On Occurrence of Certain Biotoxins along the Kerala Coast

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Occurrence of algal blooms has been reported from the coastal regions of Kerala. A study was conducted to collect detailed information on location and seasonality of the incidence of PSP and DSP toxins in the bivalves of this region for providing advance warning to avoid shellfish poisoning. A database on the hydrographic parameters in relation to algal blooms and toxic blooms at selected stations, which are sites of frequent blooms in the past years, was prepared. Data on the presence of biotoxins in mussels and water samples periodically collected from Moodadi, Tikkodi, Pallikandy, Elathur, Thalassery, Fort Cochin and Vizhinjam, which are the main mussel landing centers of the state, are reported. Apart from this, the occurrence of PSP and DSP in edible oyster, *Crassostrea madrasensis* from Central Marine Fisheries Research Institute's farm in Ashtamudi lake and the black clam *Villorita cyprinoides* from Vembanad lake, monitored regularly, are also discussed. In August 2000, the bloom of *Gymnodinium pulchellum* in Fort Cochin region was found to contain paralytic shellfish poison. Six other blooms were observed in Calicut, Chombala, Narackal, Vizhinjam, and Thankassery during the period July-September 2001. The causative species were identified as *Noctiluca scintillans, Heteroaulacus* spp. and *Prorocentrum micans* all of which were non-toxic. The study revealed that the incidence of PSP producing algal blooms are low along the Kerala coast and level of toxin is well below ($<21\ \mu g.100g^{-1}$) the toxic limit of $80\ \mu g.100g^{-1}$ of shellfish meat. In the light of the hydrographic data, the causative algal species and the occurrence of PSP and DSP toxin in the environment and bivalve meat, the safety of molluscan fishes of Kerala coast is discussed.

Key words: Biotoxins, algal bloom, bivalves, Kerala

India, being a major exporter of seafood products has to assure the quality and safety of her products. The incidence of marine biotoxins in seafood has become a threat to consumers. The two major biotoxins that cause threat to Human health are paralytic shellfish toxin (PSP) and diarrhetic shellfish toxin (DSP). Paralytic shellfish toxins are a group of neurotoxin produced mainly by dinoflagellates belonging to genus *Alexandrium, Pyrodinium,* and *Protogonyaulax.* These dinoflagellates occur both in the tropical and moderate climate zones (Hall, 1982; Krogh, 1988; van Egmond *et al.*, 1993). Shellfish grazing on these algae accumulate the toxins. The maximum permitted level of paralytic shellfish toxin in Bivalve Mollusks is $80\ \mu g.100g^{-1}$edible tissue (Council of the European Communities, 1991).

Kerala state along the west coast of India is the major producer of bivalves. Bivalves especially mussels (*Perna indica* and *Perna virides*) and clams (*Villorita cyprinoides, Paphia malabarica,* and *Meritrix casta*) are fished and marketed locally. Considering the importance of bivalves it is necessary to study the living environment of the bivalves and also the possibility of algal blooms, which may cause toxins in the bivalves. Hence it is necessary to study the existence of toxin producing algal bloom and the amount of toxin, which may be present in the bivalves growing in that environment. The present study has been taken up with this view.

Materials and Methods

Three sampling sites along the Kerala coast namely Thalassery, Fort Cochin and Vizhinjam, which had the incidence of algal blooms in previous years were selected and studied from April 2001 to September 2001. Mussel and water samples, periodically collected from these centers were analysed for PSP.

The hydrographic variation and phytoplankton composition of the coastal waters at Thalasherry, Cochin and Vizhinjam were monitored regularly. The chemical characteristics of the surface water collected were analyzed for dissolved orthophosphate (Murphy & Riley, 1962), nitrate and nitrite (Morris & Riley, 1963) and chlorophyll pigments by Spectrophotometric (Parsons *et al.*, 1984). Dissolved oxygen was measured by Winkler method (1888), and salinity using salinometer (ATAGO – S/Mill-E, Japan). Total suspended solids (TSS), total Organic Carbon (TOC), surfactants (SURF), biological oxygen demand (BOD) and chemical oxygen demand (COD) were measured by Pastel UV Spectrophotometer. Qualitative assessment of the phytoplankton at these sites was done by collecting the plankton and identifying them to the species level (Subramanyan, 1971). The occurrence of the blooms and the causative species were also identified.

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The green mussel, *Perna viridis* collected from north and central Kerala and brown mussel, *Perna indica* from the natural bed of Vizhinjam were transported to the lab in the live condition and refrigerated. The samples were later analyzed for PSP by mouse assay (AOAC, 1990). The samples were collected monthly and during bloom period samples were collected daily.

Results and Discussion

Algal blooms:

Table 1 shows the date, location and algal species notices during the bloom. The blooms were noted mainly in the post monsoon period. *Noctiluca scintillans*, *Noctiluca milaris*, *Heteroaulacus* sp. and *Prorocentrum micans* have all been responsible for harmful algal blooms. *Prorocentrum micans* has been reported to cause toxic bloom in Northern Britany (Lassus & Berthome, 1988) and in Portugal (Pinto & Silva, 1956). However in Kerala the bloom was mild and did not cause any toxic condition. In the regular monthly observations on the phytoplankton composition along north Kerala, toxic algae in substantial quantities were not observed. *Leptocylindrus dandius, Astrionella japonica, Thallassiothrix fraunfeldii, Cosinodiscus* sp., *Rhizosolenia* sp., *Thallassinema nitzchoides* are the major species found in this area.

Table 1. Date, location and algal species identified during algal bloom

Date	Location	Algal species	Intensity
25-07-2001	Calicut	*Noctiluca scintillans*	–
04-08-2001	Chombala	*Noctiluca scintillans*	Golden yellow colored bloom
09-08-2001	Fort Kochi	*Heteroaulacus sps*	Very dense bloom. Sea was brick red colored in the morning, intensities to a dark brown color by afternoon. By next afternoon coloration had disappeared. Fish mortality was reported.
26-08-2001	Vizhinjam	*Noctiluca milaris*	–
17-08-2001	Thankassery	*Prorocentrum micans*	Red patchy discoloration in the harbour area
11-09-2001	Chombala	*Prorocentrum micans*	Red discoloration as narrow streak. Fish mortality reported.

Table 2 shows the seasonal changes in the hydrographic parameters in Fort Kochi region. The bloom of *Heteroaulacus* sp. in Narackal reported of fish mortality. This might be due to clogging of algae to the gills. Along Vizhinjam, *Noctiluca milaris* bloomed in August 2001. However, in this region harmful algae were observed though they did not cause any water discolorations or mortality. The plankton blooms formed by the dinoflagellate *Noctiluca miliaris* have been implicated in mortality (Subramanian, 1985).

The gross productivity, which was 2.13 $mgC.l^{-1}.day^{-1}$ prior to the bloom, increased to 5.97 $mgC.l^{-1}.day^{-1}$ during the bloom and further rose to 10.11 $mgC.l^{-1}.day^{-1}$ after the *Noctiluca scintillans* bloom at Chombala. The total quantity of phytoplankton, namely gross productivity was increased, clearly indicating the algal growth. The net productivity (i.e. Gross productivity – Respiration) shows a slight increase. Similar trend was observed for Chlorophyll a, c and BOD. A recent investigation of remote sensing of harmful algal blooms shows high near-shore chlorophyll a in the bloom region (Yin *et al.*, 1999).

Table 2. Seasonal changes of hydrographic parameters at Fort Kochi

Parameters		April	May	June	Aug.*	Aug.**	Sept.
Productivity ($mgC.l^{-1}.day^{-1}$)	Gross	0.853	1.9395	1.7066	6.542	3.491	1.1308
	Net	0.627	0.8467	0.8524	0.9344	0.873	0.9899
Chlorophyll ($\mu g.l^{-1}$)	A	1.248	1.346	1.873	78.01	44.05	–
	B	0.645	0.327	1.055	0.00	1.047	–
	C	0.320	0.785	1.629	23.21	26.48	–
Nutrients ($\mu g.l^{-1}$)	NH_3	3.45	0.19	0.00	23.28	6.59	0.00
	PO_4	1.59	1.36	0.86	4.71	3.84	0.76
	NO_3	1.17	2.90	0.19	4.19	2.38	0.01
	NO_2	6.90	0.48	0.06	0.26	0.42	0.08
Temperature (°C)		29	28	28.2	28	28	28.3
Salinity (%)		33	34	27	33	33	32
pH		8.02	8.21	8.10	9.0	8.21	8.14
Dissolved oxygen (mg O_2 l^{-1})		4.46	3.24	5.27	5.85	4.07	4.76
Total suspended solids (ppm)		6.46	4.2	6.6	60.5	46.5	12.5
Total organic carbon (ppm)		1.8	1.4	1.6	13.2	7.2	1.6
Surfactants (ppm)		0.4	0.3	0.4	0.4	0.3	0.2
Chemical oxygen demand (ppm)		5.4	4.8	5.3	43	23.6	5.6
Biological oxygen demand (ppm)		2.4	1.4	2.2	15.6	10.6	2.1

* Represents the hydrographic parameters recorded during the bloom of *Heteroaulacus* sp. in Narackal on 9-8-2001;

** Represents the hydrographic parameters recorded during the bloom of *Heteroaulacus* sp. in Narackal on 10-8-2001;

No collection in July since the sea was rough

Table 3. Seasonal changes of hydrographic parameters at Thalassery

Parameters		April	May	June	July	Aug*	Sept**
Productivity	Gross	1.4547	1.4547	2.1337	5.974	10.114	5.67
($mgC.l^{-1}.day^{-1}$)	Net	0.4849	0.9698	1.2802	0.4267	5.2362	3.927
Chlorophyll	A	2.622	5.8036	0.3206	6.0806	10.287	5.741
($\mu g.l^{-1}$)	B	2.58	0.6221	0.0	0.928	0.9662	0.9427
	C	2.4	1.8732	0.0226	0.7658	1.764	2.2871
	NH_3	177.51	1.45	4.19	9.38	9.32	0.34
Nutrients	PO_4	13.39	1.73	1.00	2.37	2.37	2.88
($\mu g.l^{-1}$)	NO_3	0.12	1.27	0.36	2.79	0.26	4.16
	NO_2	3.77	9.37	10.65	11.87	6.97	0.04
Temperature (°C)		31.5	29	27	28	27	29
Salinity (%)		30	34	31	33	35	35
PH		7.4	8.21	8.17	7.78	7.78	8.32
Dissolved oxygen ($mg\ O_2.l^{-1}$)		9.62	5.37	5.722	7.713	7.7128	5.3424
Total suspended solids (ppm)		15.6	2.4	10.1	38.5	38.5	10.2
Total organic carbon (ppm)		13.1	0.9	10.1	38.5	2.2	2.1
Surfactants (ppm)		5.0	0.4	0.3	0.4	0.4	0.4
Chemical oxygen demand (ppm)		35.0	0.3	1.2	7.6	7.6	3.1
Biological oxygen demand (ppm)		13.2	0.4	0.3	3.1	3.1	2.8

* Represents hydrographic parameters recorded during the bloom of *Noctiluca scintillans* in Chombala on 4-8-2001.

** Represents hydrographic parameters recorded during the bloom of *Prorocentrum micans* in Chombala on 11-9-2001.

Table 4. Seasonal changes of hydrographic parameters at Vizhinjam

Parameters		April	May	June	July	August*
Productivity	Gross	1.9395	0.8534	1.1454	1.9382	3.4908
($mgC.l^{-1}.day^{-1}$)	Net	0.9698	0.4267	0.8268	0.9691	1.7454
Chlorophyll	A	0.274	1.9354	0.723	0.856	4.6296
($\mu g.l^{-1}$)	B	1.8808	0.0	0.682	0.483	0.4253
	C	2.944	3.5638	0.939	1.213	1.3663
	NH_3	0.00	0.00	0.00	0.12	3.33
Nutrients	PO_4	0.54	0.73	0.82	0.90	1.82
($\mu g.l^{-1}$)	NO_3	0.10	0.04	9.03	8.43	22.87
	NO_2	1.11	4.73	0.28	0.46	0.76
Temperature (°C)		30	29	27	28.5	29
Salinity (%)		33	34	26	28	35
PH		8.21	7.56	8.02	8.13	8.17
Dissolved oxygen ($mg\ O_2.l^{-1}$)		4.523	3.234	4.32	4.64	4.8336
Total suspended solids (ppm)		2.4	8.0	4.2	6.4	46.6
Total organic carbon (ppm)		0.9	2.5	2.5	0.9	7.2
Surfactants (ppm)		0.4	0.3	0.3	0.4	0.4
Chemical oxygen demand (ppm)		0.4	8.2	0.3	4.3	3.2
Biological oxygen demand (ppm)		0.4	3.7	0.3	2.4	0.5

* Represents the hydrographic parameters recorded during the bloom of *Prorocentrum micans* in Thankassery on 17-8-2001.

Toxicity appears to be related to the high concentration of the algal cells, a water temperature of 70° to 75°F, high pH, and length of exposure to sunlight (Jurgens, 1953; Muncy, 1963). Temperature recorded during bloom was 28-29°C. In Hong Kong, red tides higher incidence of red tides was recorded when the sea temperature was around 20-23°C (Chan & Liu, 1991).

Salinity and pH showed slight increase characteristic of the species. The dissolved oxygen, nutrients have found to be increased. A 10-fold increase in mean dissolved phosphate levels and 5-fold increase in mean dissolved nitrate levels resulted in a very large increase in phytoplankton, and increase in red tide blooms (Chan & Hodgkiss, 1987; Hodgkiss & Chan, 1983; 1986; 1987). TSS and COD values, which were low before the bloom increased at the time of bloom and decreased after the bloom. At Narakkal, similar trend was observed for these parameters and also for Gross productivity, Chlorophyll a, c, phosphate content, TOC and BOD at *Heteroaulacus* sp. bloomsite. Contrary to this, during the bloom of *Prorocentrum micans* at Chombala, the gross and net productivities, nitrite content and BOD showed a decreasing trend from the pre bloom period. This might be due to varied nutrient requirement of different algal species responsible for bloom.

The mussel samples analysed for the presence of paralytic shellfish toxins by mouse bioassay indicated no detectable toxins. However fish kills had been reported in Narackal. This could be due to the clogging of algae to the gills.

Paralytic shellfish poisoning is a worldwide problem. Phytoplankton blooms occur at the eutrophic zones. Prior to blooms, the nutrient levels will rise. The blooms, however, did not cause any danger to humans. More work is to be done in these areas to provide warning to shellfish farmers and consumers about period of incidence of blooms and effect of toxicity.

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288 Vazhavara
289 Venkalpara
418 Vairamani
292 Yellapetty

Muvattupuzha River Basin

182 Arookutty
193 Cochin
448 Karikode
419 Kulamavu
356 Malankara
358 Maniyaramkudy
299 Muvattupuzha
300 Piravam
301 Shertalai
302 Thodupuzha
303 Vaikom
304 Vellore
417 Vengannam
290 Vyttila
291 Wellington Island OBS

Meenachil River Basin

362 Erattupetta
305 Kottayam B & R
440 Kottayam OBS
306 Kottayam R.R.I.
307 Kozha
308 Kumarakam
309 Palai
310 Thanneermukkam

Manimala River Basin

311 Changanacherry
451 Kanjirapalli
312 Mundakkayam
352 Mundakkayam E.
313 Thiruvalla

Pamba River Basin

314 Alleppey
434 Alleppey P.W.D.
435 Ambalapuzha
315 Anathodu
316 Ankamoozhi
436 Chengannur
365 Mankomb
317 Maniyar
318 Moozhiyar
319 Pamba
456 Perunad
320 Perumthenaruvi
321 Seethathodu
322 Triveni
323 Wellathumoozhi

Achencoil River Basin

324 Harippad
457 Kaipattoor
325 Kayamkulam
326 Kayamkulam C.P.C.R.I.
327 Kayamkulam K.R.S.
328 Kayamkulam R.R.L.
357 Kayamkulam S.T.
329 Konni D.F.O.
330 Konni E.
331 Konni S.C.R.S.
332 Mavelikkara
333 Pathanamthitta

Pallikkal River Basin

334 Adoor
336 Karunagappalli

Kallada River Basin

335 Ariancavu
337 Kottarakkara
338 Punalur
364 Punalur K.I.P.
339 Quilon
340 Rajgiri
451 Thenmala

Ithikkara River Basin

432 Nilamal
433 Paravur

Ayroor River Basin

344 Varkala

Vamanapuram River Basin

341 Attingal
459 Baremore E.
460 Palode
342 Ponmudi
343 Vamanapuram

Karamana River Basin

445 Kovalam
345 Nedumangad
461 Thumba
346 Trivandrum Aerodrome
347 Trivandrum OBS
348 Vellayani
446 Vizhinjam

Neyyar River Basin

349 Neyyar Dam
380 Neyyattinkara
350 Parassala

Kabbini River Basin

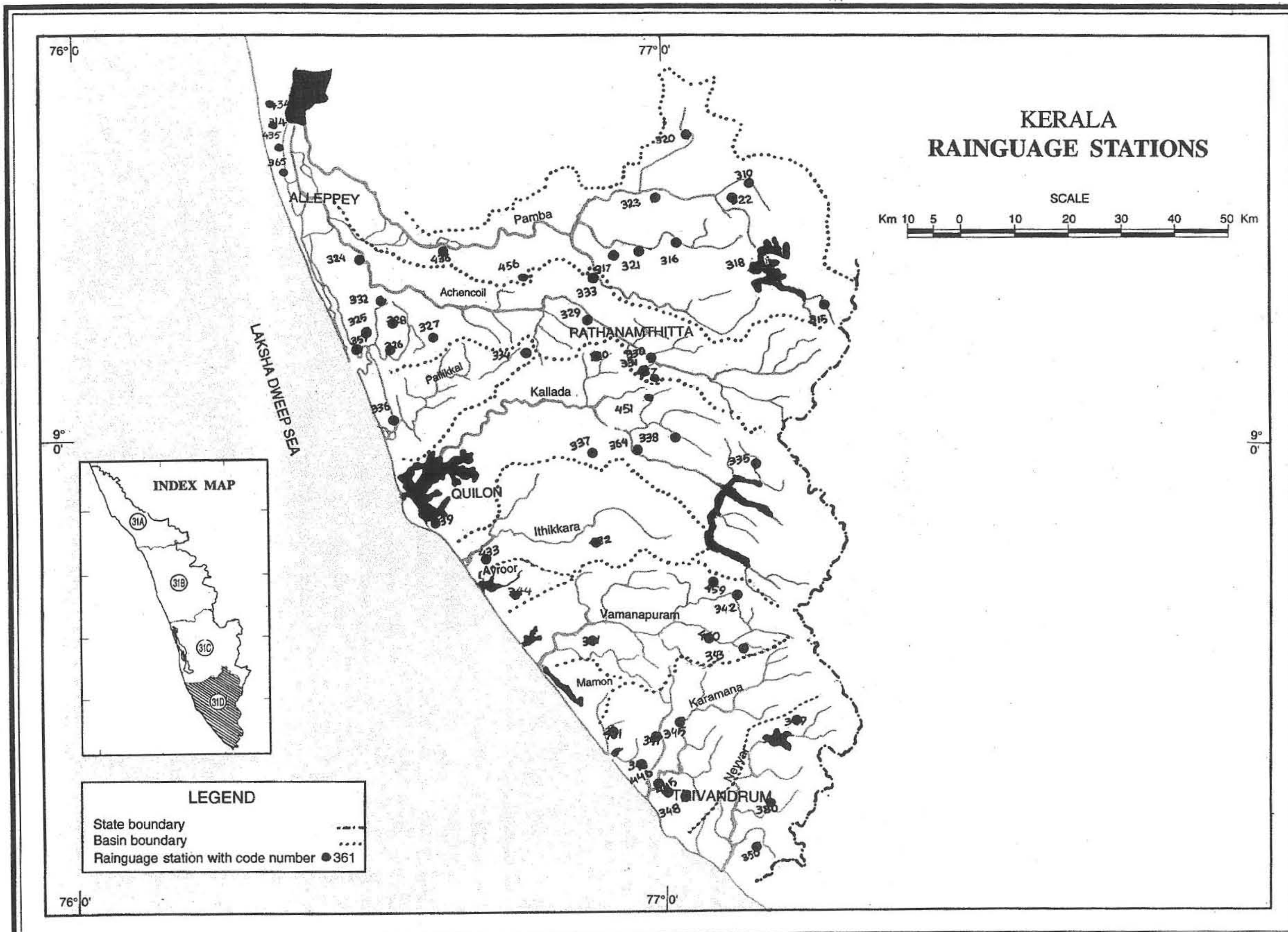
462 Achoor
22 Ashiyana
23 Chedaleth
24 Kannoth
25 Koroth
27 Kuppadi
26 Kottiyur
28 Lakkidi
29 Makkiyad
30 Mananthavady T.O.
368 Mananthavady
31 Mukki
32 Muthange
428 Nazeema E.
33 Peria
34 Thalapuzha
35 Thariyode
36 Thariyode K.S.E.B.
37 Thattamala
38 Valat
371 Vattapoyal
39 Vythiri

Bhavani River Basin

80 Mukkali
81 Panthanthodu
82 Pudur
83 Thathengalam
84 Thekkuvatta
85 Thudukki

Pambar River Basin

293 Kanthallor
294 Koilkadavu
295 Venguravai
296 Marayur
297 Sothparai E.
298 Thalayoor



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The territorial waters of India extend into the sea to a distance of twelve nautical miles measured from the appropriate base line.