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Faecal streptococci distribution in environmental and seafood samples of two different coastal environs

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Abstract : The faecal streptococci are particularly useful indicators of faecal pollution.Of concern to environmental managers is the potential threat posed by microorganisms in waste discharges to human health while bathing or through consumption of affected fishes and shellfish. The water, sediment and seafood (from landing and markets) in Pichavaram mangrove and Ennore estuary were examined for faecal streptococci. In general, the incidence of faecal streptococci was considerably high in fishes collected from marketed samples when compared to fishes from landing sites. Among the seafood samples examined, the incidence of faecal streptococci was high (30.2%) in bivalves followed by crab (23.0%), finfish (20.3%) and prawn (19.3%). Incidence was high during monsoon. Comparatively,Ennore estuary showed more counts owing to sewage discharge.

Introduction

The faecal streptococci are a group of coccoid bacteria naturally inhabiting the gut of warm-blooded animals and humans. Only 50 years ago,their practical application in water pollution research and management came into existence.They get into food through vegetation, processing equipment, processing environments or faecal contamination. Symptoms include nausea, vomiting and diarrhea, milder than those caused by other food borne illness. Also,the formation of significant levels of biogenic amines due to the prolific growth of Enterococci in many foods has been observed (Gerg and Mital, 1991;Giraffa et al., 1994). These compounds, produced from free aminoacids by decarboxylase activity of microorganisms, have various toxicological implications. Large quantities of tyramine and histamine can evoke symptoms such as hypertension, hypotension, headache, urticasia, nausea and vomiting (Edwards and Sanding, 1981).

Oliveira et al. (1997) reported that the faecal enterococci are of great sanitary as well as clinical significance and can be a better indicator organism than the total and faecal coliforms (Sinton et al., 1993).Several studies have suggested that the faecal Streptococci in recreational waters are good numerical indicators of human health risk (Cabelli, 1983; Cabelli et al., 1983). Considering the health hazards, water quality guidelineof 33 enterococci/100 ml for recreational waters and 35 enterococci/100ml for marine waters have been proposed for recreational waters. The present study was carried out to assess the distribution of faecal streptococci in environment and seafood samples of Pichavaram mangroves and Ennore Estuary.

Materials and Methods

The study was carried out in Pichavaram mangrove (Kanankeluthi canal) (Station I) (Lat. 11˚29'N; Long. 79˚49'E)andEnnore estuary (Station II)(Lat.13˚15'N; Long. 80˚19'E), located along southeast coast of India.Monthly water andsediment samples were collected from January to December 1999Surface water samples were collected using clean sterile glass bottles.Sediment samples were collected with pre-cleaned Peterson grab and storedin sterile polythene bags.The samples were immediatelytransported to the laboratory in an ice box for microbial analysis.

Faecal streptococci were enumerated by the Millipore filter technique using the *M. enterococcus* agar (M-1108,Himedia Mumbai) with the composition recommended (APHA 1989, 1992).The filter is then transferred to a selective medium in petridishes and kept in an incubator in the inverted position at the temperature of 37^{o}C for 48 hours.All colonies which appear red or maroon colour are counted as faecal streptococci. The faecal streptococci colonies were picked up and restreaked in appropriate nutrient agar plates and the pure culture was transferred into agar slants. Identification of pure culture upto species level was done following standard procedure (Starr et al., 1981).

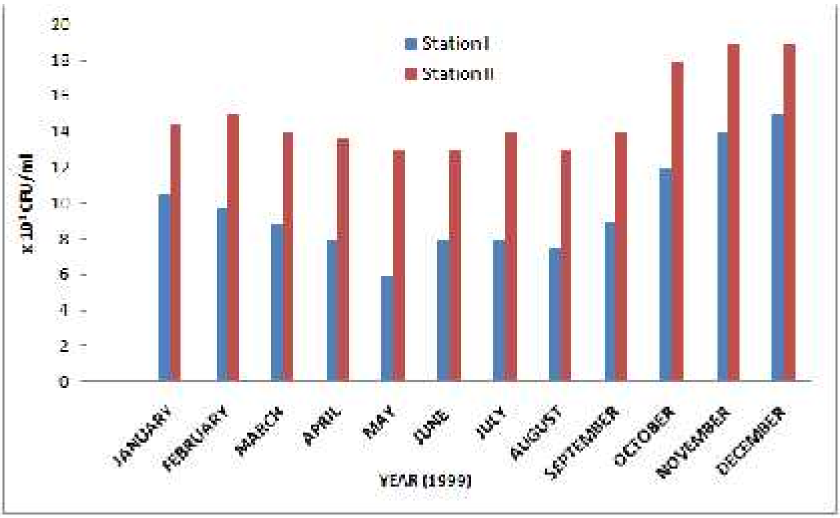
Preparation of fin and shell fish samples:

Seafood samples were collected on a monthly basis.Sampleswere identified using standard reference manualsfor finfishes,prawn,crab, oyster and clams (Fisher and White,1974; Paulpandian and Ramaswamy,1987; Sethuramalingam and Ajmalkhan, 1987; Sathyamurthy, 1956).The bacteriological analysis was carried out following the method given in AOAC (1976).

Results and Discussion

The distribution of faecal streptococci in water and sediment are given in Figs.1 and 2. Faecal streptococci population exhibited a pronounced seasonal fluctuation with well defined peaks in monsoon and postmonsoon seasons. Maximum counts were recorded during monsoon and the minimum were observed during summer season.At station I, faecal streptococci ranged from 6 to 15 $x10^1$CFU/ml in water and 2.2 to $6x10^2$ CFU/g in sediment samples.At station II, the same trend was observed and it fluctuated between 13 and $19x10^1$ CFU/ml in water and 6 and $11x10^2$ CFU/g in sediment.In both the stations,the maximum and minimum numbers were observed during monsoon and summer seasons respectively.

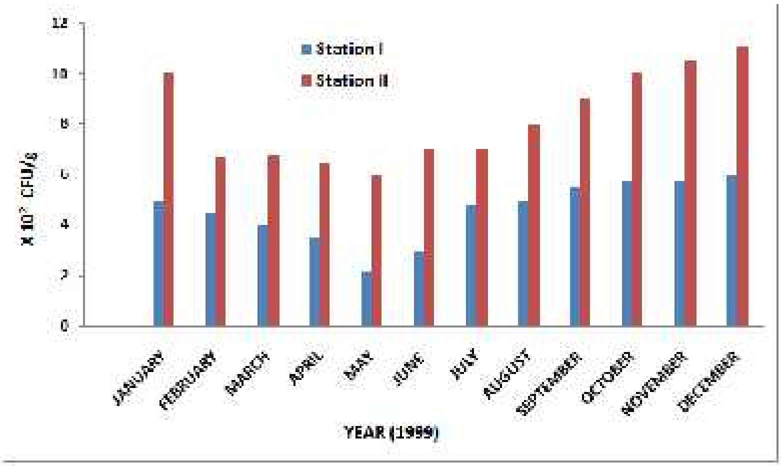
Fig: 1 Distribution of Faecal Streptococci in water samples



TheKanankeluthi canal of Pichavaram mangrove has a sandy clay bottom with low level of organic matter and always contained less population of different bacterial fractions including faecal streptococci.The significance of soil as substratum for better survival of many bacterial groups, including pathogens, has already been discussed by Klein and Casida(1970). The present study was also supported byLakshmanaperumalsamy et al. (1981) who have reported that indicator organisms and pathogens were found to be present in greater numbers in sediments than the overlying waters. Sediment can act as reservoir to these organisms.

Station II, located near the outfall of domestic wastes, showed higher levels of bacterial population in sediment samples compared to the station I. The texture of sediment at this station appears to be conducive for better survival, ultimately enhancing the occurrence of faecal streptococci and *E.coli*. This sampling site, which always contained fine clay, enriched with high nutrients, provides a good niche for the survival of these organisms.According to Greenbreg (1957), clay and slit, which constitute a major portion of the mud, are electrically charged and hence are adoptively active. This kind of adsorption by itself does not affect the survival of bacteria,but tends to remove the microorganisms from suspension and concentrate them in bottom deposits ensuring their continual existence.

Fig. 2: Distribution of Faecal Streptococci in sediment samples



Distribution of faecal streptococciin fresh fin and shellfishes

Distribution of faecal streptococci in the specimens of fresh and marketed fishes collected from Pichavaram mangroves and Ennore estuary have beenpresented in Tables 1, 2, 3, 4.

Throughout the sampling period,faecal streptococci strains could be obtained in the specimens of freshly caught finfishes and shellfishes from Pichavaram and Ennore estuary. A total of 962 specimens of fishes were collected from the landing centre and 734 specimens from the local fish market of Pichavaram. Likewise, in Ennore, 933 specimens from the landing site and 661 specimens from the local fish market were obtained and examined for the analysis.The positive numbers were found to be 19% and 27.9% from fish landing site and 28.2% and 37.9% in fish market , 172 and 261 from landing site , 207 and 251 in fish market samples collected from stations I and II respectively.

In general, the incidence of faecal streptococci was considerably high in fishes collected from marketed samples compared to fishes collected from landing sites.Among the seafood samples examined at Station I, the incidence of faecal streptococci was high (30.2%) in bivalves followed by crab (23.0%), finfish (20.3%) and prawn (19.3%) samples. The fresh finfish *Plotosuscanius* showed high percentage of faecal streptococci (20.3%), followed by *Gerresfilamentosus* (18.8%)

Liza parsia (18.7%), *Teraponputa* (16.4%), *Arius maculates* (16.2%), *Epinephalustauvina* (16.1%). The shellfish, *Meretrixmeretrix* showed the incidence of 30.2% followed by *Scylla serrata* (23%) and *Penaeusindicus* (19.3%).

At station II, the incidence of faecal streptococci was high (46%)in bivalves followed by crab (42.9%), finfish (30%) and prawn (25.2%) samples. The incidence was high (30%) in fresh finfish *Gerresfilamentosus* followed by *Epinephalustauvina* and *Teraponputa* (29.2%), *Arius maculates* and *Liza parsia* (26.7%). The shellfish, *Meretrixmeretrix* showed the incidence of 46% followed by *Scylla serrata* (42.9%) and *Penaeusindicus* (25.2%). The maximum incidence was observed in the bivalve followed by crab,finfish and prawn .

At both stations, the season-wise incidence of faecal streptococci and level revealed higher percentage occurrence during monsoon and postmonsoon seasons in fin fishes. At Station I, thefaecal streptococci ranged from 3 CFU/g (summer) to 40 CFU/g (monsoon).The maximum incidence was found in the *Arius maculatus*(23 to 40 CFU/g). The minimum incidence observed was in *Chanoschanos*(3 to 6 CFU/g).At station II, faecal streptococci counts ranged from 18 CFU/g (summer) to 40 CFU/g (monsoon). The maximum incidence was found in *Liza parsia*(20 to 40 CFU/g) and *the* minimum incidence observed was in *Chanoschanos* (18 to 25 CFU/g).

In shell fishes, the minimum and the maximum were observed during summer and monsoon seasons respectively at both stations. At station I,in fresh *Penaeusindicus*samples,faecal streptococci varied from 3 CFU/g (summer) to 12 CFU/g (monsoon)and in *Scylla serrata* , it fluctuated from 3CFU/g to 14 CFU/g and in the bivalve,*Meretrixmeretrix* (4 to 20 CFU/g). At station II,in fresh *Penaeusindicus* samples, thefaecal streptococci density varied from 3 CFU/g (summer) to 15 CFU/g(monsoon).and in *Scylla serrata* ,the counts fluctuated from 4 CFU/g (summer) to 16 CFU/g(monsoon) .In bivalve,*Meretrixmeretrix* ,faecal streptococci density varied from 5 CFU/g to 25 CFU/g .In both the stations,the specimens of fin and shell fishes ,higher incidence of faecal streptococci were recorded during monsoon season and lower incidence during summer season .

Distribution of faecal streptococci in marketed fin and shellfishes

Among the seafood samples examined at stationI (Table-II), the incidence of faecal streptococci in marketed samples were 49.1% in bivalves, 44.4% in crab, 37.7% in prawn and 34.7% in finfish samples. The high percentage of faecal streptococci was noticed in *Epinephalustauvina* (34.6%), followed by both*Plotosuscanius* and *Teraponputa* (32.6%) and *Kathelaaxillaris*(31.4%). ,*Meretrixmeritrix* showed the incidence of 49.1% followed by *Scylla serrata* (44.4%) and *Penaeusindicus* (37.7%). The order of maximum incidence was observed in the bivalve followed by crabs, prawn and finfishes.

At station II(Table-IV), among the seafood samples examined, the incidence of faecal streptococci in marketed samples was55.8% in bivalve,50% in crab,43.2% in finfish samples and 42.5% in prawn sample. The marketed finfishes, *Epinephalustauvina* showed higher percentage (43.2%) of faecal streptococci followed by *Arius maculates* (42.5%), *Chanoschanos* (42.2%) and *Siganusjavus* (39.6%). The shellfish, *Meretrixmeretrix* showed the incidence of 55.8% followed by *Scylla serrata* (50%) and *Penaeusindicus* (42.5%). The maximum incidence was observed in the bivalve followed by crabs, finfish and prawn.

At station I, the season wise incidence of faecal streptococci revealed the higher percentage of incidence during monsoon and premonsoon seasons in marketed samples. In finfish samples, faecal streptococci counts ranged from 3.14 x 10^2 CFU/g (summer) to 52.3 x10^2 CFU/g (monsoon). The maximum incidence was found in *Liza parsia*(48.2 to 52.3 x 10^2 CFU/g) and the minimum in *Siganusjavus* (3.14 to 8.3 x 10^2 CFU/g).

The faecal streptococci counts in the shellfish samples of *Penaeusindicus* varied from 5 x 10^2 CFU/g(summer) to 15 x 10^2 CFU/g(monsoon) and in *Scylla serrata* from 6 to 16.5 x 10^2 CFU/g)and in bivalve, *Meritrixmeretrix* from 9 to 17 x 10^2 CFU/g. The minimum and the maximum were observed during summer and monsoon seasons respectively.

At station II, the season-wise incidence of faecal streptococci and the level revealed the higher percentage of incidence during monsoon and postmonsoon seasons in marketed samples. In the finfish samples, faecal streptococci density ranged between 32.7 x 10^2 CFU/g (summer) and 169 x 10^2 CFU/g (monsoon). The maximum incidence was found in *Liza parsia*(132 to 168.8 x 10^2 CFU/g) and the minimum in *Epinephalustauvina* (32.7 to 56 x 10^2 CFU/g).

In the marketed shellfish samples of *Penaeusindicus*, the density of faecal streptococci varied from 60 x 10^2 CFU/g (summer) to 162 x 10^2 CFU/g (monsoon), in *Scylla serrata*from 75 to 180 x 10^2 CFU/g and in bivalve, *Meretrixmeretrix*from 110 to 176 x 10^2 CFU/g. The minimum and the maximum densities were observed during summer and monsoon seasons respectively.

Among the two stations, the Ennore estuary is the most polluted one as indicated by the highest counts of faecal streptococci in water (19 CFU/ml), sediment (11 x 10^2 CFU/g), fresh (39 CFU/g) and marketed fin fish (168 x 10^2) and fresh (25 CFU/g) and marketed shellfish (176 x 10^2). The presence of faecal streptococci was observed throughout the year and the counts were relatively higher during monsoon season. However, the streptococci were present in low numbers in water during summer (5 CFU/ml) and post monsoon (6 CFU/ml) seasons at Station I and summer (10 CFU/ml) and premonsoon (14 CFU/ml) seasons at Station II. In both the stations, the low incidence of faecal streptococci during summer, pre monsoon and post monsoon seasons may be due to prevalence of higher salinity during these seasons.

In the present study, the faecal streptococci were found at maximum during monsoon season probably due to the land drainage, low temperature and low salinity. Previous studies have demonstrated that the incidence of faecal streptococci increased with decreasing water temperature and salinity (Evison and James, 1975). From their observation with salinity and temperature tolerance, it may be concluded that faecal streptococci grew and survive better than faecal coliforms at low temperature and salinity.

The minimum density of faecal streptococci was recorded during summer season at both the stations. This might be due to the higher temperature (Station I- 33.5^{o}C; Station II- 34^{o}C) and salinity (Station I-32‰; Station II-33‰). Similar observations were made and reported by Hanes and Fragala (1976),Lessard and Sieburth (1983) and Valdes-Collazo et al. (1987). They have suggested that faecalstreptococci are incapable of surviving in seawater with higher salinity and temperature. High temperature and salinity environments are stressful to enteric bacteria and some pathogens.

Compared to Station I, maximum faecal streptococci was recordedat Station II, this may be due to the discharge of sewage which led to higher organic carbon and nutrient availability at this station.The absence of these organisms in food is generally regarded as an indication of good sanitation and proper handling conditions (Anon, 1980). Their presence in high numbers in shellfish indicates persistence of sewage contamination or insanitory handling practices (Ayres et al., 1978).

When compared with fin fishes, the shell fish collected from the both Stations showed the maximum faecal streptococci population. This might be due to the association of molluscan organism with sediment filter feeding habits. Sediment as the reservoirs of enteric microorganisms are significant sources of contaminant for shellfish and recreational waters.

In all the three categories, molluscs, crustaceans and finfishes of fresh and marketed seafood, the incidence of faecalstreptococci was high in molluscs in both the stations. Their versatile and effective water filtering gill lamellae serve as a good concentrator and reservoir of faecal streptococcus. From the present findings it is evident that *Meretrixmeretrix*(mollusc) harbor as much as 180 x 10^2 CFU/gfaecal streptococci followed by *Scylla serrata* (176 x10^2 CFU/g) and *Penaeusindicus* (162 x 10^2 CFU/g), clearly indicating that these organisms serve as reservoir for faecal streptococci.

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Table 1

Seasonal variation in distribution of faecal streptococci in fresh fin and shellfish samples collected from Station I

Name of the species	Summer			Premonsoon			Monsoon			Post monsoon			Total	% Positive
	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecals treptococci	% Positive		
Gerresfilamentosus	25	4	16	20	4	20	19	3	15.8	21	5	23.8	16	18.8
Plotosuscanius	15	3	20	20	5	25	21	4	19.1	18	3	16.7	15	20.3
Epinephalustauvina	18	2	11.1	10	2	20	15	3	20	19	3	15.8	10	16.1
Ciganusjavus	22	3	13.6	15	2	13.3	18	4	22.2	22	3	13.6	12	15.6
Arius maculatus	21	3	14.3	16	3	18.8	19	4	21.1	18	2	11.1	12	16.2
Chanoschanos	15	1	6.66	17	2	11.8	18	2	11	15	1	6.67	6	9.2
Liza parsia	18	3	16.6	20	4	20	21	4	19	16	3	18.8	14	18.7
Cynoglossusarel	10	1	10	11	2	18.2	10	2	20	8	1	12.5	6	15.4
Kathelaaxillaris	15	2	13.3	12	2	16.6	13	3	23.1	11	1	9.09	8	15.7
Theraponputa	12	2	16.6	11	3	27.3	20	3	15	17	2	11.8	10	16.7
Penaeusindicus	30	6	20	25	6	24	26	7	26.9	32	6	18.7	22	19.3
Syllaserrata	15	3	20	20	4	20	19	4	21	11	3	27.3	15	23
Meretrixmeretrix	25	6	24	25	8	32	21	7	33.3	15	6	40	26	30.2

Table 2

Seasonal variation in distribution of faecal streptococci in marketed fin and shellfish samples collected from Station I

Name of the species	Summer			Premonsoon			Monsoon			Post monsoon			Total	% Positive
	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive		
Gerresfilamentosus	15	3	20	12	4	33.3	20	4	20	13	3	23.7	14	23.3
Plotosuscanius	12	3	25	13	4	30.8	10	5	50	11	3	27.4	15	32.6
Epinephalustauvina	10	2	20	12	3	25	14	9	64.3	13	3	23.1	17	34.7
Ciganusjavus	14	1	7.14	13	2	15.4	12	7	58.3	11	2	18.2	12	24
Arius maculatus	13	2	15.4	15	3	20	17	9	52.9	19	4	21.1	18	28.1
Chanoschanos	18	2	11.1	20	3	15	15	6	40	14	3	21.4	14	20.9
Liza parsia	21	4	19	14	5	35.7	12	6	50	13	3	23.1	18	30
Cynoglossusarel	11	2	18.2	13	2	15.4	15	9	60	12	1	8.33	14	27.5
Kathelaaxillaris	15	5	33.3	13	3	23.1	12	6	50	11	2	18.2	16	31.4
Theraponputa	14	5	35.7	12	3	25	11	5	45.5	9	2	22.2	15	32.6
Penaeusindicus	20	6	30	25	7	28	30	16	53.3	15	5	33.3	34	37.7
Syllaserrata	10	4	40	12	5	41.7	13	7	53.8	10	4	40	20	44.4
Meretrixmeretrix	13	5	38.5	12	7	58.3	14	9	64.3	16	7	43.8	50.9	49.1

Table 3

Seasonal variation in distribution of faecal streptococci in fresh fin and shellfish samples collected from Station II

Name of the species	Summer			Premonsoon			Monsoon			Post monsoon			Total	% Positive
	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecals treptococci	% Positive		
Gerresfilamentosus	30	5	16.7	25	6	24	27	7	25.9	23	6	26	24	30
Plotosuscanius	23	5	21.7	13	4	30.8	15	5	33.3	21	4	19	18	25
Epinephalustauvina	15	4	26.7	18	5	27.8	17	5	29.4	15	5	33.3	19	29.2
Ciganusjavus	12	3	25	10	2	20	12	3	25	11	3	27.3	11	24.4
Arius maculatus	15	4	26.7	18	5	27.8	20	6	30	22	5	22.7	20	26.7
Chanoschanos	12	3	25	11	4	36.4	13	3	23	15	3	20	12	23.5
Liza parsia	13	4	30.8	20	5	25	22	6	27.2	21	5	23.8	20	26.3
Cynoglossusarel	18	4	22.2	17	5	29.4	15	3	20	15	5	33.3	17	26.2
Kathelaaxillaris	21	6	28.6	20	5	26.5	23	7	30.4	22	5	22.7	23	26.7
Theraponputa	17	5	29.4	18	5	27.7	20	6	30	17	5	29.4	21	29.2
Penaeusindicus	25	5	20	28	7	25	30	8	29.7	32	9	28.1	29	25.2
Syllaserrata	16	6	37.5	15	4	26.7	15	5	33.3	10	3	30	24	42.9
Meretrixmeretrix	12	7	58.3	11	4	36.4	12	6	50	15	6	40	23	46

Table 4

Seasonal variation in distribution of faecal streptococci in marketed fin and shellfish samples collected from Station II

Name of the species	Summer			Premonsoon			Monsoon			Post monsoon			Total	% Positive
	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive	No. of fishes sampled	No. of faecal streptococci	% Positive		
Gerresfilamentosus	20	6	30	19	6	31.6	18	10	55.6	17	6	35.3	28	33.8
Plotosuscanius	15	5	33.3	13	5	38.5	12	6	50	15	5	33.3	21	38.2
Epinephalustauvina	13	5	38.4	11	4	36.4	9	5	55.5	11	5	45.5	19	40.9
Ciganusjavus	10	3	30	8	3	37.5	11	6	54.5	12	4	33.3	16	34.1
Arius maculatus	13	5	38.5	12	5	41.7	12	6	50	10	4	40	20	43.2
Chanoschanos	12	5	41.7	11	5	45.5	12	5	41.7	10	4	40	19	42.2
Liza parsia	13	5	38.5	10	4	40	11	5	45.5	14	4	35.7	18	25.4
Cynoglossusarel	14	3	21.4	10	3	30	9	4	44.4	8	2	25	12	24.4
Kathelaaxillaris	13	3	23.1	11	3	27.3	12	5	41.7	13	4	30.88	13	26.5
Theraponputa	12	3	25	9	3	33.3	8	4	50	7	2	28.5	12	30.5
Penaeusindicus	15	5	33.3	14	6	42.9	12	6	50	13	6	46.2	23	42.5
Syllaserrata	9	5	55.6	11	5	45.5	10	6	60	12	5	41.6	21	50
Meretrixmeretrix	12	5	41.7	13	7	53.8	15	10	66.7	12	7	58.3	29	55.8