophilum*, providing a functional analysis of virulence mechanisms employed by the pathogen. A caveat to this method is the greater difficulty in assembly of large genomes; hence, most studies employing this method work on small genomes such as bacteria and viruses, though nematode genomes have also been sequenced (Ghedin et al. 2004).

RNA sequencing is another application of NGS technologies and is a useful molecular tool for viral discovery (e.g., Mordecai et al. 2019, 2021) and the study of pathogens and host–pathogen interactions focused on transcriptome profiling (Ozsolak and Milos 2011). Here, libraries are constructed from complementary DNA (cDNA) before sequencing (Wang et al. 2009). Following sequencing, transcripts are assembled either using reference transcript annotations or de novo assembly and then gene expression can be estimated using qPCR, and downstream applications such as differential gene expression can be compared (Kukurba and Montgomery 2015).

Genetic-based molecular methods have evolved rapidly and provide a practical way for researchers to investigate pathogens on a larger scale than previously possible. Researchers can design qPCR assays for virtually any pathogen for which sequence data are available, including microorganisms (Kralik and Ricchi 2017), or use the BLAST tool to match sequences extracted from samples with a database of genetic material (e.g., GenBank; McGinnis and Madden 2004; Soldánová et al. 2017). With today's speed and decreasing cost of gene sequencing and the development of sequence repositories such as GenBank, it is now possible to design studies that use comprehensive molecular techniques to identify suites of pathogens simultaneously.

Quantitative PCR (qPCR)

Quantitative polymerase chain reaction (qPCR) is a commonly used tool in diagnostic laboratories and has arguably become the "new norm" in diagnostics (Austin and Austin 2016; Kralik and Ricchi 2017). An excellent guide to the design, optimization, and validation of qPCR for diagnostics is provided in detail by Kralik and Ricchi (2017). In brief, primers are designed to match genetic sequences of target organism genes (in this case, infectious agents), and various qPCR methods can quantify the amount of target genetic material within each sample (e.g., probe fluorescence denoting amplification of the target sequence during thermocycling). While not directly comparable to pathogen abundance (except in the case of single copy genes assessed in a bacterium or virus), relative abundance of genetic material can be linked with pathogenicity through in situ hybridization and histopathological validation (True et al. 2009; Di Cicco et al. 2018). While histopathology remains critical to validate disease state, depending on the methodology and equipment employed, qPCR can detect the presence of infectious agents at extremely low infection levels, potentially prior to observable clinical disease (Miller et al. 2017). Such sensitivity also allows for the detection of known causative agents of disease soon after transmission events, in low abundance carrier states, or that are heterogeneously distributed within the host and thus carry a low probability of being detected by microscopy or histological examination (Cavender et al. 2004).

To determine co-infection rates, one must screen individuals for the presence of multiple infectious agents, a previously time-consuming and expensive task. This can now be achieved quickly and cost-effectively using high-throughput quantitative PCR (ht-qPCR), which runs dozens of assays against dozens of samples simultaneously. For example, assay panels for dozens of microbial pathogens (viruses, bacteria, protozoans, myxozoans, and fungi) have been created to monitor infection in salmon populations off the coast of British Columbia, Canada (Miller et al. 2016). Tissue samples from multiple organs are collected from fish sacrificed as part of the monitoring program, and sample RNA and DNA are extracted for ht-qPCR screening. As many as 80 individual samples can be screened simultaneously for the presence and relative load of 47 infectious agents (Miller et al. 2016). Based on normal working hours, ~27 000 PCR reactions can be run in 2 days when starting from isolated nucleic acids or about 360 samples screened against 47 duplicate assays (Miller et al. 2016). For a full list of strengths and weaknesses of using a platform such as BioMark, see section 7.9 in Miller et al. (2016).

Several studies have successfully applied ht-qPCR to study disease ecology in wild salmon. A survey of 45 infectious agents from adult Chinook salmon (*Oncorhynchus tshawytscha*) captured during spawning migrations from five stocks detected 20 microparasite species, several of which were new records for the species and (or) region, high rates of co-infection, and stock-specific pathogen communities (Bass et al. 2017). Comparison among hatchery and wild stocks screened for 36 infectious agents from juvenile Chinook salmon found lower pathogen diversity in hatchery-reared fish during freshwater phase; however, diversity converged with time spent in the marine environment (Thakur et al. 2018). A 10-year study of coho salmon *Oncorhynchus kisutch* of hatchery and wild origin identified 31 microparasites present of the 36 species screened; however, there were no consistent differences between hatchery and wild stocks in the marine environment (Nekouei et al. 2019). These studies together indicate that hatchery fish are not a major source of pathogens for all wild stocks and that transmission dynamics are context-specific. Perhaps more importantly, each study also provides comprehensive baseline data for infectious agent prevalence in the region, which allows monitoring for novel agents as the risk of potential spread increases with intensified aquaculture and climate change.

The high specificity and sensitivity of qPCR is both a strength and a potential limitation depending on the context within which it is applied. The often heterogeneous distribution of infectious agents among host tissues creates inherent variation in the amount of genetic material present within a given biopsy (Teffer and Miller 2019). While qPCR is superior for detecting the presence of infectious agents, histopathology remains a more reliable way to quantify infection severity and tissue damage (Cavender et al. 2004; Miller et al. 2016) and is useful for ascribing aetiology (e.g., piscine orthoreovirus and heart and skeletal inflammation; Di Cicco et al. 2018). High specificity also means it is necessary to understand what infectious agents are likely to be present and have access to gene sequence information to design effective primers to identify the full range of genetic variants. Consequently, pathogens may be missed if they are not included in the screening panel.

Molecular techniques are also being used on host tissue to determine the physiological response of fish to infectious agents. DNA microarray analysis and RNA sequencing of viral disease-infected individuals has revealed numerous genes induced during infection (Jørgensen et al. 2008; Workenhe et al. 2009; Krasnov et al. 2011). Similarly, qPCR was used to associate gene expression profiles with out-migration survival in sockeye salmon smolts, revealing infection with infectious haematopoietic necrosis virus and antiviral response were most predictive of survival (Jeffries

et al. 2014). More recently, transcriptome data collected from multiple laboratory challenge studies were used to identify a consistent transcriptional signature activated during viral disease development (Miller et al. 2017). In this study, and a similar study on human respiratory virus disease development by Andres-Terre et al. 2015, a panel of fewer than a dozen genes was capable of predicting a viral disease state and distinguish between bacterial and viral diseases. Moreover, in both studies, the tool was effective on minimally invasive tissues, like fish gill or human saliva. In salmon, this panel was then used to investigate incidences where wild fish demonstrated viral patterns in gene activation where no known virus was detected via targeted ht-qPCR; applying NGS on these samples has led to the discovery of several novel viral species in Pacific and Atlantic salmon (Mordecai et al. 2019, 2021).

While extremely powerful, there are substantial up-front costs associated with developing broad-scale qPCR screening programs. Knowledge and skill in molecular techniques is a necessity, and operational costs can be considerable depending on the number of samples being run. Moreover, samples must be collected as aseptically as possible, a feat that can be extremely challenging in field settings. Importantly, it is crucial to recognize that the relative load of infectious agent genetic material present in a tissue sample does not necessarily indicate the level of tissue damage or disease present. While further molecular advances hold promise for applications in diagnostics, at the present time necropsy and histopathology are thus still required to verify disease state.

Nonlethal sampling for infectious agent screening

Broad-scale screening of the environment: environmental DNA (eDNA)

Technological advances in gene sequencing and detection now allow researchers to screen for infectious agents noninvasively. For broad-scale detection, environmental DNA, or eDNA, is a molecular approach that has recently been developed and increasingly implemented to identify species presence from abiotic samples (i.e., sloughed genetic material in water, soil; Bohmann et al. 2014). In aquatic environments, sampling regimes are so straightforward that eDNA has been touted as "biodiversity for the lazy" (Hoffmann et al. 2016), and in some cases samples taken by trained citizen scientists are as high quality as those taken by highly experienced genetic researchers (Julian et al. 2019). For biodiversity assessment of free-living vertebrates (amphibians and fish), well-designed eDNA study protocol were more accurate for detecting aquatic species than traditional survey-based methods (Valentini et al. 2016). When surveying for pathogen distributions, extracting genetic material from water samples means that there is no need to capture and necropsy target host species and isolate diseased tissues, greatly reducing what can be intensive sampling and permitting regiments. This is particularly important when investigating rare species, either host or pathogen, that require the examination of >30 individual hosts (Julian et al. 2019). Using DNA for identification can also be highly species-specific depending on assay design, reducing error or uncertainty for species that are extremely difficult to identify based on morphology. Typically, eDNA is most often used to estimate the presence or abundance of rare or invasive species or assess overall diversity of a given habitat (Bohmann et al. 2014). One caveat, however, is that qPCR will not differentiate whether a pathogen is viable. Given that accurate and timely detection of highly pathogenic infectious agents is critical for initiating any mitigation measures, eDNA provides an extremely powerful tool for aquatic wildlife conservation in epizooic outbreaks or novel pathogen invasion.

eDNA has been successfully used to detect pathogenic nematodes (*Ribeiroia ondatrae*; Huver et al. 2015), *Ranavirus* (Miaud et al. 2019), and pathogenic bacteria (*Batrachochytrium dendrobatidis*, the causative agent of chytridiomycosis; Kamoroff and Goldberg 2017) that infect freshwater amphibians. In fish, eDNA has been successfully used to screen for the spread of the mesomycetozoean *Sphaerothecum destruens* in Europe as it is carried to new habitats by an invasive fish (Sana et al. 2018). Screening for *Gyrodactylus salaris*, a monogenean highly pathogenic in Atlantic salmon, from water samples in Norway was also successful for detecting pathogen presence (Rusch et al. 2018). In wild salmonids, eDNA sampling methods were recently validated for detecting *Ceratonova shasta*, with results supporting the method as an excellent noninvasive and sensitive way to monitor infection risk (Richey et al. 2020). For applications in aquaculture, eDNA screening for pathogens from water samples has undergone preliminary testing in both freshwater (Bastos Gomes et al. 2017) and marine (Peters et al. 2018) environments with encouraging results. A recent eDNA study using the ht-qPCR platform developed in British Columbia showed that active aquaculture farms were associated with higher levels and diversity of agents than sites that were fallowed or where farming activities were discontinued (Shea et al. 2020).

In most cases, authors suggest that further development and refinement of eDNA tools is required before eDNA alone can replace current detection methods (e.g., pathogen abundance; Rusch et al. 2018), but believe it will reduce cost, enhance broad-scale monitoring, and expedite risk-reducing measures in the event of positive detections. This is demonstrated by the screening for *B. dendrobatidis* in lakes in Sequoia King National Park in California, USA. The first detection of the agent was 4 weeks prior to large-scale die-offs of amphibians from chytridiomycosis in all systems it was detected in (Kamoroff and Goldberg 2017). This buffer between detection and observed mortality may allow for mitigative measures to be launched, such as limiting access to the infected body of water to prevent spread of the pathogen or applying treatment before transmission and (or) disease associated mortality reaches critical thresholds. eDNA may also be applied to pathogen screening in aquaculture or host species repatriation efforts by testing for the presence of infectious agents prior to transfer to open-net pens or release (Sana et al. 2018). Such efforts may restrict the transfer of infectious agents from cultured to wild fish, a factor that is currently a major conservation concern for global fisheries (Lafferty and Hofmann 2016). The use of "sentinel fish" (i.e., domesticated fish known to be pathogen-free held within sampling systems) may be used in combination with water and (or) substrate samples to confirm infection and thus ecological consequences of pathogens detected within abiotic samples (Richey et al. 2020).

There remain limitations and considerations for the use of eDNA in infectious agent monitoring. eDNA most reliably confirms only the presence or absence of target species in the environment (Hoffmann et al. 2016). However, DNA persists in the environment for as little as 48 h (Collins et al. 2018) to much as 24 days (Goldberg et al. 2018) depending on cellular material and conditions (e.g., UV exposure, temperature, and (or) water pH), limiting fine-scale spatiotemporal interpretation of species' presence. DNA can also be transported to the area naturally (e.g., predator feces, hydrological phenomena) or artificially by watercraft, waders, etc. (Goldberg et al. 2018). Many infectious agents do not have high host specificity but do demonstrate species-specific pathogenicity (e.g., *G. salaris*, Rusch et al. 2018; *S. destruens*, Sana et al. 2018). In the case where a single host species is the focus of the work, it may then be necessary to also screen for potential reservoir hosts known or suspected to be present in the system (Rusch et al. 2018). In addition, many pathogens have complex life cycles that include multiple hosts and free-living or dormant stages (Marcogliese 2004). Positive detection based on eDNA sample does not provide detail with respect to what species are infected, if infection rates are causing meaningful disease in host species, or even if suitable host species are present in the environment at all (Huver et al. 2015). This also includes trophically transmitted pathogens that may be deposited in

Fig. 2. Investigations of fish pathogens can be conducted in a variety of contexts. (A) Necropsy of mortalities collected during a large-scale "fish kill" can provide insight for zoonotic outbreaks, but difficulty preserving host tissue integrity can make identification or quantification of pathogens challenging. (B) Experimental studies remove fish from the wild and expose them to experimental conditions in captivity; however, stress associated with holding may obscure natural recovery or pathogen transmission. (C and D) Nonlethal sampling methods include small gill biopsy or sterile epithelial swab, both of which can be used for molecular genetic-based identification techniques. Photo credits: (A) Adrian Jones (2003), (B) Amy Teffer, (C) Jacqueline Chapman, (D) Lisa Kelly. [Colour online.]

the environment (e.g., *Diplostomum* spp. in bird feces; Marcogliese 2004) but unable to establish because of a missing host required for the complete life cycle. Consequently, there are limits to the conclusions that can be made based on the presence of the pathogen from water or substrate samples alone, especially in the absence of long-term sampling. Refined methodology may improve the correlation between eDNA copy number and pathogen abundance for implementation in monitoring (Huver et al. 2015; Rusch et al. 2018). Until then, the use of eDNA best complements more traditional methods (Julian et al. 2019) for quantifying species prevalence and abundance, for example by streamlining focus for biological sampling efforts.

Nonlethal biopsies of individual hosts using rt-qPCR

The incorporation of nanotechnologies such as microfluidics in rt-qPCR platforms has greatly reduced the amount of tissue that is required for DNA–RNA extraction (e.g., Fluidigm Biomark; Miller et al. 2016; Teffer and Miller 2019). For the BioMark rt-qPCR platform, tissue samples can be as small as 2 mm^3, an amount that can be taken without causing serious harm to the fish (Tavares et al. 2016; Fig. 2C). Swabs of the extracellular mucosa can also be taken from external tissues or inserted into the buccal cavity or vent to investigate the presence of pathogens in the alimentary tract (Fig. 2D). Indeed many methods currently used to collect and screen tissue lethally could be refined and transitioned to nonlethal methods (e.g., swabs; Aamelfot et al. 2015), and techniques employed to investigate fish microbiomes are directly transferrable to infectious agent screening (e.g., Hamilton et al. 2019).

While there are considerations associated with using a small tissue sample to represent the pathogen community within the entire host, recent research found a single gill biopsy contains transcripts shed by pathogens infecting internal organs (Teffer and Miller 2019). Gill swabs, used in conjunction with rt-qPCR assays, have been used for early detection of the amoeba (*Paramoeba perurans*) responsible for amoebic gill disease in aquaculture settings (Downes et al. 2017). Swabs may also be used to collect mucus from the surface of fish to identify fish pathogens. For example, epithelial mucus swabs to detect largemouth bass virus using both conventional and quantitative PCR has been successfully validated (Leis et al. 2018). Consequently, a single sample can provide a snapshot of highly detailed information regarding the infectious agent community on or within the host. This is a major advance for fisheries research by providing the first opportunity for nonlethal screening of individuals for multiple infectious agents. As a result, a myriad of research questions to better investigate broad- and fine-scale infection dynamics, host responses, and the mechanisms associated with disease outcomes can be addressed for the first time by integrating rt-qPCR screening or NGS in experimental designs and monitoring programs.

Incorporating pathogen screening in wild fish research

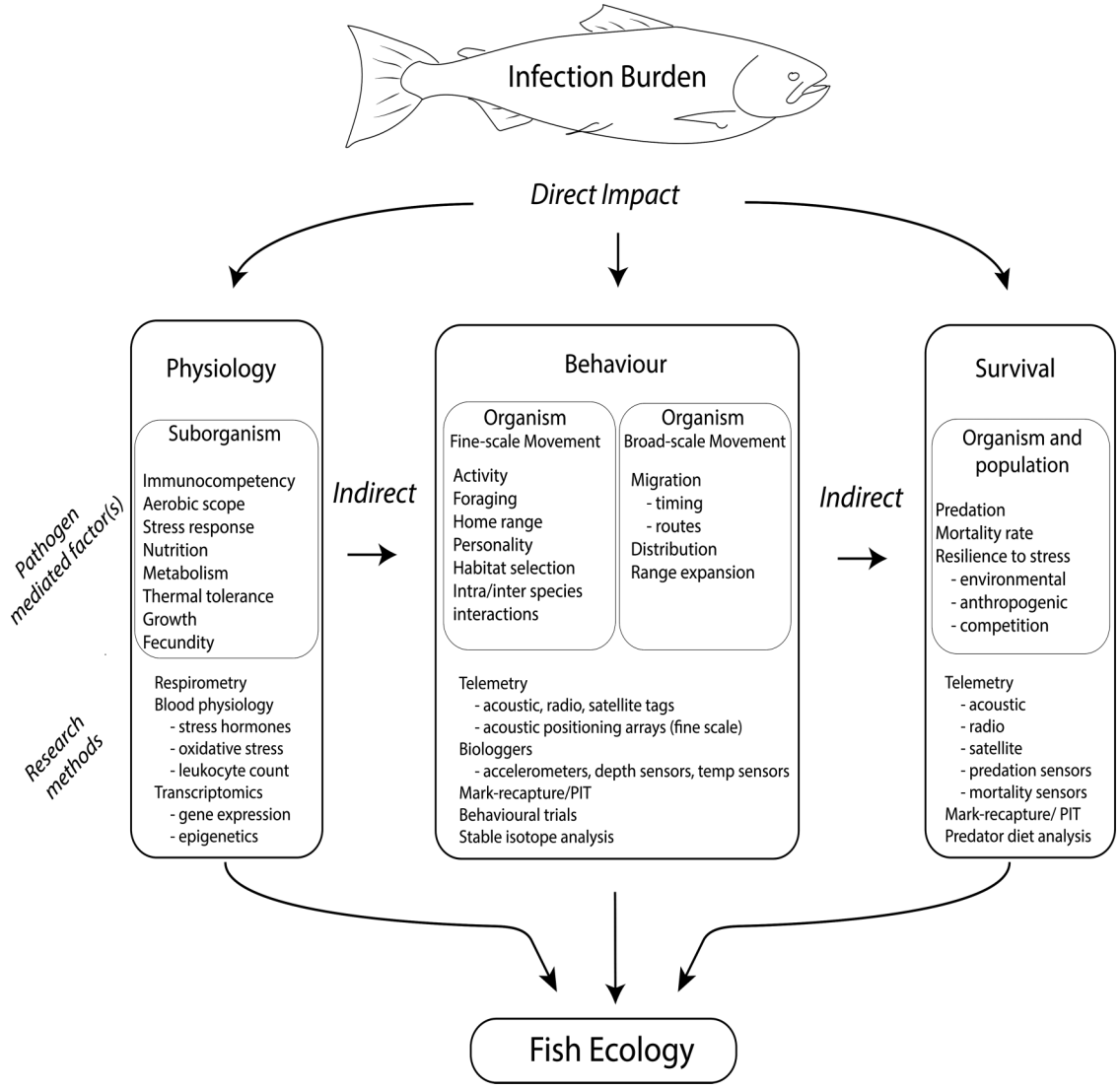
Research methods

Conventional research methods for investigating the behaviour and ecology of fish in the wild are extremely variable and context-specific. Here we provide a general summary of common research methods that may be useful for investigating infectious agents, and where screening for infectious agents may be informative and easily incorporated into current research programs. Pathogens and subsequent disease can influence host physiology, behaviour, and survival, which can be investigated either independently or in combination (Fig. 3).

In situ confinement

In situ confinement or isolation is a cost-effective way to investigate shifts in pathogens without the need to transport wild fish to artificial holding tanks. The ideal scenario for such research is

Fig. 3. Fisheries research areas and factors where individual variation may be associated with pathogen dynamics. Proposed methods can be combined with nonlethal sampling of tissues (e.g., gill biopsy or mucous swab) that is screened using molecular genetic techniques to identify the presence and relative load of pathogens. Comprehensive research can combine multiple scales of individual response to investigate broad-scale evolutionary ecology and provide a holistic understanding of observed phenomena.



isolated areas such as experimental lakes that do not require artificial barriers to separate and confine fish from the environment. Access to such facilities is unique and often not available or applicable to many fish species (e.g., pelagic or migratory species). In such cases, in-system mesocosm or net pens can be constructed to confine study organisms to a specific area that is monitored or experimentally manipulated in some way. However, artificially constraining fish can have detrimental effects associated with holding stress (see Portz et al. 2006 for a detailed review of holding stress and recommendations for enclosure design) and consequently alter natural infection dynamics. The severity of holding stress can vary depending on the size, behaviour, life stage, or biology of the study species. For example, smaller species or those with small home ranges may not be immediately affected by confinement given that fish density does not induce competition-associated stressors (Wedemeyer 1996). Confinement of migratory species in particular can exacerbate holding stress, as individuals unable to execute migratory movements may challenge the integrity of holding materials by jumping or ramming into containment structures (Donaldson et al. 2011). Indeed, preventing experimental fish from escaping is often more difficult than anticipated in such studies.

For research investigating pathogen dynamics, holding studies may increase the likelihood of transmission among confined individuals (Teffer et al. 2017). While this can be mitigated by ensuring low host densities and high rates of water exchange, it is not possible to exactly match the natural environment. For example, confinement may prevent natural prophylactic behaviours that reduce transmission under natural conditions (Binning et al. 2017). This is a major consideration when seeking to apply data obtained in holding studies to wild fishes, and the suitability of the study species for confinement and alterations to natural pathogen transmission pathways must be scrutinized to ensure study design addresses the research objectives.

Tagging studies

Characterizing ecologically relevant aspects of disease requires that there be as little intervention with normal host behaviour and physiology as possible. Currently, the best method available

to study fishes in situ is through tagging or telemetry research (Cooke et al. 2013). Tagging allows researchers to individually identify fish with uniquely coded tags that are either passive (i.e., do not emit any signal) or active, such as radio or acoustic tags. Passive tags are typically used in situations where fish will be observed or recaptured, while actively transmitting tags mean the fish can be tracked remotely using receivers (see Cooke et al. 2013 for a review of telemetry applications in fisheries research). There are many potential applications of infectious agent screening in fish tagging studies, and new stabilizing solutions such as RNA Later and the small amounts of tissue (~2 mm^3) greatly reduce the time and space required for sample storage. This provides the opportunity for samples to be taken from all tagged fish and examined in detail only if necessary, reducing cost and allowing flexibility based on findings. Below we address current knowledge and research opportunities for applying nonlethal infectious agent screening to fish tagging studies.

Passive tags include varieties of external and internal tags that do not emit a signal, requiring that the fish is either recaptured or the tag is directly interrogated by decoding equipment within short range (e.g., passive integrated transponder arrays; Castro-Santos et al. 1996; Gibbons and Andrews 2004). Research using passive tags is often termed "mark–recapture" or "capture–mark–recapture"; a number of fish are tagged and released, and then information on individuals is obtained when tagged individuals are recaptured after time at liberty (e.g., Groner et al. 2018; Chapman et al. 2020). Population estimates and demographic information can be determined based on the proportion of tagged individuals captured after a concerted sampling effort, and variation in individual factors of interest (e.g., growth, condition, or pathogens) can be investigated in response to covariates of interest. Studies involving large populations or large distributions are generally long-term, taking place over the course of months to years.

To date we are not aware of any published studies on wild fish that have combined mark–recapture data with quantitative information on fish pathogens, yet this method is suitable to combine with disease screening in a multitude of systems and research questions. In any case where the presence of infectious agent(s) is expected to be associated with mortality and (or) capture susceptibility (e.g., Groner et al. 2018), or a measurable physiological outcome expected to change over time (e.g., growth or morphometrics), screening for infectious agents can be extremely informative. Conversely, results from laboratory studies can be validated in the wild. For example, experimental research has demonstrated a positive relationship between the presence of cestodes (specifically *Schistocephalus solidus*) and three-spined stickleback (*Gasterosteus aculeatus*) growth (Arnott et al. 2000), yet whether this relationship is an artifact of laboratory conditions and access to high-quality feeds is unknown (Barber and Scharsack 2010). A simple mark–recapture study could be designed to record changes in growth and relative load of cestodes by screening using nonlethal anal swabs. When compared with other high cost tagging methods, mark–recapture studies can allow researchers to deploy large numbers of tags and thus are an excellent method for assessing spatiotemporal changes in infectious agent communities within individuals in study systems where recapture is expected.

Technological advances in hardware, software, and battery engineering has revolutionized aquatic animal tracking, making it one of the most powerful tools in fisheries ecology (Hussey et al. 2015). Tags are now smaller, last longer, emit signals farther, and can include on-board sensors (reviewed in Cooke et al. 2016) that record and transmit depth, triaxial acceleration, or environmental parameters (e.g., temperature, dissolved oxygen). Consequently, fisheries scientists are able to use remote tracking to investigate an array of research questions at broader and finer scales than ever before and relate observed movements to environmental factors and (or) experimental treatments recorded from the fish's perspective. These advancements have facilitated the transition of numerous research questions out of the laboratory and into the wild, eliminating the potentially confounding effects of holding and observing fish directly, increasing the applicability of results in wildlife ecology.

It has been well documented that aspects of movement can be correlated with individual condition such as infection dynamics; infectious agents can change the physiological state of an animal, potentially manifesting as altered behaviours associated with movement (Dall et al. 2004) or likelihood of survival (Jeffries et al. 2012). Behavioural modifications have been observed across taxa, including in many fish–pathogen interactions, and can originate from either the host's response (e.g., prophylactic or therapeutic behaviours) or be the consequence of infection (e.g., adaptive manipulation of the host by the pathogen, reduced energetic capacity; reviewed in Binning et al. 2017). How such behavioural modifications can influence the interpretation of telemetry findings is contextual, depending on the scale at which the movement is recorded and biology of the species being studied.

Factors associated with fish survival

Quantifying natural and (or) anthropogenically derived mortality rates in free-ranging fish is a central focus in fisheries conservation and management — such information is rare considering the challenges of observing mortalities in the wild. Tagging and tracking fish provides the opportunity to infer mortality based on fish movement (or more specifically, lack thereof) and investigate factors mediating natural mortality rates in the wild, including infectious agents. The level of pathogen-induced mortality that occurs in the wild is unknown. The most direct association with natural mortality occurs when infection causes fatal disease; however, sublethal disease state may interact with various stressors to influence postrelease survival (Lupes et al. 2006). The physiological and physical perturbations associated with factors such as high water temperatures or fisheries capture (e.g., exhaustive exercise, physical abrasion, compression, and lacerations; reviewed in Patterson et al. 2017) may alter fish immunocompetency (Biro and Post 2008; Van Rijn and Reina 2010), exacerbating disease potential. Incorporating screening for infectious agents in telemetry studies investigating fish survival will result in more robust and comprehensive data that capture a critical component of wild fish ecology and evolution. Even a cursory understanding of the infectious agent community of individual tagged fish at the time of release may improve the interpretation of factors influencing postrelease survival, particularly in cases where highly pathogenic agents are present. For example, a telemetry study on sockeye salmon smolts tracked individuals during their ocean-bound migration, revealing high mortality during the first riverine portion of their migration (Clark et al. 2016). Combining further telemetry study with pathogen screening revealed that the presence of infectious haematopoietic necrosis virus reduced the probability of an individual smolt surviving that same portion of migration (Jeffries et al. 2014).

Predation of tagged fish is a major consideration in telemetry studies because it results in movement or behaviour data representing that of the predator rather than the intended study animal (Thorstad et al. 2012). While it is possible to identify predated fish based on abrupt changes in movement and behaviour inconsistent with the prey species (Buchanan et al. 2013; Gibson et al. 2015), such analysis relies heavily on numerous assumptions about the consistency of species behaviours (Halfyard et al. 2017). Rather than attempting to infer predation from patterns in data, recent advances in tag technologies have incorporated mortality sensors that are trigged based on exposure to the gastrointestinal tract of the predator (Halfyard et al. 2017). Since they have been

made available, predation tags have been used in only a handful of published studies; however, major variation in predation rates has already been revealed. For example, over 50% of tagged Atlantic salmon smolts were predated postrelease in the Mirimachi River in eastern Canada (Daniels et al. 2019), while in the Great Lakes, no predation was detected in Atlantic salmon smolts released in Lake Ontario (Larocque et al. 2020).

Individuals that are weakened as a result of infection are believed to be more vulnerable to predation due to reduced swim speed and performance (Bradley and Altizer 2005), yet there is little empirical evidence demonstrating such phenomena. Laboratory research has also provided evidence for parasite-induced predation risk associated with behavioural manipulation of the host (Lafferty and Morris 1996), again something that has not been demonstrated in situ. Pairing infectious agent screening with predation tags can be used to investigate the occurrence of such phenomena in the wild. For example, for species of conservation concern, it may be critical to know whether specific infectious agents, or suites of infectious agents, alter the likelihood of predation. From a theoretical standpoint, concepts of evolutionary stable strategies and optimality in host–pathogen systems (e.g., trophic transmission) could be tested to investigate transmission rates and the potential adaptive value of pathologies associated with infection.

Factors associated with fish behavior and personality

Better quantification of fine-scale movements using telemetry has revealed high rates of individual variation in behaviours in the wild. Differences in behavioural traits can be consistent among (behavioural traits) and within (behavioural syndromes) individuals (Sih et al. 2004; Dingemanse et al. 2012; Villegas-Ríos et al. 2017) and are considered an important trait that may influence fitness outcomes and thus evolutionary processes within species or populations (Wolf and Weissing 2012). To be considered a trait or syndrome, observed variation should be repeatable and a result of both among-individual variance and within-individual consistency in behaviours measured across contexts (e.g., environmental conditions; Killen et al. 2016). Behaviours such as home range size, daily vertical migrations, and overall activity are common metrics used when defining behavioural traits from telemetry data (Taylor and Cooke 2014; Harrison et al. 2015; Villegas-Ríos et al. 2017).

Infection burden is known to influence fish movement and behaviour; thus, movement data recorded during tracking and incorporated into behavioural traits or syndromes may reflect some portions of the role infectious agents play within the study population (Poulin et al. 2012; Killen et al. 2016; Binning et al. 2017). Large ectoparasites can alter swimming behaviour, as observed in the decreased activity and increased metabolic demands in bridled monocle bream (*Scolopsis bilineatus*) infested with large ectoparasites (Binning et al. 2013). Movement that may be interpreted as "bold" or highly active behaviour is displayed by California killifish (*Fundulus parvipinnis*) infected with the trematode *Euhaplorchis californiensis* (Lafferty and Morris 1996); individuals with high infection display conspicuous swimming behaviour, making themselves 10–30 times more likely to be consumed by a predator (Shaw et al. 2010). Higher levels of "sociability" based on increased shoal cohesiveness and reduced motor activity has been related to the abundance of the sea lice *Argulus* spp., a horizontally transmitted ectoparasite infesting salmon (Mikheev et al. 2015). Activity and dispersal may also be subject to moderation by pathogens; chub (*Squalius cephalus*) infested with *Anodonta anatine* glochidia had reduced movement and dispersal compared with uninfected individuals (Horký et al. 2014). Considering that the above examples are only addressing a single macroparasite in the context of infection, a great deal remains unknown regarding host prophylactic behaviours and (or) the synergistic or antagonistic effects that co-infection and microparasites may have on fish movement.

In spite of the clear connection between movement and infectious agents, no telemetry studies specifically investigating fish behavioural traits have incorporated screening for pathogens. The effect of disease may be considered only a potential component of the contextual considerations when interpreting telemetry data rather than an important explanatory factor (Killen et al. 2016). While behavioural traits must be consistent and repeatable, the scale at which repeatability is measured may influence how changes in infectious agent dynamics are influencing observed behaviour. Infection profiles are heterogeneous within a population and likely to change over time; thus, individuals that change in infection status during the course of the study (i.e., become infected or clear infection) may exhibit high behavioural plasticity.

Considerations

Microbial infections are known to have high spatiotemporal variability, and factors driving cycles of epizootics are often unknown, complex, or unpredictable (Snieszko 1974; Herman 1990). This has presented challenges in research investigating the effects of specific agents on host biology, as repeatability among sampling events may not be possible. Host phenology, behaviour, seasonality, and environmental conditions can drive changes both for host immune function (Bowden et al. 2007) and infectious agent dynamics (Altizer et al. 2006) and should be considered during study design when logistically possible. For example, macroparasite loads are generally positively correlated with host size and age, and trophically transmitted pathogens will only be found in individuals large enough to consume the intermediate host (Marcogliese 2004). Where specific agents are the focus of research, knowledge of the pathogen life cycle, distribution, and pathogenicity should dictate the timing and locations of sampling. Fish collection methods may introduce bias (e.g., size selectivity) that skew observations, potentially leading to erroneous conclusions. Certain agents may also influence host behaviour to make them more vulnerable to capture, or behavioural types may be more vulnerable to exposure in certain habitats. Such factors should either be controlled for through study design or considered during analysis and data interpretation.

When considering implementing nonlethal sampling to screen for pathogens using genetic techniques, whether or not genetic material from the pathogen is detectible in biopsied tissue should first be validated. For example, previously authenticated identification and (or) quantification techniques (e.g., necropsy or histopathology) should at first be paired with planned sampling methods to ensure that (*i*) positive detections align with confirmed cases and (or) (*ii*) amplification curves can be associated with agent abundance. Because genomic tools are more sensitive than classical identification methods, a higher number of positive cases may be observed compared with traditional parasitological examinations (Kralik and Ricchi 2017); however, if molecular methods have lower resolution than classic screening techniques, serious consideration needs to be given to whether or not research objectives can be addressed adequately when false negatives are observed.

Knowledge of general trends and phenomena associated with disease ecology is beneficial when planning to incorporate infectious agent screening in wild fish research. Parasites are often present within host populations without causing any detrimental effects. Indeed, pathogens generally have a negative binomial distribution within host populations; carrying low loads may have little effect on survival and is thus common, and increasing loads are associated with disease and consequent mortality. In contrast, highly pathogenic infectious agents will typically be rare in wild populations because those that are infected quickly succumb to mortality. If investigating specific infectious agents,

knowledge of the expected distribution based on previous research and agent pathogenicity is useful to estimate the sample size needed to address research questions. For example, comprehensive screening that seeks to detect a large number of agents, including those that are highly pathogenic, may require a large sample size (i.e., hundreds of individuals) to reduce the probability of false negatives compared with investigations focused on highly prevalent pathogens. Collaboration between fish ecologists and epidemiologists or veterinarians should occur during study design, data analysis, and interpretation to ensure all aspects of the research plan are appropriate to address epidemiological and ecological questions and data limitations. Indeed, there is much benefit that can be derived from interdisciplinary research that combines different disciplines and techniques (Dick et al. 2017).

Conclusions

Parasites and disease of wild fish are a critical component necessary for deciphering the evolutionary drivers underlying fish behaviour and ecology. As access to highly sensitive and specific genetic-based screening techniques increases, it is our hope that wild fish ecologists consider how infectious agent screening and how collaboration with disease experts may complement their research programs. While it introduces additional complexity to research methods and analyses, incorporating data on infectious agents provides a holistic view of the study system and may illuminate factors otherwise undetected. This is particularly important for studies of fish behaviour and survival, where the exclusion of pathogens may miss the opportunity to identify phenomena important for conservation and management. Research programs may better predict the consequences of climate change, species invasions, control measures, and how factors interact for comprehensive conservation and management recommendations of aquatic ecosystems. We hope that researchers embrace the natural complexity of aquatic ecosystems by including the potential impacts of pathogens in their research when appropriate. Conversely, there are many research opportunities that may be of interest to fish epidemiologists. For example, investigating transmission pathways within and among populations based on fish movement data and (or) network analysis (Hellard et al. 2015). Nonlethal tissue sampling may not provide as comprehensive information as traditional necropsy and histological data; however, it facilitates the investigation of complex ecologically relevant theories and phenomena in situ for the first time.

The utility of interdisciplinary research programs in ecological research has been highlighted and advocated for previously (Cooke et al. 2008); however, little consideration was given to the influence of pathogens. Collaboration among epidemiologists and fish ecologists may be mutually beneficial and enable all involved to use research approaches not otherwise available. We encourage meaningful and engaging collaboration among multiple disciplines and believe doing so will result in innovative study designs that address the "real-life" complexity of aquatic ecosystems (Hellard et al. 2015; Cooke et al. 2013). Given the effort, cost, and invasive nature of most fisheries sampling, strategic collaborations may allow researchers to maximize sampling efficacy while strengthening the predictive capacity of models and enhancing the understanding of wild fish ecology and (or) epidemiology.

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