Trends in Microbiology

CellPress

Opinion

Assigning cause for emerging diseases of aquatic organisms

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Resolving the cause of disease (= aetiology) in aquatic organisms is a challenging but essential goal, heightened by increasing disease prevalence in a changing climate and an interconnected world of anthropogenic pathogen spread. Emerging diseases play important roles in evolutionary ecology, wildlife conservation, the seafood industry, recreation, cultural practices, and human health. As we emerge from a global pandemic of zoonotic origin, we must focus on timely diagnosis to confirm aetiology and enable response to diseases in aquatic ecosystems. Those systems' resilience, and our own sustainable use of seafood, depend on it. Synchronising traditional and recent advances in microbiology that span ecological, veterinary, and medical fields will enable definitive assignment of risk factors and causal agents for better biosecurity management and healthier aquatic ecosystems.

Highlights

Aquatic diseases can have serious impacts on global biodiversity, economies, and societies.

Understanding the cause of emerging aquatic diseases is a scientific challenge and governmental priority to reduce impacts and inform discovery of new treatments and management methods.

We propose a holistic approach leveraging medical criteria and traditional and modern technologies to demonstrate causal factors associated with emerging diseases of aquatic organisms.

Technological developments can increasingly enable disease emergence forecasting, high-throughput screening to rapidly shortlist suspect infectious and noninfectious agents, culture of a broader range of aquatic microorganisms, and challenge trials with whole organisms and surrogate models.

Emerging aquatic diseases in an era of global change

Healthy freshwater and marine ecosystems are foundational to global biodiversity, economies, and cultural identities. These ecosystems face mounting pressures, and emerging aquatic animal and plant **diseases** (see Glossary) are a global priority concern. Coastal intensification (e.g., coastal development, aquaculture, global shipping) adds stressors that favour disease emergence and spread in farmed and wild populations [1,2], while climate change is altering the incidence, severity, and geographic distributions of disease-causing **agents** [3–9]. Aquatic disease risks may be shifting from 'low likelihood–high consequence' toward 'high likelihood–high consequence' as a result [10], jeopardising global food security [11] and ecosystem functioning [12]. In many cases, emerging aquatic wildlife diseases may go unnoticed and unrecognised and it is critical to investigate the potential role that those emerging diseases play in population declines of unknown cause. Pre-emptive surveillance for direct (e.g., clinically diseased individuals) or indirect (e.g., unexplained population decline) signs of disease is an important consideration for aquatic systems due to the cryptic, rare, or otherwise difficult-to-observe nature of sick aquatic host organisms.

Aquatic disease investigations often take years or decades to resolve, and many are never causatively diagnosed (Figure 1). Prevailing diagnostic efforts are primarily concentrated on a small subset of aquatic **viruses**, bacteria, archaea, protists and **parasites** known to cause severe clinical diseases, principally those listed by the World Organisation for Animal Health (WOAH, formerly OIE, the Aquatic Code) [13,14]. This designated approach enables standardised diagnostic guidelines to inform animal welfare and global trade policies. However, there are no set diagnostic standards for diseases not included in the **Aquatic Code**. Complementary diagnostic pathways are urgently needed to identify and assign **causation** for new, or otherwise unexpected, **emerging diseases** [15]. Developing and implementing structured approaches to

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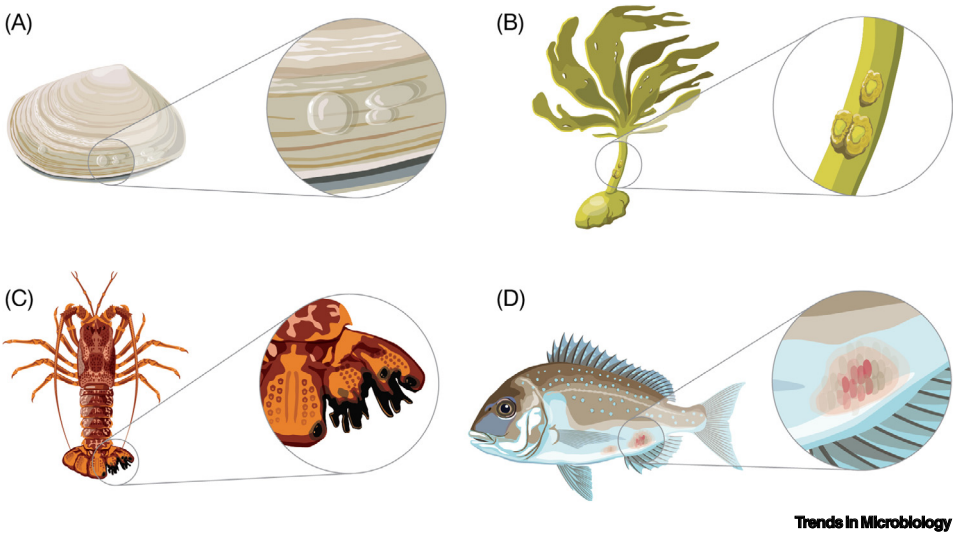

Figure 1. Examples of ongoing and unresolved disease syndromes in aquatic organisms. (A) Current hypotheses regarding gas bubble disease in surf clams, *Paphies ventricosa,* include rapid heating of sea or groundwater and intracellular bacteria. (B) Pathogens and parasites have been linked with tumour-like galls in macroalgae (bull kelp, *Durvillea* spp.). (C) Numerous bacteria have been associated with tail-fan necrosis in rock lobsters, including *Jasus edwardsii*. (D) Scale inflammation in snapper (*Chrysophrys auratus*) may be associated with ectoparasites and bacterial infection or trauma from contact abrasion with jellyfish or other fish. In each case, the unresolved cause of syndromes contributes to population declines, wild fishery impacts, declining seafood markets, and lost cultural opportunity for indigenous peoples. © Eden Cartwright, birdcircus.com.

do this, and definitively assign causation, are in the current authors' opinion the critical challenge facing aquatic microbiologists. This can be achieved through a holistic effort that bridges veterinary diagnostics and research-led approaches to reach causative, evidence-based conclusions [15–19]. Such approaches are increasingly feasible given recent advances in **culture** techniques, molecular diagnostics, and availability of purpose-built aquatic biocontainment facilities, but ultimately rely on foundational ecological frameworks and understanding [20]. (See Table 1.)

Structured approaches to assign **disease causation** are not new – Henle and Koch articulated four postulates in the late 19th century for causal relationships of an infectious agent to a clinical disease [21–23]. These postulates have been refined over time but continue to underpin the theory and practice of aetiology (e.g., [24–28]). However, demonstrating cause is rarely straightforward. This is particularly so for aquatic diseases due (at least partly) to the abundance of opportunistic microorganisms in water, **host** complexity, and the multidimensional, interconnected, and variable nature of aquatic habitats. Research with aquatic organisms is inherently costly, few aquatic microbes can be cultured at present, and purpose-designed husbandry methods are unavailable for most aquatic organisms. Using established medical (human) and veterinary (terrestrial-animal standards) aetiological frameworks to co-ordinate aquatic emerging disease investigations can help resolve the inherent complexity, rationalise the operational challenges, and drive required methodological advances [14]. Equal inclusion of laboratory and field-based research should inform cause-and-effect relationships ([20]; Figure 2, Key figure).

Using structured approaches to overcome challenges in aquatic aetiology

Several case studies illustrate the inherent challenges and pitfalls that can occur in aquatic disease investigations and showcase how structured approaches can disentangle causation and inform actionable responses when needed. For example, causes of global coral population declines

Glossary

Agent: a thing that takes an active role or produces a specified effect. In the context of 'disease agent', this may be an infectious organism (e.g., bacteria, virus), noninfectious organism (e.g., toxic algae), or abiotic factor (e.g., temperature).
Aquatic Code: the WOAH Aquatic Animal Health Code (the Aquatic Code) provides international standards to set up measures for the prevention, early detection, reporting, and control of notifiable aquatic diseases listed by the WOAH.
Bradford Hill criteria: Sir Austin Bradford Hill's nine criteria published in 1965 are widely accepted in public health research as guiding principles for investigating causality in epidemiological studies. They include 1. Strength (effect size); 2. Consistency (reproducibility); 3. Specificity; 4. Temporality (progression); 5. Biological gradient (dose–response); 6. Plausibility; 7. Coherence; 8. Experimental evidence; 9. Analogy.
Causation: the relationship between cause and effect; causality.
Challenge: trials in which participants/organisms are intentionally challenged with an infectious disease organism or factor that could induce disease.
Culture: maintenance of (tissue cells, bacteria, parasites) in conditions suitable for growth.
Diagnosis: identification of the nature and cause of disease.
Disease: a disorder of structure or function in an animal or plant.
Disease causation: the standard model of infectious disease causation under the epidemiological triad (disease triangle) theory states that an external agent can cause diseases on a susceptible host when there is a conducive environment.
Emerging disease: a new occurrence in an animal or plant of a disease, infection, or infestation causing a significant impact on animals/plants or public health resulting from: (i) a change of a known pathogenic agent or its spread to a new geographic area or species, or (ii) a previously unrecognised causative agent diagnosed for the first time.
Epidemiology: the study and analysis of the distribution and factors that determine the presence or absence of diseases.
Host: a host in the context of infectious disease refers to an animal or plant that acts as a biological refuge in which

[16,29] remain mostly unknown [19,20]. Challenges include approvals to work on protected coral species, the role of *Symbiodinium* in coral microbiomes, and limited morphological signs of disease [19,20,30]. On occasion, culture-based approaches that aimed to satisfy Koch's postulates have been successful (e.g., white pox disease caused by *Serratia marcescens* [31]) and microbial profiling has quantified dysbioses associated with diseases. Consistently applying such approaches to structure field observations, laboratory analyses, and aquarium-based experiments is continually improving success rates for assignment of coral disease causation (see [20] for review).

In the aquaculture industry, overcoming catastrophic impacts of disease can be very time sensitive. For more than 25 years the causative agent of Atlantic salmon (*Salmo salar*) amoebic gill disease in Australia was assumed to be the amoeba *Neoparamoebae permaquidensis* due to abundance in diseased gills and known disease-causing capacity (see [18]). Attempts to treat the disease were unsuccessful (cost AUD 40M/year[i]). Further research shortlisted a broader range of candidate agents and tested causation via Koch's postulates where it was discovered that the less abundant, co-occurring amoeba *N. perurans* is the primary causative agent [32–35], helping direct targeted intervention strategies [36–38]. In another case, shrimp white faeces syndrome (WFS) was assumed to be caused by a microsporidian that is abundant in characteristic white, floating faecal strings [39]. Recently, modern metagenomic (16S rRNA high-throughput sequencing) and metabolomic (liquid chromatography-mass spectometry based) techniques were applied to correlate a broad range of compositional, functional, and metabolic factors using random matrix theory. It was revealed that WFS is a noninfectious disease, not attributed to one pathogen, but a bacterial continuum in the intestinal microbiota [40]. Intestinal microbiota transplantation demonstrated a causal relationship between intestinal dysbiosis and WFS [40].

In New Zealand, unresolved diseases of IUCNred-listed species of significant cultural value include skin-reddening syndrome of kanakana (diadromous pouched lamprey, *Geotria australis*) and a respiratory disease of hoiho chicks (yellow-eyed penguin, *Megadyptes antipodes*; International Union for Conservation of Nature). Kanakana skin-reddening syndrome was first reported in 2011 and remains unexplained. There is ongoing speculation regarding infection by a shortlist of candidate pathogens versus traumatic epidermal erosion, or both [41]. Hoiho respiratory disease[ii] has been correlated to the parasite *Diomedenema dinarctos* isolated from the lungs of penguins [42]. Parasites of this genus can cause respiratory problems, malnutrition, and death [43]. Definitive causative assignment via Koch's postulates is essential to enable species management and must occur under potential conservation and cultural constraints of working with rare individuals or declining populations.

Investigating aquatic disease outbreaks of unknown cause

Accurate **diagnosis**, prioritisation, and management of aquatic diseases should embrace traditional and modern techniques that span microbiological (cell) models to whole-organism assays. This entails considerable challenges, especially in scenarios with scant information to guide investigations [44]. Further, there may be competing priorities among stakeholders related to demonstrating cause, and the need to contain or eradicate diseases[iii]. Successful eradication can be highly time-critical with significant pressure to act immediately[iv]; however, well-informed response efforts rely on accurate diagnosis (e.g., [45]) which requires improved capacity and speed of causative inference. Overcoming scientific bottlenecks and aligning societal imperatives will enable environmental risks to be managed, support greater value from natural aquatic resources, and safeguard threatened species.

The World Organisation for Animal Health's Aquatic Code provides detailed guidelines for determining susceptibility of aquatic hosts (often farmed species) to known disease agents (the

another organism (i.e., pathogen) may dwell.

Inoculate: introduce infective material, microorganisms, or vaccine into the body.

Koch's postulates: four guidelines developed by Robert Koch in the late 19th century to evaluate causation in infectious disease as follows: 1. The microorganism must be found in the diseased animal, and not found in healthy animals. 2. The microorganism must be extracted and isolated from the diseased animal and subsequently grown in culture. 3. The microorganism must cause disease when introduced into a healthy experimental animal. 4. The microorganism must be extracted from the diseased experimental animal and demonstrated to be the same microorganism that was originally isolated from the first diseased animal. The postulates have since been modified to account for new epidemiological knowledge and diagnostic technologies, but the overarching principles remain.

Parasite: an organism that lives on or in a host organism and gets its food from or at the expense of its host.

Pathogen: any organism that can cause disease (includes virus, bacteria, parasites).

Vector: an organism which spreads infection by conveying pathogens from one host to another.

Virus: an infective microbe that replicates only inside the living cells of an organism.

Table 1. Examples of traditional tools and recent advances in scientific approach to aid detection, diagnosis, and determination of causal agents

Diagnostic data sources	Key advantage of traditional tools	Recent and emerging advancements[a]	Improvements	Example applications
Field sampling	Reduces post-mortem artefacts and increases the likelihood of capturing labile infectious organisms	Artificial intelligence (AI), drone surveillance	Automate diagnosis through digital image processing	[93,94]
	Correlation of abiotic and biotic parameters associated with gross disease pathology	Modelling, hindcasting, prediction	Role infectious and noninfectious diseases can play in mass mortality events across a diversity of marine taxa	[66,95]
Pathogen culture	Sensitive, rapid, and cost-effective screening tool that detects viable organisms	High-throughput culture conditions	Microfluidics allows high-throughput generation and manipulation of isolates and cultivates microbial cells	[96,97]
Challenge trials	Clear determinations of disease causation under challenge conditions	*In vivo* challenge	Purpose-built biocontainment facilities permit inference of disease at level of the organism	[82]
		Cell lines	Informs effects of pathogens at a cellular level	[98]
Histopathology	Identify candidate agents, categorise host response, estimate infection intensity	*In situ* hybridisation, immunohistochemistry, laser microdissection, medical imaging technologies	Rectifies limitations in species identification through probe-based diagnosis	[99,100]
Characterisation and taxonomy	Identification of associated agents with hosts exhibiting clinical disease	Coevolutionary perspectives, phylogeny	Coevolutionary insights into pathogen–host associations	[101]
	High sensitivity and high specificity and possible rapid screening for the presence of a targeted pathogen	Droplet digital PCR, multiplex, magnetic beads, electrochemical biosensors, MALDI-TOF, genomics	Diagnosis of a broad spectrum of infection types and known pathogens; identify multi-agent aetiologies with specificity, sensitivity, rapid screening	[102–105]
		Metagenomics and next-generation sequencing (NGS); deep RNA sequencing	Applied early in the discovery stage to identify potential causative agents	[106]

[a]While recent advances have considerable promise, many require standardisation and validation and should be considered complementary to traditional approaches rather than replacements.

Aquatic Code Chapter 1.5; [46]). The challenge now is to build a broader framework suited for emerging disease outbreaks of unknown cause for a range of aquatic host wildlife. Revisiting basic principles is advisable to ensure that approaches can identify the possible cause of an emerging aquatic disease, which can include a diversity of bacteria and archaea (e.g., [47]), viruses [48], protists [49], parasites [5], and noninfectious agents (e.g., environmental factors, toxic microalgae, malignancies, **vectors** [50–52]). A combined approach using field-based and laboratory models is encouraged given that experimental approaches using *in vitro* and *in vivo* laboratory models may not always be achievable or relevant to natural systems.

We advocate a process with two key stages for unresolved aquatic disease investigations: (i) shortlist suspect disease-causing agents, and (ii) definitively test which suspect(s) causes the disease by directly linking the agent at the gross and cellular levels (Figure 2). An overarching consideration for shortlisting should be to avoid false negatives (type II error) to ensure that the causative agent(s) is included and to account for artefacts that can be introduced in suboptimal sampling conditions. This should be tempered against overly long lists of suspects, and decisions can be guided by the **Bradford Hill criteria**. Bradford Hill criteria are used to correlatively associate disease and agent via nine 'aspects of association', namely strength of association, consistency, specificity, temporality, biological gradient, plausibility, coherence, experiment, and analogy [53]. These aspects were developed in 1965 and remain effective following

Key Figure

Proposed investigation framework for diagnosing the cause of emerging aquatic disease

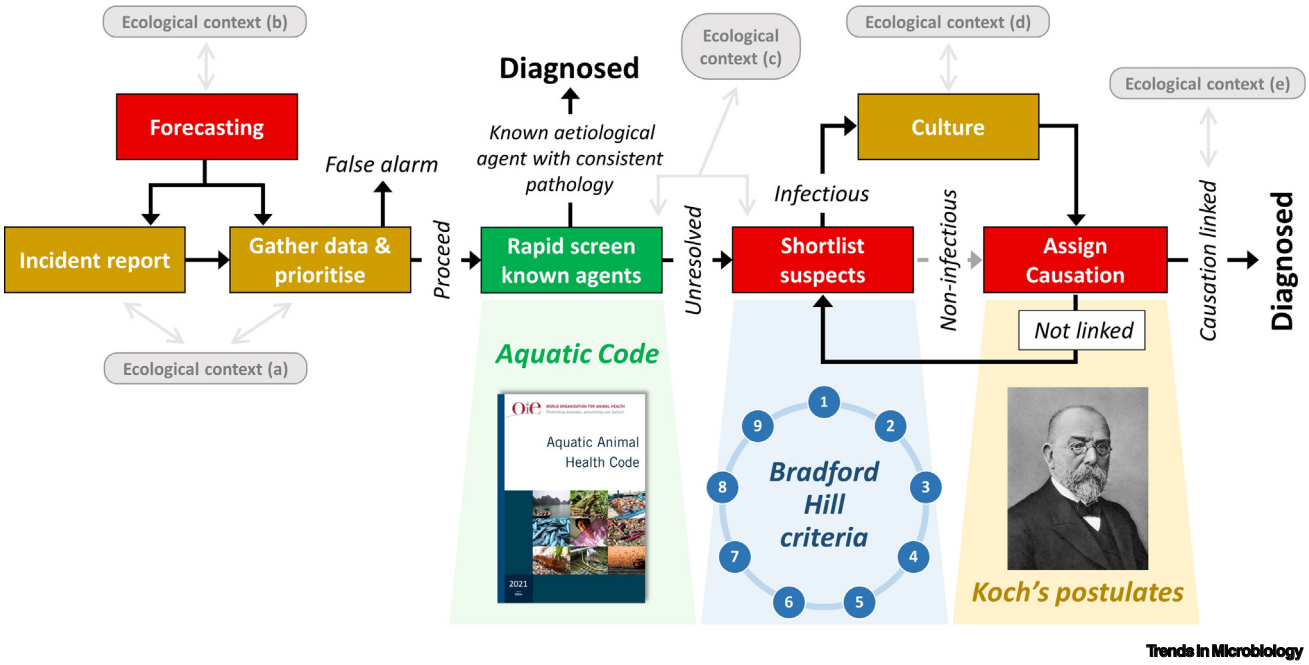

Figure 2. Areas of severe bottlenecks (red boxes), growing capability (amber boxes), and fit-for-purpose tools and methods (green box) are indicated. Aetiological advances can occur when standardised approaches using existing pillars of disease investigation (Aquatic Code, Bradford Hill criteria, and Koch's postulates) are integrated with novel methods, tools, and infrastructure. Ecological context is integral throughout the framework. (A) Environmental and ecological data are required for disease incident reporting and to inform prioritisation. (B) Ecological trends or anomalous conditions are analysed to predict disease occurrence in the field. (C) Microbiome (of healthy individuals) and pathobiome (of affected individuals) must be understood to inform screening processes and evaluate suspect agents. (D) The ecology of microorganisms (including physiological requirements) supports decision making for culture techniques. (E) Causative assignments must be cross-referenced continuously with earlier stages of the framework to understand triggers and underlying conditions for causation.

adaptation using new scientific knowledge and methods [54–56]. The reality of aquatic disease investigations usually means that only limited numbers of point samples are available (i.e., small numbers of diseased individuals with unknown histories sampled directly from the environment), making some aspects of Bradford Hill difficult to apply in their strictest sense. This is notably the case for the 'temporality' criterion, which must often be abandoned or assessed indirectly from population-level data [57]. Acknowledging the practical limitations of working with aquatic organisms, the intent should be to consider and assess available data against as many of the nine aspects of association as possible. This encompassing approach will ensure that suspect short lists are well informed via robust bodies of evidence.

Once a shortlist has been defined, **Koch's postulates** are considered pre-eminent for causal inference, having been refined over time to accommodate new medical knowledge and technologies [54,55]. While the original postulates have been adapted for new knowledge (e.g., Molecular Koch's Postulates [58], Ecological Koch's Postulates [59], Microecological Koch's Postulates [40]), the key principles remain. In combination, the postulates aim to recreate a disease state to demonstrate a direct causative link [60]. This is a robust and reliable approach to causative assignment, but it does rely on isolating and culturing the causative agent.

Methods and guidelines to inform causative inference

Implementing a structured approach to causative inference requires fit-for-purpose diagnostic methods and guidelines (Figure 3). Specific research priorities are to:

(i) forecast aquatic disease emergence to inform targeted development of new diagnostic and characterisation methods;
(ii) apply field-based research to contextualise cause and effect relationships;
(iii) establish guidelines to apply and integrate modern molecular tools and the wave of real-time environmental data for short-listing suspect infectious agents;
(iv) broaden capacity to culture marine microbes for use in causative **challenge** trials in biocontainment facilities;
(v) standardise whole-organism challenge approaches for causative linking; and
(vi) develop surrogate models, such as cell lines, for challenge trials that reduce sacrificial use of wildlife.

Forecasting aquatic disease emergence

The challenging goal of forecasting aquatic disease emergence has potential to transform biosecurity preparedness. These efforts can leverage recent advances in forecasting human disease outbreaks [61]. New approaches to epidemiological modelling are making it increasingly possible to predict the emergence and trajectory of aquatic disease events, including under climate change – for example, physical oceanographic models coupled with biological models for fish and shellfish pathogens [62]. Databases of disease incidence[v] can be exploited to parameterise models that correlate likelihood of disease emergence against a range of climatic, anthropogenic, and stochastic factors [63–65]. Models should initially aim to define taxonomic and spatial resolutions at which predictions become meaningful and reliable for aquatic systems. Even if models are reliable only at relatively high taxonomic (e.g., family level) or spatial (e.g., global eco-regions) resolution, predictions can vastly improve preparedness for disease emergence. Prioritisation of diseases to study could then be informed by the severity of similar diseases, the threat to ecosystem integrity or services, and potential financial and food security impacts. Predictions and prioritisation can inform diagnostic method development for groups of high-risk disease agents. Parameterised models with data on short-listed pathogens

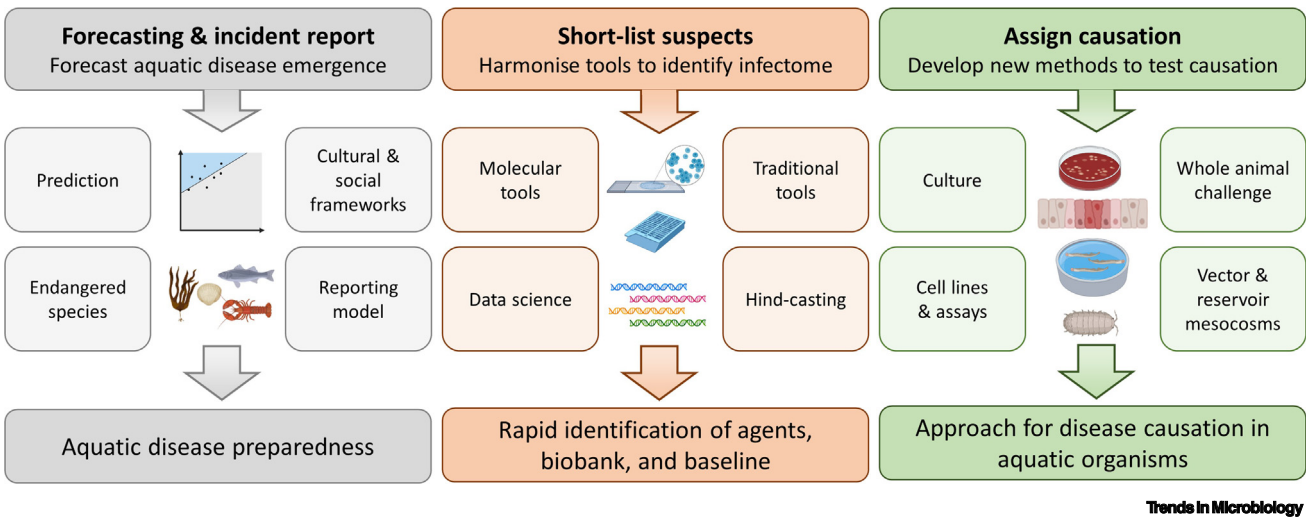

Figure 3. Key implementation pathways for approaches, methods, and tools to resolve aquatic disease causation. Traditional and emerging diagnostic methods in microbiology are central to improved preparedness, screening, and causation in aquatic disease investigations. Created with BioRender.com.

could then be used to quantify evidence for associations between pathogen prevalence and survival [66,67].

Field-based research and ecological frameworks

Effectively accounting for environmental and ecological factors is essential to implementing an effective aquatic aetiological approach [68]. As such, we advocate equal inclusion of field-based and laboratory research to inform cause-and-effect relationships, and disease impacts in wildlife and captive aquatic organisms [20]. The disease triangle theory can frame careful consideration of emerging aquatic diseases within the context of environmental stress and compromised host organisms [69]. Originally developed for understanding terrestrial plant diseases, the disease triangle conceptualises the interrelation between 'the inherent susceptibility of the host, the inoculum potential of the [agent], and the impact of the environment on […] pathogenesis'[70]. Associated factors that contribute to the onset and trajectory of a disease are the nature of the physical environment, duration of the infectious period, **pathogen** prevalence and virulence, the age (or maturity) of the host, and the host's susceptibility to disease [71]. Prioritising methods that identify underlying drivers in the environment and host susceptibility will enable more effective future aquatic disease investigations.

Guidelines to apply and integrate molecular tools with real-time environmental data

Correlative short-listing of suspects should leverage traditional diagnostic methods, modern molecular methods, and environmental hindcasting [67]. Harmonising this range of methods will provide robust bodies of evidence that encompass infectious agents (e.g., viruses, bacteria, archaea, fungi, protists, parasites) and noninfectious agents (e.g., environmental factors, harmful algae). Developing standardised guidelines is a critical step to ensure consistent and reliable data generation. This is particularly the case for modern molecular methods, which require optimised and standardised sample processing, sequencing, bioinformatics, data interpretation procedures (e.g., [72]), and potential application of artificial intelligence, such as automated diagnosis through digital image processing [15]. Traditional methods should be applied simultaneously to contextualise, ground-truth, and for subsequent isolation of short-listed suspects and rule out detections of nonclinically significant organisms [15]. Hindcasting can exploit the explosion of real-time environmental monitoring to build and validate data tools that reconstruct the environment leading up to disease incidents and identify aberrant environmental factors [67,73–75].

Broadening capacity to culture marine microbes

The ability to isolate and culture microorganisms is a major challenge for fulfilling Koch's postulates and advance research on disease causation. Isolation of species in pure culture means that they may be fully characterised for pathological properties [76,77]. Few aquatic microorganisms can be readily cultivated using standard techniques and media, and most marine microorganisms (>99%) have not been cultured [27,78,79]. However, new high-throughput methods to increase the number of culture conditions enable recovery, culture, and identification of hundreds of novel bacterial and archaeal taxa (see [77] for review). Methods to culture as-yet-uncultured aquatic microbes could target high-risk taxa identified via predictions and/or short-listing and seek to replicate unique aspects from the host/aquatic environment [80]. Alternative methods to isolate specific agents (e.g., through sucrose gradients; [81]), can enable a pure inoculum for laboratory challenge. In emerging disease situations, this approach may be inefficient and a combination of field-based traditional and molecular methods should be prioritised.

Standardise whole-organism challenge approaches

To assign causation, assays need to demonstrate causative links between exposure to a short-listed agent – or multiple agents so as not to discount polymicrobial/multifactorial diseases – and the onset of disease. Whole-organism challenge experiments in biocontainment facilities can provide tight

control of confounding variables and reliable endpoints of causation. Equivalent experiments in the field to demonstrate transmission or Koch's postulates using cultivated isolates present too serious a biosecurity threat to other organisms and humans [82]. Success requires appropriate facilities, equipment, practices, and knowledge to handle captive wildlife for infectious disease studies and an ability to mimic the aquatic environment in a laboratory setting [83]. Additional considerations include husbandry, animal welfare, veterinary care, cultural sensitivity, and biosafety to ensure scientific rigor [82,83]. More complex multifactor challenge experiments can increasingly occur in controlled laboratory environments (e.g., challenge with multiple pathogens and/or under varied temperature regimes; [84,85]). Care should be taken to recapitulate conditions in the field under which an emerging disease is most often observed to avoid falsely discounting causative agents. Additional considerations include life-stage, salinity, presence of other stressors, challenge duration, dose, and endpoints for which disease will be defined. Furthermore, vector-borne agents and reservoirs of disease in the environment, both of which are poorly understood, may play fundamental roles in aquatic disease progression [52,86,87]. Mesocosm challenge experiments that recapitulate candidate vectors and/or reservoirs alongside agent and host will be increasingly important to ensure definitive causative assignments (and possible management implications) of diverse aquatic diseases. Challenge studies that use mortality as the key endpoint can result in false negatives, especially for opportunistic or complex diseases that manifest in compromised hosts or under specific environmental conditions. Alternative measures to consider include host behaviour and pathological signs of disease combined with reisolation of the pathogen to provide a mechanistic understanding of disease development [81].

Develop surrogate models for culture-independent challenge approaches

Working with protected or difficult-to-culture host organisms is a key challenge for many aquatic disease investigations that can preclude whole-organism experiments to test Koch's postulates. Developing cell lines for aquatic hosts would enable alternate diagnostic pathways. Cell lines serve a dual purpose for disease causation research: to enable culture of intracellular infectious agents, and as high-throughput diagnostic bioassays (e.g., culture and assessment of koi herpes virus; [88]). Cell lines are particularly important as sources of biochemical products and can replace whole (host) organisms when animal ethics, cultural significance, or conservation status preclude laboratory handling – or their biology is not conducive to captivity. Cell lines from fish have been established from several aquaculture species and are being used *in vitro* for interdisciplinary research [89,90]. Developing cell lines for a broader range of aquatic host taxa and exploring the possibility for standardised cell lines susceptible to a range of taxa-specific pathogens can notably advance aquatic diagnostics [91]. In the interim, primary cultures obtained from various aquatic species and organ sources are reported with increasing frequency and may be maintained for durations ranging from 3 to 6 months [92].

Concluding remarks and future perspectives

The COVID-19 pandemic has heightened awareness of disease agents, **epidemiology**, and consequences of disease. It has also demonstrated the power of collective effort and coordinated disease research and response to build a more resilient future. It is our opinion that funding bodies and scientists engaged in aquatic disease research and management should harness this moment for a step-change advance in research on causation, ecology, and evolution of aquatic diseases.

Heightened collaboration of scientific expertise in aquatic animal health, biosecurity, microbiology (bacteriology, parasitology, and virology), ecology, epidemiology, mathematical modelling, fisheries, genomics, cell culture, aquatic animal husbandry, histopathology, and social science – alongside community, industry, and government stakeholders – can resolve critical diagnostic bottlenecks. Interconnected social frameworks can be used to translate scientific findings into lasting benefit

Outstanding questions

How do we balance structured guidance to emerging aquatic disease investigations that push beyond correlative endpoints to assign cause reliably and routinely while still allowing for stochastic factors and unprecedented events?

How do we integrate and align research-led approaches required to assign cause for emerging diseases with existing surveillance and diagnostic efforts for known diseases undertaken for routine biosecurity surveillance and by veterinary pathologists?

How can we best leverage and harmonise both traditional and modern diagnostic methods to enact effective emerging disease investigations? This includes appropriately accounting for sensitivity, specificity, masking effects, confounding results, complexity of multiple agents and microbiomes, and epidemiological progressions of individual diagnostic methods and combinations of methods.

How do we account for and overcome the unique challenges of working with host aquatic wildlife? How do we account for unknown history of individuals (i.e., 'temporality' aspect of association and naivety of organisms for challenge trials), complex disease triggers that span host, pathogen, and multidimensional environments (i.e., 'disease triangle' theory), and work with culturally sensitive or endangered species often without prior attempts to culture in captivity?

How do we integrate diagnostic efforts with communities, industries, and indigenous cultures to ensure that frameworks are in alignment with shared and conflicting values to foster collective effort to overcome emerging aquatic diseases under rapid environmental change?

for actionable conservation and fisheries management efforts (see Outstanding questions). Ultimately, this will safeguard economic and ecosystem resources to build resilience via rigorous and timely aquatic disease resolution.

Acknowledgments

This project was funded by the New Zealand Ministry of Business, Innovation and Employment, under the programmes Aquaculture Health to Maximise Productivity and Security (CAWX1707) and Emerging Aquatic Diseases: a novel diagnostic pipeline and management framework (CAWX2207).

Declaration of interests

No interests are declared.

Resources

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