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Using the Neptune project to benefit Australian aquatic animal health research

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ABSTRACT: Diseases of aquatic animals have had, and continue to have, a significant impact on aquatic animal health. In Australia, where fisheries and aquaculture are important industries, aquatic species have been subject to serious disease outbreaks, including pilchard herpesvirus, the cause of one of the largest wild fish kills ever recorded. At the same time, there is a consensus that Australia's parasite fauna are largely unknown, and that aquatic animal health information is difficult to access. Managing aquatic animal diseases is challenging because they may be entirely new, their hosts may be new to aquaculture, and specialist expertise and basic diagnostic tools may be lacking or absent. The Neptune project was created in response to these challenges, and it aims to increase awareness of aquatic animal diseases, improve disease management, and promote communication between aquatic animal health professionals in Australia. The project consists of an online database, a digital microscopy platform containing a whole-slide image library, a community space, and online communications technology. The database contains aquatic animal health information from published papers, government reports, and other sources, while the library contains slides of key diseases both endemic and exotic to Australia. These assets make Neptune a powerful resource for researchers, students, and biosecurity officials.

KEY WORDS: Biosecurity · Australia · Aquatic animal health · Database

INTRODUCTION

Fish and shellfish represent an important source of nutrition for the world's population (Tacon & Metian 2009). In 2010, 16.7% of all animal protein consumed by the global population was derived from fish and fisheries products. The demand for aquatic animal products is continuing to rise (FAO 2014b), and is expected to be met by a rapidly growing and globalised aquaculture industry. The growth of aquaculture has been extraordinary, resulting in application of the term 'blue revolution'—a comparison to the green revolution that dramatically increased agricultural yields in the mid 20th century (Coull 1993). Aquaculture production of animals for human consumption more than quadrupled between 1990 and 2012 to more than 66 million tonnes (FAO 2014b).

The growth of aquaculture has been characterised by rapid domestication of hundreds of new aquatic animal species, intercontinental translocation of species, intensification, and new production technologies and systems. These characteristics have contributed to the emergence of diseases that are new to science and to the occurrence of panzootics. Fish and fishery products are also highly traded commodities, with more than one-third of production being traded internationally. This is significantly higher than terrestrial livestock products (10% traded internationally), and increases the risk of disease spread (FAO 2014a). Diseases of aquatic animals have already taken a significant financial toll on aquaculture—

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Stentiford et al. (2012) estimate that the top 5 crustacean viral diseases alone cost 1.5 billion USD in losses annually. As aquaculture continues to grow and is increasingly relied on as a source of nutrition, the potential consequences of disease outbreaks become greater. This is also important in Australia, where the gross value of aquaculture production reached 1.1 billion AUD in 2012 (Skirtun et al. 2013).

Australian fisheries and aquaculture are characterised by a diversity of environments (tropical to temperate), and subsequently a diversity of species, including prawns, abalone, oysters, tuna, salmonids, and barramundi. This means that Australia needs to maintain expertise across a range of cultured aquatic animal species, many of which have experienced significant disease outbreaks. These include amoebic gill disease in Atlantic salmon in Tasmania (Munday et al. 2001), QX disease in oysters in New South Wales and Queensland (Adlard & Wesche 2005), abalone viral ganglioneuritis in abalone in south-eastern Australia (Hooper et al. 2007), and ostreid herpesvirus in Pacific oysters in New South Wales (Jenkins et al. 2013). After experiencing severe epizootics of herpesvirus in pilchards, which cost over 20 million AUD and resulted in one of the largest fish kills ever recorded (Whittington et al. 2005), Australia introduced a national strategic plan to improve Australia's systems for managing aquatic animal health (Bernoth et al. 2008). Although management systems have improved and scientific knowledge of aquatic animal disease has grown, new diseases continue to emerge and present significant challenges. For example, while many parasite species have been described, this represents a minority of the total species that exist (Adlard & O'Donoghue 1998, Whittington 1998, Cribb et al. 2014), and 'many more pathogenic organisms remain to be discovered' in Australia (Jones & Creeper 2006, p. 237).

In addition to the lack of understanding of many aquatic animal diseases, existing information has often remained inaccessible. This is due to several factors: large volumes of valuable data remain unpublished or are being kept as individual or local collections; there is a reliance on physical collections, such as microscope slides, that are not publicly accessible (except for those registered in state museum collections); and there is a lack of a unified platform for data sharing. Australia's aquatic animal health professionals have previously agreed that data and resources needed to be more broadly accessible to manage disease risks effectively (e.g. AQUAPLAN 2005–2010). In addition, the need for expertise across a range of production sectors, regional concentration of expertise, and the vast distances between animal health experts acted as drivers for the formation of the Australian Biosecurity Intelligence Network (ABIN), a national research infrastructure initiative.

The Neptune project was created within the ABIN initiative to enhance aquatic animal biosecurity in Australia by providing a forum for connecting aquatic animal health professionals and the considerable, but disseminated, resources that inform disease management and mitigation. While this project was focused on aquatic animal health in Australia, we are convinced that many other national jurisdictions suffer the same lack of cohesive accessibility of resources. As such, we believe the structure of Neptune may serve as a useful model for others.

DATABASE CONSTRUCTION

The concept of a national aquatic animal disease information repository had been considered in Australia for some time, and one was developed through AQUAPLAN 2005–2010. ABIN provided an opportunity to build the capabilities of the initial database so that it could better meet user needs. With that goal in mind, Neptune began as a 'proof of concept' project funded by ABIN in Canberra, Australia. The overall aim of the project was to enhance the availability and exchange of aquatic animal health information in Australia. Four online resources were created to help achieve this objective: a database, a digital microscopy platform containing a whole-slide image library, a community space, and online communications technology.

The Neptune database was created in 2011 and 2012 by Biosecurity Queensland staff in Brisbane, Australia, and IT professionals at ABIN. The purpose of the database is to store detailed information about aquatic animal health 'events', defined as scientific papers, government reports, or other data; a simplified representation of the schema can be seen in Fig. 1. The most general information, such as the title of the publication, is stored in the 'event' level, and author and citation information are stored in the 'investigation' level. The 'location' level includes habitat type and latitude/longitude data, and 'host group' includes host species and host syndrome (see below). Parasite/pathogen species, known as disease agents, are listed in 'test result', along with disease diagnosis. Finally, the affected host organ and any associated morphological changes are stored in 'test findings' (Fig. 1). Many additional fields exist that have not been mentioned for brevity. The Neptune

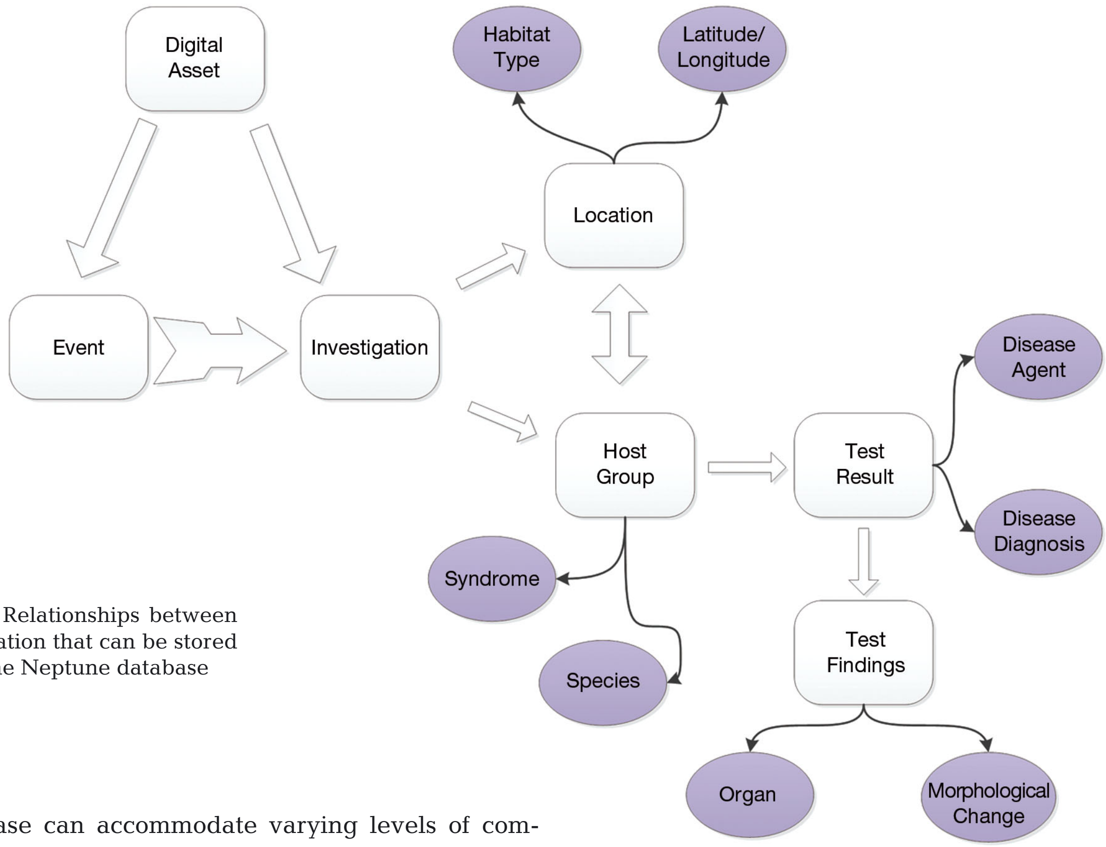

Fig. 1. Relationships between information that can be stored in the Neptune database

database can accommodate varying levels of complexity because information is stored in multiple levels. For example, multiple test results can be recorded for one host group, and multiple locations can be recorded for one host group.

A search is performed by entering criteria into 20 possible fields (Table 1) on the Neptune search page (http://www.abin.org.au). Different fields can be selected depending on the nature of the enquiry, e.g. host, pathogen, disease, location, et cetera. These fields exist either as free text or pick lists. An example of the syndrome pick list is shown in Fig. 2. Many of the pick lists were created as a collaborative effort between Neptune staff and terrestrial health experts from the Taronga Zoo in New South Wales, which promotes compatibility in data standards. For example, the taxonomic list in Neptune, which functions as the pick list for all host and agent species, currently contains 4045 species from aquatic and terrestrial taxa, with additional species being added on an ongoing basis.

Once criteria have been entered and a search is performed, the events that satisfy the query can then be viewed individually, or the information from all of the events can be exported into a Microsoft Excel spreadsheet. Computer files such as photographs, PDFs, or short videos can also be added to events as digital assets (Fig. 1). For example, photographs of fish with epizootic ulcerative syndrome have been added to a Neptune event (Fig. 3). These assets represent additional learning and teaching tools, and can be downloaded by Neptune users.

Table 1. List of fields available on the Neptune database search page. Fields are either free text or pick lists

Field	Format
Host species	Pick list
Disease agent	Pick list
Disease diagnosis	Pick list
Host morphological change	Pick list
Host syndrome	Pick list
Host organ	Pick list
Event number	Free text
Event name	Free text
Author	Free text
Author's organisation	Pick list
Location	Free text
Start date	Free text
End date	Free text
Event overview	Free text
Validation comment	Free text
Event status	Pick list
Event type	Pick list
Validation status	Pick list
Diagnostic test used	Pick list
Test remarks	Free text

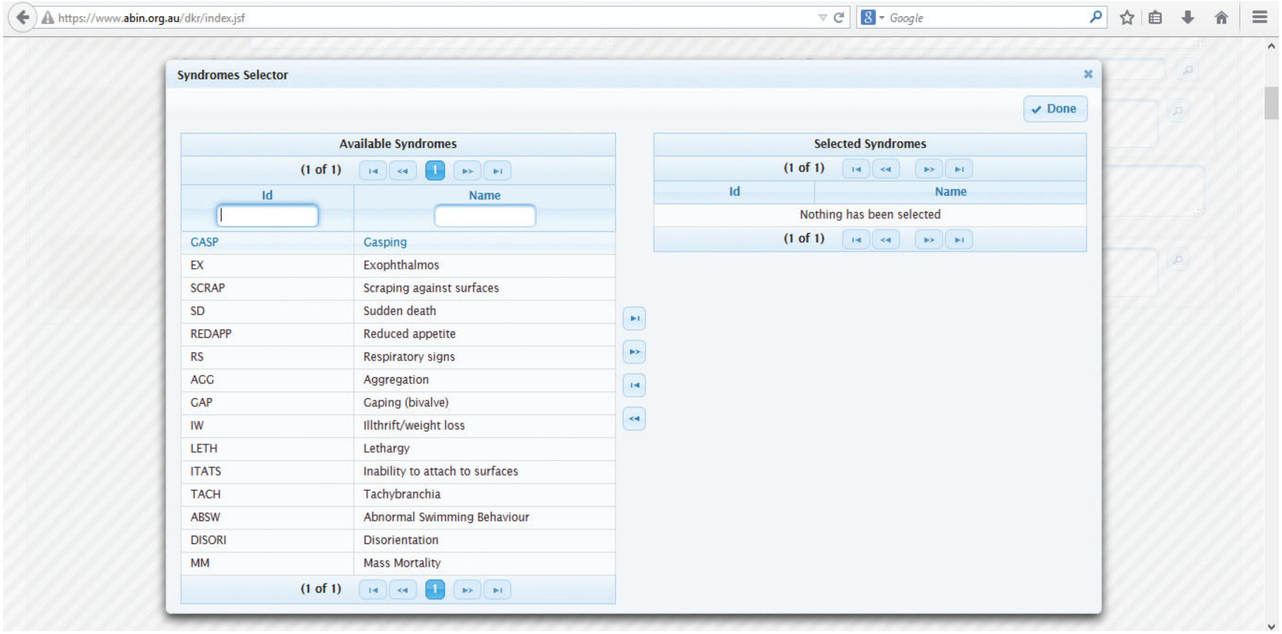

Fig. 2. The syndrome pick list in the Neptune database

Work on the whole-slide image library was initiated in 2012. Histopathology slides of key endemic and exotic parasites and diseases were collected from institutions around Australia, including the University of Tasmania, the Department of Fisheries in Western Australia, the Australian Animal Health Laboratory in Victoria, and the Tasmanian Department of Primary Industries and Water. Slides were scanned at up to 40× magnification by the Royal College of Pathologists of Australasia Quality Assurance Programs in Sydney. The Neptune slide library is on a platform hosted

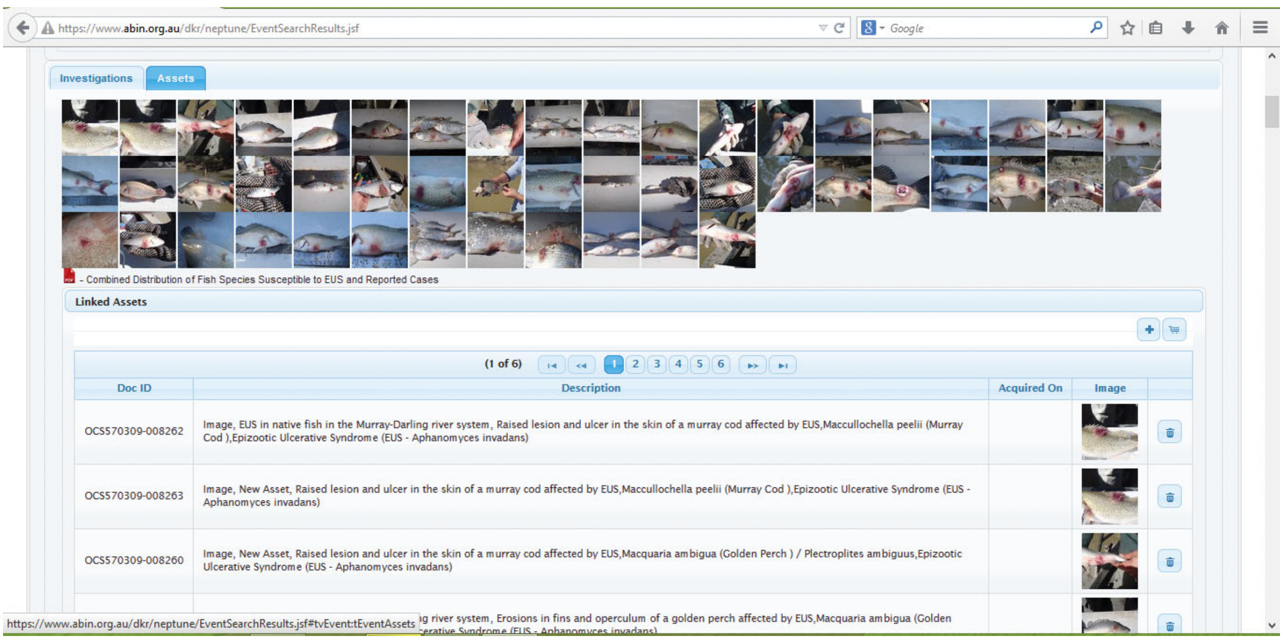

Fig. 3. An example of digital assets that have been saved with an event. These photographs show fish from the Murray-Darling river system in Australia that have been affected by epizootic ulcerative syndrome. Photographs were contributed by the Department of Primary Industries New South Wales

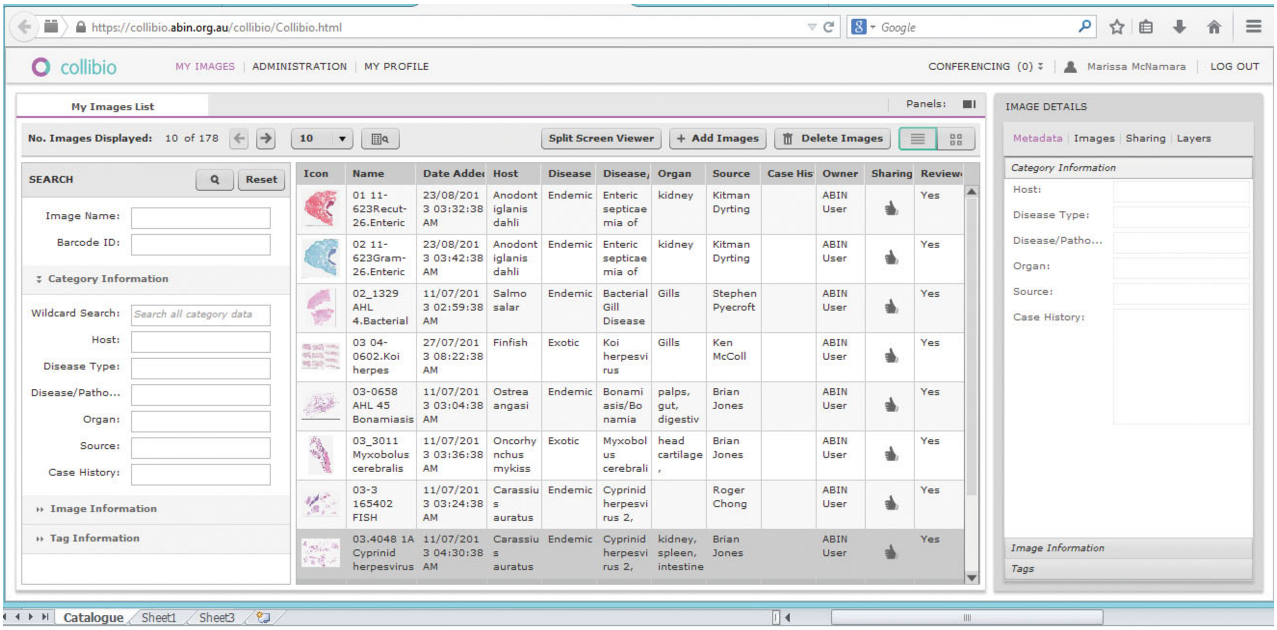

Fig. 4. The Neptune whole-slide image library

by Collibio, and currently contains 180 whole-slide images. Users who access the library start on a search page that contains general information and a thumbnail image of each slide (Fig. 4), from which individual slides can be viewed. The library is intended primarily for learning and teaching purposes, and is not meant to be used as a stand-alone tool for disease diagnosis.

A community space and online meeting technology are also part of the Neptune project. The former allows users from across Australia to post questions and comments and discuss issues, and the latter provides videoconferencing capabilities, including online conference rooms with 'whiteboards', where documents and other files can be uploaded and shared.

DATABASE ACCESS

Several user privileges have been implemented to improve security. First, all users must be assigned a profile before they can log in. The 4 profiles are viewer, submitter, validator, and administrator. Viewers can see events, but cannot change anything; submitters can add new information, but cannot delete anything; validators can add or delete information; and administrators can add or delete information, and edit pick lists. Validators and administrators can also validate events, as explained below.

The validation system represents another security measure. When a submitter adds new information to the database, by default it is 'unvalidated'. This means that it is not yet visible to other viewers or submitters, who constitute the majority of Neptune users. When this information has been reviewed and approved by a validator or administrator, its status changes to 'validated' and this information is then visible to all users who search the database. This ensures that information is reviewed by an expert before it goes live.

One final security measure merits attention. Submitters, validators, and administrators who add new information to the database can control its visibility to other users via a 'white list'. When a new event is added, the default visibility is public, meaning that all users can view it (once it has been validated). However, a submitter who is entering a new event can change the visibility, and select only particular users from the white list to be able to view their material.

The Neptune database, slide library, community space and online meeting space can be accessed at https://www.abin.org.au by clicking on 'ABIN Applications' on the bottom of the page. A username and password can be requested by emailing the contact listed on the 'Contact' section of the website. Access to Neptune is currently restricted to users within Australia, but international access may be granted in the future.

POTENTIAL DATABASE USAGES

Due to the variety of sources it can contain, the detailed information and assets that can be stored within events, the Excel export function, and the whole-slide image library, the Neptune database is a powerful online resource. It can facilitate aquatic animal health surveillance and research in ways that are either faster than or not available from a search engine such as Web of Science. Several examples are discussed below.

Example 1: the challenge of emerging diseases

New aquatic animal diseases present challenges to aquaculture industries and governments because the stakes may be high, but response actions must be taken in knowledge-poor situations. A recent example is acute hepatopancreatic necrosis disease (AHPND), caused by a strain of *Vibrio parahaemolyticus* carrying a toxin-encoding plasmid (Tran et al. 2013), which has resulted in massive declines to shrimp aquaculture in Asia. The disease was officially reported from The People's Republic of China and Vietnam in 2010, Malaysia in 2011, Thailand in 2012, and Mexico in 2013 (OIE 2013). Thailand provides an example of the impacts from AHPND: a 2014 report found that shrimp production experienced declines of 30–70% (FAO 2014b). Investigations into the disease have involved cooperation between governments, intergovernmental organisations, research organisations, and industry. The disease was initially characterised using disease signs and histopathology (NACA 2012). Research focused on identifying the causative agent and on epidemiological investigations to determine risk factors (FAO 2013). In 2013, the causative agent was identified, permitting the development of rapid and sensitive diagnostic tests (Tran et al. 2013). Neptune provides an ideal platform for investigation of new diseases such as AHPND by a distributed network of researchers. Neptune could be used to capture whole-slide digital images of case material to refine case definitions and enhance surveillance. Collaboration between histopathologists could occur in real time using Neptune's web conferencing platform. Epidemiological information could be shared and analysed collaboratively to improve understanding of risk factors. Data required for the development of diagnostic tests — such as PCR primer sequences — could be shared and the performance of assays in different laboratories collated to contribute to test validation.

Example 2: historical misidentification

Proliferative gill disease was first reported in 1981 in Mississippi (Bowser & Conroy 1985). It causes severe gill inflammation and death in channel catfish, and remains a disease concern in the southeastern USA (Mitchell et al. 1998). However, archived information indicates that the disease was probably first detected in the 1970s, but misidentified at the time (Overstreet 1990). This historical misidentification of an agent has probably occurred for other disease investigations, and may be difficult to detect. However, a researcher using the Neptune database could detect such potential errors by using only the syndrome field, which lists disease signs. For example, Bowser & Conroy (1985) list lethargy and respiratory distress as clinical signs of proliferative gill disease, and a search of the database for these signs could assist with differential diagnosis (Fig. 2). Therefore, a researcher with a suspicion that a disease may have occurred in a particular location in the past could select the relevant syndromes and search by them alone. Neptune's ability to store assets such as disease photographs is also useful in this context because images of gross pathology could assist with differential diagnosis.

Example 3: unpublished material

Unpublished material exists in a variety of forms, including case reports and surveys, and can often be useful in disease investigations. For example, Lightner et al. (1992) studied unpublished case histories from the University of Arizona to document the spread of the infectious hypodermal and haematopoietic necrosis virus in prawns in Mexico.

Unpublished material can be critical for researchers trying to detect a disease at equilibrium, as prevalences may be low (Jones 2001). For example, Hine & Thorne (1998) documented a haplosporidian in 6 of 105 pearl oyster (*Pinctada maxima*) spat from Western Australia, but this parasite was not detected in another survey of 4500 oysters (Humphrey, Norton et al. 1998). Similarly, Jones & Creeper (2006) found an unidentified protistan parasite in pearl oysters in Western Australia with a prevalence of less than 0.005%. The existence of such rare parasites is doubly hard to find if it is only informally documented; Jones (2001, p. 37) notes that 'although isolated mortalities may be known locally, such mortalities can go unreported.' Because the Neptune database is intended to store information from a variety of sources, including unpublished material, this type of information could be entered into Neptune and accessed by users. In addition, unpublished material can be stored under various event types, including 'survey' and 'mortality'. The 'mortality' event type can be especially useful because it does not require the pathogen identity to be recorded; in an examination of aquatic disease risk, Gaughan (2001) stresses the importance of historical data and asserts that case histories of epizootics should be used even when pathogen identity is unknown. The 'mortality' event label thus provides a convenient way to store information about these cases.

Example 4: compartmentalised science

Pathogens do not exist in discrete boxes, and can infect hosts from a variety of environments and taxa. However, researchers from different scientific disciplines may sometimes fail to connect. In some instances, communication breakdown has significant consequences, such as the case of West Nile virus in the USA, in which the identification of the virus in New York was impeded by a lack of understanding between veterinarians and health professionals (Tsai et al. 2009). In fact, the multidisciplinary One Health movement was created in recognition of the links between human and animal health, and the need for more collaboration between sectors (AVMA 2008). Aquatic animal diseases and parasites can only reinforce the need for this movement, as they can have surprising or unexpected connections; for example, a disease outbreak in tilapia in Australia was most likely caused by Bohle iridovirus (Ariel & Owens 1997), a ranavirus that is normally found in Australian amphibians (Cullen & Owens 2002), and this virus was also found to cause experimental mortalities in barramundi (Moody & Owens 1994). Such a broad host range is indicative of host switching, and phylogenetic analysis has shown evidence of ranavirus transitions between piscine hosts, reptiles, and amphibians via positive selection (Abrams et al. 2013).

The Neptune database is structured to help users recognise potential cases of host switching, and other unusual disease connections, due to the diversity of species it contains. Neptune has the capacity to store information about terrestrial species that may serve as intermediate or reservoir hosts, or disease vectors, because the host list in Neptune contains aquatic and terrestrial taxa, including species from more than 60 classes of vertebrates, invertebrates, and microorganisms. This ability allows Neptune users to add and connect information from potentially separate research areas.

The interconnectedness of humans and the emergence of the global economy have been responsible for many disease outbreaks as aquatic animals and their products are translocated within and between countries. The scientific literature abounds with examples of pathogens and parasites that have been introduced to new hosts and/or new locations with disastrous results (Adlard et al. 2015). However, this smaller world has a powerful upside: the interconnectedness of data. As a repository for these data, which are increasing in number every year, the Neptune project represents an impressive resource that can help researchers meet the challenge of emerging diseases to support the rapid growth of aquaculture. Neptune provides a tool for the capture of information so that investments in research and development are more fully realised. Furthermore, Neptune provides easily accessible opportunity for training the new cohort of aquatic animal health professionals as well as providing a vault that ensures knowledge is captured when key researchers retire.

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