Addressing Gaps in the Culture of Pathogen-free Polychaetes as Feed in Shrimp Hatcheries

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One of the factors that contribute to the success of shrimp hatchery operations is the availability of good quality broodstock diets. Polychaetes have been regarded as the best maturation diet for shrimps as they contain essential nutrients requisite for the reproduction of shrimps. Consequently, the demand for polychaetes increased with the intensification of shrimp farming and as a result, the natural stocks are depleting gradually and thus, could no longer provide sustainable supply for shrimp hatcheries. In addition, the issue on biosecurity concerning wild polychaetes prompted the shrimp farmers to obtain polychaetes from reputable sources, thus, the culture of polychaetes under controlled condition has become a sustainable alternative. The SEAFDEC Aquaculture Department (SEAFDEC/AQD) therefore initiated the "Refinement of rearing and feeding techniques for sustainable mass production of the polychaete *Marphysa* sp." to address the gaps in polychaetes culture and ensure the sustainability of polychaetes production to supply the shrimp hatcheries at SEAFDEC/AQD, and where the potential mass production of the polychaetes (*Marphysa* sp.) in indoor tanks is being undertaken to ensure that these are pathogen-free.

Polychaetes are aquatic multi-segmented worms which are the most abundant and diverse group of Phylum Annelida, and are found around the world from abyssal depths to shallow estuaries, rocky shores, and even free swimming in open waters. Along with sediment-dwelling mollusks and crustaceans, they are mostly abundant in mud and sand habitats. Their pencil-like bodies are soft and usually only a few centimeters long and their slow movement is aided by the retractable grip of four dense clusters of bristles and hooks on each segment called chaetae, thus the name "polychaete." There are over 80 families existing to this day and each family has distinguishing body shapes and chaetal types (Read & Fauchald, 2018).

The ecological importance of polychaetes cannot be understated. In the natural environment, they feed on detritus and smaller benthos and some species prey on other small animals using their retractable pharynx, and are fed upon by higher order predators such as fish, crustaceans, larger invertebrates, and even birds. The significant role played by polychaetes in nutrient cycling sustains the benthic environment (Hutchings, 1998). Head-down deep polychaetes are deposit feeders known for having strong effects on bioturbation and nutrient mineralization both by sediment ingestion, reworking and burrowing (Papaspyrou, Kristensen, & Christensen, 2007), and enhance organic matter mineralization and recycling (Aller, 1994). The presence of polychaetes in the sediments of organically enriched fish farms hastens the decomposition of organic matter (Heilskov & Holmer, 2006), and the burrowing formation of polychaetes creates an oxidized layer in the sediment providing optimal environment for aerobic bacteria to proliferate (Kunihiro *et al*., 2005) and, in the process, increase bioremediation.

Polychaetes have been used as bait in recreational fishing industry for many years. In 2015, the five most expensive (retail price per kg) polychaete species sold in the global market were *Glycera dibranchiata* (US$ 237), *Diopatra aciculata* (US$ 150), *Nereis (Alitta) virens* (US$ 96), *Marphysa sanguinea* (US$ 85), and *Arenicola defodiens* (US$ 82) according to the three UK-based ragworm fisheries (Watson, Murray, Schaefer, & Bonner, 2017).

In aquaculture, polychaetes can be used as feed either in pure form, combined with other natural food, or used as one of the ingredients of formulated diet for shrimp broodstock. The use of polychaetes as an effective maturation diet to shrimp broodstock has long been recognized because they contain high levels of proteins, lipids, n-3 polyunsaturated fatty acids (PUFA), and hormonally-active compounds that are responsible for the ovarian maturation of penaeid shrimps and at the same time improve the quality and viability of offspring (Lytle, Lytle, & Ogle, 1990; Luis & Passos, 1995; Naessens *et al*., 1997; Coman, Arnold, Callaghan, & Preston, 2007; Meunpol, Iam-Pai, Suthikrai, & Piyatiratitivorakul, 2007). The polychaete species commonly used as feed to shrimps are *Perinereis hellerri* (Palmer, Wang, Houlihan, & Brock, 2014), *Palola* sp. (Pamungkas, 2015), *Nereis virens* (Brown, Eddy, & Plaud, 2011), *Perinereis nuntia* (Techaprempreecha *et al*., 2011), and *Marphysa* sp. (Meunpol, Meejing, & Piyatiratitivorakul, 2005).

In recent years, however, the collection of polychaetes from the wild for aquaculture purposes declined due to biosecurity reasons (Velvizhi *et al*., 2013), as polychaetes collected from the wild are possible carriers of pathogens as they accumulate the viral pathogen in their digestive tract by consuming virus particles in the sediment. When these polychaetes from the wild are used as aquaculture feed, pathogenic diseases could be transferred to healthy broodstock shrimps leading to possible viral infection in the shrimps (Vijayan *et al*., 2005).

The collection of polychaetes in the coastal areas is considered as a major livelihood for the Irular tribal fishing community in Pitchavaram Region in Tamil Nadu, India (Velvizhi, Gopalakrishnan, Murugesan, & Kannan, 2013). The Irular fishing community collects the polychaetes by digging the areas identified with the presence of burrows and as soon as polychaetes emerge, they are handpicked and washed with water (Velvizhi *et al*., 2013). The collected polychaetes are supplied to shrimp hatcheries all over the country and it is estimated that about 6-20 MT of polychaetes are utilized annually (Vijayan *et al*., 2005).

Harvesting of polychaetes from the wild destructs the sediment bottom and thus, disturbs other benthic organisms living in the sediments. Over-exploitation of polychaetes in the natural environments could adversely affect the nutrient cycling and other biochemical processes (Kristensen & Mikelsen, 2003; Laverock, Gilbert, Tait, Osborn, & Widdicombe, 2011). However, an estimated 121,000 MT (valued at US$9.15 billion) of polychaetes were collected from the wild globally in 2015 (Watson *et al*., 2017). The increasing demand for polychaetes from leisure and aquaculture has therefore resulted to over-exploitation of the natural resources of these organisms, while presently, polychaete aquaculture is still limited to compensate the high demand.

SEAFDEC/AQD's "*OPLAN Balik Sugpo*"

SEAFDEC/AQD is currently gearing towards reviving the tiger prawn (*Penaeus monodon*, locally known as *sugpo*) industry under the banner "*OPLAN Balik Sugpo*" which entails the development of a complete and detailed operational plan that aims to bring back the booming production of tiger prawn in the Philippines. The prawn industry was considered as a sunshine industry in the country in 1980s until the occurrence of diseases brought it down. SEAFDEC/AQD is now working towards effective breeding program which ensures Specific Pathogen Free (SPF) broodstock, healthy post-larvae, and at the same time, refining the grow-out technology of intensive and semi-intensive shrimp farming. Considering the significance of polychaetes in shrimp aquaculture, there is a need to produce pathogen-free polychaetes under controlled and biosecured conditions. Thus, this study aims to showcase the potential of *Marphysa* sp. for mass production in indoor tanks to supply the needs of the shrimp hatchery of SEAFDEC/AQD, considering that this polychaete species can spawn many times throughout its lifetime making it sustainable for culture.

Culture of *Marphysa* sp. in Indoor Tanks

In order to ensure the sustainable production of polychaetes, it is necessary to address the culture gaps in every life stage of *Marphysa* sp. by understanding its biology and culture requirements. *Marphysa* sp. is one of the polychaete species commonly used as feed to shrimps and it is abundant in mangrove wetlands and fishponds in northern Iloilo, Philippines. This species belongs to family Eunicidae which is known to be gonochoric (with separate sexes), exhibit no sexual dimorphism (no difference in male and female physical attributes), and capable of multiple reproductive cycles throughout their lifetime (Giangrande, 1997; Gambi & Cigliano, 2006).

The multiple reproductive strategies exhibited by *Marphysa* sp. is considered as a sustainable advantage over the polychaete species under the family Nereididae, in which death follows right after spawning (Fischer & Fischer, 1995). Based on actual observation at SEAFDEC/AQD, *Marphysa* sp. encloses its eggs inside a gelatinous egg mass or jelly cocoon where the early stage of larval development takes place during spawning. Two days after hatching, the jelly starts to disintegrate and the trochophore larvae (early stage polychaetes) start to settle in the sediment. *Marphysa* sp. can grow up to 30 cm or more after five to six months in captivity (**Figure 1**).

Figure 1. Late juvenile (3-5 cm long) and sexually mature (10 cm and longer) *Marphysa* sp. in captivity

Nursery phase

One jelly cocoon enclosing 6,653 ± 1,606 trochophore larvae or 33,267 ± 8,032 individual/m² was stocked in each of the three nursery tanks (4 L capacity) at SEAFDEC/AQD hatchery and grown for one month in biofloc (**Figure 2**), an

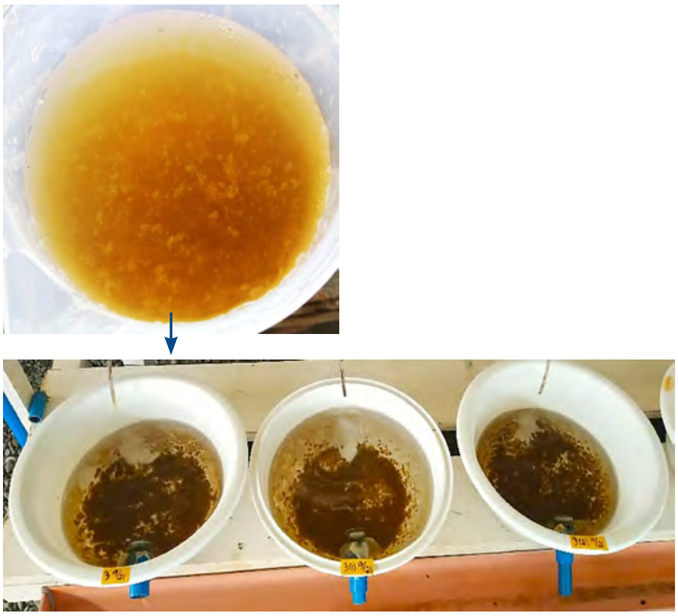

Figure 2. Biofloc as a substrate and feed in the nursery rearing of *Marphysa* sp.

aggregate of microorganisms, microalgae, zooplankton, and organic particles from uneaten feeds (Crab, Defoirdt, Bossier, & Verstraete, 2012; Ekasari *et al*., 2014) containing proteins and immunostimulants (Ju *et al*., 2008; Xu & Pan, 2013) and serves as a natural food source for shrimps (Kent, Browdy, & Leffler, 2011). Biofloc was used in this study to serve as substrate and first exogenous food source of polychaete larvae. The nutrients from the biofloc were consumed by the polychaete larvae for growth and development. For water management, a continuous flow-through seawater exchange was followed. Water temperature and salinity were monitored daily. In order to prevent the loss of polychaete larvae, a screen with a 90 µm mesh size was fitted to the drain pipe of each larval rearing tank.

Grow-out phase

After one month at the nursery, juvenile polychaetes from each of the three tanks were transferred to another tanks (0.20 m²) filled with mud and grown for another four months until harvest. Polychaetes were fed with feed mill waste (FMW) comprising feeds and feed ingredients that remained in the feed mill equipment after every operation. The water in each grow-out tank was changed every other day. Water salinity and mud temperature were measured daily.

To determine the body weight of polychaetes in each of the three grow-out tanks, the first sampling was done after two months from initial stocking and the second sampling was done after three months from the first sampling. The body weight was measured by weighing (blotted wet body weight) each of the twenty individuals from each replicate tank. Polychaetes at early stages of development were prone to damage and stress due to handling. Thus, polychaete survival was measured only at this period due to the difficulty in collecting juvenile polychaetes.

Survival and Growth of Cultured Polychaetes

At the end of the experiment, polychaete survival was computed based on the number of surviving polychaetes over the total number of trochophore larvae stocked in each replicate tank at initial stocking multiplied by 100. The biomass was computed as: biomass (g/m²) = mean body weight (g) × surviving polychaetes. The fragile nature of the polychaetes prevented the determination of survival prior to the five-month period. For the purposes of computing the biomass after three months, the survival rate after five months was used instead and it was presumed that the survival rates after three and five months would be similar. As shown in the **Table**, the average body weight after three months was 130 ± 60 mg and ranged from 70 to 250 mg. The biomass was 244 ± 70 g/m² and ranged from 163 g/m² to 384 g/m². After five months of culture, about 307-466 individuals/tank or 1,535-2,330 individuals/m² were collected. The survival rate was 8 ± 3 % which ranged from 3.47 % to 13.21 %. The mean body weight was 290 ± 30 mg with the range of 240-340 mg. The biomass was 593 ± 54 g/m² and ranged from 522 g/m² to 699 g/m².

Conclusion and Way Forward

Aquaculture of polychaetes in indoor tanks is feasible provided that optimal rearing conditions are met. The use of biofloc as a substrate and nursery feed for polychaete larvae and FMW as grow-out feed resulted to good survival and growth performances of *Marphysa* sp. Water salinity (29-32 ppt) and sediment temperature (29-30 °C) in all culture tanks were at optimum levels. The five-month culture period showed greater polychaete body weight and biomass than the three-month culture period. Extending the culture period for another month would have most likely resulted to greater body weight and biomass. With these results, fish farmers have the options on the schedule of harvesting their polychaete stocks

Table. The average survival rate (%), average body weight (mg), and biomass (g/m²) of cultured *Marphysa* sp.

	Culture period		
	Initial stocking	Three months	Five months
Number of individuals/tank	6,653 ± 1,606	-	413 ± 53
Range of individuals/tank	3,520-8,835	-	307-466
Number of individuals/m²	33,267 ± 8,032	-	2,063 ± 264
Range of individuals/m²	17,600-44,175	-	1,535-2,330
Survival rate (%)*	-	-	8 ± 3
Range of survival rate (%)	-	-	3.47-13.21
Average individual body weight (mg)*	-	130 ± 60	290 ± 30
Range of individual body weight (mg)	-	70-250	240-340
Biomass (g/m²)*	-	244 ± 70	593 ± 54
Range of biomass (g/m²)	-	163-384	522-699

** values are presented as mean ± Standard Error (n = 3)* based on the size that they need. Small size polychaete can be fed to juvenile shrimps while bigger ones can be fed to shrimp broodstock.

With regards to commercial production, the study provides knowledge on the aquaculture of mud polychaete which is an ecologically friendly feed material for shrimp production. Ultimately, this translates into a substantial improvement in the efficiency of shrimp aquaculture especially for SEAFDEC Member Countries with viable sources of *Marphysa* sp. broodstock. In addition, the development of best culture techniques for indoor mass production of polychaetes could impede the reliance on natural resources. The biosecurity issue could be addressed as well when shrimp broodstock are fed with cultured polychaetes and the transfer of diseases could be prevented. To ensure the sustainable supply of pathogen-free polychaetes, there is a need to conduct further studies on how to manipulate the spawning frequency. Increase in spawning frequency accompanied with good quality offspring will guarantee a sustainable production of *Marphysa* sp.

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