Identification and Feasibility of Nematode (*Panagrellus* sp.) Culture in Bangladesh

Al-Imran

Fisheries and Marine Resource Technology Discipline
Khulna University
Khulna-9208, Bangladesh

April, 2013

Identification and Feasibility of Nematode (*Panagrellus* sp.) Culture in Bangladesh

A Thesis

By

Al-Imran

Examination Roll No: MS 120603

Session: 2011-2012

The Thesis submitted to the Fisheries and Marine Resource Technology Discipline in partial fulfillment of the requirements for the Degree of

Master of Science
In
Fish Genetics and Biotechnology

April, 2013

Identification and Feasibility of Nematode (*Panagrellus* sp.) Culture in Bangladesh

Approved as to style and content by

Professor Dr. Md. Nazmul Ahsan

Head

Fisheries and Marine Resource Technology Discipline

Khulna University

Khulna-9208, Bangladesh

April, 2013

Declaration

Identification and Feasibility of Nematode (*Panagrellus* sp.) Culture in Bangladesh

The results presented in the thesis with the above mentioned title are entirely of the candidate's own investigations and no part of the results has been accepted for any degree nor is it being concurrently submitted for any degree

Supervisor

(Signature of the Supervisor)

Dr. Md. Golam Sarower

Professor

Fisheries and Marine Resource Technology Discipline

Khulna University, Khulna-9208, Bangladesh

Candidate

(Signature of the Candidate)

Al-Imran

Examination Roll No. MS 120603

Session: 2011-2012

April, 2013

Dedicated to...

Dr. Mohammed Mostafa

Professor
Fisheries and Marine Resource Technology Discipline
Khulna University

Acknowledgement

All the praises to the almighty Allah for blessing me to complete this project thesis work for the degree of M.Sc. in fisheries from Khulna University under the Fisheries and Marine Resource Technology Discipline. I would like to express my sincere gratitude to my supervisor, **Dr. Md. Golam Sarower**, Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, for his continuous guidance, valuable suggestions and sustained support in preparing this project thesis.

I would also like to express my gratitude to **Dr. Md. Nazmul Ahsan**, Professor and Head, Fisheries and Marine Resource Technology Discipline, Khulna University, for his valuable and sincere appreciation.

I would express my gratitude to **Dr. Mohammed Mostafa**, Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, for give me a clear concept about this study. Md. Lifat Rahi, Assistant Professor, Fisheries and Marine Resource Technology Discipline, Khulna University, Khulna for his continuous guidance. I convey my thanks to Md. Sadiqul Amin, Assistant Professor, Soil Science Discipline, Khulna University, Khulna for his valuable suggestions about measuring different soil parameters. I convey my thanks to Dr. Ghausiator Reza Banu, Professor, Fisheries and Marine Resource Technology Discipline, Khulna University and Md. Habibur Rahman, Assistant Registrar (Laboratory), Fisheries and Marine Resource Technology Discipline for their cordial help in the analytical works. I also convey thanks to all of my classmates especially for Foyez Ibn Shams Sudip Debnath, Md. Shahin Parvez, Md. Sayeduzzaman and G. M. Zillur Rahman whose inspirations were all along with me in the hard time during the course of the present study.

I want to express my gratefulness to Ministry of Education for funding the present study as a part of "Feasibility of Nematode (*Panagrellus* sp.) culture in Bangladesh and its suitability as live feed for larval rearing of giant prawn, *Macrobrachium rosenbergii*" project.

Al-Imran
April, 2013

Abstract

The free-living soil nematode *Panagrellus redivivus* is well known to be an inexpensive, standardized and permanently available food source for first feeding fish and crustacean larvae. The present study was aimed at the development of a suitable culture technique of *P. redivivus* so as to be used as live food for the larvae and prawn later on. Oatmeal, cereal, raw potato and boiled potatoes were used as culture media for the mass production of this nematode and the culture of nematode was found successful in oatmeal and boiled potato media. The local and cheap boiled potato media considered as the feasible culture media for the mass production of *P. redivivus*. The nematodes were incubated in different temperatures (22 ^{0}C, 24 ^{0}C, 25 ^{0}C, 26 ^{0}C, 27 ^{0}C and 28 ^{0}C). Mass production of *P. redivivus* was found at 26 ^{0}C incubation temperature. 1, 14,622±6,722 individuals of nematodes were harvested from 1 gm of boiled potato media. The new technology 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.

Contents

Contents		Page
Acknowledgement		I
Abstract		II
Contents		III
List of tables		IV
List of figures		V
List of abbreviations		VI
Chapters		
Chapter 1	Introduction	1-6
1.1	Background information	1-6
1.2	Objectives	6
Chapter 2	Literature review	7-10
Chapter 3	Materials and methods	11-13
3.1	Habitat identification	11
3.2	Collection and identification	11
3.3	Culture Media	12
3.4	Culture of the starter	12
3.5	Harvest and counting the cultured starter	12
3.6	Data recording and analysis	13
Chapter 4	Results and discussion	14-18
4.1	Identification of *P. redivivus*	14
4.2	Habitat of *P. redivivus*	15
4.3	Culture Media	16
4.4	Culture of the starter	16
Chapter 5	Conclusion	19
Chapter 6	References	20-25
	Photo Gallery	26-31

List of tables

Table no.	Title	Page
Table 1.	Soil parameters found suitable for nematode *Pangrellus redivivus* species	16
Table 2.	Efficiency of different media used for *Panagrellus* sp. culture	16

List of figures

Figure No.	Title	Page
Fig.1.	Tail of Male *P. redivivus*	14
Fig.2.	Tail of Female *P. redivivus*	14
Fig.3.	Mouth opening of *P. redivivus*	15
Fig.4.	Fertilized egg in female *P. redivivus*	15
Fig.5.	Genital organ of Male *P. redivivus*	15
Fig.6.	Genital organ of female *P. redivivus*	15
Fig.7.	Production of *P. redivivus* in different temperature in boiled potato based medium	17

List of Abbreviations and non-English Words

Abbreviation	Elaboration
cm	Centimeter
DoF	Department of Fisheries
DHA	Docosahexaenoic Acid
EPA	Ecosapentaenoic Acid
et al.	And Others
FAO	Food and Agricultural Organization
Fig.	Figure
GDP	Gross Domestic Product
gm	Gram
i. e./e. g.	In Effect/ Example Given/For Example
M. Sc.	Master of Science
Kg	Kilogram
KU	Khulna University
$larvae^{-1}\ day^{-1}$	Per Larvae Per Day
mL	Milliliter
mm	Millimeter
p.	Pages
PL	Post Larvae
PZ-1	Protozoea 1
viz.	Namely/That is to Say/As Follows

1. Introduction

1.1 Background Information

The world fisheries and aquaculture are mounting very frequently in recent years. Capture fisheries and aquaculture supplied the world with about 148 million tonnes of fish in 2010, of which about 128 million tonnes was utilized as food for people, and preliminary data for 2011 indicate increased production of 154 million tonnes, of which 131 million tonnes was destined as food. With sustained growth in fish production and improved distribution channels, world fish food supply has grown dramatically in the last five decades, with an average growth rate of 3.2 percent per year in the period 1961–2009, outpacing the increase of 1.7 percent per year in the world's population. World per capita food fish supply increased from an average of 9.9 kg (live weight equivalent) in the 1960s to 18.4 kg in 2009, and preliminary estimates for 2010 point to a further increase of fish consumption to 18.6 kg (FAO, 2012).

The contribution of the crustacean to the world aquaculture production values reached 22.6% in 2004. Shrimps representing 83% of crustacean production in tones and 85% in values are the largest group of species in crustacean farming. Most of these shrimp species derive from the family *Penaeidae. Litopenaeus vannamei* contributes to 47% of aquacultural shrimp production and 43% of production values (FAO, 2006). One of the major bottlenecks in aquaculture production is the rearing of fish and crustacean larvae (Lavens and Sorgeloos, 1996).

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 last Economic Review- Fisheries sector - contributes 4.43% in national GDP, 22.21% in Agriculture and 2.73% in export earning of Bangladesh. About 10.50% people of the whole population are directly involved with fisheries for their livelihood. About 921 fin fish hatcheries produce 629.175 tonnes fish fry and 140 shrimp and prawn 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

industry in the country. However, in the recent times 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 as well as creating a long lasting negative impact on the nature. In such a situation, the hatchery produced larvae is considered as an effective and sustainable biological tool for the vertical and horizontal expansion of the good quality prawn production.

The larval rearing of aquatic organisms is generally carried out under controlled hatchery conditions and usually requires intensive monitoring techniques which are normally different from conventional nursery and grow-out procedures and especially with respect to husbandry techniques, feeding strategies, and microbial control. The main reason for this is that the developing larvae are usually very small, extremely fragile, and generally not physiologically well developed. For example, their small size (i.e. small mouth size), the incomplete development of their perception organs (i.e. eyes, chemoreceptors) and digestive system, are limiting factors in proper feed selection and use during the early first-feeding or start-feeding period. Moreover, in species such as shrimp and prawn, these are not the only problems as the developing larvae also have to pass through different larval stages, eventually changing from a herbivorous filter feeding behavior to a carnivorous hunting behavior. It is perhaps not surprising therefore that larval nutrition, and in particular that of the sensitive first-feeding larvae, has become one of the major bottlenecks preventing the full commercialization of many farmed fish and shellfish species.

Prior to establishing hatcheries for seed production, it is an imperative to develop suitable feeds for the larvae to feed at first. Live feed is always better option in this regard especially for the first few days after birth. Combination of both live and nutrient rich formulated diet supports for the higher survival as well as production performance in the hatcheries and in culture farms. The development of low quality formulated diets can retard and impede growth as reviewed by Kamler (1992). Further studies reported that the high mortalities observed in carp larvae fed poor quality formulated diets which could be reduced by feeding live feed at first. This accelerates the differentiation of morphological structures and internal organs of the fish larvae and enables them to pass safely through this vulnerable period of their lives (Bryant, 1981; Dabrowski, 1984). Crustacean species have much more dependency on live feed and their culture feasibility

depends on live food during larval stages (Sorgeloos *et al.,* 2001). This live food should be easily available, reproducible, culturable and economically viable (Watanabe and Kiron, 1994). Any Problem in supplying live fed might create severe adverse impact on successful larval rearing, which in turn limits the whole production system (Guillaume *et al.,* 2001).

Live feeds are the main item in the diet of cultured fish larvae and they are of particular importance when rearing marine fish larvae of the altricial type. Altricial larvae are those that remain in a relatively undeveloped state until the yolk sac is exhausted. At first feeding, the digestive system still remains rudimentary, lacking a stomach, and much of the protein digestion takes place in hindgut epithelial cells (Govoni *et al.,* 1986). Such a digestive system in most cases incapable of processing formulated diets in a way that allows survival and growth of the larvae. Despite the recent progress in developing inert diets for fish larvae (Lazo *et al.,* 2000; Cahu and Infante, 2001; Koven *et al.,* 2001), feeding of most commercial aquatic species of interest still relies on live feeds during the early life stages. However, the low digestive capacity of altricial larvae might not be the limiting factors for aquaculture improvement. Live feeds of larvae are able to swim in the water column and are thus constantly available to the larvae. Most formulated diets tend to aggregate on the water surface or, more commonly, sink within a few minutes to the bottom, and are thus normally less available to the larvae than live feeds. In addition, since larvae are believed to be 'visual feeders', adapted to attack moving prey in nature, the movement of live feed in the water is likely to stimulate larval feeding responses. Finally, live prey, with a thin exoskeleton and a high water content (normally 80%), have a lower nutrient concentration and may be more palatable to the larvae once taken into the mouth, compared with the hard, dry formulated diet.

But the prawn larvae production in Bangladesh is solely dependent on costly live food Brine shrimp, *Artemia* nauplii for their nutrition and in the recent days, formulated custard beside brine shrimp. 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). All that is needed for incubation is the hydration of the cyst in warm, aerated seawater and illumination. This is sufficient to start the embryonic development in the dormant cysts and leads to the hatching of the

nauplii (Sorgeloos and Persoone, 1975). 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 property to consume algal feed and therefore to compete with the cultured species for food (Biedenbach *et al.,* 1989, Lavens and Sorgeloos, 1996). The main drawback for future use is that the cyst production in the Great Salt Lake, Utah, USA which is the major production site of *Artemia*, is limited and does not satisfy the growing demand of the world (Lavens and Sorgeloos, 2000). Even the '*Artemia* replacement' products increasingly used in commercial operations are normally used in co-feeding with live feeds (Curnow *et al.,* 2006; Vega- Orellana *et al.,* 2006; Hamza *et al.,* 2007; Rosenlund and Halldorsson, 2007). The lack of potential alternatives to *Artemia* may become an obstacle to a further increase of aquaculture production especially in developing countries like us. 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; Lee *et al.,* 2005).

Prawn and shrimp aquaculture have been expanding satisfyingly for the last couple of years in Bangladesh based on wild seeds initially and creating a long lasting adverse impact on nature. 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 establishes in the coastal region of Bangladesh. The hatchery owners and operators are still using costly *Artemia* as live feed followed by custard after 10 days of hatching. But the growing prawn hatchery sector is at stake in Bangladesh because of the availability of poor quality *Artemia* cyst in the market that those results sever mortality of larvae in the hatcheries. As a consequence of this, seed production has been fallen dramatically in the last 2 years in Bangladesh which is the main reason for the reduction of prawn culture area as well as total production. On the other hand, culture of prawn in the last 2 years was completely dependent on supply of natural seeds. Thus, searching of an alternative live feed and coping of that particular feed for the larvae in the hatcheries could be the most plausible solution in order to save the growing prawn industry of this country. Culture of nematodes and application as a starter feed in hatcheries can be an option to cope this particular disaster.

Free-living nematode *Panagrellus redivivus* known to many aquarium enthusiasts and fish keepers as the microworm. According to stock and Nedler, (2006) *Panagrellus sp.* has a worldwide distribution, with species described from almost every continent except Australia and Antarctica. *P. redivivus* is a bacteriophagous, ovoviviparous, free-living soil nematode, being one of the few that do not lay eggs, but hatch juveniles internally. It has four larval stages before becoming adults. The first larval stage is intrauterine, but the remaining stages are free-living. It is gonochoristic, producing equal numbers of males and females. Bacteriophagous nematodes are already known as a potential food source for fish larvae. *P. redivivus* is a nematode which is easy to rear in large quantities by using suitable culture media. The worms feed on bacteria which are pre-cultured. They have a short life cycle and a high fecundity. *P. redivivus* are a tiny nematode about 0.5 to 2.0 mm in length and 0.05 mm in diameter is suitable for fish larvae, especially those with a small mouth. 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. Their size increases by three times during the first day and five to six times during the next three days (Rainbowfish, 2008).

However, because of the high prices of *Artemia* cysts, the use of hatched *Artemia* nauplii as live food for first feeding fish larvae is too expensive. Although several studies proved that the free-living nematode *P. redivivus* is a suitable food for first feeding fish and crustacean larvae (Kahan and Appel 1975; Kahan *et al.* 1980; Biedenbach *et al.* 1989; Kumlu and Fletcher 1997; Kumlu *et al.* 1998) this species has not been used in the aquaculture industry 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 are quickly accepted or rejected on the basis of palatability (Fernandez- Diaz *et al.,*1994; Bengtson, 2003). Kahan *et al.* (1980) suggested nematodes as a potential candidate for a live food organism in rearing fish fry. Their suitable size, high nutritional values and an easy cultivation promised nematodes to be a valuable feed. 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 *L. vannamei* to be consumed and keep survive 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 is the most limiting factor to commercial application (Fisher and Fletcher, 1995) and further investigation on such techniques is recommended (Biedenbach *et al.,* 1989).

In this context, the free living soil nematode *P. redivivus* can be an alternative to the highly expensive *Artemia.* Recent investigations with free living nematodes as larval feed for various penaeid shrimps have been revealed promising (Biedenbach *et al.,* 1989; Kumlu and Fletcher, 1997). The species can be cultured in large quantities on cheap growth medium. It is ecologically and logically believed that the nematode *Panagrellus* sp. will be found available in Bangladesh. The development of mass culture of *Panagrellus* sp. will allow the production of a sufficient quantity of nematodes to deliver a standardized and permanent available live food throughout the larval rearing period of the giant prawn as an alternative to *Artemia.* Therefore, the identification and culture feasibility of the *Panagrellus* sp. has been considered as potential research in the area of fish and crustacean larval research. As the growing prawn and shrimp industries in Bangladesh have been confronting from the problem of low quality and high priced live feed, culture of a suitable nematode and later on use that nematode *P. redivivus* as live feed in hatcheries would definitely provide a breakthrough in the prawn and shrimp culture sector in Bangladesh.

1.2 Objectives

- To establish a feasible culture technique of nematode *Panagrellus. redivivus* for mass production in Bangladesh.

CHAPTER TWO

LITERATURE REVIEW

2. Literature review

As the larval nutrition is a vital stage for the further procedure of fish and shellfish culture then it's a challenge to select a suitable food for fish larvae with high growth, low mortality and cost effective.

Live food accelerates the differentiation of morphological structures and internal organs of the fish larvae and enables them to pass safely through this vulnerable period of their lives (Bryant, 1981; Dabrowski, 1984).

At first feeding the digestive system is still rudimentary, lacking a stomach, and much of the protein digestion takes place in hindgut epithelial cells (Govoni *et al.*, 1986).

The development of larvae fed formulated diets can be retarded; their growth can be impeded, and the energy and matter transformation efficiencies are often depressed as reviewed by Kamler (1992).

During fish and crustaceans early stages of development, many fish and crustacean species important for marine aquaculture rely on live food organisms (Sargent *et al.*, 1995).

For the fish and crustacean larval nutrition live food is the best choice studied by many author. The most commonly used live food is the brine shrimp *Artemia salina*, a small branchipod crustacean representing approximately 40% of the total aquaculture demand for live feeds for early stages (Lavens and Sorgeloos, 2000). This small crustacean has the advantage that its culture can be started from dried eggs (Sorgeloos and Persoone, 1975; Liao, 1992).

Artemia 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). All that is needed for incubation is the hydration of the cyst in warm, aerated seawater and illumination. This is sufficient to start the embryonic development in the dormant cysts and leads to the hatching of the nauplii (Sorgeloos and Persoone, 1975).

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 property to consume algal feed and therefore to compete with the cultured species for food (Biedenbach *et al.,* 1989; Lavens and Sorgeloos, 1996).

The main drawback for future use is that the cyst production in the Great Salt Lake, Utah, USA which is the major production site of *Artemia*, is limited and does not satisfy the growing world demand (Lavens and Sorgeloos, 2000). Even the '*Artemia* replacement' products increasingly used in commercial operations are normally used in co-feeding with live feeds (Curnow *et al.,* 2006; Vega- Orellana *et al.,* 2006; Hamza *et al.,* 2007; Rosenlund and Halldorsson, 2007).

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; Lee *et al.,* 2005).

It has been shown that the free-living nematode *Panagrellus redivivus* is a suitable food for fish (Kahan and Appel, 1975; Kahan *et al.*, 1980) and crustacean larvae (Biedenbach *et al.*, 1989; Kumlu and Fletcher, 1997; Kumlu *et al.*, 1998; Wilkenfeld *et al.*, 1984).

Bar-El *et al.,* (1980) suggested nematodes as a potential candidate for a live food organism in rearing fish fry.

The free-living nematode *P. redivivus* has desirable characteristics: the female is ovoviviparous and has a high reproductive rate; generation time is 5–6 days with up to 83 larvae in a cuticle (Cryan *et al.*, 1963).

Samocha and Lewinsohn, (1977) reported on the successful use of the free-living soil nematode *P. redivivus* in rearing post larvae of *Penaeus semisculcatus* and *Metapenaeus stebbingi*.

The proximate composition and amino acid profile of *P. redivivus* are comparable with *Artemia* (Kahan *et al.*, 1980). It has an amino acid profile that matches that of *Artemia,* while its EPA and DHA content is respectively 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 are quickly accepted or rejected on the basis of palatability (Fernandez- Diaz, Pascual and Yufera, 1994; Bengtson, 2003).

The free-living nematode *Panagrellus* sp. has been tested in *Danio malabaricus* and *Poecilia reticulata* (Kahan and Appel, 1975); common carp *Cyprinus carpio* and silver carp *Hypophthalmichthys molitrix* (Kahan *et al.*, 1980); and grass carp *Ctenopharyngodon idella* and the carp *Hypophthalmichthys nobilis* (Rottmann *et al.*, 1991).

P. redivivus in has been tested in larval shrimps *Penaeus vannamei* (Biedenbach *et al.*, 1989) and *Penaeus indicus* (Kumlu and Fletcher, 1997; Kumlu *et al.*, 1998).

Fisher and Fletcher (1995) produced nematodes by the means of a biological fermenter filled with oatmeal based culture medium, inoculated with *Escherichia coli* and *P. redivivus*.

Bedding (1981) and Bedding *et al.* (1991) developed a solid-medium technique for the production of entomopathogenic nematodes like *Neoplectana* spp. and *Heterorhabditis* sp.

With the novelty of the exploitation of the third dimension, this system enlarges the usable surface inside the growing system by using crumbled polyether polyurethane sponges to create an interstitial space. This space allowed optimal reproduction conditions and served as a living habitat for the nematodes. It also guaranteed sufficient aeration. From the solid-medium system of Bedding (1981) and Bedding *et al.* (1991), Ricci *et al.*, (2003) developed a system for the mass production of *P. redivivus*.

Compared with the biological fermenter proposed by Fisher and Fletcher (1995), the bag system by Ricci *et al.* (2003) delivers higher multiplication factors and is less complicated to apply (Schlechtriem *et al.*, 2004).

Rouse, Webster and Radwin (1992) have shown that the nutritional character of *P. redivivus* depends on the nutritional qualities of the culture medium it is grown on.

Wilkenfeld *et al.* (1984) used soft dough of corn flour and deionized water; Biedenbach *et al.* (1989) worked with a mixture (50/50) of nonbleached wheat flour, corn meal and deionized water to produce soft dough.

Kumlu *et al.* (1998) used baffled 250 ml flasks and a medium of animal protein, corn oil and yeast inoculated with *E. coli*.

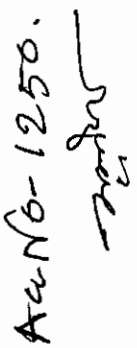
Ricci *et al.* (2003) used an oatmeal-based medium and a soluble medium made up of purified ingredients, resembling the proximate composition of oat.

Additional investigations concerning the effect of an added oil source (fish oil or sunflower oil) on body composition, average yields and multiplication factors of the nematodes were conducted by Schlechtriem *et al.* (2004).

The enrichment of culture media with further lipid sources (capelin oil, cod liver oil and marilla oil) was tested by Kumlu *et al.* (1998).

There are several studies on the use of mass-produced *P. redivivus* in the rearing of first feeding fish larvae (Santiago *et al.*, 2004; Schlechtriem *et al.*, 2004; Schlechtriem *et al.*, 2005).

Ricci *et al.* (2003) stated that it should be applicable for small-scale live feed producers in developing countries due to its simple technology.



CHAPTER THREE

MATERIALS and METHODS

3. Materials and methods

3.1 Habitat identification

Ten sites of soil ground in different areas of Khulna University were selected to see the availability of the species at different sites and thus to trace out the suitable habitat of the species. The characteristics of the soil sites (soil moisture; pH; temperature and soil depth) were recorded.

Soil moisture is measured by using gravimetric method (Evans *et al.*, 1996). Soil sample from the desired (5-15cm) soil depth was taken, 5 gm soil sample were weighted (add the unit of soil taken kg/gm) with electronic balance (Model: SHIMADZU AUY220), soil sample was then dried in a dry oven (Model: Memert, Schutzart DIN 40050-IP 20) for 24 hours at 220 degrees Fahrenheit, and then soil sample was reweighted - to determine the amount of water loss from soil. Then the moisture was calculated with following equation:

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

Soil P^H, temperature and depth were taken consequently by soil P^H meter (SPHMT-A1025 by Hanna), lab thermometer and centimeter scale.

3.2 Collection and identification

Raw potatoes were placed at different sites and at different depths (ranging from 5-15 cm depth) of soil ground and checked after 7-14 days. After 7 days, the rotten potatoes were collected from the ground and taken to the laboratory immediately and checked the presence of the species using high resolution microscope. The starters were then separated from the potato through a soft wash. The nematode *P. redivivus* identification has been done through morphological characteristics as described by Stock and Nadler (2006). The nematodes were studied under high resolution light microscope (Model: Carl Zeiss Micro imaging GmbH) at 40x and 100x zoom.

3.3 Culture Media

The starter culture media are prepared with white oat and baker's yeast as described by Ricci *et al.* (2003). The oat-based media was prepared by mixing the 10 gm oat with 20 ml warm water in a plastic container to get a paste form. Then the mixer was cooled in room temperature (26 ± 1^0C). When it cooled a pinch of bakers yeasts are spread over the paste. The mixture was incubated for 24 hours at 37 ^{0}C temperature. Then the mixer was used as starter media for the *P. redivivus* in room temperature. After getting the starter culture the key experiment was started by replacing with cereal, raw potato and boiled potato based media and observed the efficiency of media primarily with magnifying glass and then under light microscope.

3.4 Culture of the starter

The collected *P. redivivus* then placed on the culture media. The plastic containers were then placed in incubator (Model: VS-8480SL) at different temperature (22, 24, 25, 26, 27 and 28 ^{0}C). 3 replications were done for each temperature. After 7 days they started to climb up the container wall and next day they were ready to harvest. One teaspoon of old culture media was enough to start a new one.

3.5 Harvest and counting the cultured starter

The *P. redivivus* was harvested from the inner wall of the container with the scarp of a blade or scalpel. Live *P. redivivus* move very frequently and very difficult to handle it. Hence, it was fixed with 10% formaldehyde for 5 minutes in order to fix them (Ted Pell, Inc., 2010). Harvested nematodes were diluted with 10% formaldehyde 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 because the density of nematode is too much high and very difficult to count. Finally 1 ml water solution from last 10 ml was considered for counting nematode *P. redivivus*. The number of the cultured species was counted by using rafter cell under light microscope.

3.6 Data recording and analysis

All the data were recorded and analyzed by using Microsoft Office Excel 2007.

CHAPTER FOUR

RESULTS and DISCUSSION

4. Results and discussion

4.1 Identification of *P. redivivus*

The specimens were observed under light microscope where a clear layer of cuticle was observed. An adult has flagella like tail, male tail was found short while the female was comparatively long (Fig. 1, 2). Mouth is located at the front of the body tip (Fig. 3). The intestine is straight and ends shortly before the end. In male nematode there is an opening just before the end of the body and common body output for digestive and reproductive organs (Fig. 5). For female new young birth canal and excretory opening are separate. On the edge of the opening in two pockets two hook-like structures (Spicula) was present in the male (Fig. 6). The vagina is oriented by muscular sheath and this genital opening is located at the middle part of body (Fig. 3). Genital 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 (Fig. 4). These results were similar to the findings of Stock and Nadler (2006) and kakerlakenparade (2009).

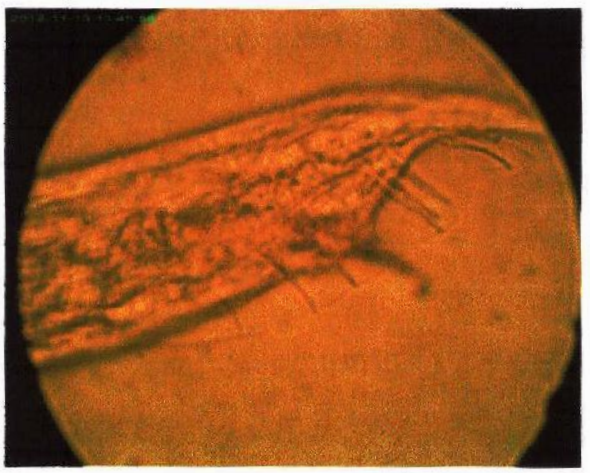


Fig. 1. Tail of Male *P. redivivus*

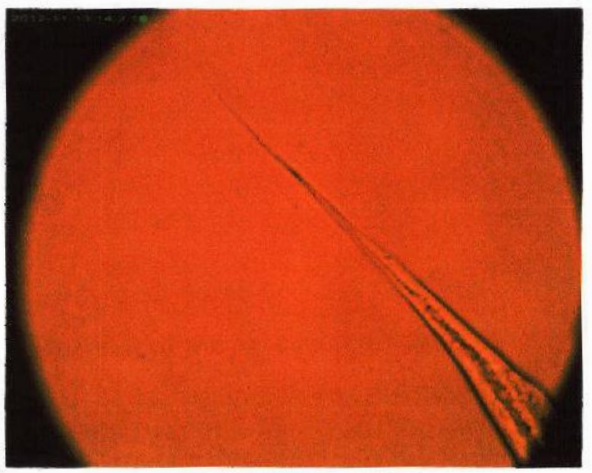


Fig. 2. Tail of Female *P. redivivus*

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

Soil parameters	Species found available	Species found few
Moisture	30±8%	14-24%
pH	7±0.5	7.5
Temperature	18±2^{0}C	<22^{0}C
Depth in soil	5±1cm	>6cm

4.3 Culture Media

Different types of media such as, oatmeal, baby cereal, raw potato and boiled potato paste were used for culturing the species. Out of these four media, oatmeal and boiled potato paste was found the best for culturing the species. Locally available boiled potatoes are considered as the as feasible media for *P. redivivus* culture in Bangladesh. The efficiency of different media for *P. redivivus* culture is summarized below in the Table 2.

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

Types of culture media	Species produced available
Oatmeal	+++
Cereal	+
Raw potato	-
Boiled potato paste	+++

The "+" sign indicates the intensity of product under light microscope while the "-" sign indicates no production

4.4 Culture of the starter

Local and cheap boiled potato paste was used to culture this species. Boiled potato paste and oat based media for culturing the species showed varied results. Number of *P. redivivus* individuals obtained was 2, 01,432±9,285 per gm media due to the use of oat based media. Boiled potato paste media on the other hand, produced approximately 1, 14,622±6722 individuals of *P.*

redivivus per gm media. 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. Fig. 7 represents the number of *P. redivivus* individuals produced with regard to temperature regimes and incubation periods. The best production was found in 26 ^{0}C temperature and at the incubation period of day 14 that ensured the maximum harvest in this study. The result showed that a 30 gm weighted boiled potato can produce approximately 3.4 million of nematodes.

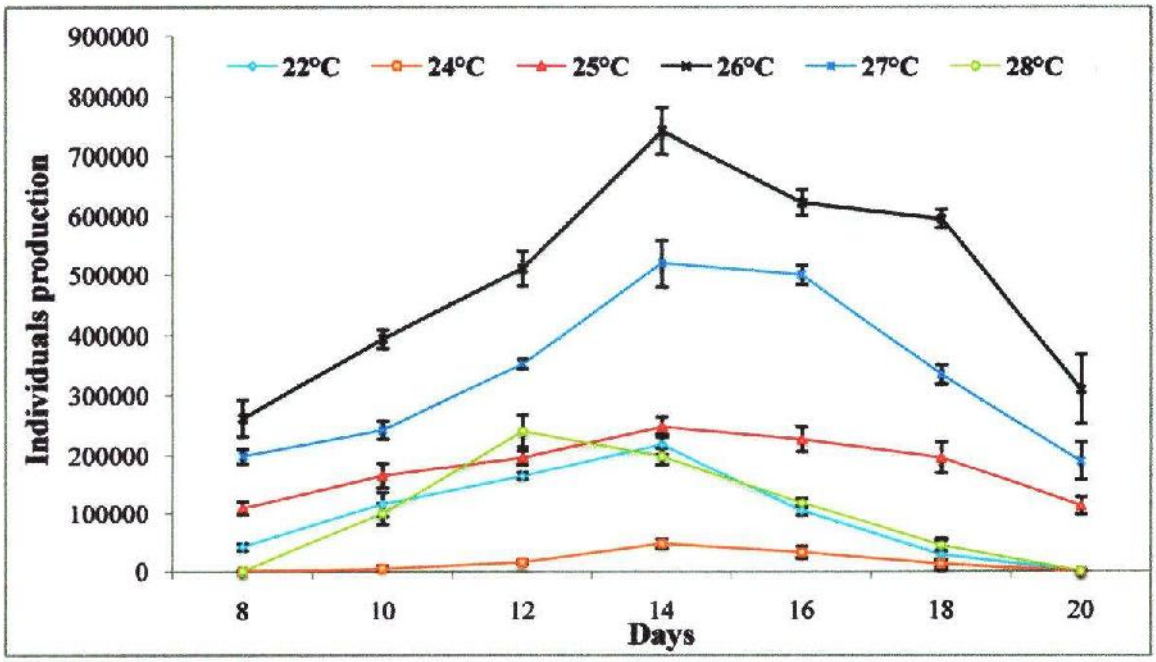


Fig. 7. Production of *P. redivivus* in different temperature in boiled potato based medium

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 of this study was comparatively low but the objectives of this study was almost fulfilled.

Boiled potato media is not enriched with enough nutrients compared with oatmeal based media enriched with sunflower oil, fish oil etc. Again maximum researcher found optimum incubation temperature at 22^{0}C but the suitable temperature for the incubation in this study was found to be at 26^{0}C. It may be an effect of the difference in

geographical location as well as genetic variation within and/or between species populations.

Actually there is a serious dearth of information on the culture and culture feasibility of *P. redivivus* in Bangladesh. Although production is comparatively low in potato based media but this could easily be used in this country because of the unavailability of an alternative live food for larvae in hatcheries. Besides, total production of *P. redivivus* in potato media was found satisfying. Thus, the boiled potato is enough for the mass production of *P. redivivus* in 26^0C with very low cost in context of Bangladesh. 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. Already 3.4 million nematodes were obtained from 30 gm potato media in small scale basis that is enough to use as food for at least 700 larvae for one day.

CHAPTER FIVE

CONCLUSION

5. Conclusion

Availability of live food is a major bottleneck for this raising sector as a considerable number of hatcheries have in the mean time been established in order to fulfill the growing demand of seeds of fish, shrimp and prawn for culture. It has already been well proven that the nematodes are a promising food source for the first feeding fish and crustacean larvae. 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 in Bangladesh, definitely it would bring a breakthrough for the hatchery sector. Further research is needed to know the suitable culture media to increase more the production and nutritional value of the nematodes; test on different fish and crustacean larvae especially shrimp and prawn; comparative study of *P. redivivus* with *Artemia* on the factor of feeding rate, survivability, growth and nutritional value of larvae. Moreover, it is not known yet whether these nematodes are parasitic or not for the larvae; their possibility to work as a vector in Bangladesh environment should also be kept into consideration. If this nematodes does not pose any threat as a parasite to fry then *P. redivivus* will be a potential candidate species as live food for the larvae of some aquatic 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.

CHAPTER SIX

REFERENCES

6. References

Bedding, R. A. 1981. Low Cost in Vitro Mass Production of *Neoplectana* and *Heterorhabditis* Species (Nematoda) For Field Control of Insect Pests. *Nematologica* 27,109-114p.

Bedding, R. A., Stanfield, M. A. and Crompton, G. V. 1991. Apparatus and Methods For Rearing Nematodes, Fungi, Tissue Cultures and the Like, and For Harvesting Nematodes. Patent, International application number, PCT/AU91/00136.

Bengtson, D. A. 2003. Status of Marine Aquaculture in Relation to Live Prey: Past, Present and Future. Live Feeds in Marine Aquaculture (ed. by J.G. Støttrup and L.A. McEvoy), pp.1-16. Blackwell publishing, Oxford, UK.

Biedenbach, J. M., Smith L. L., Thomson, T. K. and Lawrence, A. L. 1989. Use of Nematode *Pangrellus redivivus* as an *Artemia* Replacement in a Larval *Penaeid* diet. *Journal of World Aquaculture Society* 20, 61-71p.

Bryant, P. L. 1981. Adaptation of Carp (*Cyprinus carpio*) Larvae to Artificial Diets 1. Optimum Feeding Rate and Adaptation Age for a Commercial Diet. *Aquaculture* 23, 275-286p.

Cahu, C. and Infante, J. Z. 2001. Substitution of Live Food by Formulated Diets in Marine Fish Larvae. *Aquaculture* 200, 161-180p.

Charlon, N., Durante, H., Escarffe, A. M. and Bergot, P. 1986. Alimentation Artificielle Des Larves De Carpe (*Cyprinus carpio* L.). *Aquaculture* 54, 83-88p.

Curnow, J., King, J., Partridge, G. and Kolkovski, S. 2006. Effects of Two Commercial Microdiets on Growth and Survival of Barramundi (*Lates calcarifer* Bloch) Larvae within Various Early Weaning Protocols. *Aquaculture Nutrition* 12, 247-255p.

Cryan, W. S., Hansen, E., Martin, M., Sayre, F. W., Yarwood, E. A., 1963: Axenic Cultivation of the Dioecious Nematode *Panagrellus redivivus*. *Nematologica* 9, 313--319p.

Dabrowski, K. 1984. Influence of Initial Weight during the Change from Live to Compound Feed on the Survival and Growth of Four *Cyprinids. Aquaculture* 40, 27-40p.

DoF. 2012. National Fish Week 2012 Compendium (In Bengali). Department of Fisheries, Ministry of Fisheries and Livestock, Bangladesh.144p

Evans, R., Cassel, D. K. and Sneed, R. E., 1996. Measuring Soil Water for Irrigation Scheduling: Monitoring Methods and Devices. North Carolina Cooperative Extension Service Publication (AG 452-2).

Fisher, C. M. L. and Fletcher, D. J. 1995. Novel Feeds for Use in Aquaculture. Patent, International application number, A01K67/033, A23K1/18.

Fernandez-Diaz, C., Pascual, E. and Yufera, M. 1994. Feeding Behavior and Prey Size Selection of Gilthead Seabream, *Sparus Aurata*, Larvae Fed on Inert and Live Food. *Marine Biology* 118, 323-328p.

FAO. 2006. FISHSTAT Plus: Universal Software for Fishery Statistical Time Series. Version 2.3.2000 Fisheries Department, Fishery Information Data and Statistic Unit Data sets released March 2006.

FAO. 2012. *The State of World Fisheries and Aquaculture*. FAO Fisheries and Aquaculture Department, Food and Agriculture Organization of the United Nations, Rome, Italy.

Govoni, J. J., Boehlert, G. W. and Watanabe, Y. 1986. The Physiology of Digestion in Fish Larvae. *Environmental Biology of Fishes* 16, 59-77p.

Guillaume, J., Kaushik, S., Bergot, P. and Metailler, R. 2001. Nutrition and Feeding of Fish and Crustaceans. Springer Praxis, Chichester, Great Britain, 408p.

Hamza, N., Mhetli, M. and Kestemont, P. 2007. Effects of Weaning Age and Diets on Ontogeny of Digestive Activities and Structures of Pikeperch (*Sander lucioperca*) Larvae. Fish Physiology and Biochemistry 33, 121-133p.

Kahan, D., Appel, Z., 1975: The Value of *Panagrellus sp.* (Nematoda) as Food for Fish. The European Symposium on Marine Biology (Ostend, Belgium, Sept. 17–23, 1975) 1, 243–253p.

Kahan, D., Bar-El, T., Brandstein, Y., Rigbi, M. and Oland, B. 1980. Free Living Nematodes as a Dietary Supplement in the Rearing of Fish Fry and Hatcheries. General Fisheries Council for the Mediterranean. Studies and Reviews 57, 67-78p.

Kamler, E. 1992. Early Life History of Fish. Chapman and Hall, London, UK, 267p.

Koven, W., Kolkovski, S., Hadas E., Gamsiz, K. and Tandler, A. 2001. Advances in the Development of Microdiets for Gilthead Seabream, *Sparus aurata*: A Review. *Aquaculture* 194, 107-121p.

Kumlu, M. and Fletcher, D. J. 1997. The Nematode *Panagrellus redivivus* as an Alternative Live Feed for Larval *Penaeus indicus*. *Israel Journal of Aquaculture* 49, 12-18p.

Kumlu, M., Fletcher, D. J. and Fisher, C. M., 1998. Larval Pigmentation, Survival and Growth of *Penaeus indicus* Fed the Nematode *Panagrellus redivivus* Enriched With Astaxanthin and Various Lipids. *Aquaculture Nutrition* 4, 193-200p.

Lavens, P. and Sorgeloos, P., 1996. Manual in the Production and Use of Live Food for Aquaculture. FAO, Fisheries Technical Paper (361). FAO, Rome, Italy.

Lavens, P. and Sorgeloos, P., 2000. The History, Present Status and Prospects of the Availability of *Artemia* Cysts for Aquaculture. *Aquaculture*181, 397-403p.

Lazo, J. P., Dinis, M. T., Holt, J. G., Faulk, C. and Arnold, C. R. 2000. Co-feeding Microparticulate Diets With Algae: Towards Eliminating the Need of Zooplankton at First Feeding in Red Drum (*Sciaenops ocellatus*). *Aquaculture* 188, 339-351p.

Lee, C. S., O'Bryen, P. J. and Marcus, N. H., (eds) 2005. Copepods in Aquaculture. Blackwell Publishing, Ames, IA, USA.

Liao, I. C. 1992. *Penaeid* Larviculture: Taiwanese Method. In: Marine Shrimp Culture: Principles and Practices (ed. by A.W. Fast and L.J. Lester), pp.193-215. Elsevier Science Publishers B. V., Amsterdam, the Netherlands.

Masters, C. O., 1975. *Live Foods*. Tfh Publications Incorporated.

Ricci, M., Fifi, A. P., Ragni, A., Schlechtriem, C. and Focken, U. 2003. Development of a Low-cost Technology for Mass Production of the Free-living Nematode *Panagrellus redivivus* as an Alternative Live Food for First Feeding Fish Larvae. *Applied Microbiology and Biotechnology* 60, 556-559p.

Rosenlund, G. and Halldorsson, O. 2007. Cod Juvenile Production: Research and Commercial Developments. *Aquaculture* 268, 188-194p.

Rottmann, R. W., Shireman, J. V., Lincoln, E. P. 1991: Comparison of Three Live Foods and Two Dry Diets for Intensive Culture of Grass Carp and Bighead Carp Larvae. *Aquaculture* 96, 269–280p.

Rouse, D. B., Webster, C. D. and Radwin, I. A. 1992. Enhancement of the Fatty Acid Composition of the Nematodes *Panagrellus redivivus* Using Three Different Media. *Journal of the World Aquaculture Society* 23, 89-95p.

Santiago, C.B., Ricci, M. and Reyes-Lampa, A. 2004. Effect of Nematode *Panagrellus redivivus* Density on Growth, Survival, Feed Consumption and Carcass Composition of Bighead Carp *Aristichtys nobilis* (Richardson) Larvae. *Journal of Applied Ichthyology* 20, 22-27p.

Samocha, T. and Lewinsohn, C. 1977. A Preliminary Report on Rearing *Penaeid* Shrimps in Israel. *Aquaculture*10, 291-292p.

Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J. and Tocher, D. R. 1995. Requirement Criteria for Essential Fatty Acids. *Journal of Applied Ichthyology* 11, 183-198p.

Sautter, J., Kaiser, H., Focken, U. and Becker, K. 2007. *Panagrellus redivivus* (Linne') as a Live Food Organism in the Early Rearing of the Cat Fish *Synodontis petricola* (Matthes). *Aquaculture Research*, 38, 653-65p.

Schlechtriem, C., Ricci, M., Focken, U. and Becker, K. 2004a. The Suitability of the Free Living Nematode Panagrellus Redivivus As Live Food for First-Feeding Fish Larvae. *Journal of Applied Ichthyology* 20, 161-168p.

Schlechtriem, C., Focken, U. and Becker, K. 2005. Digestion and Assimilation of the Free-Living Nematode *Panagrellus Redivivus* Fed to First Feeding Coregonid Larvae: Evidence from Histological and Isotopic Studies. *Journal of the World Aquaculture Society* 36, 24-31p.

Sorgeloos, P. and Persoone, G. 1975. Technological Improvements for the Cultivation of Invertebrates as Food for Fishes and Crustaceans II. Hatching and Culturing Of the Brine Shrimp, *Artemia Salina* L. *Aquaculture* 6, 303-317p.

Sorgeloos, P., Dhert, P. and Candreva, P. 2001. Use of the Brine Shrimp, *Artemia* Spp., in Marine Fish Larviculture. *Aquaculture* 200, 147-149p.

Stock, S. P. and Nadler, S. A. 2006. Morphological and Molecular Characterisation of *Panagrellus Spp.* (*Cephalobina: Panagrolaimidae*): Taxonomic Status and Phylogenetic Relationships. *Nematology* 8(6): 921-938p.

Ted Pella, Inc. and PELCO International, 2010. Microscopy Products for Science and Industry. http://www.tedpella.com/msds_html/18510msd.htm.

Vega-Orellana, O. M., Fracalossi, D. M. and Sugai, J. K. 2006. Dourado (*Salminus brasiliensis*) Larviculture: Weaning and Ontogenetic Development of Digestive Proteinases. *Aquaculture* 252, 484-493p.

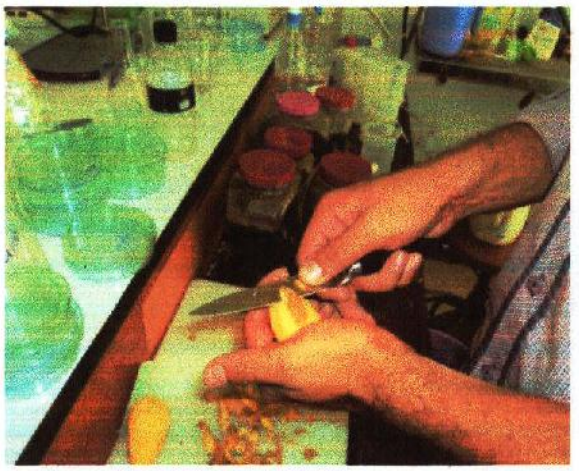
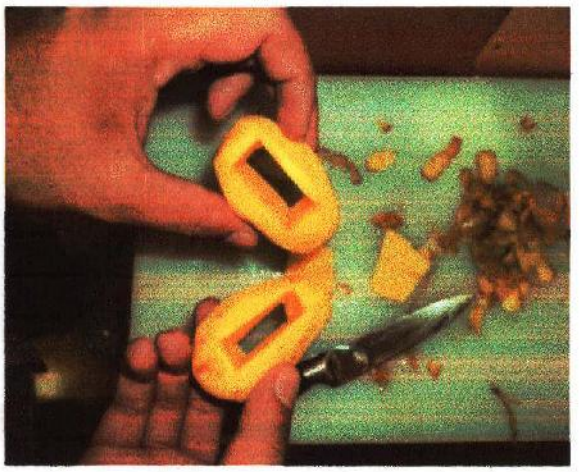
Wilkenfeld, J. S., Lawrence, A. L. and Kuban, F. D. 1984. Survival, Metamorphosis and Growth of *Penaeid* Shrimp Larvae Reared on a Variety of Algal and Animal Foods. *Journal of the World Maricultural Society*15, 31-49p.

Watanabe, T. and Kiron, V. 1994. Prospects in Larval Fish Dietetics. *Aquaculture*, 124: 223-251p.

Wickins, J. F. and Lee, D. O'C. 2002. Crustacean Farming Ranching and Culture, 2nd edn. Blackwell Science, Oxford, 446p.

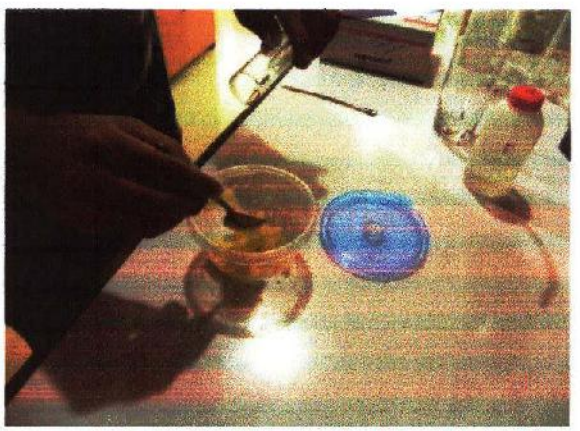
www.kakerlakenparade.de/mikrowuermchen.html

www.rainbowfish.angfaqld.org.au/microwrm.html

Raw potato were prepared for *P. redivivus* collection


The potatoes were buried under a 5 cm of soil ground




Preparation of 30 gm boiled potato paste

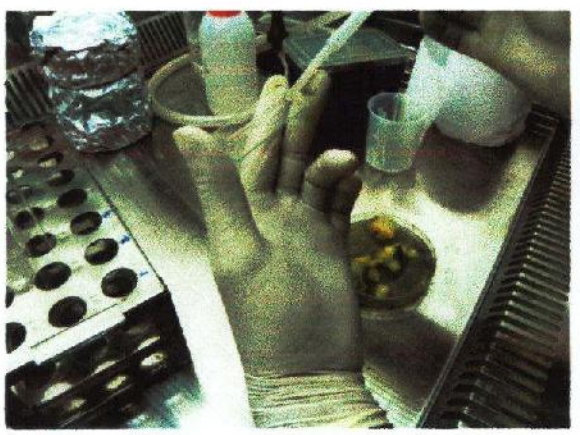
Photo Gallery

Spreading of yeast over the culture media

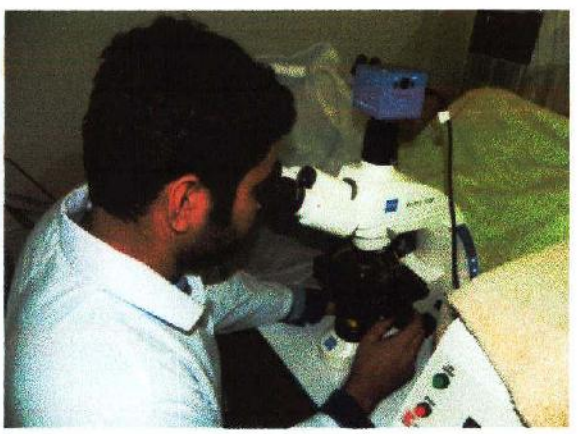
Media transferred to incubation for one day

Collection of buried potato after 7 days

Soft wash of the rotten potato

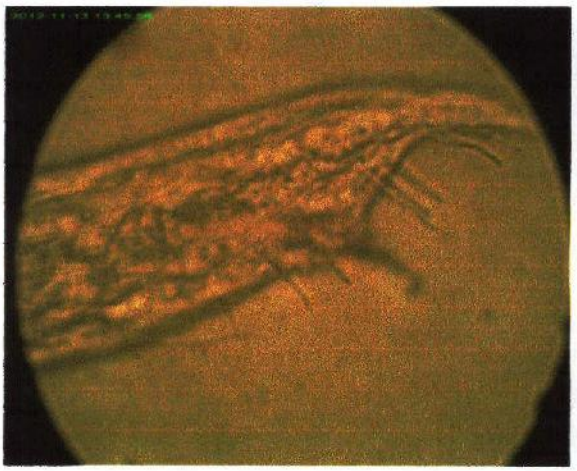


Washed water were placed in a slide

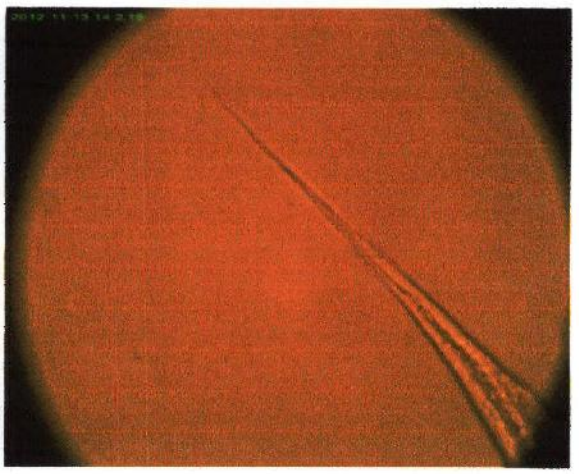


Presence and identification was done under light microscope

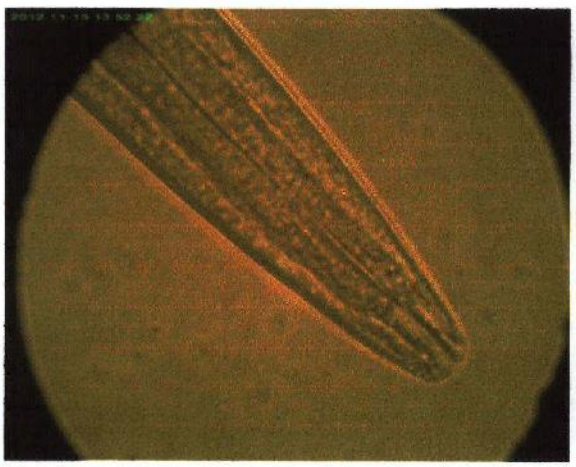
Photo Gallery



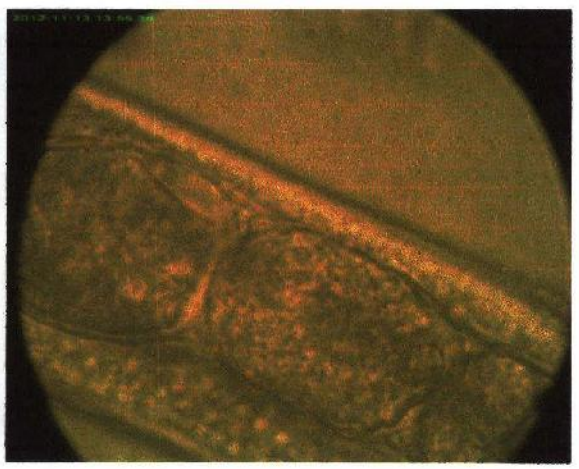
Tail of male *P. redivivus*



Tail of female *P. redivivus*

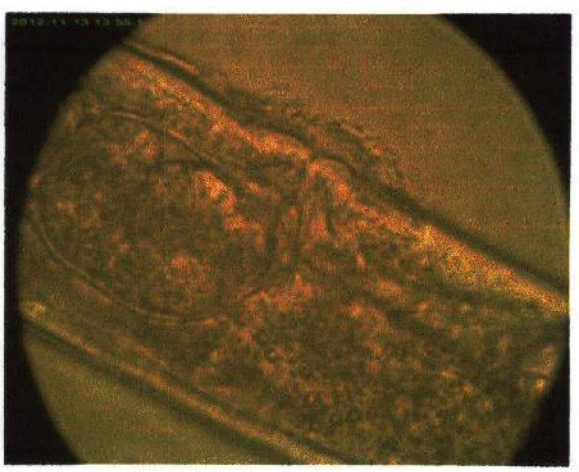


Mouth of *P. redivivus*



Egg of *P. redivivus*

Genital organ of male *P. redivivus*



Genital organ of female *P. redivivus*

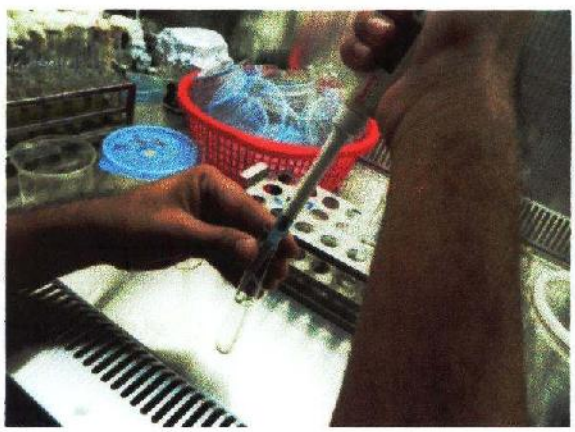
Photo Gallery

Spreading of rotten potato washed water over incubated media

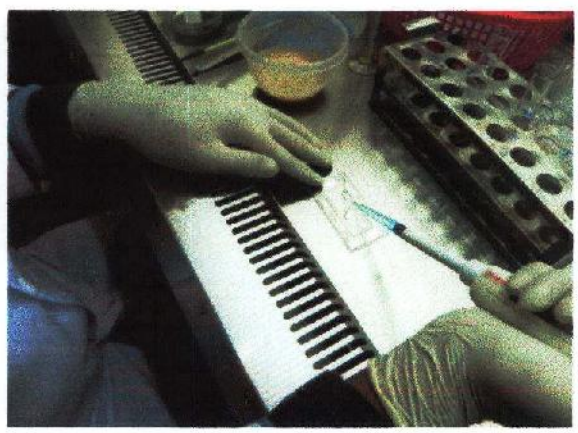
Placed the media in incubator for culture

Collection of Nematode *P. redivivus* from culture media

Fixation with 10% formaldehyde

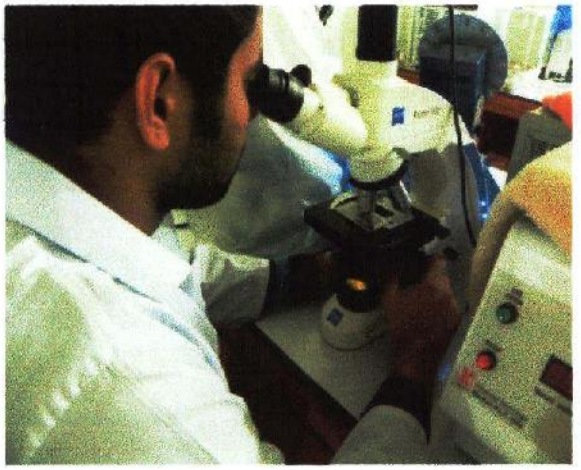


Serial dilution with 9 ml water

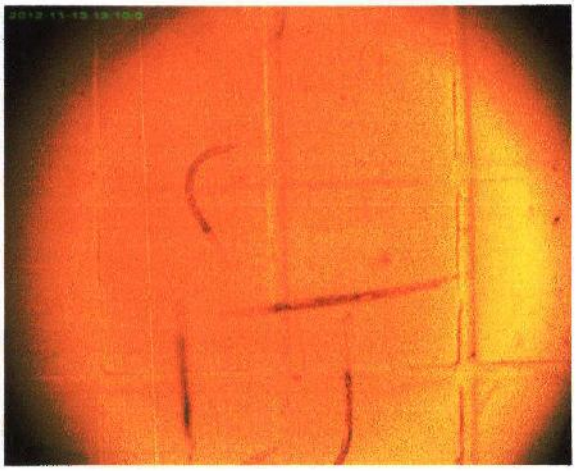


1ml diluted water placed on counter cell

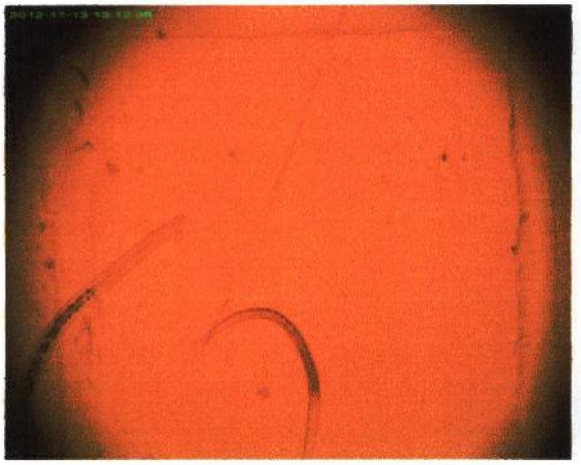
Photo Gallery



Counting the *P. redivivus* under light microscope

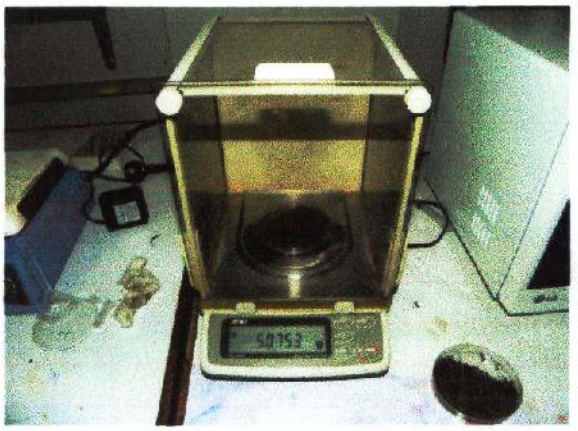


P. redivivus at 4x zoom



P. redivivus at 10x zoom

Data entry

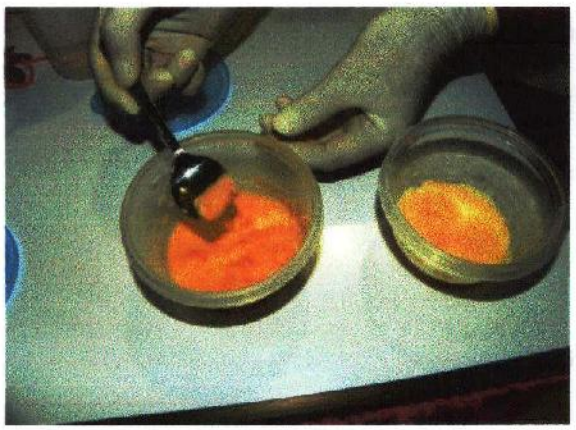


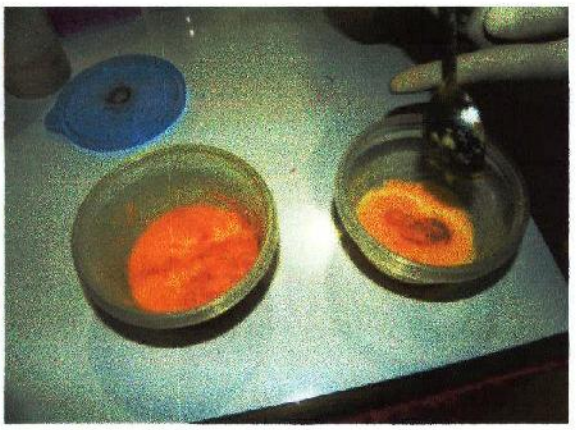
Weighting and placing of soil sample in dry oven for measurement of soil moisture

Photo Gallery

Soil sample was 105 ^{0}C for 24 hour

Soil P^{H} measurement





Starting a new culture (right) from a old culture (left) media

Raw materials for a culture media

Some mass produced culture media of *P. redivivus*