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Occurrence of parasites and *Vibrio* spp. in adult Oriental angelwing clam, *Pholas orientalis*

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Abstract. The study was conducted to determine the parasites and total counts of presumptive *Vibrio* spp. in the organs and tissues of Oriental angelwing clam, *Pholas orientalis*. Three types of parasites were recovered from the internal organs of the clam, namely ancistrocomids, unidentified circular ciliates and unidentified ciliates with short stalk. *Ancistrocoma*-like ciliates were numerous in the stomach but not in other organs of the clam. Dense populations of the unidentified circular ciliates were recovered from the gills, while the unidentified ciliates with short stalk were prevalent in low numbers in the stomach. Presumptive Vibrios were also obtained from the stomach of the clam ranging 5.07×10^4 – 3.30×10^7 colony forming units (CFU) per gram of tissue. No external manifestation of a disease was observed in all the clams that were sampled despite the presence of these parasites and *Vibrio* spp. However, there is a need to identify these parasites and to determine the *Vibrio* species that are associated with the internal organs of angelwing clam in order to ascertain whether these organisms are pathogenic to the host or not as well as if these associated organisms pose a health hazard to humans during consumption of these shellfish.
Key Words: aquaculture, bivalves, pathogens, *Pholas orientalis.*

Rezumat. Acest studiu s-a orientat spre determinarea paraziților și a numărului total de posibile specii de *Vibrio* prezente în organele și țesuturile scoicii aripă de înger orientală *Pholas orientalis*. Trei tipuri de paraziți au fost descoperite în organele interne ale scoicii: ancistrocomide, ciliate circulare neidentificate și ciliate circulare neidentificate, cu o codiță scurtă. Ciliatele de tipul *Ancistrocoma* au fost numeroase în stomacul bivalvelor dar absente în alte organe. Populații dense de ciliate circulare neidentificate au fost prezente în branhii, în timp ce ciliatele circulare cu codiță scurtă au fost predominante, în număr relativ redus, în stomac. Prezumtivele specii de *Vibrio* au fost obținute din stomac și au variat între 5.07×10^4 – 3.30×10^7 unități formatoare de colonii per gram de țesut. În ciuda prezenței acestor paraziți și a speciilor de *Vibrio* nicio manifestare externă de boală nu a fost observată la bivalvele analizate. Cu toate acestea este necesară identificarea fiecărui tip de parazit și a speciilor de *Vibrio* asociate organelor bivalvelor cu scopul de a stabili dacă aceste organisme sunt patogene pentru gazdă sau nu, precum și dacă aceste organisme asociate reprezintă un risc pentru om în urma consumului acestor bivalve.
Cuvinte cheie: acvacultură, bivalve, patogeni, *Pholas orientalis.*

Introduction. The angelwing clam, *Pholas orientalis*, locally known as "diwal", is the only endemic species of angelwing clam in the Philippines, particularly in the Western Visayan provinces of Negros Occidental, Iloilo, Capiz and Aklan. Several other species of angelwing clam are found in different parts of the world, including *Barnea dilatata*, *B. manillensis*, *Martesia striata*, *Cytopleura costata*, *Pholadidea penita* and many others. In Southeast Asia, Oriental angelwing clam is known in different local terms. In Hongkong it is called "haw chung", in Malaysia it is called "siput selat batu", in Thailand it is called "hoypim" (Laureta & Marasigan 2000). Normally its shell appears to be cylindrical and elongated in shape, and its strong foot is used to burrow in the muddy sand (Fortes 1991).

This clam has great potential in aquaculture, more than any other species of shellfish, primarily because of its white and tender meat. It was abundant throughout the Western Visayas in the past, however its population declined because of overexploitation of the clam in the wild, destructive fishing, diseases, predators, siltation and pollution (Ronquillo & Mckinley 2006).

Studies of the biology and management of angelwing clam are already available (Fortes 1991; Laureta & Marasigan 2000; Golez et al 2011) and these studies documented the aspects of the reproductive biology, ecology and stock assessment of this bivalve species. Certain aspects that are related to the physiology of this bivalve have also been explored (Tizon et al 2012, 2013). Despite of these studies done on angelwing clam, there are still other aspects on the biology of this bivalve that need to be explored, including health management and diseases.

Romalde and Barja (2006) assert that almost all molluscs, including the angelwing clam, are associated with high loads of bacteria and parasites and the repetitive mortality rates in larvae and post-larvae stages may lead to massive decline in production and serious economic consequences. Even though different water treatments and other chemotherapeutic techniques (*e.g.*, probiotics) have been applied, these methods have been proven to insufficiently eliminate cases of heavy mortality. However, these methods are still the most valid and used for many years because of the scarcity of the systematic and in-depth knowledge on the bacterial population associated with the shellfish, even though those chemotherapeutic methods are not promising.

Angelwing clam larvae are oftentimes associated with a type of ciliates that clings to their body whether they are in the wild or reared in the hatchery. This might be a causative agent for the mortality of the angelwing clam. However, there is a need to consider other parasitic organisms that are possibly associated with bivalves. On the other hand, *Vibrio* spp. are also known to be highly pathogenic to the larvae of bivalves and Genard et al (2013) have already described a species of *Vibrio* that is pathogenic to the larval stage of oysters. Through the years, the knowledge on the pathogenicity of *Vibrio* species in different marine animals has improved significantly, however, less is known about the occurrence of *Vibrio* spp. in angelwing clam. Also, there is a need to determine the different kinds of parasites that are in close contact with angelwing clam in order obtain sufficient information whether these associated parasites and bacteria are pathogenic to the shellfish. Hence, in this study, the parasites and presumptive *Vibrio* spp. that are associated with angelwing clam were identified and counted in order to provide information on the possible pathogens that are associated with the host.

Materials and Methods

Collection of samples. Three angelwing clams were used during a sampling done in September, 2011 and another three clams were sampled in November, 2011. The clams were obtained from Kalibo, Aklan, in central Philippines. The next twenty samples were obtained from San Enrique, Negros Occidental, in February, 2012. The samples were kept in a plastic box of filtered seawater for not more than a day before the examination. Prior to examination for the presence of parasites, the samples were not fed to avoid discrepancies in the result. The organisms were weighed individually and observed visually for the presence of parasites.

Parasitological Examination. Tissues were obtained from each body part of the organism, including gills, gonads, mantle, stomach and siphon. The tissues were then transferred on a glass slide and a drop of seawater was added. The slides were observed under an inverted microscope or photomicrographed. The parasites were identified following the illustrations of Halton et al (2001). The parasites from the tissues were fixed with Lugol's solution and photographed or drawn for referential purposes.

Enumeration of Vibrio species. Each of the ten angelwing samples was weighed and measured. The stomach content was aseptically removed from each clam and homogenized using a glass homogenizer. The samples were then serially diluted using sterile seawater and 100 μl from each dilution were plated onto thiocitrate bile salts (TCBS) agar to allow selective growth of *Vibrio* spp. The agar plates were incubated for 48 h at room temperature (25-28^{o}C) and the presumptive *Vibrio* colonies (both green

and yellow colonies) were counted. The number of presumptive Vibrios was expressed as colony forming units per gram of tissue samples (CFU gram^{-1}).

Results and Discussion. Table 1 shows the parasites found in oriental angelwing clam. Three kinds of parasites were recovered from the gills and stomach of the clam and all of them were ciliates and free-moving. *Trichodina*-like parasites were found and were closely attached to the gill lamellae of angelwing clam. However, it is not fully verified whether these parasites are related to trichodinids because the parasites moved erratically in a sideward direction, which was in contrast to *Trichodina*, which moves in a rotating manner. The cilia of these parasites that are found in large numbers in the gills were very pronounced.

Table 1

Types and abundance of parasites from various organs and tissues of angelwing clam

Parasite	Gills	Gonads	Mantle	Stomach	Siphon
1. Ancistrocomid	-	-	-	++	-
2. Unidentified circular Ciliate	+++	-	-	-	-
3. Unidentified ciliate with short stalk	-	-	-	+	-

Prevalence:
- : Not recovered
+ : Scarce
++ : Numerous
+++ : Too numerous to count

Two types of free-moving ciliates were found inside the stomach of angelwing clam. One is an unidentified ciliate with a short stalk and a noticeable placement of the cilia in the body. The other type is a thigmotrichous ciliate, which is similar to *Ancistrocoma sp*. Both of them were very motile and were not attached or embedded in either the inner tissue or epithelium of the stomach of the clam. Sniezko (1970) indicated that almost all ciliates that are associated with the gills of shellfish may exhibit both parasitic and commensal behavior towards the host. These ciliates utilize the gill lamellae as a substrate for attachment and therefore feed on materials that are found at the substrate (Kozloff 1946). These commensals become parasitic if they are present in large numbers and cause irritation and discomfort to the tissues of the host. The probable reason why these ciliates are mostly present in the gills is that tissues of the gills are rich sources of food supply brought about by the large amount of water that passes through this organ. Hence, the environment is favorable for the ciliates to thrive where they can obtain sufficient amount of food. Although the gills contain large number of these ciliates, no apparent symptoms of disease were observed. Additional studies are needed to determine whether these ciliates are just commensals to angelwing clam or if the tissues of the gills are resistant to the action of these ciliates. Because these ciliates were found in adult angelwing, it is interesting to ascertain whether these commensals are pathogenic to the larvae; thus this particular area of study will be focused in the future.

In the stomach of angelwing clam, ancistrocomid-like commensals as well as ciliates with short stalk were found. The identification was based on the description of ancistrocomids, which are elongated, pyriform, with pointed anterior end and possess contractile suctional tentacle for the attachment to the host (Sniezko 1970). Rows of cilia occur at the dorsolateral and ventral sides. However, to confirm the identity of the ancistrocomid that was found in this study, further taxonomical techniques are needed, such as the determination of the pattern and number of ciliary rows (Kozloff 1946).

The thigmotrichous genus of *Ancistrocoma* is very common and proven to be highly parasitic to bivalves (Sniezko 1970). They are mostly found on gills and other body surfaces of the shellfish and sometimes they invade the tissues of the host. In this study, *Ancistrocoma*-like commensals were only found in the stomach and not in other tissues or organs of the angelwing clam.

The presence of these ancistrocomids in angelwing clam may open many possibilities to find solution to the high mortality rates of angelwing larvae in the hatcheries. Based on observations, these thigmotrichs do not result in infection among adult angelwing clams, but this group of parasites may be one of the causative agents for the high mortality of larvae in the hatcheries. Further studies must be conducted to confirm this matter, such as the mode of transmission, pathogenicity, the species of the ancistrocomids, degree of presence in the larvae of angelwing clams and many others. The photographs of the various parasites obtained from the different tissues and organs of angelwing clam are shown in Figure 1.

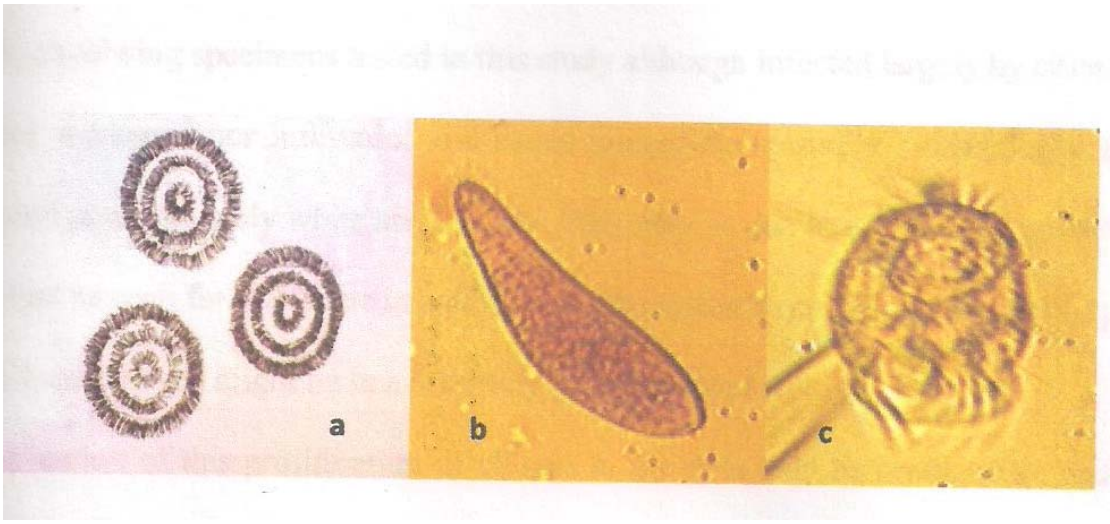

Figure 1. Photographs of parasites recovered from the tissues and organs of angelwing clam. (A) drawn image of an unidentified circular ciliate, (B) Ancistrocomid-like ciliate and (C) unidentified ciliate with short stalk.

The population of the presumptive *Vibrio* spp in the stomach of angelwing clam was also determined. The genus *Vibrio* is a member of the family Vibrionaceae, a Gram-negative and luminous bacterium. They are mostly frequent in the marine environment where water temperature is relatively high. Pathogenic *Vibrios* are fatal to many marine vertebrates and invertebrates, and have serious effects on the aquaculture industry. In the present study, the total counts of the presumptive *Vibrios* that were obtained from the stomach of angelwing clam ranged 5.07×10^4 to 3.30×10^7 colony-forming units (CFU) per gram of tissue. Table 2 shows the results.

Studies on the *Vibrio* spp. that were isolated from angelwing clam have not been established. However, recent research data suggest that various species of *Vibrio* that were identified in some bivalves pose risk to human health when these shellfish are consumed (Deepanjali et al 2005; Parveen et al 2008; Sobrino et al 2010; Yu et al 2013).

Table 2

Total counts of presumptive *Vibrios* from the stomach of angelwing clam

Sample number	Weight of the sample (g)	Counts in CFU/gram*
1	0.012	3.3×10^7
2	0.311	1.28×10^5
3	0.048	1.04×10^7
4	1.088	2.75×10^5
5	0.129	3.10×10^6
6	1.16	8.6×10^4
7	0.796	5.02×10^5
8	0.197	5.07×10^4
9	0.424	7.07×10^5
10	0.762	6.6×10^5

*CFU = Colony-forming units

Bacterial diseases in molluscs are well described in the larval stages and in the hatcheries (Gay et al 2004). The shellfish larvae are vulnerable to different kinds of pathogens at the time when these larvae attach to the bottom of the tank, where debris of uneaten food and other materials accumulate. The condition for bivalve culture favors the growth of bacteria and may cause proliferation of pathogenic *Vibrio* species in the water (Romalde & Barja 2006). Several species of *Vibrio* are well-established as recognized agents of vibriosis. These include *Vibrio anguillarum*, *Vibrio tubiashii*, *Vibrio splendidus* and *Vibrio alginolyticus.* Conversely, several species of *Vibrio*, other than the ones that were previously mentioned, have been described in the last two decades (Romalde & Barja 2006). The pathogenic potential of *Vibrio celticus* has been described recently by Beaz-Hidalgo et al (2010) even though this bacterium is considered a natural flora of cultured clam for several years.

Vibrio spp. have also been reported to be pathogenic to adult bivalves. Japanese clams were reported to acquire brown ring disease from *Vibrio tapetis* (Gay et al 2004). *Vibrio harveyi* has been found to cause severe epizootic outbreak in abalone, *Haliotis tuberculata.* In Pacific oysters, *Crassostrea gigas*, a pathogenic strain of *Vibrio splendidus*, was isolated (Gay et al 2004). The population of the total presumptive *Vibrios* in angelwing clam that was obtained in this study is useful in providing baseline data, which can be used as comparison in cases when there is a future outbreak of vibriosis in angelwing clam in the wild or in the hatcheries. Although, the pathogenicity of the presumptive *Vibrios* that were isolated from the stomach of angelwing clam was not determined, future studies will focus on establishing the dominant species of *Vibrios* in the stomach of angelwing clam and testing whether or not they are pathogenic to the different life stages of the host.

Conclusions. In conclusion, the study was conducted to determine the parasites that were associated with the organs and tissues the Oriental angelwing clam and also to confirm the presence of *Vibrio* spp. inside the stomach of the clam. Three types of parasites were isolated from the body of the clam. Two unidentified ciliates were obtained from the gills and stomach. These ciliates could either be commensals or parasites, depending on the degree of prevalence and invasion of the tissues of the host. The unidentified ciliates in the gills were present in large numbers, yet no signs of infection in the host have been observed. Another parasite was found and believed to be *Ancistrocoma*-like based on available description from literature. *Ancistrocoma*-like

commensals were present in large numbers inside the stomach of the angelwing clam and were very motile. However, no external manifestations of disease were observed in the tissues of the host brought about by the presence of these thigmotrichous ciliates. Meanwhile, *Vibrio* spp. was confirmed to be present inside the stomach of angelwing clam, hence, there is a possibility that this shellfish harbors strains of *Vibrios* that pose health hazards to the consuming public.

The data obtained from this study will serve as a reference and is helpful for more intensive research on the potential pathogens and diseases of angelwing clams. The presence of these associated organisms in the host has implications in the health management aspects during the hatchery and culture of these bivalves. The prevalence of these potential pathogens in angelwing clam should also be carefully examined in relation to the health hazards that these pathogens pose to the consumers.

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