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Research article

Detection and culture feasibility of a soil nematode *(Panagrellus redivivus)*, a potential live feed for prawn larvae in Bangladesh

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ABSTRACT

The free-living soil nematode, *Panagrellus redivivus* is recently known to be an inexpensive, standardized and permanently available food source for first feeding fish and crustacean larvae. The present study was aimed mainly at the development of a suitable culture technique of the species *P. redivivus* so as to be used as live food for the prawn larvae. The study has got the species morphologically detected in the local soil ground, which was found available under some specific environmental condition. About 30% of soil moisture, pH value of 7, 20°C temperatures and 5 cm soil depth were found better for higher availability and yield of the species. For culture feasibility, the experiments were conducted using different culture media with a combination of different incubation temperatures (22, 24, 25, 26, 27 and 28°C) and incubation periods (8, 10, 12, 14, 16, 18 and 20 days). Five different media (oatmeal, oatmeal with sunflower oil, cereal, raw potato and boiled potato) were used for the mass production of this nematode where the local and cheap boiled potato media was observed as the most successful and feasible one followed by the oatmeal media. The mass production of the species was found most suitable at the temperature of 26°C on around 14th day of culture period. A good quantity, 1, 50, 032±9, 285 individuals of nematodes were harvested highest from 1 gm of boiled potato media. This new technique for mass production of *P. redivivus* would enable fish and prawn hatchery operators to an alternative live food item to the highly expensive Artemia which is commonly used in Bangladesh.

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1. INTRODUCTION

Fisheries sector in Bangladesh is very potential for food safety and economical development of the nation. Fish provides 60% protein in the daily feeding of Bangladeshi. According to the economic review of DoF (2012) fisheries sector contributes 4.43% in national GDP, 22.21% in Agriculture and 2.73% in export earning of Bangladesh. The prawn and shrimp aquaculture have been expanding satisfyingly for the last couple of years in Bangladesh based on wild seeds initially, but creating a long lasting adverse impact on nature (Wahab, 2003; Das and Alam, 1995). Realizing this and in order to meet the emerging demand and to ensure timely supply of seeds for culture, a considerable number of hatcheries have in the recent time been established in the coastal region of Bangladesh. About 921 fin fish hatcheries produce 629.175 tonnes fish fry and 140 shrimp *(Penaeus monodon)* and prawn *(Macrobrachium rogenbergii)* hatcheries produce 7,150 million post larvae every year and it is increasing day by day (DoF, 2012). Freshwater prawn culture development in Bangladesh has attracted substantial attention for its export potential during the last two decades, where the prawn and shrimp sector as a whole is the second largest export industry in the country (DoF, 2014). However, the prawn farming sector is encountering insufficient supply of larvae; mostly depending on the wild stock which is the potential carrier of various shellfish diseases (Wahab, 2003; Deb, 1998). In such a situation, the hatchery produced larvae is considered as an effective and sustainable tool for the vertical and horizontal expansion of the good quality prawn production.

But the prawn larvae production in Bangladesh is

exclusively dependent on costly live food "Brine Shrimp", Artemia nauplii for their nutrition and growth. This small crustacean has the advantage that its culture can be started from dried eggs (Sorgeloos and Persoone, 1975; Liao, 1992). These dormant cysts can be stored for longer periods in cans and, if needed, used as a convenient off-the-shelf live food (Lavens and Sorgeloos, 2000). Although Artemia is particularly convenient to use in hatcheries (Wickins and Lee, 2002), it also has some prominent negative aspects. The most common ones are: high costs, a highly variable hatching rate, quick growth, the varying nutritional quality and the consuming of algal feed and therefore to compete with the cultured species for food (Biedenbach et al., 1989; Lavens and Sorgeloos, 1996 and Lavens and Sorgeloos, 2000). Moreover, the prawn hatchery sector is at stake because of the availability of poor quality Artemia cyst in the market that results higher mortality of larvae in the hatcheries (Curnow et al., 2006; Vega- Orellana et al., 2006; Hamza et al., 2007; Rosenlund and Halldorsson, 2007). Thus, the lack of potential alternatives to Artemia may become an obstacle to a further increase of aquaculture production especially in developing countries like Bangladesh. Owing to the obvious limitations of Artemia, other live organisms have been examined for their use in Penaeid shrimp larviculture; copepods, rotifers, daphnia, moina and nematodes have been suggested by various authors (Wilkenfeld et al., 1984; Lavens and Sorgeloos, 1996; Guillaume et al., 2001 and Lee et al., 2005). Therefore, searching of an alternative live feed and coping of that particular feed for the larvae in the hatcheries could be a very good plausible solution in order to keep the growing prawn industry in good survives.

The free-living soil nematode, *Panagrellus redivivus* is known to many aquarium enthusiasts and fish keepers as the microworm which has been popular in many countries as an alternative live feed for first feeding fish and prawn larvae (Lavens and Sorgeloos, 1996; Guillaume et al., 2001; Lee et al., 2005). The species is easy to rear in large quantities by using suitable culture media (Ricci et al., 2003). It is a tiny nematode of about 0.5 to 2.0 mm in length and 0.05 mm in diameter which is suitable for prawn/fish larvae to be feed on. They reproduce sexually and are livebearers; releasing 10~40 young every 5~7 days for a 26~36 day life span. The young reach sexually maturity in approximately three days (Biedenbach et al., 1989). According to Stock and Nedler, (2006) *Panagrellus sp*. has a worldwide distribution.

Although several studies proved that the free-living nematode *P. redivivus* is a suitable food for fish and crustacean larvae (Kahan et al. 1980; Biedenbach et al. 1989; Kumlu and Fletcher 1997; Kumlu et al. 1998), however, the species could not used in the aquaculture industry earlier due to the problems involved in its mass production. Recently, large-scale production processes have been developed for entomopathogenic nematodes. It has an amino acid profile that matches that of Artemia, while its EPA (Ecosa-pentanoic acid) and DHA (Docosa-hexaenoic acid) content is nearly a third and almost the same or a little higher of that of Artemia (Watanabe and Kiron, 1994). Again, any feed item must enter the mouth whole, i.e., feed particles have to be smaller than the larva's mouth gape, and quickly accepted or rejected on the basis of palatability (Fernandez- Diaz et al.,1994; Bengtson, 2003). Considering those criteria, Kahan et al. (1980) suggested nematodes as a potential candidate for a live food organism in rearing fish/prawn larvae. Wilkenfeld et al. (1984) stated that the nematodes were able to substitute Artemia in penaeid larval rearing diets. Their experiments showed the capability of *Farfantepenaeus aztecus*, *Litopenaeus setiferus* and *Litopenaeus vannamei* to be consumed and keep survive on the *P. redivivus* as the only food source from the Protozoea 1 (PZ-1) stage. Experiments of Biedenbach et al. (1989) with *P. redivivus* in the larval rearing of *L. vannamei* further confirmed these results. The lack of a proper mass production technology for nematodes was the most limiting factor to commercial application (Fisher and Fletcher, 1995) and further investigation on such techniques was recommended (Biedenbach et al., 1989).

The suitable size of *P. redivivus*, its high nutritional values and possible mass cultivation on cheap growth medium promised the species to be a valuable live feed for various prawn and shrimp larvae which also could be a potential alternative to the highly expensive Artemia (Biedenbach et al., 1989; Kumlu and Fletcher, 1997 and Lee et al., 2005). It is ecologically and logically believed that the nematode *Panagrellus sp*. could be found available in Bangladesh soil, and a local success of a mass culture of the species could deliver a permanent available live food throughout the larval rearing period of the prawns. As the growing prawn and shrimp industries have been confronting from the problem of low quality and high priced live feed, culture of *P. redivivus* and its use as live feed in hatcheries would provide a breakthrough in the prawn and shrimp culture sector. The main objectives of the present study were to detect the nematode, *Panagrellus redivivus* locally as well as to establish a feasible culture technique of the species for ensuring its mass production and use as an inexpensive live feed in prawn hatchery.

2. MATERIALS AND METHODS

2.1. Searching of P. redivivus and its habitat

During the period of October-November 2011, ten sites of soil ground in different areas of Khulna University campus, Bangladesh were selected randomly to search the availability of the species and

thus to trace out the habitat characteristics of the species. The characteristics of the species habitats (soil moisture; pH; temperature and soil depth) were recorded.

Soil moisture was measured by using gravimetric method (Evans et al., 1996). Five gram of soil sample was taken from the desired (5-15cm) depth by using an electronic balance (Model: SHIMADZU AUY220); then the sample was dried through a dry procedure using a dry oven (Model: Memert, Schutzart DIN 40050-IP 20) for 24 hours at 220 degree Fahrenheit; and then the soil sample was reweighted to determine the amount of water loss from soil. The moisture was calculated using the following equation:

$$\text{Moisture \%} = \frac{\text{Wet weight - Dry weight}}{\text{Wet weight}} \times 100$$

Soil pH, temperature and depth were recorded consequently by using soil pH meter (SPHMT-A1025 by Hanna), lab thermometer and centimeter scale respectively.

2.2. Collection and detection of the species

Based on the suggestions of Ricci et al. (2003), 12 raw potatoes were placed in each of the sites at the depths of 5, 10 and 15 cm, setting 4 in each of the depths of soil ground randomly and checked for the species after 7 to 14 days maintaining a 2 days interval. Thus, the checking was done on the 8^{th}, 10^{th} 12^{th} and 14^{th} day collecting 1 potato from each of the depths (5, 10 and 15 cm) of all the sites. The rotten potatoes collected from the ground sites were taken to the laboratory immediately and checked the presence of the species using high resolution microscope (Model: Carl Zeiss Micro imaging GmbH). The starters were then separated from the potato through a soft wash. The nematode *P. redivivus* detection was done following the morphological characteristics as described by Stock and Nadler (2006), using a high resolution light microscope (Model: Carl Zeiss Micro imaging GmbH) at 40x and 100x zoom.

2.3. Preparation of the culture media

The starter culture media was prepared with white oat and baker's yeast (which is also known as oatmeal media) as described by Ricci et al. (2003). The oat-based media was prepared by mixing 10 gm of oat with 20 ml warm water in a plastic container to get a paste form. The mixer was then cooled in room temperature (26 ± 10C). When it cooled, a pinch of baker's yeasts was spread over the paste. The mixture was incubated for 24 hours at 37 ^{0}C temperature, and then used as starter media for the *P. redivivus* in room temperature. After getting the starter culture the key experiments were started by replacing with oatmeal, oatmeal with sunflower oil (added 5% sunflower oil), cereal (used paste of baby cereal), raw potato (used blended paste of peeled raw potato) and boiled potato (used blended paste of boiled potato) based media. In preparing all of those media the guideline describe by Ricci et al. (2003) was followed. The efficiency of the media was observed (through estimating the abundance of the produced species) primarily with magnifying glass and then under light microscope. For each experiment (with a specific media) three replications were used.

2.4. Culture of the starter species

The collected *P. redivivus* was placed (at a rate of around 1 ml volume) on the culture media kept in plastic containers. The containers were then placed in incubator (Model: VS-8480SL) at different temperatures (22, 24, 25, 26, 27 and 28^{0}C). Three replications were done for each of the temperature tests. A gm of old culture media was used every time to start a new culture.

2.5. Harvest and counting of the cultured species

The *P. redivivus* was harvested from the inner wall of the culture container with scalpel. The live *P. redivivus* move very frequently and difficult to handle it. Hence, it was fixed with 10% formaldehyde for 5 minutes in order to fix them (Ted Pella, Inc., 2010). For counting, the harvested nematode fixed with 10% formaldehyde was used to obtain a 10 ml solution. Then another two serial dilutions were done with 9 ml distilled water each with 1 ml pre-diluted solution to reduce the high density of the cultured nematodes and thus to make easy count. Finally 1 ml water solution from the last 10 ml was considered for the counting. The number of the cultured species was counted by using "rafter cell" under light microscope.

2.6. Data analysis

Recorded data were analyzed by using Microsoft Office Excel 2007.

3. RESULTS AND DISCUSSION

3.1 Detection of P. redivivus

For the detection of the species, the collected samples were studied carefully under light microscope. An adult was observed with a clear layer of cuticle and flagella like tail. Male tail was found short while the female had comparatively long and spiky (Figure 1, 2). Mouth was located at the front of the body tip (Figure 3). The intestine was observed straight and ends shortly before the end. In male nematode there was an opening just before the end of the body and common body output for digestive and reproductive organs (Figure 5). For female, new young birth canal and excretory opening observed separate. On the edge of the opening, in two pockets two hook-like

structures (spicula) were present in the male (Figure 5). The vagina found oriented by muscular sheath and this genital opening was located at the middle part of body (Figure 6). The organ is then extended into a sex tube in which the fertilized eggs develop and provides shelter to developing nematodes; thereby helps to produce young nematodes (Figure 4). These results were similar to the findings of Stock and Nadler (2006) and Kumlu and Fletcher (1997) that confirmed the morphological detection of the species.

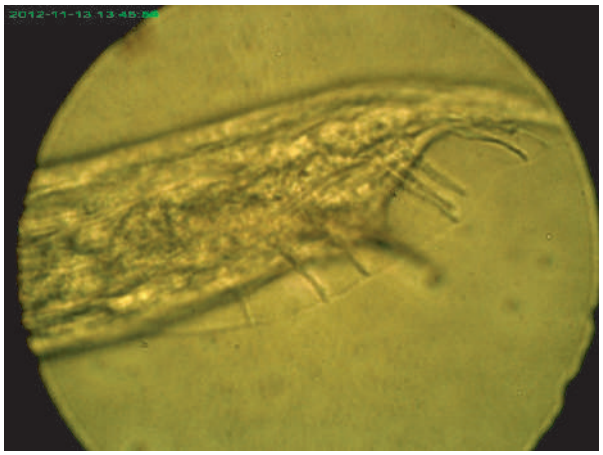

Figure 1: Tail of Male *P. redivivus*

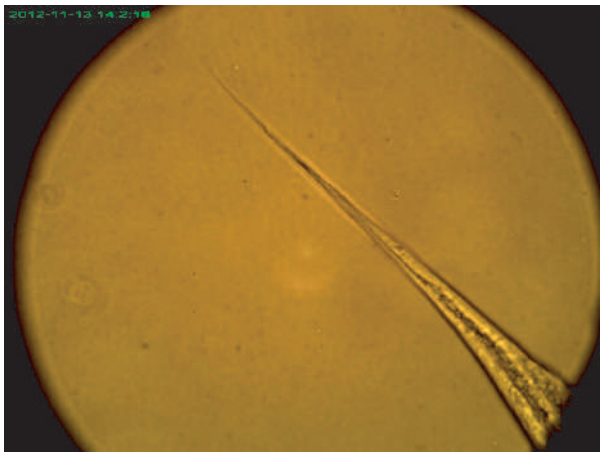

Figure 1: Tail of Male *P. redivivus*

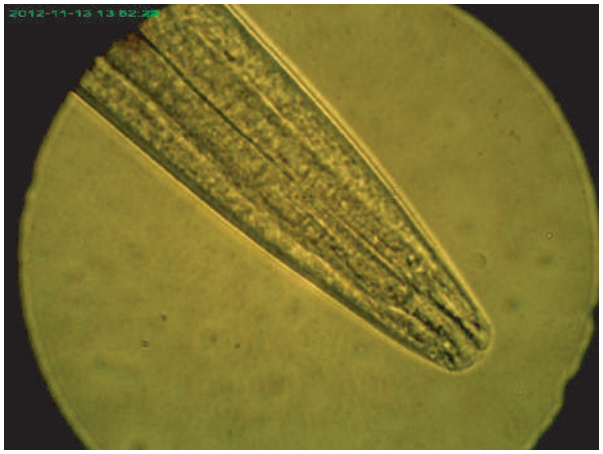

Figure 3: Mouth opening of *P. redivivus*

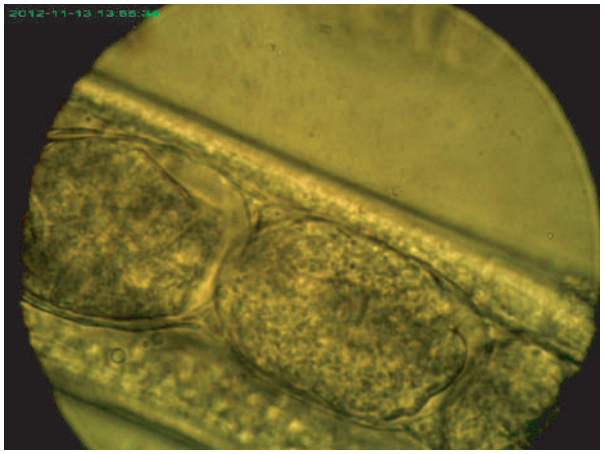

Figure 4: Fertilized egg in female *P. redivivus*

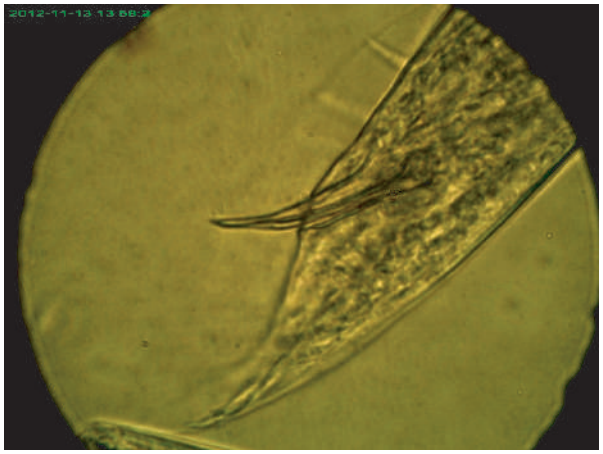

Figure 5: Genital organ of male *P. redivivus*

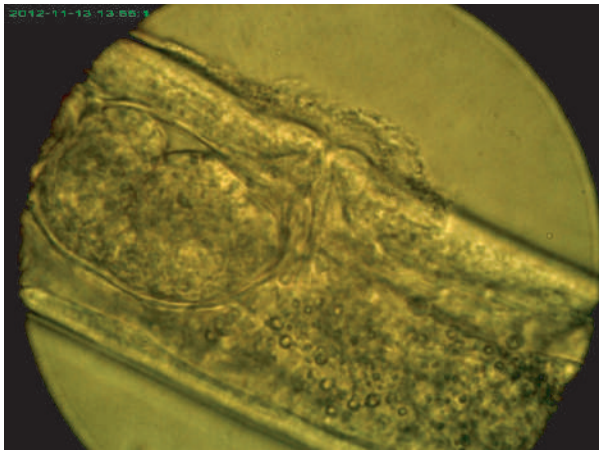

Figure 6: Genital organ of female *P. redivivus*

3.2 Habitat of P. redivivus

It was revealed that *P. redivivus* resides under some specific condition of the environment. The environmental parameters found in the habitats of the species in the local area are presented in the Table 1. This information would obviously provide a fruitful way to those who will be searching the species to start a culture or to do a further study. Habitats with around 30% of soil moisture, pH value of 7, 20^{0}C temperatures and 5 cm soil depth were found suitable for higher availability and yield of the species. These results of habitat parameters are supported by Bedding et al. (1991); Kumlu et al. (1998) and Lavens and Sorgeloos (2000) who observed more or less similar results in their studies.

Table 1. Soil parameters found suitable for the nematode, *Pangrellus redivivus*

Soil parameters	Species found available	Species found few
Moisture	30±6%	16-24%
pH	7±0.5	>7.5
Temperature	20±20C	>240C
Depth in soil	5±1cm	>6cm

3.3 Suitable culture media for the species

In this experiment five different types of media such as, oatmeal, sunflower oil enriched oatmeal, baby cereal, raw potato and boiled potato paste were used for culturing the species. Out of these five media, oatmeal and boiled potato paste were found better for culturing the species. The efficiency of different media for P. redivivus culture is summarized in the Table 2.

Table 2. Efficiency of different media used for *Panagrellus sp*. culture

Types of culture media	Species produced available
Oatmeal	+++
Sunflower oil enriched oatmeal	+
Cereal	+
Raw potato paste	-
Boiled potato paste	++++

The "+" sign indicates the intensity of production observed under light microscope while the "-" sign indicates very few or no production.

The primarily identified suitable media, boiled potato paste and oatmeal media were tested further for mass culturing of the species, and the performance of the media was observed with the view to find out the best one for the mass culture. It was observed that both of the media had a closer efficiency in producing the species. The calculated number of the produced *P. redivivus* individuals obtained from per gm of boiled potato media was 1,50,032±9,285 while the number was 1,24,622±6,722 from the oatmeal media. However, locally available boiled potato media was considered as the most feasible one for *P. redivivus* culture as it produced the highest number of individuals as well as due to its low preparation costs in comparison to the oatmeal media. Ricci et al. (2003) produced about 3,00,000 nematodes/gm of media while 5,00,000 nematodes/gm was produced by Bedding (1981). Schlechtriem et al. (2004) produced about 729 million from 200 gm oatmeal based media enriched with sunflower oil and 390 million nematodes from oatmeal based media. Although the production in this study is comparatively low, there might have scope to increase the production improving the revealed technique that needs a further detailed research.

3.4 Suitable temperature and time-length for the culture

To find out the suitable incubation temperature and incubation period for the culture, six different temperature regimes were tested separately at 22, 24, 25, 26, 27 and 28 ^{0}C, together with different incubation periods viz., 8, 10, 12, 14, 16, 18 and 20 days. Figure 7 represents the number of *P. redivivus* individuals produced with regard to temperature regimes and incubation periods. The highest production was found at 26^{0}C temperature with the incubation period of around 14th day that ensured the maximum harvest in this study; although, from the day 7 the newly grown species found started to climb up the container wall which were even ready to harvest. The result showed that a 5 gm weighted boiled potato can produce approximately 0.75 million of nematodes (Figure 7).

There are several studies on the culture and use of mass-produced *P. redivivus* in the rearing of first feeding fish/prawn larvae (Fisher and Fletcher, 1995; Santiago et al., 2004; Schlechtriem et al., 2004; Schlechtriem et al., 2005). They found optimum incubation temperature for mass culture at around 23^{0}C with the incubation period of 10 to 12 days that differs a bit with the findings of the present study. This may be due to an effect of the difference in geographical location as well as genetic variation within and/or between species populations.

There is a dearth of information on the culture and culture feasibility of *P. redivivus* in Bangladesh. Although the production has been observed comparatively low in this study still this could easily be considered for use, perhaps to overcome the unavailability of an alternative live food for larvae in hatcheries. Besides, as an initiation in this local area, the production of *P. redivivus* found in this study could be say satisfying. Thus, this boiled potato media technique which costs very low as well can be used for the production of *P. redivivus*. Sautter et al. (2007)

studied the feeding rate of fish larvae and stated that the feeding frequency of the fish larvae is about 5,000 nematode larvae-1 day 1 which is very impressive. The present study revealed that approximately 15 million nematodes can be produced just from 100 gm of boiled potato media in small scale basis that is enough to use as food for at least 3000 larvae in a day.

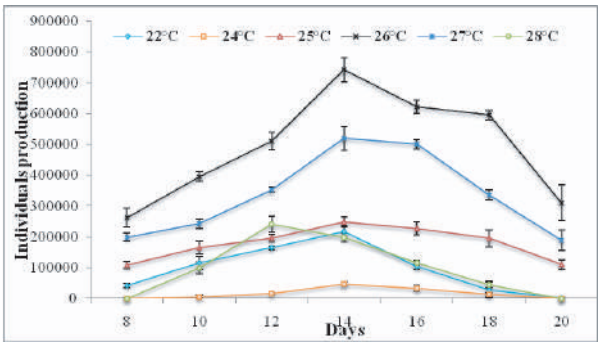

Figure 7. Production of *P. redivivus* at different temperature on boiled potato based media.

4. CONCLUSIONS

Availability of live food is one of the major bottlenecks for the raising hatchery sector worldwide. It has already been well proven that the nematodes are a promising food source for the first feeding fish and crustacean larvae. Thus, the nematode species, *Panagrellus redivivous* recently used as an inexpensive live feed in prawn hatchery industry in some countries is assumed to be a very potential one in Bangladesh as well. This required revealing a suitable mass culture technique of the species in captive condition. The present study was successful in developing a suitable and cost effective culture technique of *P. redivivus.* The results of this study clearly dictate that mass-production of nematodes in boiled potato media would hopefully be feasible in a large scale basis. If the large scale production of nematodes can be initiated, it would bring a breakthrough for the hatchery sector. However, further research is needed for the development of the culture media to increase more the production and nutritional value of the nematodes; comparative study of *P. redivivus* with Artemia on the factor of feeding rate, survivability, growth and nutritional value of larvae. Moreover, it is not yet known whether these nematodes might harmful anyway for the larvae; their possibility to work as a vector in environment should also be kept into consideration. If this nematode does not pose any threat to larvae then *P. redivivus* will be a potential candidate species as live food for the larvae of many fisheries species instead of expensive Artemia. The fisheries sector, on the other hand, will enter into a new domain specially in case of shrimp and prawn hatchery; it would also be a plausible source for betterment of feed industries.

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