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Littoral Oligochaete *Pontodrilus bermudensis* Beddard: A potential source for Arachidonic acid that stimulates maturation in penaeid shrimp

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Abstract
The littoral oligocheate *Pontodrilus bermudensis* Beddard, is widely distributed in the tropical, subtropical and warm tropical regions of the Atlantic, Pacific and Indian Oceans. Its distribution extends to 45° N and 45° S of the equator with particular abundance in the tropical and subtropical belt. As a broodstock diet along with clam meat and squid for penaeid shrimps and portunid crabs it has promoted repetitive maturation and spawning in commercially important species such as *Penaeus semisulcatus, Fenneropenaeus indicus, Penaeus monodon, Portutunus pelagicus* and *Scylla tranquebarica*. Three successive experiments were conducted to identify the diet that supports maturation with a stimulator. An experiment that was conducted to evaluate the performance of feeding the worm, clam and squid showed that *Pontodrilus bermudensis* shortens the maturation cycle significantly in penaeid shrimp compared with the clam and squid diets. The fatty acid profile of this worm (*Pontodrilus bermudensis*) was studied and compared with those of other conventional broodstock diets of penaeid shrimps. This study demonstrated that this worm contains 3-5 times more arachidonic acid than in the other conventional diets. An experimental study was conducted to evaluate arachidonic acid as a maturation stimulator in penaeid shrimp by injecting arachidonic acid at 5 µg and 10 µg / g. body weight at five day intervals against the control group. About 40 % test group animals matured and spawned within 30 days whereas none of the control group did. The present study demonstrates that arachidonic acid is the factor from this littoral oligochaete that stimulate maturation in penaeid shrimp. The present study propagate the use of arachidonic acid in commercial broodstock diets for crustaceans, and to promote *P. bermudensis* as brood stock diet in penaeid shrimp hatcheries to achieve repetitive spawning which in turn reduce the expenditure on broodstock.

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1. Introduction

1.1 Importance of the worm as broodstock diet
Repetitive maturation and spawning for prolonged period was achieved in *Fenneropenaeus indicus* (250 days), *Penaeus semisulcatus* (90 days) and *Scylla tranquebarica* (90 days), without resorting to eyestalk ablation, by feeding *P. bermudensis* that were collected from littoral region along with clam meat and squid ad libitum, in a rematuration system, exclusively developed for penaeid shrimps (Maheswarudu et al., 1996; Radhakrishnan et al., 2000; Vineetha, 2001; Maheswarudu et al., 2007). Repetitive spawning in *P. monodon* for a prolonged period (110 days), with unilateral eyestalk ablation was also achieved, and all the nauplii produced were supplied to commercial hatcheries for seed production. Experiments on domestication of blue swimming crab *Portunus pelagicus* and black tiger shrimp *P. monodon* up to F4 generation and F_3 generation, respectively by feeding *P.*

bermudensis as a supplementary diet for broodstock was successfully conducted (Maheswarudu, 2007; Maheswarudu et al., 2008). In all the above experiments, freshly collected worms from their natural habitat, chopped into small pieces, were fed along with clam meat and squid meat. After introduction of the diet in the rematuration system, broodstock of penaeids and portunids prefered worms, clam meat and squid meat in order. While chopping the worms, we noticed a fragrance that might be an attractant that stimulated the broodstock to grab the chopped worms for subsequent ingestion. This fragrance of the worm gave an idea of conducting the present study to find out the reason.

1.2 Distribution and habitat of the worm Pontodrilus bermudensis

1.2.1

The littoral Oligocheate *Pontodrilus bermudensis* Beddard, belongs to Megascolecidae family and is widely distributed in the tropical, subtropical and warm tropical regions of the Atlantic, Pacific and Indian Oceans. Its distribution extends to 45° N and 45° S of the equator with particular abundance in the tropical and subtropical belt (Rao and Ganapati, 1975). In India its distribution was recorded all along the Indian coast. Along the north-east coast it was reported from Chilaka Lake (19° 43' 00.00" N; 85° 19'00.00" E) in the salinity range 0-32 ppt. The worm also has been reported from intertidal regions in Pamban (09° 16' 36.00" N; 79° 13'34.00" E), Port Blair (Andamans) (11° 38' 50.00" N; 92° 41'15.00" E), Laccadives, Kovalam (08° 28' 26.00" N; 76° 58'42.00" E), Port Okha (Gulf of Kutch) (23° 28' 00.00" N; 69° 04'59.00" E) and Elephanta (18° 57' 45.00" N; 72° 55'55.00" E) (Beddard, 1903; Stephenson, 1914, 1915a, 1915b,1916 &1930; Aiyar,1929; Gates,1943; Menon and Sareen,1967).

1.2.2

P. bermudensis exhibits tolerance for a wide range of salinity, from 5 to 33 ppt, with the optimum at 25 ppt (Ganapati and Rao, 1972). It prefers decaying and half-decaying sea weeds, under stones, rotten logs, etc., where relatively a high carbon content is available. The bionomics of this worm from brackish water areas of the Visakhapatnam Harbour was studied and it was found that habitat of the animals was subjected to wide salinity fluctuations and heavy domestic and industrial pollution (Rao and Ganapati, 1974, 1975). Mature worms with a clitellum were found mainly during the higher salinity range 10.0 to 32.0 ppt (Panikkar and Aiyar, 1939).

1.2.3 Life stages of the worm Pontodrilus bermudensis

Cocoons were observed ranging in 3-7 mm length, spindle shaped, and milky gray in colour first and changing to green and deep pink colour subsequently with the advancement of embryonic development. The number of eggs ranges from 1-6 in each cocoon with a thick viscous albuminous fluid inside the cocoon providing nourishment for the developing worms (Rao and Ganapati, 1974).

The juveniles or the young ones of *P. bermudensis* hatching from the cocoon are from 0.9 to 1.1 cm in length and weigh around 0.006 g. They are white in colour and are usually found either entwined to the body of clitellates or in groups of 10-15 worms attached to decaying twigs or leaves. The non-clitellates are the transition forms from juveniles to mature worms. They are yet to develop a clitellum and their colour ranges from light pink to dark pink. The non-clitellates ranged from 3.7 to 7.5 cm in length and from 0.15 to 0.85 g in weight. The mature or clitellate worms ranged from 7.5 to 12 cm in length and from 0.85 to 1.00 g in weight. They were dark pink in colour with a brownish tinge. They have a characteristic clitellum between 8^{th} and 19^{th} segment. The clitellar segments are very pale in colour and thickened (Vineetha and Maheswarudu, 2013).

2. Materials and Methods

2.1 Design of the work plan successively to achieve the goal

As three broodstock diets namely *P. bermudensis*, clam meat and squid together yielded repetitive maturation and spawning for prolonged time, to evaluate the best performance diet among three, experiment on rematuration of penaeid shrimp in cages in the sea was conducted which resulted in that *P. bermudensis* shortens maturation cyle of shrimp significantly than other two diets. Then focus was made on *P. bermudensis* and fatty acid analysis of the worm studied in comparison with other annelids which resulted in that *P. bermudensis* has arachidonic acid 3-4 times more than that of in polychaetes. Then to evaluate the performance of arachidonic acid on shrimp maturation, experiment was conducted by injecting arachidonic acid to shrimp in a rematuration system exclusively developed for penaeid shrimp.

2.2 Eexperiment on Rematuration of penaeid shrimp in cages in the sea by feeding three different broodstock diets

2.2.1 Objective of research

In view of evaluating the best performance diet among three diets (*P. bermudensis*, clam meat and squid) for repetitive maturation and spawning in shrimps and crabs, an experiment on rematuration of *P. semisulcatus* was conducted in box type cages in the sea by feeding each diet separately.

2.2.2 Experiment

This experiment was conducted in box type cages (1.0 ×0.75 ×0.5 m size), in the Gulf of Mannar at 1.56 ± 0.26 m depth (09° 16' 16.7"N; 079° 07' 56.0" E) for a period of three months in 1993. The method of fixation of the cages and their subsequent maintenance was as described by Maheswarudu et al. (2011). Brood stock (Mature males and gravid females) of *P. semisulcatus* were collected from trawl net operation in the Palk Bay and transported to a shrimp hatchery, where gravid females were allowed to spawn. These animals (males and females) were divided into three groups and introduced in to cages at a stocking density 13/m^2. Triplicates were done for each group. Males and females were introduced at 1:1 ratio in each cage. Broodstock of group-1, group-2 and group-3 were fed daily with *Pontodrilus bermudensis*, clam meat and squid, at 20% of the biomass, respectively. Observations on maturity of the gonads (females) in each group were made and maturity stage of each gonad was recorded fortnightly, observing the size and colour pattern of the ovary (Rao, 1968). In the Gulf of Mannar, where maturation experiments were conducted in the cages, the bottom water temperature, salinity and pH were 29.32 ± 1.09° C, 31.42 ± 1.55 ppt and 8.38 ± 0.03, respectively.

2. 3 Fatty acid analysis of P. bermudensis

2.3.1 Objective of research

The fatty acid profile of *P. bermudensis* was analyzed by gas chromatography (Vineetha, 2001) and compared with those of other annelids which are known as broodstock diets for shrimp, to find out the variation in fatty acid profile of *P. bermudensis* from those of other annelids, and to find out the possible major stimulating factor.

2.3.2 Analysis

Live worms of *P. bermudensis* were collected from the littoral region of Gulf of Mannar, Mandapam and transported in earthen pots filled with sand and covered with wet jute cloth from Mandapam Regional Centre of Central Marine Fisheries Research Institute (CMFRI) to Central Institute of Fisheries Technology (CIFT), Kochi (76° 15' 34.5738" E; 9° 56' 21.2382"N). Extraction of total lipids, saponification, esterification and analysis of fatty acids profiles in Gas Chromatogram (GC) were done at the Biochemistry and Nutrition Division of CIFT, Kochi. Only fresh live worms were used for analysis. Total lipids were extracted from the tissues by the method of Bligh and Dyer (1959). After saponification, saponiable materials were recovered and fatty acids were converted to fatty acid methyl esters (FAME). Fatty acid methyl esters were separated and characterized by capillary gas chromatography (GC) using a Chrom Pak 9001 equipped with flame ionization detector and fitted with a 6' x 1/8" i.d. stainless steel column packed with Chromosorb H.P. (Mesh size 80-100) and liquid phase OV 275 (10%). Nitrogen was used as carrier gas (13.5 ml/min flow rate). Several precautions were taken to ensure that no degradation of the lipids occurred during storage, extraction and saponification.

2.4 Evaluation of performance of arachidonic acid on shrimp maturation

2.4.1 Objective of research

Since arachidonic acid was suspected as the compound (based on fatty acid analysis) that may stimulate maturation in female shrimps, an experiment was designed and conducted on *P. semisulcatus* in a rematuration system of 5000 L capacity that was developed for penaeid shrimp (Maheswarudu et al., 1996) by injecting arachidonic acid at two doses, 5 µg/g. body wt., and 10 µg/g. body wt. for brood stock of test groups against the control group. Each dose of arachidonic acid experiment was conducted in duplicate in Mari culture laboratories at Visakhapatnam Regional Centre of Central Marine Fisheries Research Institute, Andhra Pradesh, India (17° 41' 12.54"N; 83° 13' 6.53"E) in 2005.

2.4.2 Experiment

In experiment I about 30 live broodstock of *P. semisulcatus* (15 males and 15 females) were collected from the trawl net operation in the Bay of Bengal off Visakhapatnam and transported to the lab in 50 L jerry cans by providing aeration (battery operated aerator). The shrimp were acclimatized in the laboratory prior to experimentation. The animals were randomly distributed into three groups, five males and five females in each group, one for control and two for experimental group. Each

animal of every group was tagged with a ring tag at the base of an eyestalk. Each animal of every group was also marked by cutting the uropod tip; four animals were marked by cutting the tip of each uropod and the fifth animal was marked without cutting any uropod, to record the moulting time of each animal from the moulted exoskeleton. These groups were stocked separately in 5000 L capacity round fibreglass tanks, which were provided with a sand bed filter for recirculation of water and to simulate the natural habitat for the shrimp (Maheswarudu et al., 1996). The shrimp were fed with clam meat and squid at 1:1 ratio daily in the evening (17.00 hrs) ad libitum. Every day in the morning hours all the fecal matter was siphoned out in each tank and about 40% of the water was exchanged. If any moulted exoskeletons were in the tank, they were collected and sex of the animal that was moulted was recorded. The females of the experimental group were injected with arachidonic acid (Sigma) @ 5 µg/g. body weight at 5 days interval for a period of 25 days. Arachidonic acid was dissolved in ethanol at the concentration 1µl =25 µg. Dissolved arachidonic acid in required quantity was injected in to abdominal muscle to each female, in the middle of lateral region between first and second abdominal segments, by using micro syringe. Similarly females of the control group were injected with crustacean ringer solution.

The gonad maturity stage of each injected female was recorded, as followed in the experiment on re maturation of shrimp in cages in the sea (Rao, 1968) to evaluate the spawning performance of each female. On day 30 all the females were sacrificed and the gonads (ovaries) were isolated from each female, and the weight and ova diameters (25 numbers) were recorded from each gonad by following the method described by Browdy (1989). For ova diameter study gonads were hardened in 4% formalin prepared in sea water at least for 24 hrs and a small piece of gonad tissue was placed on a microscope slide in a drop of filtered sea water. A second slide was placed over the preparation and gently manipulated back and forth to separate individual oocytes. The maximum diameter was measured across the vertical axis under a microscope micrometer for each of twenty five oocytes and an average diameter was calculated. The gonad somatic index was calculated for each female from the gonad (ovary) weight and total weight of the animal. The mean temperature, salinity, pH and ammonia level in the experimental tanks were 29.94±1.13° C, 28.45±0.56 ppt, 8.0 and 0.00085±0.00022 mg/L, respectively.

Experiment II was conducted in a similar manner except for altering the dose of arachidonic acid at 10 ug /g. body weight of the animal.

2.5 Statistical analysis

ANOVA single factor analysis was carried out in experiment on rematuration in cage to test the significant difference in duration of maturation cycle between three brood stock groups fed with three different broodstock diets. Student T test was employed to find out the significant variation between fatty acid contents of *P. bermudensis* such as total saturated fatty acids, total mono saturated fatty acids, total poly un saturated fatty acids, ecosapentaenoic acid (EPA), docosa-hexaenoic acid (DPH), arachidonic acid (AA), Total n-3 fatty acids, total n-6 fatty acids and n-3/n-6 values and those of other polychaetes and oligochaetes (Table,3.2.1). Student T test was also employed to compare the ova diameter of control group with that of test group in experiment on evaluation of performance of arachidonic acid on shrimp maturation.

3. Results

3.1 Eexperiment on Rematuration of penaeid shrimp in cages in the sea by feeding three different broodstock diets

Distribution of gonad maturity stages (by number) of female penaeid shrimp *Penaeus semisulcatus* in cages in the sea, fed with three different broodstock diets at different periods during 90 days experimental period is presented in Table, 3.1.1. The results of this experiment has revealed that brooders fed with *Pontodrilus bermudensis* rematured and spawned after 45 days whereas brooders that were fed with clam meat and squid were able to remature and spawn only after 75 days. The duration of the maturation cycle took in three groups significantly varied (ANOVA single factor; P= 0.000596) and the results of this experiment suggested the possibility of the existence of some sort of inducing factor for shrimp maturation in *P. bermudensis*.

3.2 Fatty acid analysis of P. bermudensis

The fatty acid profile of *P. bermudensis* in comparison with those of other annelids is presented in Table, 3.2.1. Fatty acid profile of *P. bermudensis* is similar to that of *A. cristata*, but differed from those of other polychaetes and oligochaetes. Total saturated fatty acids in

P. bermudensis did not differ significantly from those of other polychaetes and oligochaetes. Total monounsaturated fatty acids also did not differ from those of polychaetes and oligochaetes except in *A. reseer* which has higher percentage of monounsaturated fatty acids. Total polyunsaturated fatty acids of *P. bermudensis* did not differ from those of five listed polychaetes but significantly differed from those of three oligochaetes which have two times higher. *P. bermudensis* has low percent of total n-3 fatty acids which significantly differed from those of other polychaetes and oligochaetes. Percent of total n-6 fatty acids of *P. bermudensis* is higher than *G. dibranchiata*, *A. reseer*, *N. viridens* and lower to that of *L. rubellus* and *E.euginae*. The percent of EPA in *P. bermudensis* is very low compare to other worms. DPH is higher in *G. dibranchiata* and *A. reseer* and all oligochaetes have low percent. The percent of AA in all earth worms (>10 %), including *P. bermudensis* is higher than those (< 5%) of polychaetes (*G. dibranchiata, A. resser, N.viridens*), 6-11 times higher. From this study it was suspected that arachidonic acid is the compound that may stimulate maturation in female shrimps.

Table 3.1.1: Distribution of gonad maturity stages (by number) of female penaeid shrimp *Penaeus semisulcatus* in cages in the sea, at different periods, fed with three different broodstock diets

Day	Maturity stage-5 (spent recovery stage)	Maturity stage-1 (Early maturing stage)	Maturity stage-2	Maturity stage-3	Maturity stage -4	Diet
1	24	0	0	0	0	*Pontodrilus bermudensis*
15	0	24	0	0	0	
30	0	0	24	0	0	
45	0	0	0	24	0	
60	24	0	0	0	0	
75	0	24	0	0	0	
90	0	0	16	8	0	
1	24	0	0	0	0	*Anadora grannosa*
15	0	24	0	0	0	
30	0	0	24	0	0	
45	0	0	24	0	0	
60	0	0	24	0	0	
75	0	0	0	24	0	
90	24	0	0	0	0	
1	24	0	0	0	0	Squid meat
15	0	24	0	0	0	
30	0	0	24	0	0	
45	0	0	24	0	0	
60	0	0	24	0	0	
75	0	0	0	24	0	
90	24	0	0	0	0	

Table 3.2.1: Comparison of fatty acid profile (% by weight) of *P. bermudensis* with those of some polychaetes and oligochaetes (compiled from literature)

	Fatty acid	*P. bermudensis*	*G. dibranchiate* (Lytle et al,1990)	*A. reeser* (Lytle et al,1990)	*A. cristata* (Lytle et al,1990)	*Neries viridens* (Lytle et al,1990)	*N. diversicolor* (Luis &Ponte,1993)**	*Pheretima sp* (Lytle et al,1990)	*Lumbricus rubellus* (Lytle et al,1990)	*Eudrilus euginae* (Lytle et al,1990)
	Saturates									
1	C 14: 0	12.93	0.52	6.93	1.26	1.12	0.9	2.5	1.62	3.516

2	C 15:0	4.22	0	0	0.00	0.00	0	0	0	0
3	C 16:0	9.6	8.69	4.13	27.24	13.25	17.9	4.98	3.16	4.912
4	C 17:0	2.29	0	0	0.00	0.00	0	0	0	0
5	C 18:0	6.14	6.45	4.06	6.07	5.20	5.6	9.11	8.86	9.85
6	C 20:0	0	1.11	0.17	1.13	1.32	0	0.56	0.37	0.629
7	C 22:0	0	1.97	2.16	0.72	0.43	0	1.5	1.02	1.508
8	C 23:0	0	0	0	0.00	0.00	0	1.64	1.22	1.465
	Total	35.18	18.74	17.45	36.42	21.32	24.4	20.29	16.25	21.88
	Monounsaturates									
9	C16:1	4.27	8.61	40.59	11.08	8.41	1	5.03	3.82	4.844
10	C18:1	11.48	9.96	10.09	16.97	12.59	15.8	14.29	14.37	12.157
11	C 20.1	6.68	10.69	7.97	11.40	9.11	3.8	6.58	9.58	8.1
12	C 22.1	0	6.56	4.35	1.79	6.10	0	0.05	0.08	0.069
13	C24:1	2.2	0	0	0.00	0.00	0	0	0	0
	Total	24.63	35.82	63*	41.24	36.21	20.6	25.95	27.85	25.17
	Poly unsaturates (PUFA)									
14	C 16:3n3	0	0	0	0.00	0.00	2.8	0	0	0
15	C 18:2n6	8.19	0.61	0.48	3.25	0.56	13.6	13.47	12.63	15.586
16	C 18:3n3	0.62	0.57	0.57	2.33	2.10	1.5	0.97	1.77	2.506
17	C 18:4n3	1.58	0	0	0.00	0.00	0	0	0	0
18	C 20: 2n6	0	1.08	1.4	2.12	0.66	6.6	4.51	5.6	5.043
19	C 20:3n3	0	0.46	0.25	0.87	0.11	0	8.64	4.9	4.376
20	C 20:4n6 (AA)	10.01	1.49*	1.48*	8.56	1.34*	4.3	12.11	17.31	14.713
21	C 20:5n3 (EPA)	2.48	25.44*	5.54*	1.89	25.34*	0	10.78*	12.51*	9.6*
22	C 22:2n6	0	1.02	0.8	0.00	0.56	0	0.32	0.31	0.225
23	C 22:3n3	0	2.69	2.76	0.00	2.94	0	0	0	0
24	C 22: 5n3	0	6.91	2.78	0.71	5.96	9	1.74	0.88	0.704
25	C 22: 6n3 (DHA)	1.19	5.17*	3.47*	2.61	2.89	1	1.19	0.25	0.182
	Total	24.07	45.44	19.53	22.35	42.47	38.8	53.73*	56.16*	52.935*
26	Unidentified/unlisted	16.12	0	0	0.00	0.00	16.2	0	0	0
27	Total	100	100	99.98	100.00	100.00	100	99.97	100.26	99.985
28	Total n3	5.87	41.24*	15.37*	8.41	39.34*	14.3*	23.32*	20.31*	17.37*
29	Total n6	18.2	4.2*	4.16*	13.93	3.12*	24.5	30.41	35.85*	35.57*
30	n3/n6	0.32	9.83*	3.71	0.60	12.6*	0.6	0.77	0.57	0.49
31	DHA/EPA	0.47	0.2	0.63	0.11	28.23	0	0.11	0.02	0.02
32	Total fatty cids ug/g.wt.tissue	3360.3	2590	5470	613	3020		7870	1670	1600
*= significant at 5% level (P < 0.5), ** = only data equal to or above 1% of total fatty acids are listed.										

3.3 Evaluation of performance of arachidonic acid on shrimp maturation

The results of the two experiments that were conducted by injecting arachidonic acid at two doses, 5 µg/g. body wt., and 10 µg/g. body wt. for brood stock of test group females of *P. semisulcatus* against the control group to evaluate the performance of arachidonic acid on shrimp maturation is presented in table, 3.3.1.

It was found that 40% of the brood stock of test group I, which were injected with arachidonic acid (5 ug/ g. body weight) attained maturity and spawned within 30 days; whereas the brood stock of test group, in experiment II, those injected with arachidonic acid at 10 µg/g. body wt., about 40 % of females matured and spawned, 20% once and 20% twice, within 30 days. None of the broodstock in the control group attained maturity within 30 days in both experiments. In both experiments gonad somatic index was higher in test group of not spawned females than those of control group. Similarly ova diameters of test groups were significantly higher (P=3.59052E-15) than those of the control groups (Table, 3.3.1). However, mortality was recorded in both the experiments, both in control and test groups.

Table 3.3.1: Effect of arachidonic acid on maturation of penaeid shrimp *Penaeus semisulcatus*

	Parameter	Control group	Test group
	Arachidonic acid dosage (5µg/g. body weight)		
1	Mean total length (mm) of females	179+16.56	196.0+ 10.12
2	Mean weight (g) of females	56.0+ 15.5	72.9+13.3
3	Number of moults undergone/female	1.2	1.2
4	Mortality (%)	40	30
5	Matured and spawned once (%)	0	40
6	Matured and spawned twice (%)	0	0
7	Not spawned (%)	60	30
8	Mean gonad somatic index of not spawned	0.933+0.84	1.09+0.429
9	Mean ova diameter of not spawned	108.84+7.55	135.95+56.5
	Arachidonic acid dosage (10µg/g. body weight)		
1	Mean total length (mm) of females	195.0+24.49	194.7.0+15.57
2	Mean weight (g) of females	72.2+26.54	74.7+19.08
3	Number of moults undergone/ female	1.1	1
4	Mortality (%)	40	40
5	Matured and spawned once (%)	0	20
6	Matured and spawned twice (%)	0	20
7	Not spawned (%)	60	20
8	Mean gonad somatic index of not spawned	0.757+0.078	1.205+0.601
9	Mean ova diameter of not spawned	102.37+2.702	158.705+81.18

4. Discussion

These three successive experiments of the present study confirm that arachidonic acid from the *P. bermudensis* is a major factor which triggers and promotes maturation in penaeid shrimp.

4.1 Achievements

4.1.1

The study on rematuration of penaeid shrimp in cages has revealed that *P. bermudensis* as a broodstock diet shortens shrimp maturation cycle by 40% compare to other broodstock diets such as clam and squid, suggesting to use this worm as mandatory broodstock diet to maximize reproductive outcome. In penaeid prawns such as *Metapenaeus monoceros, Metapenaeus affinis, Penaeus indicus* and *Parapenaeiopsis stylifera* the gap between successive spawnings was reported two months i.e the time taken for the development of the immature ova to the final stage of maturity (Rao, 1968; Sudhakara rao, 1989). In *Penaeus duorarum notialis* the interval between successive spawnings was reported as 2-3 months in the Atlantic waters (Burukovski, 1970). In the present study broodstock fed with clam meat and squid could remature and spawn in 90 days whereas those fed with *Pontodrilus bermudensis* could remature and spawn in 60 days, duration for completion of maturation cycle significantly reduced by 40% from those other two groups, suggesting that *P. brmudensis* as most suitable diet for broodstock of penaeid shrimps.

4.1.2

In penaeid shrimp, during maturation, stored lipids are mobilized from the hepatopancreas and dietry lipids are rapidly processed for transport to the developing ovaries (Ravid et al., 1999). The importance of lipids in maturation has long been recognized, and more focus was made on lipid nutrition especially on highly unsaturated fatty acids (Wouters, 2004).

Fatty acids, especially n-3 PUFAs, in particular ecosapentaenoic acid (20:5n-3) and docosa-hexaenoic acid (22:6n-3), as essential components for shrimp maturation, play a significant role in induction of shrimp maturation in captivity (Wouters et al., 2001). Xu et al. (1994) while evaluating the anchovy oil, linseed oil, corn oil and fork lard for fatty acid source for broodstock diet of *F. chinensis*, found that anchovy oil was best performance diet, and anchovy oil resulted in shrimp eggs containing high n-3 PUFA levels. He also found that positive correlations between egg 20:5n-3 levels and fecundity and between egg22:6 n-3 levels and hatching percentage. It is evident from the table 3.2.1 that *P. bermudensis* is source for these two essential fatty acids for promoting shrimp maturation.

4.1.3

The Maine bloodworm *Glygera dibranchiata* has been used as successful maturation diet in reared Penaeus vannami to trigger the maturation. Lytle et al.(1990) compared the fatty acid profile of blood worm with other annelids, polychaetes, oysters and squid, and considering parameters such as total n-3

PUFA, n-3 PUFA/ n-6 PUFA, and percentage of DHA+EPA in total n-3 PUFA, they reported that *Glygera dibranchiata* was successful maturation diet. These parameters are low in *P. bermudensis* when compared to those of *Glygera dibranchiata*. However, the percentage of AA is high in *P. bermudensis*, compared to that of Glycera dibranchiata, and all oligochaetes (earth worms) have high AA values than those in polychaetes (Table, 3.2.1). The high value of AA in *P. bermudensis* compare to that of blood worm, suggests that AA is playing a significant role in promoting shrimp maturation besides sourcing essential fatty acids (n-3 PUFAs) for rapid maturation in the penaeid shrimp.

4.1.4

Fatty acid profile of *P. bermudensis* is similar to that of *A. cristata,* and *P. bermudensis* has high percent of total saturated fatty acids and mono unsaturated fatty acids (35.18+24.63=59.81%), more than half of total fatty acids. Saturated fatty acids serve as source of energy during oogenesis and vitellogenesis (Guary et al., 1974; Teshima et al., 1988). Clake et al., (1999) suggested that saturated and mono unsaturated fatty acids serve as a source of energy during embryogenesis and early larval development in *Macrobrachium rosenbergii*. The fatty acid profile of *P. bermudensis*, rich in saturated and mono unsaturated fatty acids, supports gonad development and subsequent six non feeding Naupliar larval stages in penaeid shrimp as energy source.

4.1.5

The study on evaluation of performance of arachidonic acid on shrimp maturation has confirmed that arachidonic acid is the major factor from *P. bermudensis* that stimulates maturation in penaeid shrimp. In both the experiments 40% of test group females matured and spawned within 30 days where as none of the control group did. The gonad somatic indexes as well as ova diameter of not spawned females of test groups were higher than those of control groups. Higher dose of arachidonic acid (10 ug/g.body weight) has resulted in second time spawning as well as higher values of gonad somatic index and ova diameter (Table, 3.3.1). The results of these two experiments suggest that arachidonic acid in *P. bermudensis* is playing a significant role, may be as an endocrinological factor, for promoting shrimp maturation. The role of arachidonic acid in shrimp maturation has been suggested as precursor for prostaglandins (Middleditch et al., 1980) and prostaglandins may involve in reproduction as it does in mammals, certain fishes and insects (Sargent et al., 1989). Tahara and Yano (2004) analysed total lipid, fatty acids and prostaglandins (PGF (2 alpha) and PGE(2)) in the ovary of kuruma prawns (*Marsupenaeus japonicus*) during ovarian development and suggested that prostaglandins (PGF(2 alpha) and PGE(2)) and arachidonic acid are deeply involved in ovarian maturation. To find out the path ways of arachidonic acid metabolism and to find out the targeted organ in shrimp where it is acting to stimulate and rapid the maturation process further studies are required by injecting radio labeled arachidonic acid and tracing the path way.

4.1.6

In both experiments on evaluation of performance of arachidonic acid on shrimp maturation mortality was recorded in both groups (Control and test) of females. This may be attributed for stress caused by intra muscular injecting and handling and this type of demerit can be avoided, either by incorporation of arachidonic acid in to artificial diets or by feeding fresh worms of *P. bermudensis*.

4.2 Scope for further research

4.2.1

In the present study 40 % females of *P. semisulcatus* that were injected with arachidonic acid at 5µg/g.body weight and 10 µg./g. body weight matured and spawned, yielding second time spawning in 20% of females with higher dose (10 µg/g.body weight). Optimum dose of arachidonic acid can be perfected by injecting different higher doses, than the two doses of the present study and observing reproductive performance.

4.2.2

As the present study resulted in mortality due to intra muscular injection of arachidonic acid It is advisable to use arachidonic acid orally by incorporating in to commercial broodstock diets. When arachidonic acid is used orally some amount of arachidonic acid is lost because the entire quantity will not be assimilated in to body. To standardize the optimum levels of arachidonic acid in commercial diets further experiments are to be conducted on the same line.

4.2.3

The present study confirms that arachidonic acid is stimulating maturation in penaeid shrimp, may be as an endocrinological factor. Experiments are needed to find out the path

way of arachidonic acid metabolism, and on which endocrine organ it is acting to stimulate rapid maturation, by injecting radio labeled arachidonic acid and tracing the path way of it.

4.2.4
In fatty acid profile of *P. bermudensis* about 16.12 % of the fatty acids were not identified. Further analysis can be made to draw complete profile by identifying un identified fatty acids, and this complete profile can be used to prepare artificial diets.

4.2.5
Since arachidonic acid induces maturation in penaeid shrimp at low doses (5 µg/g. body wet. and 10 µg/g. body wt.) Its possible role is suspected as endocrinological factor. Studies are required on these lines by crustacean endocrinologists to explore appropriate method to make it for more commercial application.

4.2.6
Middleditch et al. (1980) suspected that arachidonicacid is the precursor of prostaglandins, and Tahara and Yano (2004) suggested that both prostaglandins and arachidonic acid are deeply involved in shrimp maturation. The present study reveals that arachidonic acid alone stimulates rapid maturation, which needs detailed study to explain functional role of arachidonic acid.

Conclusion

The present study yielded the following merits of *P. bermudensis* to use as successful brood stock diet for penaeid shrimps.

1. *P. bermudensis* as a broodstock diet shortens shrimp maturation cycle by 40% compare to other broodstock diets such as clam and squid, suggesting to use this worm as mandatory broodstock diet to maximize reproductive outcome.
2. *P. bermudensis* is source for two essential fatty acids (ecosapentaenoic acid (20:5n-3) and docosa-hexaenoic acid (22:6n-3) which improve fecundity as well as hatching rate in penaeid shrimp.
3. *P. bermudensis* has high percent of total saturated fatty acids and mono unsaturated fatty acids (35.18+24.63=59.81%) which serve as source of energy for gonad development and subsequent six non feeding Naupliar larval stages in penaeid shrimp.
4. The high content of arachidonic acid (AA) in *P. bermudensis* promotes rapid maturation in penaeid shrimp.

In conclusion the present study is scoping to incorporate arachidonic acid in commercial broodstock diet and to use *P. bermudensis* as fresh brood stock diet in shrim/crab hatcheries for achieving repetitive maturation and spawning for prolonged period. Usage of same batch of broodstock for repeated spawning for prolonged period will reduce the expenditure on brood stock. Penaeid shrimps and portunid crabs prefer *P. bermudensis* compare to clam and squid and targeted goals in domestication programmes of commercially important species of crustaceans can be achieved by using this worm as fresh broodstock diet (Maheswarudu, 2007) for achieving rapid maturation.

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Author's Contribution and Competing Interests

To evaluate the best diet among three namely *Pontodrilus bermudensis*, clam meat and squid meat for stimulation of maturation in penaeid shrimp experiments were conducted in cages in the sea by the corresponding author. Fatty acid profile of *P. bermudensis* was studied by the contributing author as a part of Ph.D thesis under the guidance of the corresponding author. Then experimental study to evaluate the arachidonic acid as maturation stimulator for penaeid shrimp was studied in a rematuration system exclusively developed for penaeid shrimp by the corresponding author. Preparation of manuscript was done by the corresponding author, and both reviewed the manuscript.

The authors are not competing for financial interests.

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