The Bioscan 7 (3) : 533-538, 2012
AN INTERNATIONAL QUARTERLY JOURNAL OF LIFE SCIENCES
www.thebioscan.in

EFFECTS OF TWO LEAF LITTER SPECIES ON THE COLONIZATION OF MACROINVERTEBRATES IN A TROPICAL STREAM OF INDIA

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KEY WORDS
macroinvertebrates
Riparian species
Environmental variables
Functional feeding groups
Leaf litter

Received on :
14.03.2012

Accepted on :
11.08.2012

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ABSTRACT
We examined the effect of two dominant leaf litter species of *Pongamia pinnata* and *Syzygium cuminii* on the colonization of macroinvertebrate community in a tropical stream of south India. The result of the leaf fall pattern of stream revealed that leaves entering regularly in to the stream for a year, and a greater proportion of leaves occurred between January and June. Out of 4 riparian vegetation features, tree shade and abundance of *S. cuminii* were significant predictors of macroinvertebrate assemblage variation The rate of colonization of macroinvertebrates was higher in *S. cuminii* than *P. pinnata*. Assemblages were composed of collector- filterers (38.3%), and to a lesser extent predators (36.5%) and collector-gatherers (23.4%). Of the 12 environmental variables, water temperature, pH and stream substrates were the significant predictors of macroinvertebrates assemblage colonization.

INTRODUCTION
Inputs of coarse particulate organic matter (CPOM) from riparian vegetation are a source of nourishment for aquatic organisms in streams (Conners and Naiman, 1984; Benefield, 1997), which strongly influence the aquatic food chain (Cortes *et al.,* 1995). Streamside vegetation has the capacity to affect stream function through interactions mediated through leaf litter fall (Petersen and Cummins, 1974; Cummins and Kulg, 1979; Cummins *et al.,* 1989). For example, leaf litter from different tree species decomposes at significantly different rates in streams (Ostrofsky, 1997; Webster *et al.,* 1999) and supports different microbial (Wallace *et al.,* 1997; Hieber and Gessner, 2002) and invertebrate assemblages (Graca, 2001; Leroy and Marks, 2006). Aquatic macroinvertebrates and microbes that colonize fallen leaves can discriminate among leaf litter of different species (Webster and Benfield, 1986; Petersen *et al.,* 1989). Leroy *et al.* (2006) hypothesized that aquatic macroinvertebrates could also discriminate among leaves at a genetic level due to differences in phytochemistry among and within cotton-woody hybrids (Findlay and Jones, 1990; Driebe and Whitham, 2000; Schweitzer *et al.,* 2004).

The relationship between litter species diversity and breakdown dynamics in streams have been conducted in temperate streams (Swan and Palmer, 2004; Leroy and Marks 2006; Kominoski *et al.,* 2007). Given that many stream food webs are dependent upon allochthonous litter as a source of energy, nutrients and habitat (Vannote *et al.* 1980; Wallace *et al.,* 1997), understanding the relationship between litter species diversity and breakdown dynamics in streams draining forested watersheds is of considerable ecological importance. A few works have been studied in tropical streams of India that influence of leaf litter on freshwater fauna by Gopal (1988); the effect of riparian vegetation and macroinvertebrate assemblages in Karanataka stream (Dinakaran and Anbalagan, 2007a); spatio-temporal pattern of leaf litter associated macroinvertebrates in Western Ghats streams (Anbalagan *et al.,* 2012). Previously work on macroinvertebrate assemblage structure of Gadana river basin have shown that leaf litter in riffles of different stream order affects the composition of aquatic macroinvertebrates (Anbalagan and Dinakaran, 2006). However, we aimed to analyze the effect of two dominant leaf litters of *Pongamia pinnata* and *Syzygium cuminii* on the colonization of macroinvertebrate community in a tropical stream of south India.

MATERIALS AND METHODS

Study area
Gadana river is a perennial stream with its tributaries forms a sub-basin in the major Tamiraparani river basin in Peninsular India, which is a part of Western Ghats of south India (Fig. 1). It has a 33km river, which joins to the Tamiraparani near Truppudaimarudur village in Ambasamudram taluk of Tirunelveli district. It has its origin in the eastern slopes of Western Ghats at an altitude of 1,564 m msl, (latitude 8°48'

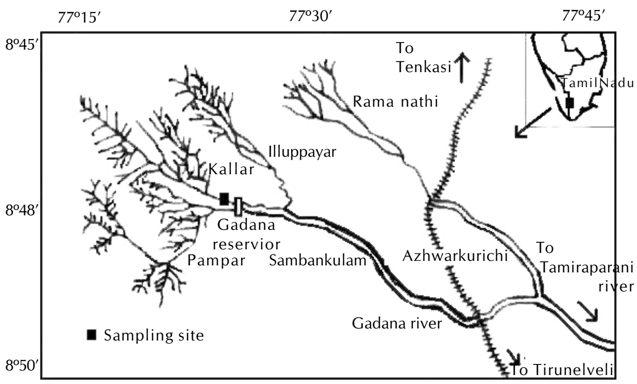

Figure 1: Study area

and longitude 77°19') and flows down the eastern slopes of Western Ghats. Three tributaries such as Pampar, Kallar and Iluppaiyar join to form the Gadana river. Pampar and Kallar streams are regulated into a reservoir called Gadana reservoir. After the confluence the river flows about 10km and merges with Ramanathi in the Kila Ambur village.

Sampling methods

The month-wise sampling was done for12 months. 5 sampling sites were selected from fourth order stream of this river basin. Each site was divided into 10m units for evaluation, each of which was independently visually assessed. The results of these assessments were averaged across the site. The percentages of total tree cover, total herbaceous cover and tree shade (overhanging trees) were determined using densiometer (model C). Bank full width was estimated from 3 transects of a 100m stretch. To quantify the leaf litter fall or input source of organic matter in stream, at selected habitat namely near the tree at spaced 1m, 2 m, 3 m, 4m and 5m, a wooden square frame ($50cm^2$) with nylon mesh was placed under these trees and the leaf litter enclosed within it was collected once for a month and transferred to the polythene cover, subsequently weighed and counted in the laboratory. Water and air temperature were recorded in the field using thermometer. Dissolved oxygen, total dissolved solids, conductivity and pH were measured using a water analysis kit (Naina Solaris Limited, www.indianindustry.com). Latitude, longitude, elevation and basin location were determined by Global Positioning System (GPS). Substrates were classified according to the following criteria: <0.5 mm mud/silt, 0.5-2mm sand, 2-64mm gravel, 65- 256mm cobbles, and >256 mm boulders (Jowett *et al.,* 1991). The average stream width and depth were calculated from three measurements with a calibrated stick from one transect across the channel. Current velocity of the stream was obtained by a flow meter.

The mass of decaying leaf litter was collected from the stream under the respective riparian species of *P. pinnata* and *S. cuminii* in different habitats (riffle, pool and edge) by using Dipper net. They were packed into a tight polythene bags and brought to the laboratory. Macroinvertebrates were removed from leaf litters using fine brush and forceps and they were preserved in the field using a 70% ethanol, subsequently identified in the laboratory to generic level and counted. Richness, abundance, Shannon's diversity and the Biological Monitoring Working Party (BMWP) index (Armitage *et al.,* 1983) were calculated. Macroinvertebrates were assigned to five major functional feeding categories, according to Dudgeon (1999).

Data analyses

Two sets of environmental data were used to in multivariate analyses. The first set had 5 variables (total tree cover, total herbaceous cover, overhanging tree shade, tree debris, and abundance of the dominant tree species), and the second set had 8 abiotic variables (water temperature, pH, conductivity, dissolved oxygen, surface water flow, average depth, average width and stream substrates). The distribution of the invertebrate taxa over the different substrates and their seasonal variation in densities was analysed with Redundancy Analysis (RDA) using program CANOCA. RDA is a constrained form of Principal Component Analysis (PCA). Using this technique the abundance of all species is simultaneously related to the environmental variables. Prior to this analysis the species abundances were standardized to zero mean and unit variance in order to prevent the most abundant species from dominating the results. RDA minimizes the residual sum of squares over all species under the constraint that the subsequent axes of the ordination are linear combinations of the environmental variables and sampling dates. The results are presented as a biplot of species loadings and as centroids of the environmental variables (mean scores of the samples from a certain substrate). The resulting vectors indicate increasing abundance in the direction where they point. A Monte Carlo permutation test was performed to detect significant relationships. The best riparian and abiotic predictors (those retained for analysis) were identified by a forward selection procedure.

RESULTS

Six riparian woody species (eg., *Pongamia pinnata*, *Mangifera* sp. *Syzygium cuminii, Erythrozylum* sp., *Aglanda rheede* and *Tectona grandis*) were found in the river corridors of the stream. Only *Pongamia pinnata,* and *Syzygium cuminii* occurred in a large part of the studied sites (>75%). Other tall tree species included *Erythrozylum* sp., *Aglanda rheede* and *Tectona*

Table 1: Mean, standard deviation (SD) and minimum (min) and maximum (max) values for selected environmental variables of stream

Environmental variables	Mean	Min-max
Riparian variables		
Tree richness per site	4±1	3 – 5
Herbaceous richness per site	14±2	12 – 16
Total tree cover (%)	40±20	20 – 60
Tree shade (%)	48±10	40 – 60
Abiotic variables - local		
Water temperature (°C)	24±1	19 – 21
Conductivity (μmhos/cm)	122±27	92 – 145
Dissolved Oxygen (mg/L)	8±1	7 – 9
pH	7±0.1	6.9 – 7.1
Stream width (m)	11±7	3 – 17
Stream depth (cm)	15±5	5 – 10
Surface water flow (sec/m)	16±3	14 – 20
Bedrock (%)	7±5	0 – 10
Boulders (%)	20±10	10 – 30
Pebbles (%)	23±6	17 – 29
Gravels (%)	18±8	10 – 28
Sand (%)	5±2	3 – 7

Table 2: Mean ±SD and minimum (min) and maximum (max) values of macroinvertebrate assemblages of stream

Macroinvertebrates	Mean	Min-max
Richness per site	26.3±13.3	24 – 29
Abundance	228.6±7.5	220 – 233
Shannon- Weiner Index	2.7±0.04	2.6 - 2.7
BMWP	167.3±28.6	147 – 200
Collector-Gatherer (%)	23.4±13.0	4.6 – 41.8
Collector-filterers (%)	38.3±11.0	1-24
Predators (%)	36.5±9.03	1 – 30
Scrapers (%)	6.1±1.9	1 – 5
Shredders (%)	23±3.5	3 – 5

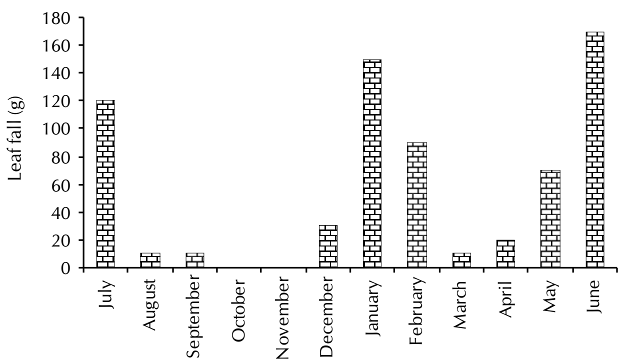

Figure 2: Average leaf litter fall (dry mass) of stream between July 2004 and June 2005. (Leaves were collected in 6 traps spaced at 1m² intervals along the length of the study reach)

grandis, but the occurrence was sparse and therefore not included as riparian variable. Tree cover of the riparian vegetation and bankfull width varied among the sites studied and along with a given stretches of river (Table 1). At most locations, land surfaces was occupied by herbaceous plants, which were generally dominated by *Cyperus bulbosus,* *Cyperus dubius, Fimbristlis schoenoides, Cyanoris cristata* and *Commelina clarata.*

Table 3: Summary statistics for the principal component analysis (PCA) relating macroinvertebrate assemblages to riparian and abiotic variables. Eigen values were 3.599 for the PC1 and 0.401 for the PC2 in the riparian PCA and 8.34 for the PC1 and 4.66 for the PC2 in the abiotic PCA. * = < 0.05

	Factors loadings		Contribution of the variables	
	PC1	PC2	PC1	PC2
Riparian Variables				
Pongamia pinnata	0.850	0.527	20.072	69.261
Tree cover	0.999	0.031	27.756	0.244
Syzygium cuminii	-0.969*	0.247	26.086	15.248
Tree shade	-0.969*	0.247	26.086	15.248
Abiotic variables				
Water temperature (ºC)	-0.539*	0.842	3.485	15.218
Conductivity (µmhos)	0.999	0.046	11.968	0.045
Dissolved Oxygen (mg/L)	0.888	-0.460	9.457	4.537
PH	-0.539*	0.842	3.485	15.218
Stream width (m)	0.565	0.825	3.834	14.594
Stream depth (m)	0.926	-0.377	10.285	3.055
Surface water flow (m/sec)	0.806	-0.592	7.784	7.528
Bed rock (%)	-0.842*	-0.539	8.507	6.234
Boulders (%)	0.531	0.847	3.385	15.396
Pebbles (%)	-0.842*	-0.539	8.507	6.234
Gravels (%)	0.972	0.234	11.338	1.172
Sand (%)	-0.842*	-0.539	8.507	6.234

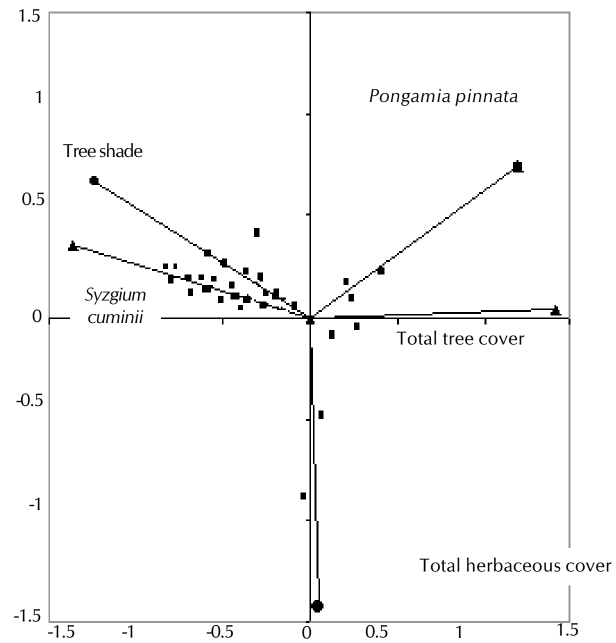

Figure 3: Principal component analysis of macroinvertebrate and riparian variables

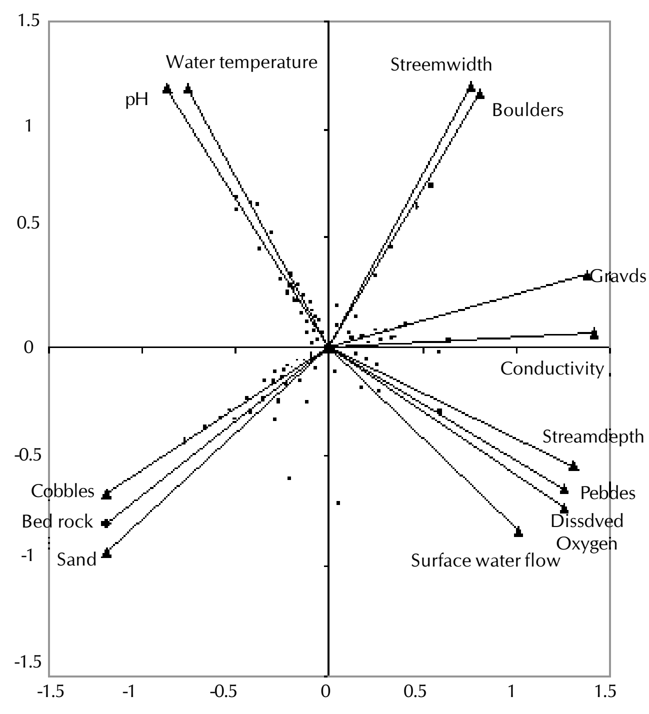

Figure 4: Principal component analysis of macroinvertebrate and abiotic variables

The sampled sites included generally shallow, slow moving, fast moving, small to medium sized channels. Water temperature, pH, conductivity, dissolved oxygen, stream width, stream depth and stream substrates are given in Table 1. The riverbed was usually composed of hard substrates, but the amount of small substrates was frequently high Fallen leaves were collected from the wooden framed nylon mesh. Leaves enter the stream in all months, while a substantial proportion of the annual input from streamside forest of stream occurs during January and June (Fig. 2).

Taxa richness, Shannon diversity values, BMWP score and the percentage of functional groups are given in Table 2. Hydropsychidae was one of the most abundant groups among the macroinvertebrate community. Baetidae, Leptophlebiidae and Simuliidae were also present. *Hydropsyche*, *Thiara* and *Simulium* were enormously colonized with *S. cuminii*. *Baetis* and *Choroterpes* were colonized with *P. pinnata*. The BMWP score in the investigated sites was ranging from 147 to 200. In most of the sites collector-filterers were dominating more than the other functional groups (Table 2). Four riparian variables namely tree cover, tree shade and abundance of *P. pinnata,* and *S. cuminii* were retained by the forward selection procedure. The macroinvertebrates showed the strong relationship with *S. cuminii* and they were weakly related with *P. pinnata* (Table 3; Fig. 3). Water temperature, pH and stream substrates had high relationships with macroinvertebrate assemblages (Fig. 4).

DISCUSSION

Environmental variables generally exert an important influence on macroinvertebrate assemblages (Collier 1995; Wright, 1995). Graca *et al.* (1989) showed significant relationship between 17 groups of co-occurring taxa and variations in water chemistry, substrate particle size, yearly temperature and flow. Although 16 environmental variables (riparian plus abiotic) were considered in this study, only 3 were significantly related with macroinvertebrate assemblages. Similar trend was observed in the Iberian river basin where macroinvertebrates were poorly grouped and poorly related to environmental variables (Aguiar *et al.,* 2001). Multivariate analysis in the Iberian basin did not show spatial patterns of macroinvertebrate assemblages related to stream hierarchy variables (stream order). Trophic groups also do not have relationships with the habitat descriptors, although a similar species composition and the same proportion of feeding groups occurred in basin (Aquiar *et al.,* 2001).

Deciduous leaves are clearly a major food input to forested stream. The rate at which these leaves decompose and enter the food web depends on a number of factors and processes: resistance to physical abrasion, chemical composition and susceptibility to chemical leaching, ambient stream temperature following inundation, microbial activity and the feeding activity of aquatic macroinvertebrates and substantial amount of organic matter (leaf litter) entering into the stream during October and November in White Clay Creek stream of Eastern North America (Sweeney, 1993). In the present study, organic matter enters into the stream for all months but a substantial proportion of the leaves occur during January and June.

Riparian variables significantly accounted to the macrobenthos distribution in the present study area. Moreover, the tree cover is the most important variable. Tree cover and tree shade contribute habitat heterogeneity and within stream patchiness and, thus to the distribution of benthic organisms (Downes *et al.,* 1993; Hall *et al.,* 1994). It is well known that riparian features influence macroinvertebrate assemblages and many studies have addressed the consequence of different riparian composition and disturbance on macroinvertebrates (Hawkins *et al.,* 1982; Rundle *et al.,* 1992; Tait *et al.,* 1994). Results of this study reinforce assertions that riparian-reach variables influence macroinvertebrate structure and functions more than land use catchment variables (Subramanian *et al.,* 2005). The significant relationship between riparian vegetation and the macroinvertebrate community indicate that forest canopy strongly influenced the structure of macroinvertebrate community. Forest shade and coverage appeared to increase EPT, total richness and diversity taxa. Riparian forests in stream corridors further increase the value of large numbers of sensitive macroinvertebrates and abundances than adjacent habitats (Naiman *et al.,* 1993; Tate and Heinly, 1995).

The macroinvertebrates showed the strong relationship with *S. cuminii* than *P. pinnata*. The results reflected that the importance of litter as a source of food and total densities of macroinvertebrates of major taxa were greater in leaf litter of *S. cuminii*. Similar results have been reported in other studies where the colonization of palatable and non-palatable leaves has been compared in streams (Dobson *et al.,* 1992; Dudgeon and Wu, 1999). This indicates that form a palatable litter, which served mainly as a food source and thus supported high densities of macroinvertebrates. Contrarily lower densities of macroinvertebrates associated with *P. pinnata* indicated lesser palatability of its leaf and therefore may be useful as a substrate. This result reflects the fact that leaf litter in a tropical stream may serve as food or substrate for macroinvertebrates (Dudgeon and Wu, 1999). In tropical countries where many leaf types of varying palatability and defensive compounds are present (including a greater proportion of condensed tannins: Stout, 1989), the patch – specific response of faunal densities to changes in the total amounts of these compounds can be expected to be rather weak, and macroinvertebrate abundance is unlikely to correlate closely with litter biomass.

In general, riffles areas (cobble, leaves, gravel, macrophytes) supports more taxa than structurally simple substrates (bedrock and sand) and are less affected by water flow allowing a greater period for colonization and processing of benthic organic matter by macroinvertebrates. Also, the community established in stony substrates becomes more stable serving as refugia for younger organisms (Bapista *et al.,* 2001). Similarly community structure of aquatic insects was influenced by substrates. According to Macan (1978), the behavior of stream species should be related to the velocity of flow, as fast waters should transport more nutrients in a determined period and it would allow many filter-feeding species to occur. Besides, the preferences for these substrates are also related to a high availability of dissolved oxygen concentrations. Temperature and pH and also related with macroinvertebrate assemblages in the present study stream. This is probably due to the influence on canopy cover and stream size. Headwater streams in mountain areas have low temperature, high gradient and are often bordered by riparian forest. In downstream, the temperature increases, gradient decreases and the importance of shading from riparian canopy declines enabling aquatic macrophytes to develop (Larsen *et al.,* 2000). Temperature has an overall influence on life processes and may play a major role in controlling composition, development and function of Ephemeroptera, Plecoptera and Trichoptera taxa in hill stream of south India (Dinakaran and Anbalagan, 2007b). Minshall and Robinson (1998) recorded temperature determinants of species richness among other physico-chemical variables.

There is a high proportion of collector-filterers and a low proportion of shredders in this study. The high proportion of collector-filterers is probably related to the abundant of organic matter, which is supplemented by fine particulate allochthonous inputs and algae that usually offer considerable nutritional value (Shepard and Minshall, 1981). Ash and alder leaves are known to have leaves that decompose easily and provide good quality food for macroinvertebrates (Barlocher, 1985), whereas the *S. cuminii* was significantly related to shredder abundance in this study. Ross (1963) was the first to notice an association of the distribution of some North American shredder taxa, especially Trichoptera, with the riparian vegetation. Shredders such as Lepidostomatidae (Trichoptera) and Tipulidae (Diptera) are abundant in the riparian zone in perennial streams of temperate regions (Grafius and Anderson, 1979; Dudley and Anderson, 1987), while Thiaridae (molluscs) and Lepidostomatidae (Trichoptera) were the most in the riparian zone of this stream.

This study evidenced that leaf litter diversity has the capacity to affect in-stream decomposition rates and stream invertebrates, but that effects depend on both litter quality and stream characteristics (Leroy and Marks, 2006; Kominoski *et al.,* 2007). Thus, litter diversity and quality strongly influence the colonization of macroinvertebrates assemblages and determine the food chain in stream.

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