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Original Research Paper

Feeding Habitats of Mosquito Larvae and Their Gut Flora at Mysore

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ABSTRACT

Algae are important food sources for aquatic insects including mosquitoes. Eight mosquito species were collected in and around Mysore city in order to examine the gut contents of the larvae. A total of sixty five species of algae were identified from different larval habitats of mosquito larvae, out of which 55 were encountered in the larval guts. From these algal species identified in the larval gut, 21 (38.18%) belonged to Bacillariophyceae, 12 (21.8%) to Chlorophyceae, 9 (16.36%) to Cyanophyceae, 7 (12.72%) to Desmidaceae and 6 (10.9%) to Euglenophyceae. From the larval breeding sources, out of the 65 identified species, 26 (40%) belonged to Bacillariophyceae, 16 (24.6%) to Chlorophyceae, 10 (15.3%) to Cyanophyceae, 7 (10.76%) to Desmidaceae and 6 (9.2%) to Euglenophyceae. Identification of food sources and their importance to larval development and survival is particularly relevant to the development of novel vector control methods, especially in the study area where malaria, chikungunya and dengue are endemic.

INTRODUCTION

Mosquitoes occupy an important place among various vectors of communicable diseases. This is the largest group of insects of public health importance in the world as they can transmit diseases such as malaria, chikungunya, dengue, encephalitis and yellow fever. Studying the mosquito ecology including larval habitats and food sources is important from disease epidemiology point of view. In this regard studies done by Coggleshall (1926), Hinman (1930), Howland (1930), Kaufman et al. (2006), Larid (1998), Merritt et al. (1990, 1992) and Rettich et al. (2001) reveal the importance of algae as the larval food source.

Mosquito control requires a thorough knowledge on the ecology of the local species with respect to breeding sources, feeding and behaviour. Proliferation of mosquitoes is determined by the availability of suitable and sufficient food sources with suitable physicochemical parameters in the habitat for the larval stages and blood feeding sources for adult females. The larval food items include microbial flora, fauna and particulate matter. Some of the microbes in the habitat act as biocontrol agents as well. It is in this regard identification of food sources is important for the development of novel vector control methods such as encapsulation of bacterial toxins into larval food items or genetic modification of bacteria/algae to spread parasite inhibiting genes (Theiry et al. 1991). Further, enhancing the persistence of bacterial toxins in larval feeding zones is an important target as these organisms which propagate in the breeding sites and serve as food for mosquito larvae. In light of the above information, the present study was undertaken in a few areas of Mysore and Mandya districts of Karnataka State to generate information on the mosquito larval diversity, breeding habitats and natural algal flora as such studies have not been undertaken in this region earlier.

MATERIALS AND METHODS

Mosquito larvae were collected in and around Mysore city (12°30' N, 76°55' E) and rural parts of Mandya district (13°14' N, 77°20' E). The climate is quite congenial for proliferation of mosquitoes in both the sampling areas. The sampling sites included paddy fields, ground pools, ponds, tree holes, coconut shells, garden pots, plastic containers, cement tanks, fountains and sewage tanks/canals. The larvae were collected employing standard method with enamel dipper in ground pool habitats and Pasteur pipette for tree holes and coconut shells following the method of Silver (2008). Late third or early fourth instar larvae were taken and identified following the taxonomic keys of Christophers (1933), Barraud (1934) and Ramachandra Rao (1984).

Mosquito larval gut dissection: Fourth instar larvae were individually taken on a glass slide and washed with distilled water. The gut which is covered by the peritrophic membrane was dissected out carefully and washed with distilled water. The gut contents were then teased from the membrane with minuten needles into a drop of water and glycerol kept on the slide and observed under microscope after placing a cover glass.

Collection and preservation of water samples: One litre of water samples was collected from each breeding source, transferred into a plastic container and 25 mL of 4%

formaldehyde and a few drops of Lugol's iodine solution were added (Welch 1948). This was kept undisturbed for sedimentation and finally 50 mL of the sediment was preserved in vial for further analysis.

The algae in the larval gut contents and breeding sources were photographed and identified using taxonomic keys of Deshikachary (1959), Prescott (1982), Sarode & Kamath (1984) and Scott & Prescott (1961).

RESULTS

Pooled data obtained from different breeding habitats surveyed during the year 2008 showed that nine species of mosquito larvae belonging to three genera viz., *Aedes* (22%), *Anopheles* (11%) and *Culex* (67%) were encountered in the study area.

Table 1 provides the list of algae encountered in the larval gut of different mosquitoes along with the species of mosquitoes collected. Sixty five species of algae were identified from different breeding sources of mosquitoes, out of which 55 could find place in the larval gut of mosquitoes. From the 55 algal species encountered in the mosquito larval gut, 21 (38.18%) belonged to the family Bacillariophyceae, 12 (21.8%) to Chlorophyceae, 9 (16.36%) to Cyanophyceae, 7 (12.72%) to Desmidaceae and 6 (10.9%) to Euglenophyceae. Algae which were identified and recorded in different mosquito larvae are given in the Table 1. Twelve algal species were recorded from the gut of *Aedes aegypti*, 13 from *Ae. albopictus*, 10 form *An. stephensi*, 15 from *Cx. fuscocephala*, 11 from *Cx. gelidus*, 21 from *Cx. quinquefasciatus*, 19 from *Cx. vishnui* and 27 from *Cx. tritaeniorhynchus*.

Table 2 enlist the 65 algal species recorded from different breeding sources of the mosquitoes. Out of these, 26 species (40%) belonged to Bacillariophyceae, 16 (24.6%) to Chlorophyceae, 10 (15.3%) to Cyanophyceae, 7 (10.76%) to Desmidaceae and 6 (9.2%) to Euglenophyceae.

Out of 65 algal species recorded from different larval habitats, 34 were from paddy fields, 24 from ground pools, 13 from the pond, 6 each from tree holes and coconut shells, 12 from garden pots, 9 from plastic containers, 16 from cement tank, 12 from fountains and 28 species from sewage canals/tank.

DISCUSSION

The mosquito survey conducted during the present study for their gut flora in and around Mysore and Mandya districts reinstates the prevalence of widespread larval breeding habitats and associated breeding of different mosquito species. The observations made here are in line with the earlier studies conducted from the Vector Biology Research lab, Department of Zoology, University of Mysore with regard to the mosquito species (Sathish Kumar & Vijayan 2005, Urmila et al. 1999). However, they have not investigated the flora in the breeding sources and larval guts. Out of 9 mosquito species collected, 8 are reported to be incriminated vectors of diseases. Therefore, it is important to monitor the prevalence and density of various vector species in different foci periodically. This will provide a baseline data on the possible epidemiology of vector-borne diseases.

Larval source management is a strategy which includes larviciding and source reduction by environmental manipulation, which includes modification and elimination of aquatic habitats and food sources present in it. In the present study, algae and other unspecified inorganic materials, spores, fungi and insect scales were observed in the gut contents of different mosquito species. Mosquito larvae collected from all habitats had a greater proposition of Bacillariophyceae compared to Chlorophyceae, Cyanophyceae, Desmidaceae and Euglenophyceae. By knowing the kind and composition of food that makes habitat particularly favourable for mosquito survival, it might be possible to manipulate the habitats to eliminate breeding. It has been found that algae are generally represented in the gut in proportion to their abundance among the microflora and microfauna in larval habitats (Marten 2007). In line with this, present data showed that algae represented in the larval gut are fairly in proportion to their abundance in the larval habitats (Tables 1 and 2).

Perusal of the literature reveals that Anopheline larvae are strongly associated with algae in natural habitats studied at Michigan (Wallace & Merritt 1999). In the present study too *An. stephensi* was found associated with *Spirogyra*, a filamentous alga that serves as food which is in agreement with the report of Bond et al. (2004) at Chiapas, Mexico. They have also reported that *An. pseudopunctipennis* breeding has been reduced by removing *Spirogyra* from their breeding sites. *Oscillatoria* species were encountered more in larval gut and breeding sources of *Cx. quinquefasciatus* which matches with the experiments conducted by Marten (1986). Further, Marten (2007) has reported that many species of *Scenedesmus* were found to kill the larvae. In the present investigations also *Scenedesmus* species were encountered in the larval gut as well as in the larval habitats, but its larvicidal property is yet to be confirmed at Mysore.

It was found at Mysore that Bacillariophyceae was in high proportion in majority of the mosquito larval guts and breeding sources, followed by Chlorophyceae and Cyanophyceae. So, it is evident from these data that Bacillariophyceae serves as a major source of food for many species of mosquito larvae followed by Chlorophyceae. Cyanophyceae

Table 1: Specieswise survey of phytoplankton in the gut of different mosquito larvae.

	Mosquito Species	*Aedes aegypti*	*Aedes albopictus*	*Anopheles stephensi*	*Culex fuscocephala*	*Culex gelidus*	*Culex quinque-fasciatus*	*Culex tritaeni orhynchus*	*Culex vishnui*
I.	**Chlorophyceae**								
1.	*Cladophora insignis*		+						
2.	*Coelastrum cambricum*	+	+				+	+	
3.	*Dictyosphaerium ehrenbergianum*				+				
4.	*Oedogonium epiphyticum.*	+							
5.	*Scenedesmus caudate acculantus*								+
6.	*Scenedesmus obliques V. torters*				+				
7.	*Scenedesmus perforatus*								+
8.	*Scenedesmus quadricauda*						+		
9.	*Schroederia indica*	+							
10.	*Spirogyra* (conjugation)			+					
11.	*Spirogyra crassa*			+				+	
12.	*Zygnema indicum*				+				
II.	**Desmidaceae**								
1.	*Closterium lunula*	+		+					
2.	*Closterium calasporium*		+					+	
3.	*Closterium intermedium*		+					+	+
4.	*Closterium turgidum*		+					+	
5.	*Cosmarium decoratum*				+				
6.	*Cosmarium lundelli Var. circulare*			+					
7.	*Staurastram crenulatum*	+							
III.	**Bacillariophyceae**								
1.	*Achnanthes microcephala*		+				+	+	
2.	*Achanthes* sp.					+		+	+
3.	*Cyclotella catenata*						+		+
4.	*Cyclotella* sp.						+		
5.	*Cyclotella striata*							+	+
6.	*Cymbella powaiana*					+		+	+
7.	*Diploneis subovalis*			+					
8.	*Gomphonema acquetoriale*						+	+	
9.	*Gomphonema agustatum*	+		+			+	+	
10.	*Gomphonema gracile*		+						
11.	*Gomphonema spiculoides*		+		+			+	
12.	*Nitzschia apiculata*				+			+	+
13.	*Nitzschia intermedia*	+	+	+	+	+	+	+	+
14.	*Nitzschia obtusa*	+	+		+	+	+	+	+
15.	*Nitzschia regula*	+	+	+	+	+	+	+	+
16.	*Pinnularia gibba*				+	+		+	+
17.	*Pinnularia lundelli*		+		+				
18.	*Pinnularia viridis*	+	+	+					
19.	*Rapalodia gibba*				+			+	+
20.	*Stauroneis phoenicenteron*				+	+	+	+	+
21.	*Synedra ulna*						+		
IV.	**Euglenophyceae**								
1.	*Lepocinclis fusiformis*							+	
2.	*Lepocinclis ovum*					+		+	+
3.	*Phacus chloroplastus*					+		+	+
4.	*Phacus orbicularis*			+		+	+	+	+
5.	*Phacus tortus*					+	+	+	+
6.	*Trachelomonas hispida*								+
V.	**Cyanophyceae**								
1.	*Anabaena spiroidis*				+			+	
2.	*Arthrospira platensis*							+	
3.	*Merismopedia glauca*						+		
4.	*Merismopedia tenuissima*	+					+	+	
5.	*Oscillatoria limosa*						+		
6.	*Oscillatoria nigra*				+		+		

Table cont...

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7. *Oscillatoria prolifica*		+
8. *Oscillatoria willei*	+	+
9. *Phormidium fragile*		+

Table 2: Correlation of phytoplankton recorded from different larval habitats.

Breeding Sources	Paddy Field	Ground pool	Pond	Tree holes	Coconut shells	Garden pots	Plastic contai-ners	Cement tanks	Fountains	Sewage tank
I. Chlorophyceae										
1. *Cladophora insignis*				+	+					
2. *Closteriopsis longissima*	+									
3. *Closterium ehrenbergii*	+									
4. *Coelastrum cambricum*	+			+	+	+		+		+
5. *Dictyosphaerium ehrenbergianum*	+									
6. *Oedogonium epiphyticum.*		+						+		
7. *Pediastrum duplex Var. reticulatum*								+		
8. *Pediastrum duplex Var. subgranulatum*								+		
9. *Scenedesmus caudato acculantus*		+								
10. *Scenedesmus obliques V. tortus*	+									
11. *Scenedesmus perforatus*		+								
12. *Scenedesmus quadricauda*										+
13. *Schroederia indica*								+		
14. *Spirogyra* (conjugation)									+	
15. *Spirogyra crassa*	+								+	
16. *Zygnema indicum*	+									+
II. Desmidaceae										
17. *Closterium lunula*								+	+	
18. *Closterium calasporium*	+				+					
19. *Closterium intermedium*	+	+		+	+	+	+			+
20. *Closterium turgidum*	+				+					
21. *Cosmarium decoratum*	+									
22. *Cosmarium lundelli*									+	
23. *Staurastram crenulatum*								+		+
III. Bacillariophyceae										
24. *Achnanthes microcephala*	+	+	+			+	+			+
25. *Achanthes* sp.	+		+			+			+	+
26. *Anamoensis spharophora*		+								+
27. *Cyclotella catenata*	+									+
28. *Cyclotella* sp.										+
29. *Cyclotella striata*		+		+						
30. *Cymbella powaiana*		+	+							
31. *Diploneis subovalis*									+	
32. *Fragilaria construens*		+								
33. *Gomphonema acquetoriale*		+								+
34. *Gomphonema agustatum*		+						+	+	+
35. *Gomphonema gracile*				+		+	+			
36. *Gomphonema spiculoides*	+					+				
37. *Nitzschia apiculata*	+	+				+	+			
38. *Nitzschia intermedia*	+	+	+			+	+	+	+	+
39. *Nitzschia maharashtrensis*	+							+		
40. *Nitzschia obtusa*	+	+	+			+	+	+		+
41. *Nitzschia regula*	+	+	+	+		+	+	+	+	+
42. *Pinnularia gibba*	+	+	+							
43. *Pinnularia lundelli*	+				+					
44. *Pinnularia viridis*						+	+	+	+	
45. *Pleurosigma hippocampus*	+									

Table cont...

...Cont. Table

46. *Rapalodia gibba*	+	+			+	+	+	+
47. *Stauroneis phoenicenteron*	+	+	+					+
48. *Surirella ovata*				+				
49. *Synedra ulna*								+
IV. Euglenophyceae								
50. *Lepocinclis fusiformis*	+		+					
51. *Lepocinclis ovum*		+	+					
52. *Phacus chloroplastus*	+	+	+					+
53. *Phacus orbicularis*	+	+	+				+	+
54. *Phacus tortus*	+	+	+					+
55. *Trachelomonas hispida*		+						
V. Cyanophyceae								
56. *Anabaena aphamizomenoides*	+							
57. *Anabaena spiroidis*	+							
58. *Arthrospira platensis*	+							
59. *Merismopedia glauca*								+
60. *Merismopedia tenuissima*	+					+		+
61. *Oscillatoria limosa*								+
62. *Oscillatoria nigra*	+	+						+
63. *Oscillatoria prolifica*								+
64. *Oscillatoria willei*						+		+
65. *Phormidium fragile*								+

(blue-green algae) have many advantages in biological control of mosquitoes when compared with other mosquitocidal bacteria. For example, the cyanobacterium *Oscillatoria agaridhii* and *Anabaena circinalis* were found to be highly toxic to larval stages of *Aedes aegypti* (Kaviranta & Abdel-Hameed 1994) in Finland. In the present study too *Oscillatoria* and *Anabaena* species were found in the larval gut, but further study has to be carried out in this regard to know the larvicidal property.

It is common in nature for mosquito larvae to die before completing their development which may be because of poisoning by algal toxins or they starve to death while feeding on algae that are indigestible (Marten 1987). So mosquito indigestible phytoplankton have good field characteristic as a biological control agent as they are present as suitable food in the breeding habitats. Another major advantage of phytoplankton for mosquito control is that the vectors have not developed resistance to these toxins. Thus, encouraging toxin producing and indigestible micro algal growth is a good alternative for mosquito control. The first important step in this kind of control is the identification of suitable algae present in natural mosquito breeding habitats. Our analysis suggests that due to the wide range of microorganisms available in natural habitats, mosquito larvae may feed on any available groups, toxic or otherwise. Thus, this study improves our understanding of the larval ecology of local mosquitoes to facilitate the development of new mosquito control tools. Further, the present study was carried out for the first time in this region and the findings may gain operational value.

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