E-ISSN: 2347-5129
P-ISSN: 2394-0506
(ICV-Poland) Impact Value: 5.62
(GIF) Impact Factor: 0.549
IJFAS 2018; 6(6): 380-386

www.fisheriesjournal.com
Received: 06-09-2018
Accepted: 11-10-2018

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Water quality enhancement potential of the mangrove clam *Polymesoda erosa* on the survival of *Barnea manilensis* reared for laboratory experiments

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DOI: https://doi.org/10.22271/fish.2018.v6.i6e.2659

Abstract
Due to its known filtering power and ability to survive in estuarine environments teeming with microbes and rich organic matter, it was investigated whether *Polymesoda erosa* can also maintain water quality of small-scale culture systems in the laboratory. In this study, it ensured the survival of another estuarine bivalve species, *Barnea manilensis* which is observed to be very sensitive to the build-up of waste especially when water change is not carried out constantly. Fifteen (15) *B. manilensis* clams were reared together with ten (10) *P. erosa* clams in a small basin and they survived without water change for 5 days while those reared without the presence of *P. erosa* died after 2 to 3 days. This was repeated in 3 runs and this result was consistent. The use of "*P. erosa*-filtered water only" was also tested, but it did not increase the survival of *B. manilensis* reared in it. This suggests that live *P. erosa* clams must be present in the culture containers for the water-enhancement effect to manifest.

Keywords: *Polymesoda erosa*, *Barnea manilensis*, clam filtration, polyculture, bioremediation

Introduction
The mud clam *Polymesoda erosa* are hardy bivalves that exhibit excellent water filtration abilities and is commonly found in estuarine environments such as mangrove swamps. This species was mentioned in several studies to play an important role in the food web and in nutrient cycling (Clemente, 2007; Mhatre and Kunde 2014; Elvira *et al*., 2016) [5, 14, 7]. Some unpublished reports claim that they can survive in artificial culture without adding additional food input as long as they are exposed to light. They can also thrive in cultures that are poorly managed, expecially when it comes to the maintenance of water quality. This suggests that they probably have formed associations with microbial populations that contributed to these survival mechanisms. This is expected for any mangrove clam species considering the nature of their natural habitat (Roeselers and Newton, 2012) [26]. One good example is *Anodontia edentula* that was found to contain sulphide-oxidizing bacteria in their gills (Lebata, 2001) [12]. *P. erosa's* antibacterial activity (Chatterji *et al*., 2002) [4] or inhibitory effects on some known pathogens (Sugesh and Mayavu, 2013) [29] were also observed. Thus, mangrove clams that show high survivability in artificial systems possess properties that can support the integrity of their culture environment. The possibility of using these clams as filtering agents to improve water quality in polyculture systems has great potential.

In general, the maintenance of water quality is a must in the culture of aquatic species used in laboratory experiments. There are some conditions however that can limit this practice. Some experimental organisms taken from the wild are usually sensitive to water quality changes and they die before the experiments are completed. An efficient recirculating system is not possible under certain circumstances; thus, manual water changes are required to keep the cultures healthy through constant thinning of opportunistic microbial populations (eg. Araneta, 2016) [1]. But this can disturb the experimental conditions. Also, many of these indigenous microbes are heterotrophs that can grow fast when supplied with excess organic material- usually coming from unused feeds and waste material. It would be useful if there is a "helper" organism present that can control these potential threats without the need to agitate the experimental setup.

For example, the black clam, *Chione fluctifraga*, was used by Cordova *et al*. (2011) [6] to process effluents produced by shrimp aquaculture. Given the initial observations of *P. erosa's* survivability and filtering capability after grown in isolation for a year, its potential in ensuring the survival of a relatively weaker clam species was tested. Both clams were reared together so that any observable effect on *B. manilensis* survival can really be attributed to the presence of *P. erosa*. Another line of inquiry is whether the observed survival can happen even without the presence of *P. erosa* in the culture. There is a possibility that the water quality enhancement factor is not strictly associated with *P. erosa*. This means, the water filtered by them is already enough to produce the same effects. This can suggest the practicality of providing a separate water-treatment area where these clams can manage the water without competing for space. It is on this light that this study was conducted.

Materials and Methods

This study utilized a completely randomized experimental design (CRD), where the initial set of culture environments namely, 1. With *P. erosa*; 2. Without *P. erosa*; and 3. Using *P. erosa* filtered water only, were randomly assigned to 45 clams in 2 experimental runs. This allowed the researcher to compute survival rates of *B. manilensis* cultured in these conditions.

Fig 1: One-year old *P. erosa* aquarium culture

Barnea manilensis used in this study were purchased from a local shellfish collector/vendor at Brgy. Tiwi, Barotac Nuevo, Iloilo, Philippines.

Fig 2: *Barnea manilensis* collected from the wild

Both clams were cultured in a 30-liter container containing artificial saltwater at 15 ppt salinity. Ten (10) *P. erosa* clams and fifteen (15) *B. manilensis* clams were reared for 2 days in identical conditions prior to the formal experimental runs. Due to limited time to culture microalgae food, the researcher used an artificial food mix made of commercial algal and seaweed wafers mixed with rice bran. The clams were initially fed with this mix at 1% of their live body weight and this was slightly reduced after several days when excess food start to accumulate in the culture containers. The experimental setup was summarized in Fig. 3 below.

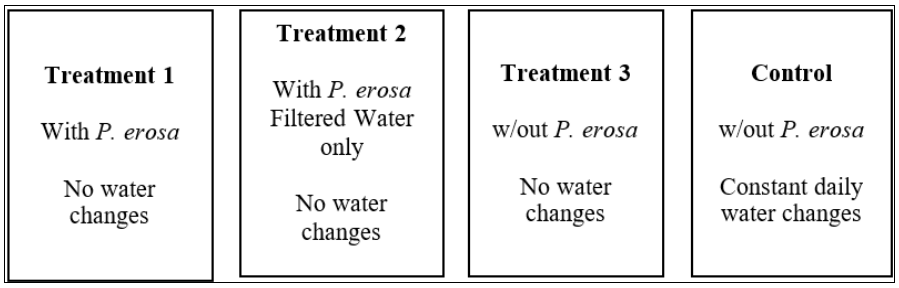

Fig 3: The experimental setup carried out in 2 runs and 3 trials each.

The above setup was carried out in 2 runs. Each run repeated thrice. The entire experiment lasted 27 days in total at 3 different instances. On the first run, one 30-liter plastic box containing 10 *P. erosa* was laid side by side with another box without *P. erosa*. 15 *B. manilensis* clams were placed in each box and were reared without water change until considerable mortalities were observed.

Fig 4: First Run of *B. manilensis* culture "with" (left) and "without" (right) the presence of *P. erosa*.

In the second run, water was siphoned using a small pump from a *P. erosa* culture into a culture container where another batch of *B. manilensis* is being reared. In this manner, the water was filtered by *P. erosa* at least 1 day before they are introduced into the *B. manilensis* culture. Both clams however are not physically close to each other instead in the previous run where they are cultured together in one experimental box. Mortality rates were computed using the online Schneider-Orelli's formula (Puntener, 1981) [21] by simply keying in the percentage mortality in both the treatments and the control. All computations were encoded into SPSS v.20 software and mean comparisons were carried out using One-way Analysis of Variance at 0.05 level of significance.

The ability of *P. erosa* to filter a suspension of decaying organic material was also tested. The culture container was filled with chopped *Azolla*, rice bran, food scraps and fish food and was left for 3 days for microbial action to take effect. *P. erosa* was then introduced to the culture and observed if it can make the cloudy water clear again.

Data and Results

Table 1 shows the mortality rates in the different treatments in 3 trials. It is obvious upon first inspection that *Barnea manilensis* survived better when reared with *P. erosa* clams. In the control containers where water change was done regularly, there are still mortalities.

Table 1: Mortality Rates of *Barnea manilensis* reared in 3 experimental conditions

Trials	Treatments			Control
	T1 (*P. erosa* present)	T2 (*P. erosa*-filtered water only)	T3 (*P. erosa* absent)	
1	0%	78.57%	100%	6.66%
2	0%	100%	78.57%	6.66%
3	6.66%	100%	88.84%	13.33%

Fig 5: Healthy *B. manilensis* clams (Right) reared with *P. erosa* acquired black organic material from the culture container.

It is also clear in Table 1 that filtered water alone has not decreased the mortality in a rearing environment. One-Way Analysis of Variance showed significant differences ($p=0.00$, $\alpha=0.01$) in the mortality rates of *B. manilensis* in 3 treatments. Post hoc test (LSD) at 0.01 level of significance showed that mortalities in treatment 2 (mean=92.86%) and treatment 3 (89.14%) are significantly higher than in treatment 1 (mean=3%).

Fig 6: *Barnea manilensis* (in orange bowl) are dead after 2 days without water change and without *P. erosa* in its culture container

Barnea manilensis clams (Fig. 5) survived 3 days without water change when cultured together with *P. erosa* and they appear healthy and responsive to touch (they extend/retract their siphons). There is no significant difference between those reared with clam-filtered water only and those reared in the absence of *P. erosa* at 0.05 level of significance. Both treatments resulted to high mortalities (see Fig 6). This means that the water-quality enhancement factor is not found in surrounding water. Also, the filtering of water by *P. erosa* does not guarantee any lowering of the mortality rates. It was greatly supported by the results above that *P. erosa* is needed in the culture containers to be able to exploit its water-enhancement capabilities.

Furthermore, a "dirty" suspension made up of decaying organic matter from fish food, food scraps and plant material were cleared by 16 *P. erosa* clams in 5 days. It took that long because maybe, the microbial population is difficult to control due to the abundance of food in the culture container for them to keep their numbers.

Fig 7: Cloudy Organic Suspension before it was cleared by *P. erosa*

Nevertheless, the organic material was disposed eventually by the clams and the suspended particles were converted to waste pellets at the bottom of the culture container. The clam survived the poor water quality of such an environment and even cleared it up.

Fig 8: The Cloudy Organic Suspension was cleared by *P. erosa* after 5 days

It is hypothesized that there may be beneficial bacteria that are associated with *P. erosa*. It may be a probiotic with antagonistic effects on other microbial population or it may be some kind of an ammonia or phosphate oxidizer. Together with the filtering capabilities of this species, the combined efforts of associated heterotrophic microbes add more synergistic effects to the maintenance of water quality by *P. erosa*. This still need to be proven though in future investigations.

Discussion

The ability of *Polymesoda erosa* to improve the water quality where they thrive is not new in nature. There are several mechanisms mentioned in the literature. For example, they can act as biofilters (Buttner, 1986, Pirzan & Tjaronghe, 1997) [3, 19] or possess the ability to metabolize ammonia and nitrates (Li *et al*., 2010) [13]. Associated microbial symbionts are responsible for these nitrogenous-waste elimination capacity as well as their antagonistic effects on potentially-harmful free-living organic oxidizers. It is also well-known that microbial activities/ processes are the primary driving force in mangrove ecosystems (Holguin *et al*., 2001) [9]. Without these so-called microbial-mediated nutrient cycling processes, it is hard to transport nutrients within aquatic ecosystems (Sjoling *et al*., 2005) [28]. The removal of toxic wastes by microbes obviously makes the environment more habitable to all organisms. This and the innate feeding behavior of clams as suspension feeders have potential in cleaning "dirty" water such as aquaculture effluents or laboratory experimental cultures.

Microbe- regulated nitrogen cycle within mangrove ecosystems usually include dinitrogen-fixation, nitrification, denitrification, ammonification, anaerobic ammonium oxidation, and nitrate reduction to ammonium (Purvaja *et al*., 2008) [22]. In a closed system such as an experimental container, ammonia build-up can affect the outcome of experiments. It was long observed prior to this study that *P. erosa* can thrive without any water change in an environment as small as a 30-liter container. Ammonia has a very strong smell and it is not observed in the cultures with *P. erosa* on it. The other two treatments not subjected to water change have that strong characteristic urine-like smell that obviously indicate deteriorating water quality in just a few days. This observation opens the possibility that *P. erosa* harbors

ammonia-metabolizing bacteria that is responsible for reducing ammonia levels in the cultures. This needs to be verified in future studies by focusing directly on ammonia content of the cultures before and after *P. erosa* was introduced. It is also established that bivalves ingest suspended particles, keeps the organic component and discards the inorganic (Troell & Norberg 1998, Pfeiffer *et al*., 1999, Kasai & Nakata 2005) [31, 18, 11]. The inorganic component can actually be separated via the filtration mechanism/defecation of bivalves (Ramos *et al*., 2009) [23]. In this study, *P. erosa* produce an oily, semi-solid waste material that settles at the bottom of the container. This greatly contributed to making the water clear. It is also possible to siphon out these submerged materials without reintroducing them in the water column. These fecal materials can have a variety of applications and beneficial microbes thrive in it.

Decomposition and reutilization of nitrogenous compounds by clams such as *P. erosa* can also be applied in recirculating aquaculture systems. Ammonia and nitrite are toxic while nitrate can increase the chance of hypertrophication of the environment (Buhmann, 2013) [2]. All potential water treatment techniques must be applied in intensive fish production. Constructed wetland technology has been used for wastewater treatment since the early 1970s. Wetlands are resilient and economical for treating wastewater, such as domestic sewage, industrial and agricultural wastewater, landfill and storm water leachate and runoff (Webb *et al*., 2012) [33]. Ecological approaches such as aquasilviculture- a type of polyculture system in mangroves have been re-established and integrated with new technologies, such as the simultaneous production of fish with filter feeders such as clams and plants or algae (FAO, 2012) [8]. These practical methods are also adopted in standard aquaculture systems, such as ponds, net cages, and even in laboratory settings.

Up to the present, aquaculture effluents always pose negative impacts on the environment. The use of filtering organisms such as clams can be implemented as one of the sustainable strategies especially when low or zero water exchange systems or recirculation methods are needed. Reintegration of nutrient inputs through polyculture practices, the use of water treatment sinks such as mangroves or artificial sheds (Rivera-Monroy *et al*., 1999, Primavera *et al*., 2007) [25, 20] and bioremediation of effluents (Troell *et al*., 2003) [32] are all sustainable and less taxing to the environment. *Polymesoda erosa* thrives in wetland ecosystems and therefore expected to perform well in bioremediation polluted water such as aquaculture effluents. If fact, many farms have integrated these approaches for different types of cultures (Neori *et al*., 2007) [16]. Bioremediation using bivalves rely mainly on the use of its filter-feeding ability. Plants such as microalgae and seaweed (Jones *et al*., 2002, Shpigel *et al*., 2005, Muangkeow *et al*., 2007) [10, 27, 15] can also be used because they also support the filtering heterotrophs and at the same time also extract nitrogenous waste from the system. Many mollusk species can also bioremediation aquatic environments and at the same time provide extra income to farmers (Rawson *et al*., 2002, Peharda *et al*., 2007) [24, 17]. Some have been observed to control luminous bacteria in shrimp ponds (Tendencia, 2007) [30]. Yokoyama *et al*. (2002) [34] studied the effect of green mussels, *Perna viridis*, on the water quality of a series of attached ponds that received shrimp effluents. The green mussels effectively removed excess food particles and improved water quality. It is therefore important to evaluate the performance of every potential organism if they will be used as Bioremediation in specific effluent waters.

All-in-all, there are few (if not none) investigations where the survival of a relatively sensitive organism such as *Barnea manilensis* in laboratory culture is maintained by co-culturing it with a bioremediation species. This is however similar in principle in the wild and in large polyculture systems. It is only the application that differs. When water quality in the laboratory is hard to control due to some experimental requirements or circumstances, utilizing support organisms that also offer the same solution in terms of increasing survival can be very useful. This study was able to ensure the survival of another species by utilizing the water-quality enhancement properties of *P. erosa.* It is for sure not only limited to *B. manilensis* and future investigations can be made to confirm this.

Conclusion and Recommendation

The potential of *P. erosa* as a water quality enhancer in aquaculture systems has been confirmed in this study. It was shown to clear “dirty water” in a couple of days and was able to ensure the survival of *Barnea manilensis* culture even without regular water changes. The water enhancement ability can be attributed directly to the presence of *P. erosa* because the same effects are not observed if they are removed from the culture. Several lines of inquiry can be followed from this simple investigation. It is recommended that the ammonia-reducing ability of this species is studied even with basic test kits. It is also interesting to try and isolate associated bacteria in these clams as they can have potential biotechnology applications such as using them as probiotics or in bioremediation of toxic pollutants. Samples can also be checked via molecular phylogeny to identify the microbial groups that are associated with these organisms. A large-scale deployment of these clams in a grow-out pond can also be tested and observed if it has substantial effect on maintaining water quality for other cultured species. Moreover, mangrove ecosystem studies should consider this clam as one of the keystone species in such ecosystems.

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