Tropical Biomedicine 23(2): 164–171 (2006)

Microalgae associated with *Leptoconops* breeding sites in selected sandy beaches of Malaysia

Rohani A., Khadri M.S. and Lee H.L.
Medical Entomology Unit, Infectious Disease Research Centre, Institute for Medical Research, 50588 Kuala Lumpur, Malaysia.
Email: rohania@imr.gov.my
Received 8 May 2006; received in revised form 25 September 2006; accepted 25 September 2006.

Abstract. A study was carried out to investigate correlation between presence of specific microalgal species and *Leptoconops* biting midge larvae in its breeding habitats. Sand samples containing microalgae were collected from the beach where the midges were most commonly biting and from sand beaches which are potential as breeding habitats of *Leptoconops*. The survey covered sand beaches from 12 seperate islands. At all sites, the Bacillariophyta constituted the largest representatives of the microalgae community with the majority from the Naviculaceae family. A total of 24 microalgal species were identified from the sand samples collected from the study sites. Sand samples from Kentot Kecil Island had the highest number of algal species (11.0) and the highest algae species diversity (Shanon-Weiner Diversity Index, H' = 0.884). Besar Island (Johor) had the lowest number of algal species (2.0) whereas Tengol A Island had the lowest algae species diversity (H'=0.234). Highest similarity index was recorded between sand samples collected from Tengol A Island and Tengol B Island (75.0%) followed by Besar Island (Melaka) and Tengol B Island (62.0 %). The variation between other islands were relatively high. Virtually many kinds of algae were found where *Leptoconops* were breeding but *Fragilaria intermedia*, *Mastigloia minuta* and *Navicula advena* were particularly common.

INTRODUCTION

Biting midges are common nuisance insects which bite throughout the day and breed in beach sand located above the high tide level near creeping vegetation (Aussel, 1993a). It is an unbearable pest, its bite often producing lesions that degenerate into infected wounds, sometimes accompanied by oedema and lymphademopathy which could remain intensely itching for months. Besides creating this serious public health problem, a number of tropical species have been known to prevent the development of tourism associated with beaches and is considered an important detriment to economic development (Strickman *et al.*, 1995). In Malaysia, biting activities of *Leptoconops spinosifrons* were reported in sandy beaches of Pulau Aur and Pulau Besar in Johor (Khadri *et al.*, 2002). In certain months of the year, these biting midges can be very abundant and disturb outdoor activities.

The breeding area of *Leptoconops* was found to be the storm beach shelf, often sloping to the landward side, between high tide level and the landward edge of the beach. The breeding area included parts of the beach invaded by beach morning glory and the associated grasses (Brenner & Wargo, 1984) but also included open sand, shaded and unshaded and sand covered by seaweed and other sea drift (Laurence & Mathias, 1972). The immatures were restricted to a narrow strip and most occurred in the upper 6 cm of sand. Within a given breeding site, larvae were aggregated in their distribution. Eggs were laid on the bare sand surface, from which the larvae apparently dispersed to the plant-covered sand and to deeper strata, before returning to the surface for pupation and emergence

(Aussel, 1993b). The immature stage feeds on algae, fungi and bacteria. Small fragments of organic materials including recognizable fragments of mycelium, amorphous and diatoms were found in the gut of *Leptoconops* larvae (Laurence & Mathias, 1972).

The objective of this study was to screen the presence of specific microalgal species living in Leptoconine habitat compared to microalgal species living in non-Leptoconine habitat.

MATERIALS AND METHODS

Collection and identification of microalgal samples

Study areas, sampling methods and procedure for isolation and identification of the immature stages have been reported in a previous paper (Khadri *et al.*, 2002). Sand samples containing microalgae were collected from the beach where the midges were most commonly biting and from sand beaches, which are potential breeding habitats of *Leptoconops*. At each study area, 3 sites of the beach were selected randomly at 200 to 500 m intervals and a single 6-12 cm-deep layer was sampled at each site. Forty g of the sand samples were mixed with 100 ml sterile seawater. The mixture was sieved (mesh size-1mm) to get rid of the sand. The water samples (20 ml) were transferred into 50 ml sample bottle and preserved using 4% formalin. The microalgae present in the water samples were identified using several taxonomic keys (Whitford & Schumacher, 1973; Wah & Wee, 1988; Lokman 1990; Wah *et al.*, 1992; Tomas, 1997). A Double-Neubauer haemocytometer counting chamber was used for microalgal identification and counting under standard light microscope at the magnification of 400X.

Data analysis for biological parameters

Cell count of the microalgae was conducted and the results processed into three parameters, namely cell density, species richness and the Shannon-Weiner Diversity Index. Cell density was expressed as number of cells per mL and was calculated by totaling the cell density for all species in each sample. The Shannon-Weiner Diversity Index, H' was calculated using the formula:

$$H' = -\sum_{i=1}^{n} pi \ln pi$$

where
pi = proportion of species in the samples
n = number of species in the sample

Microalgal cell count

The Double-Neubauer haemocytometer chamber, a counting cell that limits the volume and area for ready calculation of population densities, was used. The haemocytometer chamber was approximately 50 mm long by 20 mm wide by 1 mm deep and has grid lines marked on the glass slide. The total area of the bottom was approximately $1000mm^2$ and the total volume approximately $1000mm^3$ or 1 mL.

Microalgal samples were appropriately diluted and homogenized for cell count. Before filling the haemocytometer counting chamber, cover glass was placed diagonally across the cell and sample transferred with pipette. The number of cells per mL were multiplied or divided by a correction factor to adjust for sample dilution of concentration. The number of microalgae per mL were calculated as follows:

$$No./mL = \frac{C \times 1000\ mm^3}{A \times D \times F}$$

Where
C = number of organisms counted
A = area of a field, mm^3
D = depth of a fields (haemocytometer depth), mm
F = number of fields counted

Environmental parameters

Various environmental parameters were also recorded at the study sites using specific equipments. This includes salinity (ppt), dissolved oxygen (mg/l), pH, temperature (°C) and conductivity (µS/cm). The specific equipment was calibrated accordingly before use. Environmental parameters were determined by stirring each sand sample with 500 ml of sterile sea water. The specific meter probe was immersed in the fluid and parameters were measured. Although the environmental parameters were measured in the supernatant fluid and not in the sand, the results remain comparable, since the same operation was used for each sample.

RESULTS AND DISCUSSION

Biting activity of *Leptoconops*

Some islands are heavily infested with the Leptoconine biting midges while other islands with almost similar features are not infested. *Leptoconop*s and its biting activity were only positive in Besar Island (Johor), Aur Island (Johor) and Tenggol A Island (Terengganu) and Tenggol B Island (Terengganu) with average biting activity of 75, 7, 11and 8 bites/man/hour, respectively. Detail results were reported elsewhere. At all sites of Kentot Kecil Island (Kedah), Datai Island (Kedah), Teluk Belimbing (Kedah), Singa Island (Kedah), Tengkorak Beach (Kedah), Beras Basah Island (Kedah) Dayang Bunting Island (Kedah) and Besar Island (Melaka) none of the research workers experienced any bite of *Leptoconops* neither did they notice any landing on their body.

Environment parameters of study sites

Table 1 give results of conductivity, salinity, pH and temperature of the sand samples during the survey. Water temperature for the sandy beaches ranged between 25.5ºC – 35.2°C. The variations in the water temperatures were due to the different sampling times, usually lower temperature in the morning and higher temperature at noon. The overall range of pH varied from 7.19 to 8.05. Mean dissolved oxygen ranged between 4.5-14.0 mg/l. The conductivity of the sand samples ranged from 17.0 – 25.0 Ω. The salinity was fairly uniform for all the sandy beaches; lying within a arange of 17.9 – 35.9 ppt.

Larva distribution depends on physio-chemical parameters such as conductivity, particle size and sorting of substrate. Muddy substrates with high conductivity and coarse particle size are apparently suitable as breeding sites. Size and sorting of substrate do not directly influence the presence and distribution of *Leptoconops*, but, in most cases, they have a major effect on the distribution of food organisms (Aussel *et al.*, 1993b). Differences of humidity or temperature between morning and afternoon, or seasons, did not influence larval behaviour within a breeding place (Aussel *et al.*, 1993b), however, the available amount of water is an ecological factor influencing microathropod behaviour (Linley, 1969).

Table 1. Physico-chemical parameters of sand sample from various islands collected during the survey

Island	Mean pH	Mean Temperature (ºC)	Dissolved oxygen (mgL^{-1})	Salinity (ppt)	Conductivity ($mscm^{-1}$)
Kentot Kecil Island (PKK)	7.64	25.5	5.0-13.7	33.9	24.0
Datai Island	7.63	26.3	5.5-10.4	27.9	18.1
Teluk Belimbing	7.64	25.9	10.0-14.0	17.9	19.1
Besar Island (Melaka)	7.85	30.3	6.9-11.3	35.7	17.0
Singa Island	7.89	32.9	5.5-9.8	30.8	19.6
Tengkorak Beach	7.81	27.6	6.0-7.2	35.1	17.2
Beras Basah Island	8.04	32.9	5.9-7.1	33.1	25.0
Dayang Bunting Island (PDB)	8.02	25.5	5.2-6.0	30.5	17.6
Aur Island	7.19	31.9	8.6-11.4	33.7	17.9
Besar Island (Johor)	8.05	26.4	4.5-5.6	35.3	20.8
Tengol A Island	8.01	35.2	9.5-10.6	35.9	21.8
Tengol B Island	7.75	30.9	8.2-9.9	34.7	19.6

Correlation between the *Leptoconops* larval habitats and distribution of algae. The survey covered sand beaches from 12 seperate islands (Figure 1). Sand samples were taken from the beach close to where the midges most commonly biting or at the potential breeding sites. A checklist of the microalgae identified in the survey is summarised in Table 2. Table 2 also shows the mean values of species richness (number of species) and species diversity (Shannon-Weiner Index, H') for each sand beach. At all sites, the Bacillariophyta constituted the largest representatives of the microalgae community with the majority from the Naviculaceae family. A total of 24 species were identified from the sand samples collected from the study sites. The five most abundant species detected were *Diploneisis gravelleana*, *Mastigloia minuta*, and *Achnanthes longipes* from Telok Belimbing; *Cylcotella kutzingiana* from Kentut Kecil Island and *Fragilaria intermedia* from Datai Island.

Sand samples from Kentot Kecil Island had the highest number of algal species (11.0) and the highest algae species diversity (Shannon Diversity Index, H' = 0.884). A total of 11 species were found in the sand samples from this island. The three most abundant species detected were *C. kutzingiana*, *M. minuta* and *Coscinodiscus curvatulus*. Besar Island (Johor) had the lowest number of algal species (2.0) whereas Tengol A Island had the lowest algae species diversity (Shannon Diversity Index, H'=0.234). In

Note: Kentut Kecil Island, Datai Island, Teluk Belimbing, Singa Island, Tengkorak Island and Dayang Bunting Island are located surrounding or nearby Langkawi Island.

Figure 1. Map of Peninsular Malaysia to show locations where *Leptoconops* larvae and sand samples were collected.

Table 2. Checklist of phytoplankton and abundance (cells L^{-1})

Taxon / Island	PKK	Datai Island	Teluk Belim-bing	Besar Island Melaka	Singa Island	Teng-korak Beach	Beras Basah Island	PBD	Aur Island	Besar Island Johor	Tengol A Island	Tengol B Island
BACILLARIOPHYTA												
Achnanthaceae												
Achanthes brevipes Agardh	33,334	–	–	–	12,500	12,500	–	–	–	–	–	–
Achnanthes longipes Agardh	–	–	400,000	–	12,500	–	–	–	–	–	–	–
Total	33,334	0	400,000	0	25,000	12,500	0	0	0	0	0	0
Biddulphiaceae												
Odontella aurita (Lyngbye) Agardh	–	–	–	10,000	–	–	–	–	–	–	–	–
Total	0	0	0	10,000	0	0	0	0	0	0	0	0
Chaetocerotaceae												
Bacteriastrum sp.	16,667	–	–	–	–	–	–	–	–	–	–	–
Coscinodiscaceae												
Coscinodiscus curvatulus Grunow	83,335	–	50,000	–	–	–	16,667	–	–	–	–	–
Cylcotella kutzingiana Thwaites	200,004	16,667	–	10,000	37,500	–	16,667	10,000	–	–	–	–
Total	300,006	16,667	50,000	10,000	37,500	0	33,334	10,000	0	0	0	0
Cymbellaceae												
Amphora ovalis Kutzing	66,668	–	–	–	–	–	–	–	–	–	–	–
Amphora lanceolata Grunow	–	–	25,000	–	–	–	–	–	–	–	–	–
Total	66,668	0	25,000	0	0	0	0	0	0	0	0	0
Diatomaceae												
Synedra sp.	33,334	–	–	–	71,430	–	–	–	–	–	–	–
Total	33,334	0	0	0	71,430	–	0	0	0	0	0	0
Fragilariaceae												
Fragilaria intermedia Grunow	16,667	200, 004	75,000	10,000	–	–	283,339	–	–	12,500	133,336	16,666
Grammatophora marina Grunow	33,334	150, 003	–	70,000	14,286	75,000	50,001	–	200, 000	–	–	33,332
Total	50,001	350, 007	75,000	80,000	14,286	75,000	333,340	0	200,000	12,500	133,336	49,998

PKK – Kentut Kecil Island
PDB – Dayang Bunting Island

Table 2. Checklist of phytoplankton and abundance (cells L^{-1}) …. *continued*

Taxon / Island	PKK	Datai Island	Teluk Belim-bing	Besar Island Melaka	Singa Island	Teng-korak Beach	Beras Basah Island	PBD	Aur Island	Besar Island Johor	Tengol A Island	Tengol B Island
Naviculaceae												
Amphiprora gigantean Grunow	–	–	–	30,000		–	–	–	–	–	–	–
Diplonei gravelleana Hagelstein	–	66,668	900,000	–	–	–	16,667	–	–	–	–	–
Mastigloia minuta Grev.	150,003	183,337	950,000	30,000	14,286	12,500	–	–	25,000	–	16,667	8,333
Navicula sp. 1	–	16,667	–	–	–	–	–	–	–	12,500	–	–
Navicula abrupta Gregory	33,334	–	100,000	–	42,286	12,500	16,667	–	12,500		–	–
Navicula advena A. Schmidt	–	–	–	–	–	–	–	–	25,000	–	–	–
Navicula elegans W. Smith	–	33,334	–	–	–	–	250,005	–	–	–	666, 680	8,333
Navicula serians Brebisson	16,667	–	–	–	–	–	–	–	–	–	–	–
Pleurosigmaelongatum W. Smith	–	–	–	10,000	–	–	–	20,000	–	–	–	16,666
Total	200,004	300,006	1,950,000	70,000	57,144	25,000	283,339	20,000	62,500	12,500	683, 347	33,332
Nitzschiaceae												
Nitzschia commutata Grunow	–	33,334	175,000	–	–	12,500	–	–	–	–	–	–
Niztschia lanceolata W. Smith	–	–	–	10,000		–	–	–	–	–	–	–
Nitzschia panduformis Grunow	–	–	–	–	14,286	–	–	–	–	–	–	–
Total	0	33,334	175, 000	10,000	14,286	12,500	0	0	0	0	0	0
CYANOPHYTA												
Oscillatoriaceae												
Oscillatoria sp.	–	–	–	–	–	62,500	–	–	–	–	–	–
Total	0	0	0	0	0	62,500	0	0	0	0	0	0
TOTAL	683,347	700,014	2,675,000	180,000	178,575	187,500	650,013	30,000	262,500	25,000	816,683	83,330
SHANON –WIENER DIVERSITY INDEX	0.884	0.712	0.624	0.770	0.716	0.634	0.567	0.276	0.347	0.302	0.234	0.539

PKK – Kentut Kecil Island
PDB – Dayang Bunting Island

terms of both species richness and Shanon's diversity index, it was clearly indicated that sand samples from Kentot Kecil Island had the most diverse phytoplankton flora. On the contrary, sand samples from Besar Island (Johor) had lowest number of algal species. The coarse sand in Kentot Kecil Island supported a luxuriant growth of microalgae and this could be due to it been more developed and polluted compared to Besar Island (Johor).

Table 2 shows the microalgae associated with *Leptoconops* at Besar Island (Johor), Aur Island (Johor) and Tenggol A Island (Terengganu) and Tenggol B Island (Ternggamu). Eight algal species belonging to Fragilariaceae, Naviculaceae and Nitzchiacea were encountered in sand samples from these islands. Virtually many kinds of algae were found where *Leptoconops* were breeding but *F. intermedia*, *M. minuta* and *N.advena* were particularly common. Reports from Jamaica also reveals that sand inhabited by *Leptoconops* larvae was frequently characterized by a green fringe on the immediate surface which was caused by the presence of algae (Davis & Linsley, 1971). However, our study showed that, microalgae species were very low in infested islands compared to the sand beaches without *Leptoconops*. This may be because *Leptoconops* infested islands are normally clean and not polluted. The sand from the larval habitat at these beaches are composed of shell and coral and are highly calcacerous. Further sampling of microalgae and *Leptococonops* might have confimed the results. It is difficult to compare present results with previous work because only very limited data are found in the literature.

Some genera like *Achantes*, *Odetella*, *Cosconodiscus*, *Cytotella* and *Amphora* are found on non-infested islands but are not present in the infested islands. The Sorensen's Similarity Index of algal species in various sandy beaches are presented in Table 3. The similarity between alga taxa found in different sandy beaches ranges from

Table 3. Sorensen's Similarity Index of microalgal taxa in sandy beaches

	1	2	3	4	5	6	7	8	9	10	11	12
1	100											
2	42.0	100										
3	32.0	50.0	100									
4	42.0	42.0	25.0	100								
5	53.0	32.0	25.0	38.0	100							
6	56.0	28.0	29.0	029.0	57.0	100.0						
7	55.0	44.0	53.0	40.0	40.0	15.0	100					
8	8.0	10.0	0.0	40.0	10.0	0.0	0.0	100				
9	40.0	33.0	33.0	33.0	60.0	60.0	36.0	0.0	100			
10	8.0	40.0	40.0	10.0	0.0	0.0	11.0	0.0	0.0	100		
11	23.0	54.0	36.0	36.0	9.0	11.0	40.0	0.0	14.0	20.0	100	
12	38.0	62.0	23.0	62.0	27.0	36.0	46.0	14.0	44.0	20.0	75.0	100

Note: 1 – Kentut Kecil Island (PKK)
2 – Datai Island
3 – Teluk Belimbing
4 – Besar Island Melaka
5 – Singa Island
6 – Tengkorak Island
7 – Beras Basah Island
8 – Dayang Bunting Island (PDB)
9 – Aur Island
10 – Besar Island Johor
11 – Tengol A Island
12 – Tengol B Island

Sorensen's Similarity Index

$$S = \frac{2C}{A+B} \times 100$$

where
A = number of species in one site
B = number of species in another site
C = number of species common to both sites

0.0 to 75.0%. Highest similarity index was recorded between sand samples collected from Tengol A island and Tengol B island (75.0%) followed by Besar Island (Melaka) and Tengol B Island (62.0 %). The variation between other islands were relatively high. Only a study of several years would provide reliable data to confirm a relation between distribution of microalgae and the *Leptoconops* larval habitats. Further investigations are needed to determine the relevance of these microalgae to the survival of the *Leptoconops* in the sand beaches. This can be carried out by looking at the gut contents of Leptoconops larvae.

REFERENCES

Aussel, J.P. (1993 a). Ecology of the biting midge *Leptoconops abiventris* in French Polynesia. II. Location of breeding sites and larval microdistributiuoin. *Medical and Veterinary Entomology* **7**: 80-86.

Aussel, J.P. (1993 b). Ecology of the biting midge *Leptoconops abiventris* in French Polynesia. III. Influence of abiotic factors on breeding sites. Towards ecological control?. *Medical and Veterinary Entomology* **7**: 87-93.

Brenner, R.J. & Wargo, M.J. (1984). Observations on adult bionomics and larval ecology of *Leptoconops torrens* (Diptera:Ceratopogonidae) during an outbreak in the Coachella valley of southern California, USA. *Journal of Medical Entomology* **4**: 460-469.

Davies, J.B. & Linley, J.R. (1971). Sand flies and tourism in Florida and the Bahamas and Caribbean area. *Journal of Economic Entomology* **64**: 264-278.

Khadri, S.M., Lee, H.L., Abdullah, A.G., Azahari, A.H. & Abu Hassan, A. (2002). A first Note on nuisance biting-midges *Leptoconops spinosifrons* (Diptera: Ceratopogonidae) at sandy beaches in Malaysia. *Tropical Biomedicine* **19(1&2)**: 127-129.

Linley, J.R. (1969). Seasonal changes in larval populations of *Leptoconops becquaerti* (Kieff.) (Diptera:Ceratopogonidae) in Jamaica, with observations on the ecology. *Bulletin of Entomological Research* **59**: 47-64.

Laurence, B.R. & Mathias, P.L. (1972). The biology of *Leptoconops (Styloconops) spinosifrons* (Carter) (Diptera: Ceratopogonidae) in the Seychelles islands, with descriptions of the immature stages. *Journal of Medical Entomology* **9**: 51-59.

Lokman, S. (1990). Diatom Marin di Perairan Malaysia. Dewan Bahasa dan Pustaka, Kuala Lumpur, 260 pp.

Strickman, D., Wirtz, R., Lawyer, P., Glick, J., Scottwell, S. & Perich, M. (1995). Metrological effects on the biting activity of *Leptoconops americanus* (Diptera: Ceratopogonidae). *Journal of the American Mosquito Control Association* **11**: 15-20.

Tomas, R.C. (1997). *Identifying Marine Phytoplankton*. Academic PressSan Dieago, California, 856pp.

Wah, T.T. & Wee, Y.C. (1988). Diatoms from mangrove environments of Singapore and Southern Peninsular Malaysia. *Botanica Marina* **31**: 317 – 327.

Wah, T.T., Wee, Y.C. & Phang, S.M. (1992). Diatoms from marine environments of Peninsular Malaysia and Singapore. *Gardens' Bulletin* **44(2):** 73 – 125.

Whitford, L.A. & Schumacher, G.J. (1973). *A Manual of Freshwater Algae*. Sparks Press, Raleigh,North Carolina, 324 pp.