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Minireview

Transboundary movement of shrimp viruses in crustaceans and their products: A special risk?

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ABSTRACT

Shrimp and shrimp products form the most valuable internationally traded fisheries commodity, and the volumes are huge, estimated to be about 3.6 million tonnes. However, despite the existence under the General Agreement on Tariffs and Trade, of the Sanitary and Phytosanitary Measures (SPS Agreement) and the activities of the World Organisation for Animal Health (OIE), viral shrimp epizootics have spread and continue to spread, affecting world production. Though most attention has focussed on the movement of live shrimp product, the spread of new and emerging diseases through other crustaceans and their nonviable products is of increasing concern. The risks associated with the unrestricted movement of nonviable product will be outlined and measures that can be taken to mitigate the risk are discussed. Ultimately, for crustacean diseases, the paradigm under which the OIE has operated for the past 80 years needs to change.

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1. Introduction

The transboundary movement of shrimp is a major world trade activity since shrimp are the most valuable internationally traded fisheries commodity (Anderson, 2003). A 2008 report by the FAO estimated that the world wild-capture and aquaculture sectors produce a combined 6 million tonnes of shrimp product, of which about 60% is traded on the world market and of which 95% of the aquaculture product comes from Asia (Gillett, 2008; Walker and Winton, 2010). Shrimp has been America's major seafood item, in terms of per-capita consumption, since 2001 when it surpassed canned tuna. In 2009, imports amount to some 230 000 tonnes (FAO Globefish, 2009). It is clear that consumer demand for safe

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seafood will grow driven by the general population increase as well as demand for the health benefits of seafoods.

A major concern for shrimp production has been the series of viral diseases that continue to severely affect production of shrimp, world wide. There are currently over 20 pathogenic viruses of shrimps described (Biosecurity Australia, 2009), and the list is still growing. Lightner (2003) estimated that the costs, to 2001, of White Spot Syndrome Virus (WSSV), Taura Syndrome Virus (TSV), Yellowhead Virus (YHV) and Infectious Hypodermal and Hematopoietic Necrosis Virus (IHHNV) exceeded \$US 7 billion. Briggs et al. (2005) estimated that disease losses due to shrimp viruses probably exceeded \$US 1 billion per year. Such losses are continuing, for in June 2011 there were newspaper reports of a new shrimp epizootic of unknown aetiology causing losses estimated at US\$48 million in Vietnam (Vietnam News, June 10, 2011). While the economic impact of these viruses on aquaculture can be readily documented, their biological and economic effect on

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the wild capture fisheries to which they have spread, is almost impossible to determine (Lightner, 1996). Viruses undoubtedly affect the natural mortality of populations and thus impact on estimates of "F", the fishing mortality, but the impacts are difficult to measure and are often dismissed due to the "old school of thought" that significant epizootics rarely if ever occur in wild populations (Anon, 1995). The question then is how do we increase shrimp production, global trade and at the same time mitigate the impacts of disease on production.

Transfer of viruses due to international trade in live aquaculture animals has been well documented as a major underlying reason for the major epizootics including WSSV, IHHNV, TSV, YHV and more recently white-tail disease of Macrobrachium (Lightner, 1996; Lightner et al., 1997; Sahul Hameed et al., 2004; Bonami et al., 2005). However, not all of the spread can be explained by movements of live shrimp for aquaculture. WSSV is known to experimentally or naturally infect (give positive PCR results) for about 90 species of arthropod, including crabs, lobsters, copepods and insect larvae as well as polychaete worms (Vijayan et al., 2005) and rotifers (Yan et al., 2007). Only decapod crustaceans appear to support replicating virus but the ability for the virus to spread through transmission in non-replicating hosts is still unclear. Fortunately, results for the crustacean brine shrimp Artemia salina, a common food source in shrimp hatcheries, suggests that they do not play a role in the transmission of viruses (WSSV and HPV) (Chang et al., 2002; Li et al., 2003; Zhang et al., 2010). Other shrimp viruses are probably more host specific than WSSV, however, most survive freezing and increasingly concern has been expressed at the potential for fresh processed and frozen crustacean products to contribute to the spread (Nunan et al., 1998; Durand et al., 2000; McColl et al., 2004; Reddy et al., 2011).

The international movement of animals and their products is governed by the General Agreement on Tariffs and Trade (GATT), and the associated Sanitary and Phytosanitary Measures (SPS Agreement). Responsibility for the technical issues associated with application of the SPS agreement is vested in the World Organisation for Animal Health (OIE). The OIE itself came into existence because of transboundary movements of diseased animals. In 1920, rinderpest occurred unexpectedly in Belgium, as a result of zebus, originating from India and destined for Brazil, transiting via the port of Antwerp. By 1924, twenty-eight States had obtained an "international agreement" creating the Office International des Epizooties (OIE) based in Paris, and the International Committee of the Office held its first General Session in 1927. The basis for the agreement was the principle "only sanitary documents emanating from nations with correctly organised veterinary services can be considered as providing importers with sufficient guarantees" (OIE, 2011).

Unfortunately, while shrimp and crustacean products fall under the mandate of the OIE, invertebrate fisheries biologists and pathologists have historically had little interaction with veterinarians and that has been compounded in many countries where fisheries management is administratively separate from the terrestrial agricultural departments that report to the OIE. That has made it difficult to detect and report new and emerging diseases in a timely manner, for example, Koi herpesvirus was identified in 1998 (Pokorova et al., 2005) but was only listed as reportable by the OIE in May 2006 – some 8 years after the first outbreaks, and listing was far too late to prevent the global spread of the disease.

Secondly, while the major production diseases are known for most terrestrial production animals, which are generally mammals or birds that have been domesticated for centuries, crustaceans in general and shrimp in particular have only been farmed intensively since the early 1970s and are still an unknown quantity, not only in terms of their diseases but also their physiology including their immunology. The first report of the occurrence of a virus in

Crustacea was in a crab, *Macropipus depurator* in 1966 (Vago, 1966). Viral diseases are often overlooked when the mortalities and production losses start to occur, providing a window of opportunity for unintentional spread of disease. An example of this is Taura syndrome, which was first described in Ecuador in 1992 and thought to be a disease resulting from agricultural pollution. By the time a viral aetiology was demonstrated, in 1995, the disease had spread through South America (Chamberlain, 1994; Hasson et al., 1995; Brock, 1997).

Finally, shrimp, and perhaps crabs appear to be unusual among aquatic invertebrates in the number and variety of viruses that they carry. Lobsters, for example, are known to host only one virus (Shields and Behringer, 2004). It is probably fair to say that there are no virus free shrimp populations in the wild, and individual animals often carry more than one virus type (Madhavi et al., 2002; Flegel et al., 2004; Natividad et al., 2006: Umesha et al., 2008). The reason that crustaceans and insects, which lack an acquired immune mechanism should do this is unknown, but it has been hypothesised that "sequestering" or "accommodation" of virus may be linked to the existence of an as yet unknown mechanism allowing for specific memory of pathogens and the dampening of viral triggered apoptosis in response (Flegel, 2007). Whatever the reason, translocation of shrimp is a high risk activity with respect to transfer of disease, particularly viral diseases.

2. The risk from shrimp products - is it real?

Apart from the direct movement of infected crustaceans from one region to another for aquaculture, which is the most common means of virus spread (Subasinghe and Bondad-Reantaso, 2008) there have been a number of outbreaks of shrimp diseases or shrimp viruses in other crustaceans, where a direct translocation of live animals cannot be demonstrated. This has raised concerns that viruses are being spread through shrimp and crustacean products or processing wastes. Examples include the outbreak of WSSV in Darwin, Australia in 2000 (East et al., 2004) and the 2009 outbreak of WSSV in Louisiana crawfish ponds (Baumgartner et al., 2009). A number of studies have shown that frozen shrimp contain viable virus and that the virus can be transmitted to other crustaceans through feeding frozen infected product (Nunan et al., 1998; Durand et al., 2000; McColl et al., 2004; Reddy et al., 2011). Apart from the direct use of frozen product as aquarium/farm feed, other identified pathways of infection include: discarded waste from crustacean processing plants; use as bait and berley. Industrial sabotage is also a possibility, often overlooked in risk assessments.

The use of frozen crustacean product included in natural food maturation diets for crustacean broodstock is a problem. Though recognised as a disease risk, and banned in some countries, such diets are considered important for successful post-larval production (Wouters et al., 2001; Coman et al., 2007). This route may have been the source of WSSV in crayfish held at the Washington Zoo (Lightner, 1996) and was the cause of the WSSV outbreak in Darwin, Australia in 2000 (East et al., 2004).

Processing plants, particularly those that use imported product and which then discharge into the environment, have been implicated in the spread of WSSV and YHV in Texas (Lightner et al., 1997) and into crawfish (*Procambarus clarkii* and *P. zonangulus*) ponds in Louisiana (Baumgartner et al., 2009).

The use of product for bait and berley has been identified as a risk pathway although it is extremely difficult to prove that a disease has entered a wild capture fishery by this means. However, diversion of shrimp packaged for human consumption as well as the sale of small shrimp (bait shrimp) is common (Biosecurity Australia, 2009).

Movement of shrimp viruses via transmission through wild stocks is also common. The introduction of IHHNV into stocks in the Gulf of Mexico in the 1980s was followed by epizootics in aquaculture farms in Mexico in 1990 (Lightner, 1996). Taura became established in wild stocks in the Gulf of Fonseca off Honduras and El Salvador and from there via wild caught broodstock to Florida (Lightner, 1996). Wild fish stocks can become infected and suffer high mortalities through the release of pathogens in untreated hatchery effluent or through the stocking or escape of diseased fish into aquatic systems. In Australia, there is a zoogeographic barrier at the Torres Strait. Shrimp stocks to the west of the Torres Strait have a distinctive parasite fauna (Owens, 1990). Historically, Gill Associated Virus (GAV) does not occur on the western side of the Australian continent (Jones, 2004), however, the recent detection of GAV in wild shrimp fisheries in the Joseph Bonaparte Gulf is believed to be due to the escape of infected shrimp from Northern Territory shrimp farms that obtained their post-larvae from Queensland (Humphrey pers. com.). As a result, Western Australia prevents shrimp farmers from sourcing broodstock shrimps from

Industrial sabotage becomes a very real possibility when many of the known exotic shrimp viruses are available at the local supermarket (Ueda et al., 2008), there are commercial pressures for sabotage and it is a simple matter for someone to throw frozen product into a farm, thus establishing an infection. That remains a possible but unprovable scenario for the detection of IHHNV in some shrimp farms in Australia. Testing of Australian shrimp failed to detect the presence of Philippine strain IHHNV, though the noninfectious integrated or Madagascar strain, had been confirmed (Krabsetsve et al., 2004; Tang and Lightner, 2006). During the period 1992-2007, Queensland shrimps were exposed to intensive study, resulting in the discovery of a number of novel viruses (but not infectious IHHNV). The draft import risk assessment reflected this understanding and proposed that imports should be subject to testing for IHHNV (Biosecurity Australia, 2007). This was a contentious decision. In early 2008 allegations were made to the federal government that Australian farmed shrimp were infected with IHHNV. Subsequent testing proved that that was the case and that the shrimp from the infected farms in Queensland had a 94-99% similarity to infectious Asian strains of IHHNV (OIE Ref: 7166, 11 July 2008; Saksmerprome et al., 2010). As a result, the requirements for import testing were relaxed, removing the requirement to test for IHHNV (Biosecurity Australia, 2008).

The presence of viable infective pathogen is not sufficient, of itself, to establish an infection. A susceptible host has to become infected, and then the infection has to spread within the new population. That is not a simple issue, and is very hard to quantify. There is a high probability that crustacean tissues used as bait and berley will be eaten by non-crustacean hosts, and that even if the tissue is eaten by a crustacean, that the dose will be insufficient to establish an infection or that the crustacean will become ill and in turn be eaten by a non-crustacean, thus breaking the cycle. As "r-selected species", shrimp are prone to predation, exhibiting high mortality rates through their life cycle with instantaneous mortality rates of up to 94% (Biosecurity Australia, 2009). It is intriguing that when WSSV was discovered in the Darwin aquarium facility in Australia in 2000, the initial surveys of wild crab populations at the outlet of the facility tested positive for WSSV by PCR but that subsequent testing was negative (East et al., 2004). Were the original test results simply false positives (which are possible with crabs (pers. obs.; Claydon et al., 2004) or did WSSV simply fail to establish? It is also known that the immunology of crustaceans is strongly influenced by environmental conditions including temperature and that this too can affect the outcome of an infection (Le Moullac and Haffer, 2000; Rahman et al., 2006).

3. Potential mitigation measures

It is usual when performing a risk assessment to establish the hazard and then to assess the risk that it poses. That approach works reasonably well with recognised pathogens, but it is very difficult to assess the risk of an "unknown disease" (Murray and Peeler, 2005) and it is highly likely that any attempt to restrict trade based on an unknown risk would be challenged.

As was done in the Australian risk assessment (Biosecurity Australia, 2009), there are a range of measures that can be implemented against identified risks posed by named pathogens. The problem is that new diseases are emerging all the time and conventional risk assessment soon become obsolete, or risk never being completed at all (the Australian risk assessments for live mollusks and for freshwater crayfish products both began in 1996 and have still not been completed). In addition, because frozen product is freely available on supermarket shelves, it is highly probable that the unknown diseases will emerge and be traded before the OIE notification process can lumber into action.

Assuming the risk of introduction, establishment and spread requires management, what can be done? Sourcing from virus free stocks is not an option because, outside of a high health hatchery, it would seem that all shrimps carry viruses. Likewise pathogen inactivation is not an option because shrimp tissues do not survive well through the processes required to inactivate all of the potential viruses. It is interesting that the effectiveness of chilling, freezing, cooking and high pressure treatment are very poorly researched and documented in the literature yet the information about these steps is critical to assessing the "risk". For example, Sritunyalucksana et al. (2010) showed that YHV injected into shrimp which were then processed, frozen and fed to naive shrimp resulted in a much reduced ("negligible") transmission risk. However, Reddy et al., (2011) found that frozen Indian P. monodon, (whether whole or headless and peeled) PCR positive for WSSV caused 100% mortality in naive shrimp.

Measures which may reduce the risk, including the risk posed by unknown viruses, centre on codes of practice, packaging that avoids diversion to high risk activities (pack size, presence of marinades, and little waste), public education (labelling, point of sale education) and introduction and enforcement of end-use regulations such as prohibitions on use as bait. The question which is, to date, unanswered is who is going to pay for such consumer oriented education programs, and should the education materials be developed and coordinated at an international level? The consequences, when we spread a new disease can be severe and affect the whole distribution chain, not just the producer.

4. Conclusions

In order to further increase both the quantity and quality of the shrimp being grown, the international community needs to have much more transparency over emerging disease issues and a willingness of jurisdictions to act to manage emerging risks without waiting for "disease listing" by the OIE. At present the OIE requires notification (aquatic code article 1.1.3.5) that "for diseases not listed by the OIE, if there is a case of an emerging disease or pathogenic agent should there be findings that are of epidemiological significance to other countries". Unfortunately, by the time "epidemiological significance" is recognised, the disease has long spread. It is sobering to consider that none of the mollusc diseases listed by the OIE were recognised as "diseases of epidemiological significance" until they were translocated and created trouble in their new environment, often in a novel host.

It is also problematic that the OIE requires notification of the presence of the pathogen, not necessarily the disease, but all of the six crustacean viruses currently notifiable to the OIE may be freely purchased, conveniently frozen to $-20\,^{\circ}\text{C}$, at supermarkets in so-called disease free zones. This issue needs to be addressed.

This is not just an issue over disease. Movement of aquatic organisms either deliberately, for aquaculture or fisheries enhancement, or by accident including through ballast water and hull fouling affects biodiversity. As a result, the International Council for the Exploration of the Sea (ICES) adopted a Code of Practice in 1979 in an attempt to reduce the risks of adverse effects of introductions and transfers of marine organisms. The Code was updated in 2005 (ICES, 2005). While requiring that the OIE protocols be consulted, the Code has a much stricter requirement, recommending that "Only progeny of the introduced species may be transplanted into the natural environment, provided that: (1) a risk assessment indicates that the likelihood of negative genetic and environmental impacts is minimal: (2) no disease agents, parasites, or other nontarget species become evident in the progeny to be transplanted: and (3) no unacceptable economic impact is to be expected (ICES, 2005). This recommendation that no disease agents be transferred is also reflected in the 1992 Convention on Biological diversity, Article 8(h) that requires signatories to: "Prevent the introduction of, control or eradicate those alien species which threaten ecosystems habitats or species" (UN, 1992); "Alien species" of course can include parasites. While these codes refer to live organisms the principle is equally applicable to shrimp product carrying live alien species (i.e., viruses).

Perhaps the OIE needs to increase the rigor of their reporting requirements such that all new and emerging diseases causing, or with the potential to cause, significant mortalities should be tabled and made available to trading partners. While that requires more work it allows countries to respond to new threats in a reasonable timeframe without having to rely on media reports of emerging diseases.

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