Polychaetes as Potential Risks for Shrimp Pathogen Transmission

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Abstract

Polychaetes comprise the benthic meiofauna of the soft-bottom intertidal zone and of shrimp ponds in coastal areas. While polychaetes provide benefits to the shrimp farming industry as (i) natural food in traditional shrimp ponds, (ii) nutrient regenerators through bioturbation and removal of organic waste in the sediment through feeding, and (iii) feed supplement to enhance maturation of shrimp brooders, the conditions present in aquaculture ponds may increase the opportunity for polychaetes to transfer pathogens to shrimp through the food chain. There is growing concern that internationally traded polychaetes, which are fed to shrimp brooders, are potential vectors for the transmission of other shrimp pathogens. The detection of the aetiological agents of two newly emerging diseases of shrimp in polychaetes, Enterocytozoon hepatopenaei (EHP) and Vibrio parahaemolyticus causing hepatopancreatic microsporidiosis (HPM) and acute hepatopancreatic necrosis disease (AHPND), respectively, suggests that these worms can be a host or passive carrier of these pathogens. This review discusses the benefits of polychaetes to shrimp farming, the risk of shrimp pathogen transmission by polychaetes at the pond, hatchery and global level, and calls for closer observation on shrimp pathogens in polychaetes used as shrimp feed.

Keywords: AHPND, EHP, pathogen transmission, risk, polychaetes, shrimp, WSSV

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Introduction

Polychaetes are ubiquitous benthic-meiofauna in shrimp ponds and the soft sediments of coastal areas. As a group, polychaetes provide ecological services for the sediment environment through bioturbation and the removal of organic wastes during feeding (Brown et al. 2011) and by being prey for animals at higher trophic levels such as shrimp, fish and birds (Hutchings 1998). Eutrophication of the pond bottom during shrimp culture results in hypoxia and entrapment of inorganic phosphorus, nitrogen and organic matter in the sediment. Polychaetes are beneficial for the pond bottom environment by recycling nutrients, making these partially available to primary producers and consumers in the pond, and by reducing the anaerobic area at the sediment-water interface through movement. Certain species of polychaete are traded at the global level as favoured baits for anglers (Arias et al. 2013, Carregosa et al. 2014) and as supplemental feed to shrimp brooders to improve spawning performance and enhance maturation (Leelatanawit et al. 2014). Polychaetes are highly adapted to a broad range of environmental conditions (Çinar 2013) and have remarkable reproductive plasticity and adaptability (Arias et al. 2013). The latter may assist polychaetes to colonize new areas (Çinar 2013) and to thrive in ponds and estuaries which are rich in organic matter.

Sediment may act as a sink to pathogens, assisting in their survival, hence becoming a pathogen reservoir or resource. Research on the link between the presence of white-spot syndrome virus (WSSV) in the sediment, benthic polychaetes and WSSV infection in shrimp (Vijayan et al. 2005; Desrina et al. 2013; Haryadi et al. 2015) sheds some light on the different facets of the role of polychaetes in shrimp farming. Burrower and detritofeeder polychaetes live in shrimp production systems such as ponds – including coastal areas receiving effluents from the ponds – where they are exposed to and potentially acquire pathogens present in the sediment. In the case of WSSV, the port of entry of the pathogen into polychaetes is most likely per os, and the worm in turn transfers the pathogen to shrimp after being fed upon. These findings raised interest in the possible role of polychaetes in the spread of two newly emerging shrimp diseases, acute hepatopancreatic necrosis disease (AHPND) caused by the bacterium *Vibrio parahaemolyticus* (VP_AHPND) and hepatopancreatic microsporidiosis (HPM) caused by the microsporidian *Enterocytozoon hepatopenaei* (EHP) (Thitamadee et al. 2016). The pathogenic form of *V. parahaemolyticus* carries a plasmid encoding two toxins, PirA and PirB, which, when expressed, are responsible for the disease in shrimp (Lee et al. 2015; Han et al. 2015). Here we not only review the benefits of polychaetes for shrimp culture, but also discuss the potential risks for further spread of pathogens based on experiences gained from studying WSSV infection in the polychaete *Dendronereis* spp., and finally suggest enhanced surveillance for shrimp pathogens in polychaetes used for shrimp feed as a starting point to mitigate or control the disease.
Overview of the Biology and Ecology of Polychaetes Relevant to Shrimp Culture

Polychaetes form a class of segmented worms in the Phylum Annelida; are highly varied in shape, size, and reproduction strategy; and occupy a variety of ecological niches (Hutchings 1998). Many polychaete species are ubiquitous macro-invertebrates in coastal habitats including rocky coastal areas and soft-bottom estuaries (Sarkar et al. 2005). They are considered opportunistic species, as these animals are the first to inhabit defaunated soft sediments with high organic matter (Kanaya 2014). The ability of polychaetes to inhabit estuaries shows their high adaptability and environmental plasticity, because estuaries and coastal areas are highly dynamic ecosystems. Naturally, polychaetes living under these conditions in estuaries have high tolerance to a broad range of salinities (8–19 ppt in water and 0–2.5 ppt in soil) (Roy and Nandi 2012), high concentrations of organic matter and to pollution. Since most shrimp ponds are built in estuarine areas, it is expected that polychaetes will be abundant in shrimp ponds and in most cases, will become the dominant benthic invertebrate species (Fujioka et al. 2007; Ngqulana et al. 2010). The ability of polychaetes to live in various ecological niches and benthic conditions has resulted in their broad geographical spread by accidental and intentional transportation. The distribution and abundance of polychaetes are affected by sediment conditions, including texture (Sarkar et al. 2005), organic content (Gowda et al. 2009), water depth, salinity, temperature (Hutchings 1998) and predation (Abu Hena et al. 2011).

Aquaculture activities produce a bulk of organic wastes that may cause the accumulation of nutrient-rich sediment and patches of hypoxial areas on the pond bottom. While shrimp avoid pond areas having low dissolved oxygen content, in contrast, polychaetes live (some even thrive) under such conditions. Polychaetes provide ecological services for the pond environment and for animal life in it through movement and transport processes, feeding activity and by being prey for animals at a higher trophic level. Errant polychaetes such as the nereids move horizontally and vertically for foraging and burrowing, causing considerable bioturbation. The mixing of sediments and pore water in the sediment-water interface during bioturbation facilitates the degradation of organic matter in a density-dependent manner (Kristensen et al. 1985; Papaspyrou et al. 2010). Polychaetes ventilate their burrows (Kristensen 1984), thereby stimulating metabolism of aerobic microbes in the sediment. This helps to restore the living area for cultured shrimp by increasing the availability of nutrients while reducing anaerobic conditions (Hutchings 1998). Since polychaete burrows can reach 30 cm depth below the sediment surface, burrower polychaetes may also help in recirculating some nutrients that may accumulate during shrimp culture. This is very relevant for traditional ponds where sediment removal is limited and spaced in time. Our observations on two burrower nereidids (Dendronereis spp. and Hediste diversicolor) is that they stay in the burrow with a vertical or horizontal orientation and that they move actively to grab their food and pull it into the burrow.
The feeding activity of polychaetes facilitates the removal of organic matter from the sediment while the nutrients are utilized for polychaete development, hence assisting in nutrient recycling in the sediment. Two species, *Nereis virens* and *N. diversicolor*, showed the ability to metabolize the nitrogenous faecal waste of clams (Batista et al. 2003a) and faecal and feed waste of halibut (Brown et al. 2011). These studies indicate that polychaetes can be a solution for aquaculture waste management, thus promoting sustainable aquaculture. Most polychaetes that live in the soft sediment are suspension and deposit feeders (Hutchings 1998), although the mouth of some worms, for instance nereidid polychaetes, is equipped with cuticular structures called jaws. Accordingly, the feeding strategy of nereidid species can change in accordance with the types of food available. For example, wild *N. diversicolor* living in estuaries mainly feed on a mucous complex containing organic matter, bacteria and fungi (Fidalgo e Costa et al. 2006), although they also feed on sediment and predate other nereids. The flexibility in feeding strategy and the ability to live under oxygen-poor conditions in organic-matter-rich sediments make polychaetes such as *N. diversicolor* (Batista et al. 2003b) and *N. virens* suitable bottom scavenger species in integrated multitrophic aquaculture (IMTA) systems (Brown et al. 2011; Van Geest et al. 2014). Large detritofeeder polychaetes such as eunicids can increase recycling of proteinaceous waste produced from aquaculture activities by enhancing enzymatic degradation (Santander-De Leon et al. 2010). Although no studies have been done on the impact of polychaetes on nutrient cycling in shrimp ponds, integrated production systems of shrimp and polychaetes in ponds should be explored as a means to raise nutrient utilization efficiencies. On the other hand, filter and detritus-feeder polychaetes are exposed to pathogens that are present in the sediment and can acquire the pathogens through feeding, as in the case of WSSV (Vijayan et al. 2005). They can be passive or active vectors of pathogens, or both, as is probably the case for WSSV. In turn, polychaetes, potentially carrying pathogens, are preyed upon by shrimp (Nunes and Parsons 2000). At present, information on the contribution of infected polychaetes to the transmission of shrimp diseases is limited, and more research is required.

**Polychaetes as the Natural Feed of Shrimp in Grow-Out ponds**

Three genera of polychaete have been reported as feed of shrimp in ponds and hatcheries: *Dendronereis* (Haryadi et al. 2015), *Perinereis* (Poltana et al. 2007; Meunpol et al. 2010; Leelatanawit et al. 2014) and *Marphyssa* (Vijayan et al. 2005). Members of these genera are burrowers and have a broad geographical distribution (Hutchings and Karageorgopoulos 2003; Glasby and Hutchings 2010; Ngqulanana et al. 2010). *Perinereis* spp. prefer sandy sediment, whereas *Marphyssa* spp. and *Dendronereis* spp. are mostly abundant in soft and muddy sediments. The species of polychaete found in shrimp ponds may vary from one area or region to another, but also depends on the environment and the pond management. Polychaetes and other benthic invertebrates are important components of the shrimp diet in traditional shrimp ponds. Shrimp inherently rely on natural food in the pond, and polychaetes having a high protein and fatty acid content are an attractive food source. Polychaetes occur in ponds rearing giant tiger prawn (*Penaeus monodon* Fabricius 1798) all the time (Abu Hena et al. 2011).
However, the density of polychaetes tends to decrease towards the end of the shrimp-culture period (Nunes and Parsons 2000; Abu Hena et al. 2011), due to predation, as shown by gut content analysis of *P. monodon* reported by Varadharajan and Soundarapandian (2013). At low shrimp density, the predatory pressure on benthic prey is low (Balasubramanian et al. 2004), which may explain the abundance of polychaetes in traditional extensive ponds and throughout the culture time, as we observed in our own study, albeit that the dominant species may vary (Desrina 2014). Although no systematic studies have been done on the growth and weight gain of grow-out shrimp resulting from feeding on polychaetes in Indonesia, during interviews with the first author, farmers indicated that shrimp grow faster and are healthier when shrimp ponds contain lots of *Dendronereis* spp. Furthermore, in laboratory observations, shrimp show a high preference for polychaetes relative to formulated diet, and this preference might reflect the situation in ponds (Desrina 2014).

Being a benthic invertebrate, the well-being of polychaetes is also determined by pond bottom condition. The bottom of a traditionally managed pond is likely to be a selective environment for polychaetes because of the high concentration of organic matter. Many ponds are not completely dried after harvest and sediments are seldom completely removed. Nevertheless, even when the incoming water is first passed through a settlement pond, sediment accumulation in the shrimp pond will remain substantial. Accretion of organic matter-rich sediment over a 5–10 year period can result in a 30 cm thick semisolid layer of dark, highly reduced, sediment. Some polychaetes are adapted to this condition, having the ability to live in a low-oxygen niche and to feed on organic waste (Brown et al. 2011). The *Dendronereis* spp. we observed were most abundant in sediment having a soil organic carbon concentration between 5–10 % (Desrina 2014), a condition unfavourable for most other benthic organisms.

**Polychaetes as Natural Feed of Shrimp Brooders**

Feeding fresh feed to shrimp brooders is widely practiced in shrimp hatcheries. Because they are highly nutritious, polychaetes as a group are an important component to promote spawning performance of shrimp broodstock (Chung et al. 2011). Polychaetes contain high levels of unsaturated fatty acids such as arachidonic acid (Hoa et al. 2009; Leelatanawit et al. 2014) and the reproduction hormones progesterone (P4), 17α-hydroxyprogesterone (Meunpol et al. 2007) and prostaglandin E2 (Meunpol et al. 2010), which enhance gonad maturation of female and male shrimp brooders. Polychaetes reported to be used in hatcheries are the mud worm *Marphyssa* spp. (Vijayan et al. 2005) and the sandworm *Perinereis* spp. (Poltana et al. 2007). In addition, *P. cultrifera* has been investigated for boosting the reproductive performance of captive sole (Cardinaletti et al. 2009), indicating an increased interest in exploring the use of polychaetes not only to condition shrimp brooders but also for fish broodstock. Although polychaetes alone are nutritious enough to ensure good reproductive performance, studies on combining polychaetes and immunostimulant sodium alginates produced even better results in terms of the amount of eggs produced by spawners, total larval production and hatching rate of *P. monodon* as compared to polychaetes alone (Chung et al. 2011).
This shows that the reliance on polychaetes and other fresh feed may be reduced by using supplements that compliment the nutrition provided by the polychaetes. In our laboratory, shrimp prefer live polychaetes, although they also eat frozen ones. Freezing and thawing can result in loss of body fluid that leaks during the process and loss of odor, hence, making them less attractive to shrimp. Most companies advertise nereid polychaetes in the form of freeze-dried or frozen material. The commercial use of polychaetes as a replacement for fishmeal in the shrimp feed has been initiated.

**Polychaetes as Potential Risk for Shrimp Disease Transmission**

So far, only three species of polychaete have been reported to carry natural infections of shrimp pathogens: WSSV in *Marphyssa* spp. (Vijayan et al. 2005), *P. nuntia* (Supak Laoaroon et al. 2005) and *Dendronereis* spp. (Desrina et al. 2013; Haryadi et al. 2015). WSSV transmission from polychaete to shrimp was reported only for *Dendronereis* spp. and *Marphyssa* spp. However, *Dendronereis* spp. is a replicative host for WSSV (Desrina et al. 2013), while *Marphyssa* spp. appears to be only a passive vector (Vijayan et al. 2005). Recently, the DNA of two newly emerging pathogens of shrimp, EHP and VP<sub>AHPND</sub>, was detected in imported polychaetes in Thailand using polymerase chain reaction (PCR). These worms were suspected as the route of entry of the AHPND agent into shrimp hatcheries in Thailand (Thitamadee et al. 2016), indicating that polychaetes may act as hosts, carriers or vectors that play a role in the spread of these pathogens at the global level. However, there is no information about the species of polychaete involved or the body parts that were positive for the AHPND aetiologic agent. Further systematic studies are needed to verify the role of polychaetes in the epidemiology of AHPND and EHP. The niche, feeding strategies and position of polychaetes in the food chain accentuate the risk of shrimp pathogen transmission by polychaetes, although the pathway may be different for grow-out ponds and hatcheries.

There are several reasons why polychaetes pose a potential risk as vectors, carriers and/or hosts of shrimp pathogens in the pond environment. First, polychaetes permanently reside in burrows in the pond sediment, and hence this provides opportunities for polychaete and shrimp-pathogen encounters over an extended period of time. For generalist pathogens, continuous exposure to potential hosts is an important factor driving pathogen adaptation and broadening of the host range (Woolhouse et al. 2001), which could be the situation for WSSV and VP<sub>AHPND</sub>, since both are multihost pathogens. Further, polychaetes' contact with the pathogen will become more intense during a disease outbreak when the pathogens are more abundant in the pond.

Second, the niche and feeding guild of polychaetes facilitate the acquisition of pathogens which settle in the sediment. Ponds act as sediment and organic matter traps during shrimp production. High concentrations of DNA of viral pathogens to humans, terrestrial animals (Staggemeier et al. 2015) and fish (Honjo et al. 2012) in pond sediment indicates that sediments can be a sink or reservoir for pathogens. Furthermore, sediment may provide a suitable niche for persistence of viruses, prokaryotes and parasites.
For example, WSSV retained its viability and infectivity in the sediment for 35 days (Satheesh Kumar et al. 2013), presenting opportunities for the pathogen to enter susceptible benthic inhabitants (hosts and/or vectors), such as polychaetes. Although there are as yet no reports of the presence of EHP spores in the pond sediment, the observation that faeces of shrimp suffering from white faeces syndrome (WFS) contained EHP spores (Rajendran et al. 2016; Tang et al. 2016) suggests that the spores may sink and reside in the sediment. Likewise, *V. parahaemolyticus* is ubiquitous in the sediment (Darshanee Ruwandeepika et al. 2012). For a generalist pathogen, sediment-associated pathogen transfer through the food chain increases the survival of the pathogen in the environment and its maintenance in the pond environment through transfer in the food chain. As filter feeders and detritofeeders, polychaetes may acquire pathogens present in the sediment, as reported for WSSV (Vijayan et al. 2005), although it is not known how long it took to replicate in the polychaete. Moreover, our observations with *Dendronereis* spp. indicate that this polychaete can carry a heavy infection of WSSV without showing any behavioural or gross external signs, suggesting that viral-host adaptation may exist (Haryadi et al. 2015). In the laboratory, *Dendronereis* spp. and *Hediste diversicolor* fed on formulated shrimp feed use their jaws to grab food and drag it into their burrows. However, by microscopic examination we also found sand and soil in the respective guts, suggesting that they also eat detritus. If we consider the condition in the pond, these worms can also feed on infected shrimp carcasses; thus, polychaetes may ingest pathogens directly from diseased shrimp.

Third, burrowing polychaetes can possibly avoid the chemicals used to control pathogens and pests in ponds by retracting into their burrows, allowing pathogens to survive within their host. For example, we detected WSSV with 1-step PCR from some *Dendronereis* spp. obtained from the pond bottom at up to 30 cm depth and from *Marphyssa* spp. from up to 40 cm depth, following chemical treatment to eradicate the virus after an outbreak. Oral transmission through the food chain and cohabitation are the two most important situations for shrimp pathogen transmission. Taken together, polychaetes can contribute to the epidemiology of diseases in the shrimp pond.

World shrimp production is projected to increase and as a consequence, the demands for broodstock will also increase, and thus the demand for polychaetes as an ingredient in broodstock diets. For this reason, polychaetes traded globally may pose a biosecurity risk in shrimp hatcheries. Some hatcheries raise their own polychaetes to prevent disease transmission to their facility. However, it is quite often that the polychaete production is not enough to meet the demand, forcing the company or local farm to rely on polychaetes collected from the wild. Often, polychaetes fed to brooders in hatcheries are collected from the estuary adjacent to the farm. In turn, surface waters adjacent to shrimp farms receive farming effluents, establishing a type of permanent contamination loop. Further, it is common for wild polychaete populations to have co-infection of more than one pathogen with different host exploitation and transmission strategies, or with one species of pathogen with different genotypes (Ben-Ami et al. 2011). For example, some of the *Dendronereis* spp. we examined had WSSV and haplosporidian cysts in the body cavity. This makes reliance on polychaetes captured in the wild an even more risky venture.
AHPND is caused by a strain of *V. parahaemolyticus* that has acquired at some point a specific plasmid carrying toxin genes (PirA and PirB). As the plasmid is transmissible by horizontal gene transfer and *V. parahaemolyticus* is ubiquitous in the brackishwater environment, the presence of the AHPND agent in polychaetes should not be surprising. We isolated sucrose and non-sucrose fermenting *Vibrio* from the coelomic fluid of healthy looking *Dendronereis* spp., and this suggests that this bacterium might be a normal inhabitant in the polychaetes. The ecology and biology of EHP is largely unknown, including whether shrimp is the sole host to this parasite, or whether there are secondary hosts among meiobenthic animals such as polychaetes and bivalves. It is even not clear if EHP is dependent on an animal host at all. Nevertheless, the detection of toxin-containing plasmid DNA in polychaetes (Thitamadee et al. 2016) strongly suggests that the pathogenic agent or parts of it have been in close contact with polychaetes.

Findings from a previous study on WSSV in *Dendronereis* spp. (Desrina et al. 2013) showed that the polychaete *Dendronereis* spp. with WSSV was widely distributed in Indonesia and that this polychaete can harbour the virus without notable signs of disease, such as white spots under the epidermis or sluggishness. Also, the occurrence of WSSV in *Dendronereis* spp. correlated positively to the WSSV infection in shrimp (Desrina 2014). We may infer, somewhat tentatively, that a similar situation may be applicable to AHPND and EHP, considering the ubiquity of *V. parahaemolyticus* in shrimp culture operations and the plasticity of the plasmid and the nature of Microsporidia. The occurrence and growth characteristics of the disease agents of AHPND and HPM in polychaetes need further investigation, and as do the species and source of the polychaete(s). Most importantly, transmission studies to show that the disease is indeed transmitted from polychaetes to healthy shrimp are needed. This information is important to determine the strategy and method of control. For example, if polychaetes are only a mechanical vector, then depuration for 48 hr until the gut is cleansed may be applicable.

Having said all this, there are potential vectors of pathogens in the pond environment other than polychaetes, such as crabs and crayfish. However, they are not permanent residents in ponds and can move to neighboring shrimp ponds, increasing the risk of horizontal transmission.

**Conclusion**

Our current knowledge on the involvement of polychaetes in shrimp pathogen transmission is limited by: (i) the few studies that have been conducted, (ii) the scant knowledge of the life history of the pathogen (especially in the case of AHPND and HPM), (iii) the absence of knowledge on the defense responses of polychaetes important for shrimp farming, (iv) the biology and ecology of polychaetes in ponds, and (v) the distribution of pathogens in polychaete host tissue(s). When reporting pathogen occurrence, it is advisable to identify the polychaetes to the lowest taxonomic level possible, because polychaetes form a large class of annelid worms and species susceptibility to a given shrimp pathogen may thus vary.
However, the mere presence of the pathogen DNA in the polychaete does not prove that: (i) the whole pathogen is alive, it might just be an inert residue, (ii) the pathogen develops in the polychaete, or (iii) that the pathogen will be transferred to the shrimp and cause disease. Experiments need to be carried out to investigate these issues. It may be concluded that the ecological niche in pond settings and the feeding habits of polychaetes allow these animals to acquire shrimp pathogens and transmit them to shrimp upon feeding. However, this cycle may start with an unnatural abundance of the pathogen in the environment (e.g. due to incomplete, inappropriate or inaccurate pond cleaning), resulting in the accumulation of the pathogen in the polychaete, or that the pathogen is undergoing (epi)genetic changes adapting to the polychaetes. Further studies on the epidemiology of shrimp pathogens and the role of polychaetes and pond management strategies in influencing this multifaceted interaction are needed. Since HPM and AHPND are caused by otherwise normal inhabitants of the pond, control measures may include sound shrimp health management (e.g. better management practices, BMPs), crop rotation to break the pathogen cycle and lowering stocking density. Excluding polychaetes altogether from the pond environment might be a way forward to lower the risk of shrimp pathogen transmission by polychaetes, but is unrealistic.

Nevertheless, the way forward is the rigorous screening of polychaetes used as feed for shrimp for the presence of pathogens, more specifically WSSV, VP_{AHPND} and EHP, as is currently being done for shrimp. Specific nested PCR tests are available and in place to detect these pathogens, for WSSV since 1995 and for VP_{AHPND} (Flegel and Lo 2014; Sirikharin et al. 2015) and EHP (Tangprasittipap et al. 2013) since 2013 and 2014, respectively. Even differential PCRs are available to differentiate pathogenic and benign strains of \textit{V. parahaemolyticus} (Sirikharin et al. 2015). It is also important to check the resident polychaetes in shrimp ponds for the presence of these pathogens, in particular for VP_{AHPND}, as this bacterium can also multiply outside a host. Early detection and monitoring are the first steps in mitigation or control of pathogens such as WSSV, APHND and EHP in shrimp ponds.

In summary, polychaetes have been "under the radar" for quite some time as vectors of shrimp pathogens and are often not part of biosecurity regimens and regulatory frameworks. However, recently there is increased interest in polychaetes, not in the least because important pathogens such as WSSV, APHND and EHP are found in and possibly transmitted by these organisms. The lack of fundamental insight into the biology of polychaetes, their behaviour and vectorial competence in ponds, as well as the lack of hygiene in polychaete-producing farms requires the increased attention of scientists, practitioners and regulators in filling in this void. Hopefully this review is an incentive and encouragement for such a venture.

References


