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Culture of the littoral oligochaete *Pontodrilus bermudensis* Beddard

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Abstract

The use of the littoral oligochaete *Pontodrilus bermudensis* Beddard as a dietary supplement promotes maturation and spawning for a prolonged period in *Feneropenaeus indicus*, *Penaeus monodon*, *P. semisulcatus*, *Portunus pelagicus*, and *Scylla tranquebarica*. Thus, production of these worms in captivity will be useful for successful management of shrimp/crab hatcheries. To develop a culture protocol for this worm, different organic amendments were tested. Experiments conducted in plastic trays with a 2-cm-thick sand base, have revealed that out of four organic amendments (cow dung; cow dung + seaweeds; cow dung + leaf litter; and cow dung + hay) tested first three promoted multiplication of worms by 108.5%, 280.0%, and 230.0% , respectively, after 30 days. Subsequent experiments conducted in wooden crates with verminbed (12 cm intertidal sand + 5–6 cm coarse sand + 2–3 cm basal layer of broken bricks and pebbles) as a base, have shown that three organic amendments (cow dung; cow dung + seaweeds; and cow dung + leaf litter) promoted growth of the worms in terms of number as well as biomass up to 90 days. Growth of the worms in the latter two amendments differed significantly ($P< 0.003$) from that of cow dung alone. Finally, a twin culture system was designed in wooden crates with verminbed as base and mixture of organic amendment (cow dung + seaweeds + leaf litter) and then tested for 240 days by employing partial harvest of worms and up loading organic amendment, at regular intervals from each culture system alternately. In total 5.526 kg of worms were harvested from the 9.380 kg of organic amendment, resulting in an organic amendment conversion ratio of 1.7: 1. Thus, this twin culture system can provide an uninterrupted supply of worms for use in shrimp/crab hatcheries to ensure successful broodstock management by increasing number of culture units as per the requirement of worms. The present protocol, developed for culture of P. bermudensis, ecofriendly in nature by conversion of organic resources in to worms through bioprocess without any chemical involvement, easy to adopt, can be employed to lower operational cost of shrimp/crab hatcheries by adopting cultured P. bermudensis as a broodstock diet.

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1. Introduction

1.1 Need of the culture

When used as a supplementary diet along with clam and squid meat, *P. bermudensis* has been shown to promote repeated spawning for a prolonged period in *Feneropenaeus indicus* , *Penaeus semisulcatus*, *P. monodon*, *Portunus pelagicus* and *Scylla tranquebarica* (Maheswarudu et al., 1996; Radhakrishanan et al., 2000; Vineetha, 2001; Maheswarudu et al., 2007). Experiments on the domestication of the blue swimming crab Portunus pelagicus and the black tiger shrimp *P. monodon* up to the F_4 generation and F_3 generation, respectively, through inbreeding by feeding them *P. bermudensis* as a supplementary diet also were successfully conducted (Maheswarudu, 2007; Maheswarudu et al., 2008). Experiments conducted in cages with *P. semisulcatus* in the Gulf of Mannar to evaluate the diet that best supports maturation revealed that worms induced maturation in a shorter time compared to clam meat and squid meat (Maheswarudu per. com). Because *P.*

bermudensis is a prospective supplementary diet for captive broodstock of penaeid prawns and portunid crabs, production of this worm through culture is of great importance to the aquaculture industry.

1.2 Maturation stimulator and maturation diet
Maheswarudu et al. (1995) attempted to isolate the factor from *P. bermudensis* that induces maturation of shrimp/crabs in an experiment using the red swamp crayfish *Procambarus clarkii* as a test animal, and subsequent study to identify the factor yielded successful results (published elsewhere). Vineetha (2001) studied the biochemical composition of *P. bermudensis* at three stages of the life cycle and compared the fatty acid composition of this worm versus those of other annelids. *P. bermudensis*, being rich with polyunsaturated fatty acids that are required for attaining shrim/crab maturation (Lytle et al., 1990; Woutre et al., 2001) and also having maturation stimulating factor for shrimp/crab, is most indispensable broodstock diet for successful management of shrimp/crab hatcheries. Of late, all commercial shrimp/crab hatcheries are dependent on captive broodstock to produce disease free/ disease resistant seeds. Seed quality is fully dependent on condition of the captive broodstock (Browdy, 1998; Racotta et al., 2003). Domestication of shrimp/ crab is an absolutely essential requirement to produce specific pathogen-free /disease resistant brood stock. P. bermudensis will play a significant role as a broodstock diet in domestication programme to achieve successive generations of desired commercial species for improving targeted traits of economic importance (Maheswarudu, 2007; Maheswarudu et al., 2008).

1.3 Protocol for culture
Ganapati and Rao (1972) conducted salinity tolerance studies and were able to maintain P. bermudensis in large glass troughs filled with filtered brackish water for 96 hours only. Information on the culture of *P. bermudensis* in land-based culture systems is not available. To develop a protocol for culture of this worm, different organic amendments were tested. The present paper describes attempts to culture these worms in trays and wooden crates with a sand substratum and different organic amendments such as cow dung; cow dung+ sea weeds; and cow dung+ leaf litter. The feasibility of long-term culture of this worm with periodic partial harvesting and uploading organic amendment also was assessed to ascertain the harvestable biomass and sustainability of the remaining population.

2. Literature review

2.1 Earthworm culture
Annelids have been cultured in laboratories for many years, mainly for use in pollution assays. Their small size, short life cycle, and high reproductive potential make culture easy. Annelids also are used as fish bait and food for cultured invertebrates and fishes. Several species of annelids belonging to the classes Polychaeta and Oligochaeta are used as food for marine animals (Swingle, 1961). Commercial production of polychaetes such as *Glycera dibranchiata* (Maine blood worm) and *Americonophus reesi* (Panama blood worm) is practised in the US. Several oligochaetes, like the white worm *Enchytraeus* sp. (Blount, 1937; Loosanoff, 1937), the redworm *Tubifex* sp. (Swingle, 1961; Kosiorek, 1974; Marian and Pandian, 1984; Marian et al., 1989; Ahamed and Mollah, 1992), and the terrestrial earthworm *Lumbricus terrestris* (Hess, 1937) are mass cultured as food for cultured marine fishes and invertebrates. Of late, a comprehensive review on the production of earth worm protein for animal feed from organic wastes, giving account on methods of earth worm culture, harvesting earth worms from organic wastes, processing earth worms for animal feed, different methods for processing earth worm pastes, and out come of animal feeding trials (fish, Chicken and pig) with earth worm protein was documented (Edwards and Niederer, 2010). The important role of earth worm as a protein source for fish feed and its use in augmenting fish production from aquaculture industry has been documented (Ghosh, 2004; Sogbesan, 2007; Sogbeasan and Madu, 2008; Medany, 2011; Olele and Okonkwo, 2012; Bag et al., 2012).

Earthworms, which are oligochaetes, have a worldwide distribution (Jameison, 1971). Species such as *Eisenia foetida*, *E. hortensis*, *Eudrilus eugeniae*, *Amynthas gracilus*, *Perionyx excavatus*, and *Lampito mauritii* are cultured for organic waste recycling and for improving soil fertility. The culture of earthworms, known as vermiculture, and its usage and importance in agriculture have been discussed by several authors (Bhawalkar and Bhawalkar, 1992; Ismail, 1997; Gajalakshmi and Abbasi, 2004; Sharma et al., 2005; Sinha et al., 2008; Kale, 2007 &2010; Pandit et al., 2012).

2.2 Distribution

Five species belonging to the genus Pontodrilus (*P. bermudensis*, *P. litoralis*, *P. gracilis*, *P. matsushimensis*, and *P. phosphorus*) have been reported from littoral regions (Beddard, 1895; Michaelson, 1900; 1910; Stephenson, 1915 b; Gates, 1943). *Pontodrilus bermudensis* Beddard occurs in tropical, subtropical, and warm tropical regions of the Atlantic, Pacific, and Indian Oceans. Its distribution extends to 45˚ N and 45˚ S, and it is particularly abundant in the tropical and subtropical belt (Rao and Ganapati, 1975). In India, P. bermudensis has been reported from Chilka Lake (19 0 43' 00.00" N; 850 19'00.00" E), Pamban (09 0 16' 36.00" N; 790 13'34.00" E), Port Blair (Andamans) (11 0 38' 50.00" N; 920 41'15.00" E), Kovalam (08 0 28' 26.00" N; 760 58'42.00" E), Port Okha (Gulf of Kutch) (23 0 28' 00.00" N; 690 04'59.00" E), and Elephanta (18 0 57' 45.00" N; 720 55'55.00" E) (Beddard, 1903; Stephenson, 1914; 1915a; 1916, 1930, Aiyar, 1929; Gates, 1936; Menon and Sareen, 1967).

2.3 Biology

Pontodrilus bermudensis exhibits tolerance to a wide range of salinities (5 ppt to 33 ppt), with an optimum at 25 ppt (Ganapati and Rao, 1972). Its preferred habitat is decaying and half decaying seaweeds and under stones and rotten logs. Bionomics of *P. bermudensis* from the brackish water areas of the Visakhapatnam Harbour have been reported (Rao and Ganapati, 1974, 1975). This worm also has the luminescence system, contained within (14.4 /lm mean diameter) granule-filled coelomic cells. The bioluminescence of these cells can be stimulated by agitation, by addition of hypotonic hydrogen peroxide and by addition of hypotonic "synthetic earthworm luciferin, but hypotonicity alone stimulates little luminescence (Wampler and Jamieson, 1986). The freshly laid cocoons of *P. bermudensis* are spindle-shaped and green in colour (3-7 mm in length and 2-3.5 mm in diameter) and the colour of the cocoon changes to deep pink with the development eggs and embryos inside. The number eggs in the cocoon varied from 1 to 6 but majority with 1to 3 eggs (Rao and Ganapati, 1974).

3. Materials and Methods

The present study was conducted in the shrimp hatchery at the Regional Centre of Central Marine Fisheries Research Institute (CMFRI), Mandapam Camp (9º.18´ N; 79º.08´ E) using three successive experiments. All experiments were conducted under the natural photo period (light 12: dark 12 h) at room temperature (24–27° C).

3.1 Collection of worms

Pontodrilus bermudensis were collected from the littoral area of the Gulf of Mannar, behind the shrimp hatchery of the CMFRI Mandapam Camp (09o 16' 16.7"N; 079o 07' 56.0" E).The worms were cleaned with ambient sea water, counted, and weighed before being transferred to the culture systems. As the worms are very delicate, they were handled carefully during transfer to the culture systems.

3.2 Organic media

Seaweeds: mixture of seaweeds that has washed ashore along the coastline of the Gulf of Mannar.

Leaf litter: mixture of leaves collected in the campus.

Hay: Oryza sativa hay.

Cow dung: fresh cow dung collected from local cattle.

Granite soil: powder collected from the granite stone crusher operation and sieved through 200 µ mesh.

Semi-dried seaweeds (moisture content: 38.6 ± 11.7%), leaf litter, and hay were pounded into small pieces before application as an organic manure.

3.3 Experiment 1: Culture in plastic trays

This experiment was designed to evaluate the suitability of organic amendment for growing the worms by testing four organic amendments namely cow dung, cow dung + seaweeds, cow dung + leaf litter, and cow dung + hay. Experiment was conducted in four setups, each run in triplicate: One was the control (cow dung) and each of the other three used a different organic amendment (cow dung + seaweeds, cow dung + leaf litter, and cow dung + hay). Twelve plastic trays (40 × 30 × 5 cm) perforated at the base to facilitate drainage of water were used for the culture. A fine mesh (200 µm) nylon cloth lining on the bottom of the tray prevented escape of worms through the holes. In each tray a 2cm thick layer of sand from the seashore was spread over a 0.5 cm thick coarse sand (river) layer. Twenty non-clitellate (immature) worms (0.016 worm/ cm2), whose biomass was recorded, were introduced into the sand in each tray.

The following amendments were added at a

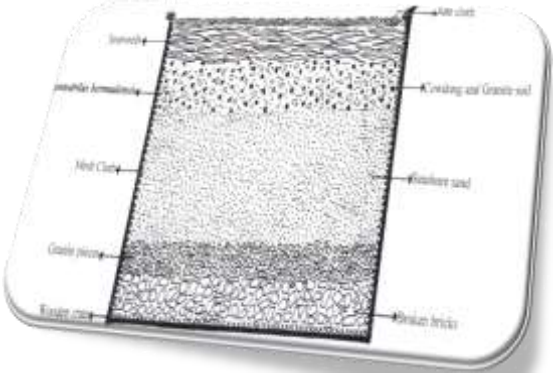
rate of 0.5 g/ cm^2 to each of the trays on top of the sand layer (each organic medium as a separate layer): Control (amendment 1) was fresh cow dung (100%); amendment 2 was cow dung (25%) + seaweeds (75%); amendment 3 was cow dung (25%) + leaf litter (75%); and amendment 4 was cow dung (25%) + hay (75%). The trays were sprinkled with ambient seawater (30 ppt salinity) daily (about 250 ml of sea water per tray) over the course of 60 days and were covered with a moist jute cloth to retain the moisture at 65-70%. The total number and biomass of the worms in each tray were recorded on day 30. All worms in each tray were collected and counted individually, and the weight of the worms (group) was measured to the nearest 0.001 g using a single pan electronic balance. Before being weighed, worms were placed on tissue paper for 2 mins to remove as much moisture as possible. After measurement, worms were transferred back to their respective tray for continuation of the experiment. After 50 days all trays were infested with insect larvae, so they were treated with neem oil (2000 ppm: 2g of oil in 1000 ml of sea water). Neem oil was mixed with sea water (250 ml), sprinkled on each tray, and mixed into the organic amendment gently with a fork to ensure uniform distribution. Because all of the worms and the insect larvae died and disintegrated by day 60, the experiment was terminated.

3.4 Experiment 2: Culture in wooden crates

The theme of this experiment is to ensure sustainability of worms for long duration in three organic amendments those promoted growth in experiment 1 (3.3) by providing vermibed; and to arrest infestation of fly larvae in organic amendment by adding granite soil to the cow dung.

This experiment was conducted in rectangular wooden crates (45 × 36 × 30 cm) with three organic amendments; each amendment was tested in triplicate. The inner sides and bottom of each crate were lined with fine mesh (200 µm) nylon cloth to prevent the escape of worms. A vermibed was prepared in each of the crates following the method described by Ismail (1993; 1997). The vermibed consisted of a 5–6 cm-thick layer of coarse sand covering a basal layer of broken bricks and pebbles (approximately 2–3 cm thickness) to ensure proper drainage of water. A layer of intertidal sand up to a height of not less than 12 cm (after moistening) topped this coarse sand layer. About 100 non-clitellate worms (0.06 worm/cm^2) were added to the sand layer in each crate after their biomass was recorded (Figure. 3.1). Granite soil was added to fresh cow dung at a ratio of 1:5 (100 g granite soil to 500 g cow dung) to avoid infestation by fly larvae (Bhawalker pers. comm.). The granite soil contained high levels of potassium; although release of potassium is very slow, it raises the pH of the organic amendment, which might explain how it kills insect larvae in cow dung. Granite soil also provides minerals to promote the growth of worms.

Figure 3.1: Diagrammatic representation of the vermibed prepared for culturing worms in wooden crates. Sea weeds are represented for supplementary amendments.



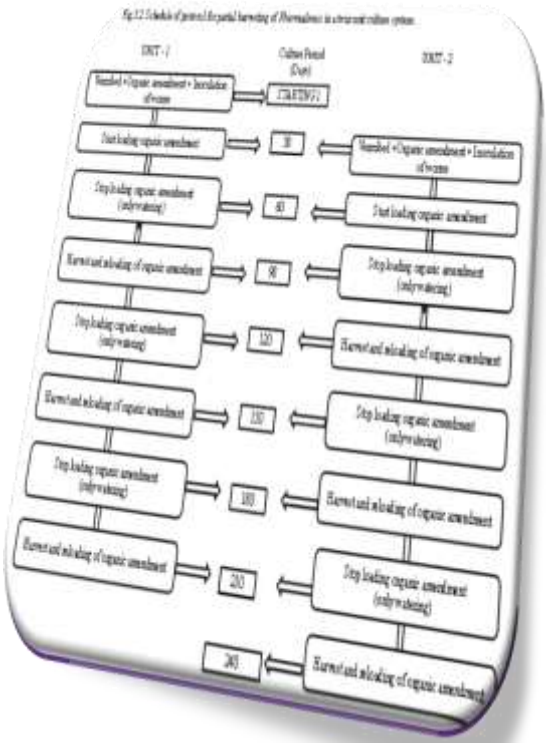
The following amendments (at 1.0 g/cm^2) were placed on top of the intertidal sand layer (each organic medium as a separate layer): Amendment 1 (control) was cow dung (100%); amendment 2 was cow dung (40%) + seaweeds (60%); and amendment 3 was cow dung (40%) + leaf litter (60%). Once a day over the course of the experiment 1 l of seawater was sprinkled into each crate, and the crates were covered with a moist gunny cloth to retain moisture at 65-70%. The total biomass of the worms in each crate was recorded on days 30, 90, and 180. To make the biomass estimation the whole crate was watered profusely with sea water so that all of the worms congregated in the top layer. Three 4 × 4 × 4 cm samples of from each crate were selected randomly and the total number of worms and biomass was recorded; these values were used to estimate figures to the whole crate area (1620 cm^2). After recording biomass worms were transferred back to their respective crate, and the same organic amendment was continued until the end of the experiment. On day 180 all worms in each crate were collected, washed with ambient sea water, counted individually, and total weight (to the nearest 0.001 g) was recorded. Prior to being weighed, worms were placed on tissue paper for 2 minutes to remove moisture.

3.5 Experiment 3: Twin culture system with periodic harvesting

This experiment was designed to ascertain the harvestable biomass and the sustainable capacity of the population of *P. bermudensis* remaining after partial harvesting at regular intervals in a continuous culture system treated with the addition of organic amendment at regular intervals. Two culture systems (two units), each composed of two wooden crates (45 × 36 ×30 cm), were selected for conducting this experiment. Vermibeds were prepared in the wooden crates in a manner similar to that described in experiment 3.4. About 100 non-clitellate worms (0.06 worm/cm^2) were introduced into the sand layer of each crate (unit 1) after their biomass had been recorded. Granite soil was added to fresh cow dung at a 1:5 ratios. Cow dung (40%) + seaweeds (30%) + leaf litter (30%) were added to each crate on top of the sandy layer (1.0 g/cm^2), each organic medium as a separate layer. Sea water was sprinkled into the crates and the units were covered with a moist gunny cloth, as described in experiment 3.4. From day 31, once every 10 days up to day 60, organic amendment in the same ratios, was spread (at a concentration of 0.05 g/cm^2) in a thin layer on top of the substrate in each crate. The top layer of decaying organic matter (mulch) periodically was turned gently using a fork to mix the organic matter with soil.

On day 90, standing biomass of the worms was estimated and a partial harvest was conducted from each unit 1 crate. On the day of harvesting the whole crate was watered profusely so that the worms moved to the upper layer. The top layer of mulch and sand were collected separately and worms were hand picked and separated into juveniles, non-clitellates, and clitellates. After collecting the worms the mulch and sand were placed back into their respective crates. The total biomass of juveniles, non-clitellates, and clitellates was recorded separately for each crate, and total biomass of these three categories was considered to be the standing biomass. The biomass of non-clitellates was considered to be harvestable biomass, so all non-clitellates were harvested. Juveniles and clitellates were transferred back to their respective crates to build up the population for further culture, and total biomass of these two categories remained as the standing biomass. The mixed amendment was added once every 10 days from day 91 to day 120 at a concentration of 0.05 g/cm^2 as a thin layer on top of the substrate in each crate. A second harvest was conducted on day 150, and the amendment was added once every 10 days for days 151-180. A third harvest occurred on day 210.

The procedure was the same for the unit 2 crates of the twin culture systems, except all procedures were offset by 30 days to facilitate harvesting of worms from the two units alternately every 30 days. Figure 3.2 shows the schedule followed in this experiment.



3.6 Study of the growth stages of P. bermudensis

The three growth stages of *P. bermudensis* are juveniles (thin and white in colour), non-clitellates (pink in colour but without the clitellum or sexual development), and clitellates (pink in colour and with the clitellum and sexually mature). At the time of harvest in experiment 3.5, the length, weight, and appearance of the different growth stages of *P. bermudensis* were recorded. Length was measured for each individual worm after it sat on tissue paper for some time to reduce wriggling. Total weight of 10 worms was measured for each stage, from which mean weight of the worm was worked out.

3.7. Statistical analyses

An analysis of variance (ANOVA) was conducted to look for differences between population number and biomass of the worms reared in different organic amendments at different time intervals. Student t test was used to find out the difference in population number

and biomass between control (cow dung) and each test organic amendment at different times. For day 90 in experiment 3.4, the live weight of the worms reared in the three organic amendments was compared using ANOVA. The same analysis was used to look for differences between the harvestable biomass at different periods and between standing biomass at different intervals in the twin culture system.

4. Results

4.1 Experiment 1 Culture in plastic trays

Tables 4.1 and 4.2 give the results of experiment 3.1. The increase of worms by number in amendments 1, 2, and 3 was 108.5%, 280%, and 230%, respectively, by day 30. In amendment 4 (cow dung + hay), all worms had died by day 30. A marginal increase in the weight of the worms—6.5% in amendment 2 and 4.9% in amendment 3—also occurred, where as in amendment 1 the weight of the worms decreased by 22.9%. On day 30 in amendments 1–3, almost all of the worms were in the juvenile stage; most of the non-clitellates (inoculated) were dead, and only a few mature (clitellate) worms were alive. All of the culture trays were infested with the larvae of an unidentified insect by day 50; after treatment with neem oil at 2000 ppm, all worms and insect larvae were dead and disintegrated by day 60.

Table 4.1: Population by number (mean ± SE) of *P. bermudensis* on days 30 and 60 during culture in trays with four organic amendments

Amendment No.	Amendment	Day 1	Day 30	Day 60
1	Cow dung (control)	20 ± 0	41.7 ± 4.06	0
2	Cow dung + seaweeds	20 ± 0	76.0 ± 6.24**	0
3	Cow dung + leaf litter	20 ± 0	66.0 ± 6.98*	0
4	Cow dung + hay	20 ± 0	0	0

* = Mean differed from that of control at P < 0.05 level: ** = Mean differed from that of control at P < 0.01 level

Table 4.2: Biomass in grams (mean ± SE) of *P. bermudensis* on days 30 and 60 during culture in trays with four organic amendments

Amendment No.	Amendment	Day 1	Day 30	Day 60
1	Cow dung (control)	19.65 ± 0.53	15.15 ± 2.45	0
2	Cow dung + seaweeds	20.31 ± 0.84	21.62 ± 1.24**	0
3	Cow dung + leaf litter	19.35 ± 0.08	20.30 ± 1.62*	0
4	Cow dung + hay	19.78 ± 0.7	0	0

* = Mean differed from that of control at P < 0.05 level: ** = Mean differed from that of control at P < 0.01 level

4.2. Experiment 2: Culture in wooden crates

Tables 4.3 and 4.4 summarize the results of experiment 3.2. A gradual increase in number and biomass of worms occurred in all three amendments up to day 90, with maximum values on day 90. By day 30, the number of worms had increased 22.75 times, 31.22 times, and 30.7 times in amendments 1, 2, and 3, respectively. The rate of increase in amendments 2 and 3 differed significantly (P = 3.02E-07) from that of amendment 1 (control). By day 30, biomass had increased 1.24 times, 1.54 times, and 1.46 times in amendment 1, 2, and 3, respectively, with a significant difference (P = 0.005) in the rate of growth between the control and the other two amendments.

4.3. Experiment 3: Twin culture system with periodic harvesting

Table 4.5 shows the standing biomass (juveniles and clitellates) and yield (non-clitellates) in the twin culture system at different intervals during the 240-day experiment. In this experiment, worms were harvested first on day 90 and then every 30th day thereafter. The yield on days 90, 150, and 210 was 0.284 g/cm2, 0.276 g/cm2, and 0.273 g/cm2, respectively, from unit 1; the yield on days 120, 180, and 240 was 0.298 g /cm2, 0.289 g/cm2, and 0.282 g/cm2, respectively, from unit 2. The harvested biomass (443.75–483.29 g) taken at monthly intervals did not differ significantly (P = 0.09), nor did the standing biomass (retained population) (152.76–178.12 g) (P= 0.059). An average of 450.8 g/crate and 470.2 g /crate of worms was harvested from units 1 and 2, respectively, every 2 months, resulting in an average yield of 460.5 g/crate every month from this twin culture system (6,480 cm2). In total, this experiment used 9,380 g of organic amendments and yielded 5,526 g of worms; the organic amendment conversion ratio was 1.7: 1 (1 g worms for 1.7 g of organic amendments). To produce 1 g of worms the required organic media were 0.11 g of granite soil, 0.57 g of cow dung, 0. 51 g of seaweeds and 0.51 g of leaf litter.

Table 4.3: Population by number (mean ± SE) of *P. bermudensis* on days 30, 90, and 180 during culture in wooden crates with three organic amendments

Amendment No.	Amendment	Day 1	Day 30	Day 90	Day 180
1	Cow dung (control)	100 ± 0	2275.67 ± 45.19	2697.33 ± 16.79	321.67 ± 12.86
2	Cow dung+ seaweeds	100 ± 0	3122.00 ± 34.17**	4021.33 ± 186.57**	594.67 ± 10.93**
3	Cow dung+ leaf litter	100 ± 0	3073.67 ± 37.15**	3947.00 ± 116.84**	548.33 ± 22.58**

* = Mean differed from that of control at P < 0.05 level;** = Mean differed from that of control at P < 0.01 level

Table 4.4: Biomass in grams (mean ± SE) of *P. bermudensis* on days 30, 90, and 180 during culture in wooden crates with three organic amendments

Amendment No.	Amendment	Day 1	Day 30	Day 90	Day 180
1	Cow dung (control)	98.52 ± 0.25	122.64 ± 9.26	241.99 ± 2.25	90.58 ± 9.21
2	Cow dung+ seaweeds	98.62 ± 0.26	152.29 ± 7.76**	577.20 ± 21.37**	123.20 ± 2.25*
3	Cow dung+ leaf litter	98.85 ± 0.55	144.39 ± 2.48*	539.37 ± 14.03**	127.46 ± 10.23**

* = Mean differed from that of control at P < 0.05 level: ** = Mean differed from that of control at P < 0.01 level

4.4. The growth stages of P. bermudensis

The juveniles or young worms hatching from the cocoon ranged from 0.9 to 1.1 cm in length and weighed ~6 mg. They are white in colour and in this study were usually found either entwined with the body of a clitellate or in groups of 10–15 worms attached to decaying twigs or leaves. The non-clitellates are the transition stage from juvenile to mature worm. They have not yet developed a clitellum and their colour ranges from light pink to dark pink. When juveniles reach ~3.7 cm in length, they change to non-clitellates and are light pink. The non-clitellates ranged from 3.7 to 7.5 cm in length and 150 to 850 mg in weight. The mature or clitellate worms ranged from 7.5 to 12 cm in length and 850 to 1,000 mg in weight. They were dark pink with a brownish tinge. Mature worms have a characteristic clitellum between the 8^{th} and 19^{th} segment. In this study, the clitellar segments were thickened and very pale in colour.

Table 4.5: Standing biomass (g), harvested biomass (g), and biomass of retained population (g) of P. bermudensis in twin culture system after 240 days. Values are mean ± SE

	Unit 1			Unit 2		
Culture period (days)	Standing biomass1 (g)	Harvested biomass (g)	Retained population biomass (g)	Standing biomass2 (g)	Harvested biomass (g)	Retained population (g)
Stocking time	100.45 ± 0.65					
30				102.93 ± 1.58		
90	614.98 ± 10.61	461.07 ± 5.03	153.91± 5.58			
120				653.15 ± 10.15	483.29 ± 6.20	169.86 ± 3.95
150	600.26 ± 11.14	447.5 ± 6.5	152.76 ± 4.64			
180				647.76 ± 11.35	469.64 ± 5.47	178.12 ± 5.88
210	612.15 + 9.55	443.75+ 5.75	168.41± 3.79			
240				628.93 ± 13.07	457.61± 6.89	171.32 ± 6.18

5. Discussion

5.1 Achievements

The present study illustrates the feasibility of culturing the littoral oligochaete *Pontodrilus bermudensis* in a vermibed with organic amendments such as cow dung, cow dung + seaweeds, and cow dung + leaf litter. Such a system can provide a supplementary diet to support successful broodstock management of penaeid shrimp and portunid crabs.

5.1.1

Successful cultures of earthworms such as

Lampito mauritii (Grace and Ismail, 1995) and *Enchytracus albidus* (Blount, 1937; Loosanoff, 1937) in small containers have been reported. In the present study, *P. bermudensis* cultured in the plastic tray system with cow dung, cow dung + seaweeds, and cow dung + leaf litter amendments produced a first generation of juveniles by day 30, whereas all of the worms died in the cow dung + hay amendment. Thus, the first three amendments were favourable media for culturing the worm. All plastic tray systems were infested with insect larvae by day 50, and the subsequent treatment with neem oil killed all of the worms and larvae by day 60. Neem oil might be toxic to *P. bermudensis* at 2000 ppm.

5.1.2
In the present study of culture in plastic trays with 2 cm of sand as a base, the majority of worms (inoculated) died by day 30. In contrast, successful long-term culture for 180 days was achieved when the worms were cultured in wooden crates with vermibed, which might have provided enough depth for the worms to move. In their natural habitat, *P. bermudensis* are epigeic and live as deep as 5 cm in the sediments in the Pondichery region (12º N and 79º E) along the southeast coast of India (Sathianarayanan and Khan, 2006). Similarly, Hess (1937) successfully cultured the common earthworm, *Lumbricus terrestris*, a preferred food item for cultured marine invertebrates, in large boxes with a sand substratum.

5.1.3
When cultured in wooden crates, *P. bermudensis* survived and reproduced for up to 180 days. This may be due to the increased thickness of the substratum and to the prevention of infestation by insect larvae via addition of granite soil to the cow dung. Although the worms grew in all the three organic amendments, significantly better growth occurred in the amendments with seaweed and leaf litter compared to cow dung alone. *Pontodrilus bermudensis* grew best in the seaweed amendment, as shown by the highest biomass values and population numbers in the crates with this amendment. In its natural habitat, *P. bermudensis* aggregates in large numbers under decaying seaweed beds that are washed ashore along the Gulf of Mannar coast (Maheswarudu, per.com). The leaf litter amendment also promoted growth, which was not significantly different from that of the seaweed amendment. Seaweeds and leaf litter might provide trace elements that cow dung alone do not have (Dhargalkar and Pereira, 2005). Gut contents of *P. bermudensis* collected from its natural habitat have included diatom frustules and flocculent detrital material (Rao and Ganapati, 1975). From the day of inoculation through days 30 and 90, a gradual increase in population numbers and biomass occurred in all three amendments. Biomass and numbers declined after day 90, probably because the organic matter that was provided at the onset of the experiment was exhausted by that time. A similar trend was reported for the laboratory culture of *L. mauritii* (Grace and Ismail, 1995; Grace, 1996).

5.1.4
In experiment 4.2, the doubling time for density and biomass of the worms was achieved in 30 days and 31-90 days, respectively. NIIR Board reported doubling time for density and biomass for *L. mauritii* and *P. excavatus* in different organic amendments (Table 5.1). The doubling time for density and biomass of *P. bermudensis*, in cow dung amendment, are comparable those of *L. mauritii*. In same organic amendments (biogas slurry, biogas slurry+ leaf litter, biogas slurry+ sawdust, biogas slurry+ bagasse) the reported mean doubling time for density and biomass of *L. mauritii* to be 26–42 days and 35–39 days, respectively, and of *P. excavatus* to be 8–11 days and 11–23 days, respectively. Because doubling time varies based on the strength of the organic amendment and the efficacy of the worm in terms of reproduction and growth (Parthasarathi, 2007; Suthar, 2007: Suthar and Singh, 2008) these results shows the high reproductive potential and fast growth of P. exacavatus compared to *L. mauritii*. The reproductive potential and growth of *P. bermudensis* are comparable to those of *L. mauritii* (Table 5.1).

5.1.5
The twin unit culture system used in this study permitted sustainable periodic harvesting of non-clitellates at day 90 and then every 30th day thereafter without adversely affecting the standing biomass. The standing biomass recovered within 60 days in each unit, which yielded biomass for further harvest. Because the two units were harvested alternately, enough time was provided to replenish the standing biomass of the culture units. For a cocoon to hatch, grow, and in turn produce a cocoon takes about 60 days in *L. mauritii* and 54 days in *P. excavatus* (Ismail, 1997). In the present study, harvesting only non-clitellates every 60th day and leaving juveniles and clitellates allowed juveniles to become clitellates and clitellates to shed cocoons; this

Table 5.1: Comparison of doubling time of *P. bermudensis* with those of other earth worms

Parameter	*Pontodrilus bermudensis*	*Lampito mauritii* (NIIR Board)	*Perionyx excavatus* (NIIR Board)
Doubling time (days)			
For density	< 30 in cow dung < 30 in Cow dung+ sea weeds < 30 in Cow dung+ leaf litter	42.26 in biogas slurry 36.28 in biogas slurry+ leaf litter 26.96 in biogas slurry+ sawdust 40.77 in biogas slurry+ bagasse < 30 in sand+ cow dung	9.36 in biogas slurry 8.78 in biogas slurry+ leaf litter 9.28 in biogas slurry+ sawdust 11.26 in biogas slurry+ bagasse
		< 80 in sand+ cow dung+ Cellulose	
		<30 in sand+ cow dung+ paper	
		< 80 in sand +cow dung+ saw dust	
For biomass	<90 days in Cow dung <90 days in Cow dung + sea weeds <90 days in Cow dung+ leaf litter	35.00 in biogas slurry 30.53 in biogas slurry+ leaf litter 39.38 in biogas slurry+ sawdust 33.32 in biogas slurry+ bagasse <80 in sand+ cow dung < 80 in sand+ cow dung+ cellulose < 80 in sand +cow dung+ paper < 80 in sand+ cow dung+ saw dust	11.68 in biogas slurry 11.55 in biogas slurry+ leaf litter 23.24 in biogas slurry+ sawdust 19.70 in biogas slurry+ bagasse

Table 5.2: Comparison of organic amendment conversion ratio to biomass of *P. bermudensis* with those of other oligochaetes

S. No.	Species	Organic amendment conversion ratio to bio mass of worms	Gross food conversion efficiency (%)	Reference
1.	*Pontodrilus bermudensis*	1.69: 1 in cow dung (40%) + sea weeds (30%) + leaf litter (30%)	58.9 in cow dung (40%) + sea weeds (30%) + leaf litter (30%)	Present study
2.	*Hyperiodrilus euryaulos*	1.76:1 in soil substrate 1.43 :1 in cellulose 1.64 :1 in dry neem leaves and soil substrate	56.8 in soil subtrate 69.9 in cellulose 60.9 in dry neem leaves and soil substrate	Sogbesan and Ugwumba (2006)
3.	*Eudrilus eugeniae*	10:1 (dry weight) (=3.03:1) in cattle waste solids	33 in cattle waste solids	Dominguez et al. (2001)
4.	*Eisenia fetida*	5:1 (dry weight) (= 1.51: 1) in potato waste 6:1 (dry weight) (=1.81:1) in activated sea wage sludge	66.22 in potato waste 55.24 in activated sea wage sludge	Edwards (1983) Hartenstein (1983)

scenario supported the culture system and allowed partial harvesting in a sustainable manner. Addition of the same organic amendment was continued, which promoted the hatching and subsequent growth of existing cocoons in the culture system (Dominguez et al., 1997).

The results of this study indicate that this twin unit culture system can be successfully used to commercially culture *P. bermudensis*. The harvested worms can be frozen and stored for daily feeding of broodstocks of penaeids and portunids. The number of vermiculture units can be increased depending on the need for worms. For example, worms could be harvested every 15 days by setting up four

culture units and inoculating each unit with worms with a gap of 15 days.

5.1.6

The twin culture system with periodical harvest resulted in an organic amendment conversion ratio of 1.7:1 [= 5.6: 1 (dry weight)]. Similar results were reported for *E. fetida* in potato waste (5:1 (dry weight)) and in activated sewage sludge (6:1 (dry weight)) by Edwards (1983) and Hartenstein (1983), respectively. Sogbesan and Ugwumba (2006) reported conversion ratios for *Hyperiodrilus euryaulos*, in three organic amendments (soil substrate - 1.76:1; cellulose - 1.43:1; dry neem leaves and soil substrate-1.64: 1). The result of *P. bermudensisis* is also comparable with that of *Hyperiodrilus euryaulos*. However, low conversion ratio (10:1) was reported for *Eudrilus eugenia* in cattle waste solids (Dominguez et al., 2001). The conversion ratio of organic amendment to biomass of worms is dependent on the nutritious value of the organic amendment as well as growth rate of the species (Table 5.2). The cost of organic amendments is low for producing *P. bermudensis*. At present, the conventional and mandatory broodstock diet for penaeid shrimp, polychaetes, demands high price (Rs.1,000 to 1,500/ kg. = $20- 30/ kg.), which makes broodstock management expensive. This cost can be reduced by replacing polychaetes with *P. bermudensis*.

5.2 Scope for further research

5.2.1

Because all shrimp hatcheries are located in coastal regions, seaweeds and sea grasses are readily available and can be used as amendments for culturing *P. bermudensis*; thus, the culture of this worm could be incorporated as part of the hatchery operation. However, although the sustainable production of worms was achieved in the twin culture system, the present study was conducted at a single stocking density with a particular ratio of organic amendment. In the natural habitat, the maximum recorded density of worms was reported to be 5 worms/cm^2 (Ganapati and Raman, 1973; Rao and Ganapati, 1975). In the present study the maximum density recorded was 2.5 worms/cm^2, indicating that the possibility exists to increase the production of worms per unit area by manipulating the stocking density to establish optimal stocking density as well as the concentration and ratio of organic media to find optimal feeding rate (Ndegwa et al., 2000).

5.2.2

The information on the reproductive biology of *P. bermudensis* is limited to the description of cocoons (Rao and Gnapati, 1974), and description of three growth stages namely juveniles, non-clitellates and clitellates in the present study. Detailed study on biological aspects such as life cycle span, onset of maturation, cocoon shedding and the potentiality of worm to produce number of cocoons, and development time for embryos to hatch (Bhattacharjee and Chaudhuri, 2002; Chaudhuri and Bhattacharjee, 2011; Vijaya et al., 2012) is inevitable to manipulate the biological parameters to achieve higher production in captivity.

5.2.3

Vineetha (2001) studied the biochemical composition, including the fatty acid profile, of *P. bermudensis* collected from the littoral region. Whether any difference exists in the composition of worms from the natural habitat versus those grown in the different organic amendments remains to be studied, as does the effect of cultured worms on the reproductive performance of penaeids and portunids.

5.2.4

The earthworms produce cocoons by cross fertilization, self fertilization or through parthenogenesis (Chaudhuri and Bhattacharjee, 2011; Vijaya et al., 2012). Some species have ability for self fertilization or parthenogenesis without undergoing mating process which is more suitable for manipulation of reproductive performance in view of enhancement of production in captivity in short time. There is necessity of exploring possibility of using biotechnological tools to induce self fertilization/ parthenogenesis in earth worms to produce more cocoons from an individual worm in stipulated time. The detailed study on mode of fertilization in *P. bermudensis* is inevitable in view of using biotechnological tools for inducing maturation.

5.2.5

A small oligochaete, *Enchytraeus japonensis*, reproduces asexually by fragmentation and subsequent regeneration (Myohara et al., 1999) in laboratory condition. The possibility of this application in *P. bermudensis* has to be explored to make use it in large scale culture for mass production of worms.

Conclusion

In conclusion, the present study shows the

feasibility of culturing *P. bermudensis* in vermibed with sand as a base and with three organic amendments (e.g., cow dung, cow dung + seaweeds, and cow dung + leaf litter) for use as feed for shrimp/crabs. Cow dung + seaweeds and cow dung + leaf litter yielded the best results. The twin culture system, with the periodic addition of organic amendment, allowed for the partial harvest of worms every month; thus, this system is a dependable vermiculture system for providing food for maintenance of broodstock of penaeids and portunids. Because all shrimp/crab hatcheries are located in the coastal region, seaweeds can be utilized for production of worms through organic farming. The merits of culturing *P. bermudensis* for use as a supplementary diet item for broodstock of penaeids and portunids are as follows:

1. It is easy to culture *Pontodrilus bermudensis* by following the protocol developed in the present study.

2. It has a high organic amendment conversion ratio.

3. Maintenance of culture systems is easy, as the cocoons to adult stages are benthic.

4. Its doubling time for density and biomass are comparable to those of *Lampito mauritii.*

5. It has maturation stimulator besides having essential poly unsaturated fatty acids that together yield repetitive spawning in penaeids and portunids.

6. Cost of broodstock management in shrimp/crab hatcheries can be lowered by adopting cultured *P. bermudensis* as broodstock diet.

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