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The use of large scale DNA/RNA sequencing has become an integral part of biomedical research. Reduced sequencing costs and the availability of efficient computational resources has led to a revolution in how problems concerning genomics and transcriptomics are addressed. Sequencing-based pathogen discovery represents one example of how genetic data can now be used in ways that were previously considered infeasible. Emerging pathogens affect both human and animal health due to a multitude of factors, including globalization, a shifting environment and an increasing human population. Fish farming represents a relevant, interesting and challenging system to study emerging pathogens. This review summarizes recent progress in pathogen discovery using sequence data, with particular emphasis on viruses in Atlantic salmon (Salmo salar).

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1. Introduction

Throughout medical history, the study of emerging pathogens has depended on technological advances. Visual identification of infectious agents was one of the earliest challenges to be addressed, and due to his improvements on the optical microscope, Antonie Philips van Leeuwenhoek is often credited as being the first scientist to observe bacteria (Dobell, 1932). Researchers now have powerful techniques such as electron and super-resolution microscopy at their disposal when working on pathogenic agents. Early epidemiological studies showed correlations between disease and exposure to contagious materials (Eyler, 2001); today influenza outbreaks can be forecasted using collective intelligence algorithms based on internet search query mapping (Carneiro and Mylonakis, 2009) and even changes in complex viral communities can to some extent be predicted (Anthony et al., 2015).

In order to establish models for infectious diseases a limiting factor has always been the ability to isolate and propagate the pathogen in a laboratory setting. By using defined media, many species of bacteria and other microorganism can be cultured, allowing detailed biological and biochemical studies. In permissive cell lines, viruses and obligate intracellular microorganisms can be propagated, passaged and thus kept in culture indefinitely. Material from such purified and enriched cultures may be used in experimental transmissions and if disease characteristics are reproduced, a causal relationship between pathogen and clinical parameters may be established (Koch, 1884). Unfortunately, a significant number of bacterial species will not grow (as pure cultures) in defined media (Stewart, 2012), and for viruses, the fraction of species that can be cultivated is likely to be significantly lower, given the incredible diversity found using metagenomics and other methods that are not dependent on laboratory propagation (Bergh et al., 1989; Paez-Espino et al., 2016).

Abbreviations: CMS, cardiomyopathy syndrome; DDBJ, DNA Data Bank of Japan; ENA, European Nucleotide Archive; EST, expressed sequence tags; GSS, genome survey sequence; HSMI, heart- and skeletal muscle inflammation; HSS, haemorrhagic smolt syndrome; IPN, infectious pancreatic necrosis; IPNV, Infectious pancreatic necrosis virus; ISA, infectious salmon anemia; ISAV, Infectious salmon anemia virus; PD, pancreas disease; PDG, proliferative gill disease; PMCV, Piscine mycocarditis virus; PRV, Piscine orthoreovirus; RACE, rapid amplification of cDNA ends; SAV, Salmon alphavirus; SGPV, Salmon gill poxvirus; SPDV, Salmon pancreas disease virus; SSH, suppression subtractive hybridization; VACV, Vaccinia virus; VHSV, viral hemorrhagic septicemia; VHSV, Viral hemorrhagic septicemia virus.

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For the characterization of pathogens that resist culturing, a plethora of molecular tools has been developed. The great majority of these make some assumptions about the microorganism in question; broad range PCR (Relman et al., 1990) assumes a high degree of sequence similarity in primer-regions, detection using immunological methods assumes cross-reactivity of antibodies, and molecular subtraction methods such as suppression subtractive hybridization (SSH) (Diatchenko et al., 1996) will not work efficiently on complex mixtures of nucleic acids and requires a matching, pathogen-free, isogenic sample. The advent of large-scale DNA sequencing has allowed for the indirect, unbiased description of both the species composition and the transcriptional activity in a biological sample. If total nucleic acids are extracted, DNA and RNA libraries may be sequenced and the data compared with annotated databases such as GenBank (Clark et al., 2016), the European Nucleotide Archive (ENA) (Leinonen et al., 2011) and the DNA Data Bank of Japan (DDBJ) (Kaminuma et al., 2010).

In the early 2000s, Dr. Matthew Meyerson and colleagues pioneered using sequence data alone for pathogen discovery (Weber et al., 2002). The method, referred to as ‘Computational subtraction’, was used to identify several known human pathogens in a proof-of-principle analysis using human transcripts sequenced downloaded from GenBank. This was accomplished through a set of computational filtering steps where accesses likely to originate from the host (human) as well as sequences of poor quality, low complexity etc. were removed, enriching for transcripts that appeared to be of an allochthonous nature. The approach was later used on clinical specimens, again showing the ability to detect pathogens by relying solely on sequence data (Xu et al., 2003). Due to the relatively high cost of DNA sequencing at the time, sequence tag-based cloning methods and statistical tools were also developed in order to increase the feasibility of using this strategy on diseases of unknown etiology (Laframboise et al., 2004; Tengs et al., 2004). A competing suite of methods for pathogen discovery that also took advantage of the increased availability of sequence data (including the human genome, (Lander et al., 2001; Venter et al., 2001)) as well as technical developments, were microarray-based strategies. Here, extracted nucleic acids were labeled and hybridized to high-density microarrays with probes complementary to genomes and transcripts of known pathogens (Ksiaszek et al., 2003; Wang et al., 2002).

In spite of significant efforts, few new pathogens of clinical relevance were discovered using this first generation of high-throughput sequence-based pathogen discovery tools. Cost associated with sequencing was a limiting factor, as well as the computational resources needed and the software available for handling large amounts of data. In addition, many of the early strategies did not actually interrogate total nucleic acids, as the protocols used in effect led to a subsampling of the genome/transcriptome by for instance reverse transcribing only polyadenylated transcripts or by just using the DNA fraction. As many viruses have RNA genomes and no DNA intermediate forms in the replication cycle, and many viruses do not polyadenylate their transcripts or have polyadenylated genomes, these pathogens will go unnoticed regardless of sequencing depth. In bacteria, poly(A) tails are in some instances very short and might not contain only adenosine residues (Mohanty and Kushner, 2011), making transcripts unsuitable for reverse transcription/amplification using oligo(dT)-based protocols. However, independent of the genomic architecture and characteristics of any RNA produced, there will be pathogen-associated transcriptional activity at the site of an ongoing infection. Using a protocol for total RNA reverse transcription (Palacios et al., 2007) in combination with pyrosequencing, Dr. William I. Lipkin expanded the potential for sequencing-based pathogen discovery with an unbiased genomics/metatranscriptomics approach (Cox-Foster et al., 2007). This combination of next generation sequencing, powerful clusters of supercomputers and sophisticated algorithms for handling billions of sequence reads has now led to the development of a multitude of pipelines for computational subtraction and in silico pathogen discovery (Kilianski et al., 2015; Kostic et al., 2011).

2. Aquaculture and infectious diseases of Atlantic salmon

Most of the food we eat originates from a limited number of terrestrial domesticated animal and plants species, and even for fish and other aquatic animals, the major source today is aquaculture, as the capture of wild fish has started to level off (www.fao.org/3/a-i3720e.pdf). Domestication of fish for large-scale industrial aquaculture production started only in the last century. This implies that most of the currently farmed fish species have not changed, or only slightly so, compared to their wild counterparts. This differs from the terrestrial domesticated species with approximately 10,000 years of breeding for production beneficial traits. Furthermore, aquaculture relies on production of selectively bred fish where the entire life cycle is completed in captivity. Farming conditions are often based on observation of wild fish only kept in captivity for a part of their life cycle.

Rearing of the salmonids Atlantic salmon (Salmo salar) and Rainbow trout (Oncorhynchus mykiss) is one of the most lucrative branches of aquaculture (www.fao.org/3/a-i5555e.pdf). Fish production is industrialized and animals are kept in extremely dense populations compared to their wild counterparts. The large ecological change from wild to culture leads to strong selection pressure on fish pathogen evolution (Miller et al., 2014). Low-pathogenic agents causing persistent infection with intermittent shedding might not have a significantly negative effect on wild populations of fish, and, if the pathogen kills the host and thus interferes with its own transmission to a new host, it will be counter-selected. The surplus of hosts in an aquaculture setting ensures that transmission continues independent of virulence. Agents with fast replication and shedding, traits that are often related to virulence, would have a selective advantage. Therefore, in dense animal populations, like trout and salmon farms, the negative effect of pathogens tends to increase over time partly because new susceptible hosts cannot escape the infection. Furthermore, wild and farmed fish share the same body of water, which gives many opportunities for interactions and renewal of the pathogen flora for both wild and farmed fish (Johansen et al., 2011). Crossing of the species barrier and successful establishment in a new host can also lead to increased virulence (Longdon et al., 2015). To switch species, a pathogen has to overcome host barriers such as mucus and macrophage cells, enter the cells and propagate in the new environment. Some viruses infecting fish are rather promiscuous regarding host species, and often exist in geographically defined rather than species-related subtypes (Walker and Winton, 2010).

Atlantic salmon farming appeared as a modern industry in the early 1980s and several bacterial diseases soon became threats due to losses and excessive use of antibiotics. However, oil adjuvanted intraperitoneally administrated vaccines were successfully introduced in the late 1980s to control these diseases. Some of these bacterial pathogens have been described primarily using molecular methods, for instance ‘Candidatus Branchiomonas cyrtocila’ (Toenshoff et al., 2012), though culturing strategies have been developed for the most important species, such as Aeromonas salmonicida, Flavobacterium psychrophilum and Yersinia ruckeri.

Viruses have short generation times and large population sizes, and viruses with RNA genomes have particularly high mutation
rates. RNA viruses are common among emerging infections in aquaculture (Walker and Winton, 2010), and the genetic diversity may expand host species range. Due to the intracellular replication and the hijacking of host cell machinery, there are also numerous virus-host cell interactions that may modulate virulence. While infectious pancreatic necrosis (IPN) and the associated IPN virus (IPNV) was already known, several other viral diseases emerged in Norwegian salmon farming after the control of bacterial diseases. Infectious salmon anemia (ISA) was recorded in 1984 (Thorud and Djupvik, 1988), cardiomyopathy syndrome (CMS) in 1985 (Amin and Trasti, 1988), pancreas disease (PD) in 1989 (Poppe et al., 1989), heart- and skeletal muscle inflammation (HSMI) in 2004 (Kongtorp et al., 2004b) and viral hemorrhagic septicemia (VHS) has been known since the 1930s to affect many different species of farmed and wild fish, including salmonids (Skall et al., 2005).

For several of these viral diseases, detailed analysis of the relevant infectious agent and the associated pathogenesis has allowed for the development of efficient countermeasures. An early observation was that ISA spread like a contagious disease, and the viral nature of the agent was indicated by experimental transmission in fish (Dannevig et al., 1994). This was verified by electron microscopy, demonstrating the presence on enveloped virus particles of approximately 100 nm in tissue sections of diseased fish (Iovland et al., 1994). Yet, the virus resisted cultivation in common fish cell lines. However, in 1994, a long-term cell line developed from Atlantic salmon head kidney (SHK-1) could support the propagation of ISAV (Dannevig et al., 1995). Cloning of ISAV nucleic acids from cell culture soon followed (Mjaaland et al., 1997), including characterization of important genes and molecular characteristics (Rimstad et al., 2001; Sandvik et al., 2000). The full-length nucleotide sequence of the ISAV genome became available 18 years after the first clinical observation of the disease (Clouthier et al., 2002). Historically, PD in farmed Atlantic salmon was first recognized in Scotland in 1976 (Munro et al., 1984), while the first isolation of a virus in cell culture, as well as an experimental reproduction of the disease, was published in 1995 (Nelson et al., 1995). Partial genome sequencing indicated that SPDV (also called Salmon alphavirus; SAV) was a member of the genus Alphavirus (Weston et al., 1999). This was verified when the full-length genome sequences of SPD virus and its close relative Sleeping disease virus became available in 2002 (Weston et al., 2002). However, in spite of great efforts, culturing and identification of the causative agents responsible for HSMI and CMS remained unsuccessful.

3. Heart- and skeletal muscle inflammation (HSMI)

HSMI is an inflammatory disease of the heart and skeletal muscle of farmed Atlantic salmon. The disease commonly affects fish 5–9 months after sea transfer and may develop over a period of several months before clinical signs are observed (Kongtorp et al., 2006). Affected fish have severe inflammation of the epicardium, endocardium and of the red skeletal muscle (Kongtorp et al., 2004a, 2004b). Initial experimental transmissions of HSMI supported an infectious etiology for the disease and that a non-enveloped virus most likely was the relevant agent (Kongtorp et al., 2004a; Kongtorp and Taksdal, 2009). However, laboratory propagation of the virus was not successful.

In order to identify the causative agent of HSMI, high-throughput sequencing of total RNA from an experimental transmission was performed using 454-type pyrosequencing (Palacios et al., 2010). As the cardiac tissue appeared to be the main site of infection, serum and heart muscle samples were used from an Atlantic salmon that had been inoculated with cardiac tissue from field outbreaks of HSMI. Through multiple rounds of pyrosequencing, Sanger sequencing, cloning and both 5′ and 3′ rapid amplification of cDNA ends (RACE), the complete genome sequence of a novel reovirus could be elucidated (Markussen et al., 2013; Palacios et al., 2010). Piscine orthoreovirus (PRV) is the first described fish virus belonging to the genus orthoreovirus (Markussen et al., 2013) (International Committee on Taxonomy of Viruses; Release 2015). Quantification of viral RNA loads using realtime PCR in diseased versus healthy fish and immunohistochemical staining of heart tissue support a causal relationship between the presence of PRV and the clinical signs of HSMI (Finstad et al., 2012; Lovoll et al., 2012). The virus also appears to be associated with the formation of melanized spots in the white muscle of farmed salmon (Bjorgen et al., 2015). PRV is unusual in that erythrocytes are a major target cell type (Finstad et al., 2014; Wessel et al., 2015b); in contrast to mammalian erthrocytes, fish erythrocytes are nucleated and support virus replication. The salmon erythrocytes have a functional transcriptional and translational machinery, and infected erythrocytes mount an innate immune response (Dahle et al., 2015). PRV replication occurs in cytoplasmic structures called virus factories, which are organized by non-structural virus proteins (Haatveit et al., 2016) (Fig. 1). The viral factories protect the viral molecular patterns that would otherwise alarm the innate immune response, and, additionally, the viral structural protein α3 binds

Fig. 1. A) Atlantic salmon erythrocyte with nucleus and cytoplasmic inclusion bodies. B) Close up of inclusion bodies showing Piscine orthoreovirus (PRV) particles (image courtesy of Dr. Øystein Wessel).
dsRNA in a sequence independent way, also restricting the activation of an innate immune response (Wessel et al., 2015a).

4. Cardiomyopathy syndrome (CMS)

CMS mainly appears in fish 12–15 months after transfer to seawater and pathological signs include inflammation of the endocard and spongiosum of the atrium and ventricle (Brun et al., 2003). Early reports of lesions compatible with CMS indicated that the disease was present in several European countries and on both sides of the Atlantic (Brocklebank and Raverty, 2002; Rodger and Turnbull, 2000; Sande and Poppe, 1995). CMS-like lesions were also observed in wild Atlantic salmon (Brun et al., 2003).

Independent experimental transmissions of CMS were published, but no specific candidate pathogens were found (Bruno and Noguera, 2009; Fritsvold et al., 2009). Based on these initial observations, attempts were made to identify the causative agent using high-throughput sequencing and conventional molecular methods combined with virus culturing (Haugland et al., 2011; Lovoll et al., 2010). For the computational subtraction approach, total RNA was extracted from both heart and kidney tissue and material from field outbreaks as well as experimental transmissions were used (Lovoll et al., 2010). A sequence with weak similarity to totiviruses was detected, and the complete genome has subsequently been published (Haugland et al., 2011; Lovoll et al., 2010). There appears to be a strong link between the totivirus found (Piscine myocarditis virus; PMCV) and CMS pathology (Haugland et al., 2011; Lovoll et al., 2010), and PMCV is almost exclusively found in myocardial lesions (Wiik-Nielsen et al., 2012). Immunologically, fish with CMS and expressed pathology coupled with high viral loads seem to have higher lymphocyte infiltration in heart tissue than fish with less virus (Timmerhaus et al., 2012). It has also been suggested that the composition of fish feed, in particular the lipid content, may be optimized for modulating the inflammatory response of Atlantic salmon so that the pathological consequences of a PMCV-infection become less severe (Martinez-Rubio et al., 2014). Genetically, the strains of PMCV found in Atlantic salmon along the coast of Norway seem to be quite homogenous (Wiik-Nielsen et al., 2013), although a distinct virus isolate has been found in an unrelated fish species, Atlantic argentine...
Argentina silus (Bockerman et al., 2011; Tengs and Bockerman, 2012).

5. Salmon gill poxvirus (SGPV)

Gill diseases of farmed Atlantic salmon are generally thought to be complex disorders where multiple (prokaryotic/eukaryotic/viral) factors are involved, and very few pathogens have been linked specifically with outbreaks of for instance proliferative gill disease (PDC) (Mitchell and Rodger, 2011). Early electron micrographs of gill tissue from diseased Atlantic salmon indicated that a poxvirus-like agent might be involved in disease development (Nylund et al., 2008) and high-throughput total RNA sequencing of gill tissue obtained from a PDC outbreak (Fig. 2) produced a handful of reads that seemed to stem from an atypical member of the Poxviridae family of viruses (Gjessing et al., 2015) (Fig. 3). Again, in situ methods and quantitative PCR support a causal relationship, albeit attempts on propagation of the virus in a laboratory setting have thus far proven unsuccessful.

Using a quantitative PCR assay, a sample with high load of the suspected virus (Salmon gill poxvirus; SGPV) was identified. This sample was used as template for a round of Illumina sequencing, generating sufficient data for construction of an SGPV genome scaffold. The sequence gaps were then be closed using PCR and conventional sequencing (Gjessing et al., 2015). Analyzing the genomic sequence, several unique molecular features were observed, including loss of genes thought to be essential for virus biogenesis/host-virus interactions and a large number of open reading frames with unknown functions (Gjessing et al., 2015). Poxviruses have some of the largest genomes reported, allowing for the introduction significant amounts of transgenic material using reverse genetics techniques. Vaccinia virus (VACV), for instance, has been widely used for recombinant antigen expression (Siciliano et al., 2013), and if a recombinant version of SGPV could be designed to produce functional antigens against diseases relevant for the production of Atlantic salmon, it is possible to envision that the administration of such a (non-pathogenic) version of SGPV could provide efficient protection against a large number of salmon pathogens.

It is likely that a large fraction of fish that show signs of PDC and are positive for SGPV also will contain other (pathogenic) agents if gills are thoroughly investigated. To what extent SGPV represents a primary pathogen remains to be further investigated, but an intriguing possibility is that SGPV has the ability to suppress the immune system of infected fish, facilitating secondary infections by for instance ‘Candidatus Branchiomonas cysticola’ (Mitchell et al., 2013; Toenshoff et al., 2012). There are several examples of poxviruses that can modulate the immune branch of the JAK/STAT signaling pathway, for instance through dephosphorylation of activated STAT1 (Najarro et al., 2001), blocking the activation of TYK2 (Wang et al., 2009) or by mimicking the anti-inflammatory cytokine IL-10 (Fleming et al., 1997).

6. Other diseases of unknown etiology in Atlantic salmon

In an artificial setting with a high density of potential hosts, such as a fish pen, novel pathogens, or (more virulent) new versions of known pathogens, seem to emerge more or less continuously. Resistance to measures currently in place to limit disease outbreaks is fairly common and the great majority of virus vaccines administered to farmed Atlantic salmon are not 100% effective. Currently, few diseases of unknown etiology that significantly affect the health of farmed salmonids and/or lead to financial losses have been described in the literature, though an exception could be haemorrhagic smolt syndrome (HSS), where virus-like particles have been observed (Nylund et al., 2003). An active area of research is the health of wrasses (and lumpsucker (Cyclopterus lumpus)) currently used as cleaner fish to reduce the burden of sea lice on
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farmed salmon. Here, multiple pathogens have been studied and several atypical genotypes of known bacterial species described (Alarcon et al., 2016; Gulla et al., 2015, 2016). The interplay between pathogens found on the farmed fish and pathogens that affect the cleaner fish is also being studied by several groups (for review, see (Treasurer, 2012)). As the use of cleaner fish becomes more widespread it can be expected that these fish will present with diseases of unknown origin, especially if the industry continues the current practice of long-distance movement of cleaner fish populations between farms.

7. Pathogen discovery in aquaculture

The diversity of species and the overall production volume of aquaculture can be expected to grow significantly in the future, and the fish farming industry is already considered the fastest growing food production sector in the world (www.fao.org/3/a-i5555e.pdf). Emerging pathogens is one of the risk factors that needs to be addressed for the industry to be sustainable. As sequencing-based pathogen discovery has allowed researches to solve several longstanding problems in the production of Atlantic salmon, as well as other species (Bacharach et al., 2016), we believe this will be an important tool in future investigations of infectious diseases in aquaculture. Sequencing costs have dropped by several orders of magnitude in the last 10 years, and emerging technologies such as nanopore sequencing can be expected to further facilitate pathogen discovery (Greninger et al., 2015).

Before postulating that a disease is of an infectious nature, alternative hypotheses should always be considered. Toxins, water quality, oxygen levels, health-issues associated with particular batches of juvenile fish and even the physical fitness of the fish (Castro et al., 2013) may be linked with increased morbidity, mortality, pathological changes and decreased production volumes. Epidemiological observations and pathological findings may be combined with gene expression profiling of diseased fish to look for signs of an inflammatory response (Johansen et al., 2015; Krasnov et al., 2011), and controlled experimental transmissions will obviously also support the presence of an infectious agent. It is equally important to ensure that the disease in question can be defined specifically enough to avoid lumping together animals that may not suffer from the same condition. PD, CMS and HSMM share many of the same pathological features (Ferguson et al., 1986, 1990), but are caused by viruses that are completely unrelated.

Another crucial step when identifying pathogens using high-throughput sequencing is the acquisition of appropriate sample material. Ideally, tissue from experimental transmissions should form the basis. A successful experimental transmission would clearly support an infectious etiology and the inoculum used might reasonably also support the presence of an infectious agent. It is equally important to ensure that the disease in question can be defined specifically enough to avoid lumping together animals that may not suffer from the same condition. PD, CMS and HSMM share many of the same pathological features (Ferguson et al., 1986, 1990), but are caused by viruses that are completely unrelated.

The first step is to identify potential outbreaks instead need to be identified. Pathogens are analyzed by sequencing the genome, and the results are compared to known sequences in databases. If a match is found, the pathogen is identified. If no match is found, new sequences are generated and compared until a match is found. This process is repeated until all potential pathogens are identified.

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Saprolegnia spp. can also act as primary pathogens (Willoughby and especially in areas with compromised epithelial integrity. However, lesion in order to establish itself. In salmonid secondary infections or additional factors. In other case, the microbe discovered will when combined with stressors, such as movement of lates (Koch, 1884), is able to cause disease without the need for prove to be a primary pathogen, which, in line with Koch’s postulates (Koch, 1884), is able to cause disease without the need for secondary infections or additional factors. In other case, the microbe might just weaken the host’s immune system or physical integrity in a way that facilitates secondary infections, or the proposed pathogen can be a secondary agent, requiring a primary lesion in order to establish itself. In salmonid fish, water molds of the genus Saprolegnia are very common secondary pathogens, especially in areas with compromised epithelial integrity. However, Saprolegnia spp. can also act as primary pathogens (Willoughby and Pickering, 1977). It also possible that the pathogen is only associated with increased morbidity/mortality, lower growth rates etc. when combined with stressors, such as movement of fish cohorts or delousing. Linking the presence of a particular microbe with a set of specific clinical signs of disease can be very challenging and it is important not to prematurely extrapolate from correlation to causation.

Koch’s postulates are recognized as the gold standard for defining a disease as unequivocally being of an infectious nature (Koch, 1884). As sequencing-based pathogen discovery does not necessarily lead to the isolation of the infectious agent, several of these postulates will be impossible to fulfill (Fredricks and Relman, 1996). Consequently, extra caution needs to be shown when implementing a pathogen in disease development if the association has been made primarily using molecular data. Although a strictly molecular approach to pathogen discovery and detection has some shortcomings compared to strategies relying on culturing and visual identification, the increased availability of sequence data, extreme reduction in cost of sequencing and the continuous development of bioinformatics tools has rendered this suite of methods extremely powerful. If the appropriate tissue can be sampled at the time of infection, even minute amounts of pathogen-derived nucleic acids can be detected. The molecular data acquired can immediately be used to develop (quantitative) detection assays for screening and epidemiological studies. Sequence information will also provide a molecular toehold for a more comprehensive description of the pathogen’s genome/trascriptome and potential antigens. Any type of infectious agent may be detected (virus, fungi, bacteria, parasites etc.) without any prior knowledge about the molecular makeup or visual appearance. With an increasing number of uncultured microorganism being described in the literature, we believe that molecular pathogen discovery will play an important role not only the future of aquaculture, but also in human and veterinary medicine in general.

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