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Breeding patterns of the JE vector *Culex gelidus* and its insect predators in rice cultivation areas of northern peninsular Malaysia

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Abstract. Japanese encephalitis (JE) virus activity is an important cause of viral encephalitis in Southeast Asia. In Malaysia, JEV activity has been first detected in *Culex gelidus* in 1976. Since then, no study has fully addressed the seasonal dynamics of this mosquito. As irrigated rice production expands, the incidence of JEV vectors, particularly *Cx. gelidus* is expected to increase. We surveyed Penang Island to determine the breeding patterns of *Cx. gelidus* and their potential insect predators, in relation to habitat/niche and rice growing period. Six rice fields proper (RFP) and related drainage canals (DC) were visited through three cultivation cycles (CCs) over 17 months. Weekly visits were performed to each of the 36 sites and mosquito larvae and aquatic insects were sampled from RFP and DCs using dippers. *Culex gelidus* was abundant in RFP and almost absent in DCs. Its densities usually were high during the first and 3rd CC and when the RFs were in Fp, Pp and Gp. In DCs, the mosquito was abundant during Mp, e.g., 2nd CC. Predators, especially those belonging to the families Corixidae, Coenagrionidae and Dytiscidae, were more present in RFP. Predator numbers usually were high during the first CC; in some cases predator abundance peaked during other CCs, e.g., corixids and dysticids. In RFP, neither corixids nor coenagrionids showed any positive correlation with densities of *Cx. gelidus*. However, dytiscids' population peaked when the mosquito densities were on the rise. These observations suggest that *Cx. gelidus* is active during the period of rice cultivation. Operational vector control through bio-control or with insecticides near the end of the rice cultivation season in RFP may prove beneficial in reducing the density of *Cx. gelidus*, but also the amount of bio-agent or insecticide applied on riceland.

INTRODUCTION

Mosquito-borne diseases are a major threat to public health worldwide. Frequent outbreaks of diseases such as dengue (Rahman *et al.*, 2002; Enserink, 2006) are reminders of the continued threat of diseases transmitted by insects, as does the rise in cases of Japanese encephalitis virus (JEV) infection during the 2000s in Asia (Ayukawa *et al.*, 2004; Parida *et al.*, 2006; Wang *et al.*, 2007).

Several species of *Culex* that are potentially competent for JEV transmission (Yap & Ho, 1977; Vythilingam *et al.*, 1994; Abu Hassan *et al.*, 1998), are found in Malaysia, where rice is the third most important economic crop covering an area of about 209,300 hectares (Karim *et al.*, 2004). The amount of land devoted to rice cultivation by irrigation, and consequently the incidence of related mosquito-borne diseases, are both expected to increase with the growing need for rice to feed the expanding human population. With this expansion of irrigated rice production, JE vectors most likely to increase in importance include *Culex gelidus* and *Culex tritaeniorhynchus*. In Malaysia, JEV activity has been detected in both species, but is most common in the former species (Heathcote, 1970). *Culex gelidus* is found in many Southeast Asian countries (Lee *et al.*, 1989), where it is considered important as an important vector of Japanese encephalitis (Williams *et al.*, 2005). Native to Southeast Asia, this species has proven to be invasive. It has been reported as a voracious biter of humans, but also as having a preference for large domestic animals (Whelan *et al.*, 2000). In Malaysia, *Cx. gelidus* is considered important in maintaining JE in pig-mosquito cycles. As Malaysia is not that devoted to pig farming, it is possible for *Cx. gelidus* to acquire an increased human biting behavior, with respect to reduced number of pig host.

Rice fields are inhabited by the immature stages of mosquitoes including *Cx. gelidus* and *Cx. tritaeniorhynchus* in addition to heterospecific insects that include mosquito predators (Sunahara *et al.*, 2002; Carlson *et al.*, 2004). In fact, rice growth creates microclimates that provide a variety of habitats and niches conducive to a variety of organisms (Edirisinghe & Bambaradeniya, 2006). When irrigated, rice fields are connected to a network of irrigation and drainage canals (DCs). In addition to being the origin of most of the aquatic flora and fauna in rice fields (Fernando, 1993), these sub-habitats contiguous to the rice field proper (RFP) also provide another environment for colonising insects (Amerasinghe *et al.*, 1991; Edirisinghe & Bambaradeniya, 2006). However, there has been relatively little research addressing the insect fauna of these non-rice subhabitats. There has been a great deal of research regarding the effects of rice growth on the population dynamics of insect communities. Overall, initial plant growth increases population density of both mosquito and aquatic insect (Chambers *et al.*, 1979; Mogi & Miyagi, 1990), while later plant growth has a negative effect (Chandler & Highton, 1975, 1976). The arthropod community of RF is a dynamic system in which each member has a preference for a particular phase of rice field (RF) development (Takagi *et al.*, 1996; Victor & Reuben, 2000).

Efforts to combat rice field mosquito vectors still rely heavily on insecticides (Rose, 2001; Russell & Kay, 2008). However, the increased use of chemical insecticides has led to adverse effects on the environment, paddy ecology and human health (Pingali & Gerpacio, 1997). Therefore, there is increasing emphasis on reducing the amount of insecticide applied on agricultural land. The spatial distribution of mosquitoes in RFs and the related seasonal variations are central to the successful application of an insecticide-based control programme and have important environmental implications. Accurate information regarding the spatial distribution of the target has the potential to reduce the amount of product sprayed onto the crops. This information also is important for identifying reservoirs of infectious agents. Surprisingly, the role of space as it affects population dynamics of RF insects remains largely unexplored.

This study was set out to investigate the breeding patterns of *Cx. gelidus*, and coexisting aquatic insect predators in north peninsular Malaysia, taking into account habitat/niche and rice cultivation phase.

MATERIALS AND METHODS

Study area and sampling sites

The survey was carried out in Penang, Malaysia within the rice growing areas of Jalan Penanti and Seberang Perai from February 2003 to October 2004. These areas are mostly flat, and cover 8.75 hectares located 5.417ºN 100.417ºE (5º24'N 100º14'E). The area has a year-round equatorial climate, and is warm with high levels of sunshine. The average annual rainfall is about 2600 mm, and the daily relative humidity varies from 60% to 96% with ambient temperatures ranging from 23ºC to 35ºC (Ong, 1993; Ahmad *et al.*, 2006). The high levels of rainfall associated with high relative humidity and the warm temperatures provide ideal conditions for mosquitoes. All rice fields (RFs) in the area are irrigated by large irrigation canals from rivers and rainfall. The water is received from a main reservoir and supplied to smaller canals present throughout RFs where two varieties of rice (MR219 and MR220) are cultivated. For the purpose of the survey, RFs were divided into three stations, with two plots at each station, two replicates in each plot and three sampling sites in each replicate. Plot surface areas ranged from 0.76 to 1.89 hectares. All 36 sites were visited during the rice growing season. In the year and half study period, typical cultivation included a first cycle (February 2003 to July 2003), a second cycle (August 2003 to April 2004) and a third cycle (April 2004 to October 2004). Except for the brief drainage period during these cycles, RFs were flooded continuously. As there were no other ecotones in the vicinity, e.g. marshes, RFs proper (RFP) and drainage canals (DCs) were the main breeding habitats for aquatic insects. The categorization of rice cultivation phases was adopted from Mogi & Miyagi (1990), Abu Hassan (1994) and Che Salmah *et al.* (1999). RFs were classified into one of six phases of cultivation: plough phase (Pp), germination phase (Gp), young phase (Yp), tiller phase (Tp), mature phase (Mp) and fallow phase (Fp). All phases were present at the 36 sites.

Sampling

Since this study was aimed at only *Cx. gelidus*, corixids, coenagrionids and dytiscids, other aquatic insects were not enumerated thus following Das *et al.* (2006). During each rice cultivation cycle (CC), mosquito larvae and aquatic insects were sampled from RFP and DCs adopting others (Chambers *et al.*, 1979; Takahashi *et al.*, 1982; Stewart *et al.*, 1983; Service, 1993; Abu Hassan, 1994). Sites for sampling were marked in both RFP and DCs using wooden stakes numbered according to the replications. Sampling was performed each week from February 2003 to October 2004. Metal dippers of 350 mL capacity fitted with handles *ca.* 1.2 m long. At each sampling occasion, dips were taken along the margin of RFP and DCs for 10 minutes per site. Dips of water were strained through a nylon bag (13 x 18 cm) and sampled insects brought to the laboratory.

Data collection and analysis

In the laboratory, some samples were preserved in bottles with 75% ethanol, separated by stages and counted. In some cases, first and 2nd instar larval stages were reared at room temperature (26ºC±1ºC, relative humidity 75%±10% and photoperiod 13:10 h, 1 h dusk) until fourth stage and then identified to species under a dissecting microscope (Olympus CX41; Olympus, Tokyo, Japan). Aquatic insects were identified morphologically to families, genera and species using appropriate taxonomic keys (Usinger, 1956; Yano *et al.*, 1981; Mores *et al.*, 1984). Mosquito immature stages (larvae and pupae) were identified to subgenera or species using the keys of Stojanovich & Scott (1966), Reid (1968) and Rattanarithikul & Panthusiri (1994). Density was defined as the number of individuals per dip. This parameter was determined for (i) *Cx. gelidus* collected from RFP and DC and (ii) corixids, coenagrionids and dytiscids from RFP. To assess temporal variations of population levels, densities were related to CCs. The interactions of *Cx. gelidus* with the predators were examined in RFP when there were increased numbers of L1-L2 and of predators. Analyses were performed using Kendall's tau-b correlation analysis from the statistical software package SPSS for Windows v.11.0 (SPSS Software, Inc., New York, USA) (George & Mallery, 2003).

RESULTS

Culex gelidus

A total of 20,913 individuals of mosquito immature stages (L1-L2, L3-L4, and pupae) were collected, of which 99.9% were collected from RFP and 0.1% were from DC.

All stages were found in RFP, but only the two last were collected from DC. L3-L4 was the most common stages, comprising more than 45.7% of the total. Its abundance was higher in RFP than in DC (98.8% *vs.* 1.2%, respectively).

During the first CC, the density of *Cx. gelidus* in RFP was 4.48 individuals (larvae + pupae) per dip during Gp. It increased and remained almost constant throughout the Yp with small peaks (11.32 and 9.71 individuals per dip) during the 3rd and 6th weeks. The mosquito was almost absent (0-19 individuals per dip) when RFs were in Tp and Mp. The density was high and almost constant during the Fp with a major peak (325.9 individuals per dip) during the 17th week. In DCs, this species was absent (Figure 1).

During the 2nd CC, the density of *Cx. gelidus* was highest when RFP were in Pp with a major peak (116.08 individuals per dip) during the third week. It decreased sharply during Yp and Tp. The mosquito was almost absent (0-1.5 individuals per dip) during the 11th and 13rd weeks, but reappeared at the end of Mp.

During Fp, the density of the mosquito increased from 21.49 and 32.99 individuals per dip between the 17th and 19th weeks and then decreased to its minimum (0 individuals per dip) during the 21st week. From this week, the density of the mosquito increased to 91.57 individuals per dip during the 23rd week and then decreased sharply in the 25th week. In DCs, *Cx. gelidus* first appeared during the end of Tp and the beginning of Mp with a small peak during the 12th week. The mosquito was absent the following 6 weeks. During Fp, the density of the mosquito increased to a maximum at the 19th week and then decreased during the following week. The mosquito exhibited a third peak during the 21st week and was absent thereafter (Figure 1B). During the 3rd CC, *Cx. gelidus* was almost absent (0-0.66 individuals per dip) during Gp, Yp, Tp and Mp. During Fp, its density increased from 11.66 and 22.57 individuals per dip between the 15th and 16th weeks. The mosquito was not encountered during from the 17th to the 19th weeks. The density of the mosquito peaked during the 20th week (302.15 individuals per dip) and then decreased sharply thereafter. In DCs, this mosquito was not encountered (Figure 1C).

Insect predators

A total of 13,176 corixids were collected, of which 71.8% were collected from RFP. They were most common during the first and 2nd CCs, comprising 43% and 33% of the total, respectively. A total of 1,508 coenagrionid larvae were collected, of which 64.6% were found in RFP. Coenagrionids were more abundant during the first and 2nd CCs, representing 60.8% and 25% of the total, respectively. A total of 1,470 dytiscid larvae were collected, of which 64.6% were found in RF proper. Dytiscids were mostly encountered during the 2nd CC, comprising 46.3% of the total (Table 2). Other taxa encountered were Baetidae, Libellulidae, Belostomatidae, Nepidae, Notonectidae, Pleidae, Hydrophilidae and Hydrochidae.

Coenagrionid population dynamics and interactions with *Cx. gelidus*

The genera encountered were *Pseudagrion*, *Ischnura*, *Aciagrion* and *Cercion*. The density of *Coenagrionids* was low during Gp and Yp. From the end of the latter phase, their density gradually increased attaining 3.9 larvae/dip at the 12th week and its maximum (7.3 larvae/dip) at the beginning of Mp. It decreased to less than three individuals thereafter. Coenagrionid larvae exhibited a significant negative correlation (r = -0.344, $P<0.05$) with densities of *Cx. gelidus* especially during Fp. The peak in the population of the mosquito coincided with a decrease in that of the predator (Figure 2).

Corixid population dynamics and interactions with *Cx. gelidus*

Only the genus *Micronecta* was found. The densities of corixids were low throughout Gp and Yp. From the end of Yp, the density of this insect increased progressively and reached its major peak (95.8 indiv./dip) at the 9th week when rice field were in Tp. A second but minor peak also was observed during this phase. The population decreased gradually during the next phases, but corixids tended to rebound at the end of Fp. There were strong negative correlations between the population densities of the two insects during the first (r = -0.477, $P<0.01$) (Figure 3A) and the 2nd (r = -0. 367, $P<0.05$) (Figure 3B) CCs. The peaks in the population of the mosquito

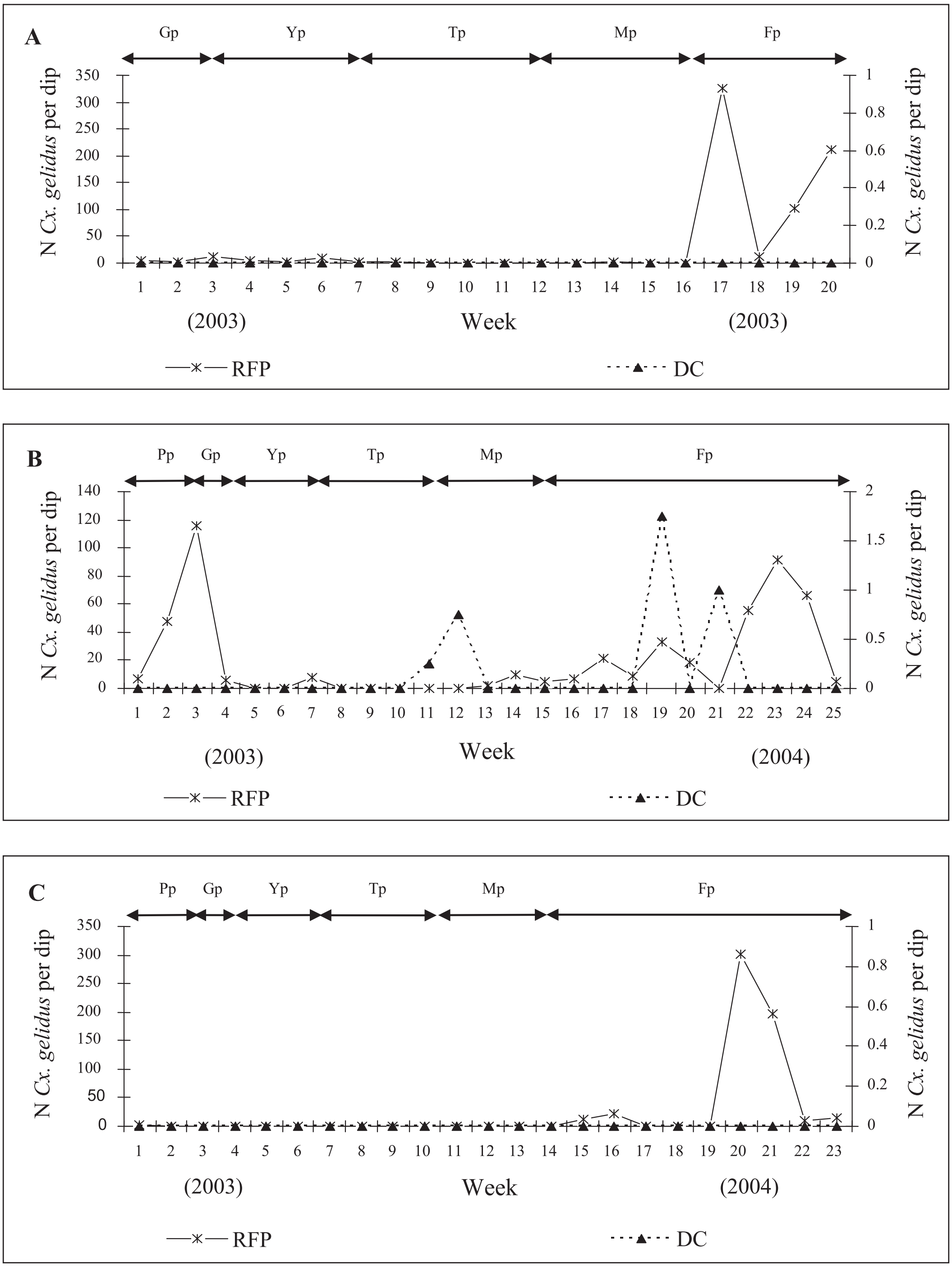

Figure 1. Seasonal densities of *Cx. gelidus* and aquatic insects at different rice growing phases during first (A), second (B) and third (C) cultivation cycles

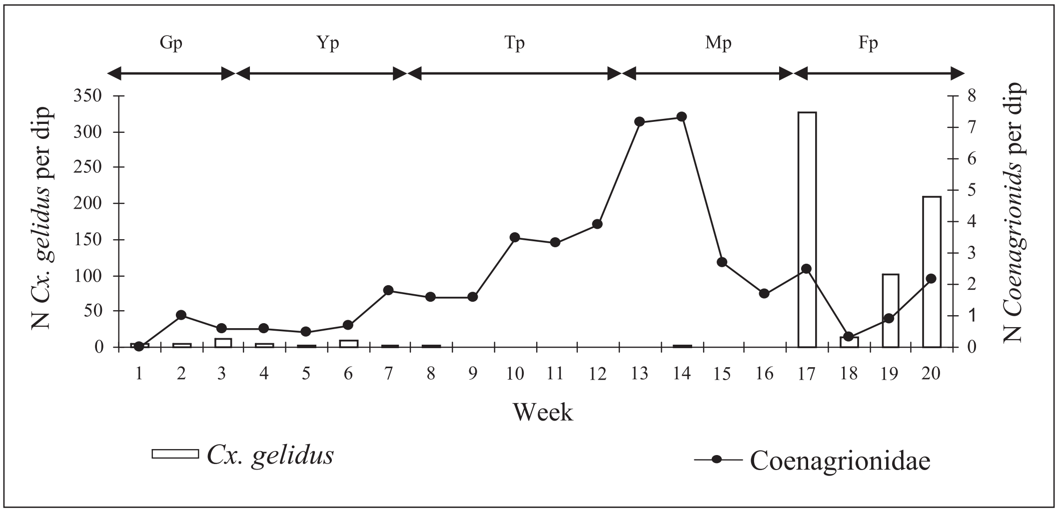

Figure 2. Interactions of *Cx. gelidus* immatures stages with Coenagrionidae in rice fields proper (RFP) during the first rice cultivation cycle

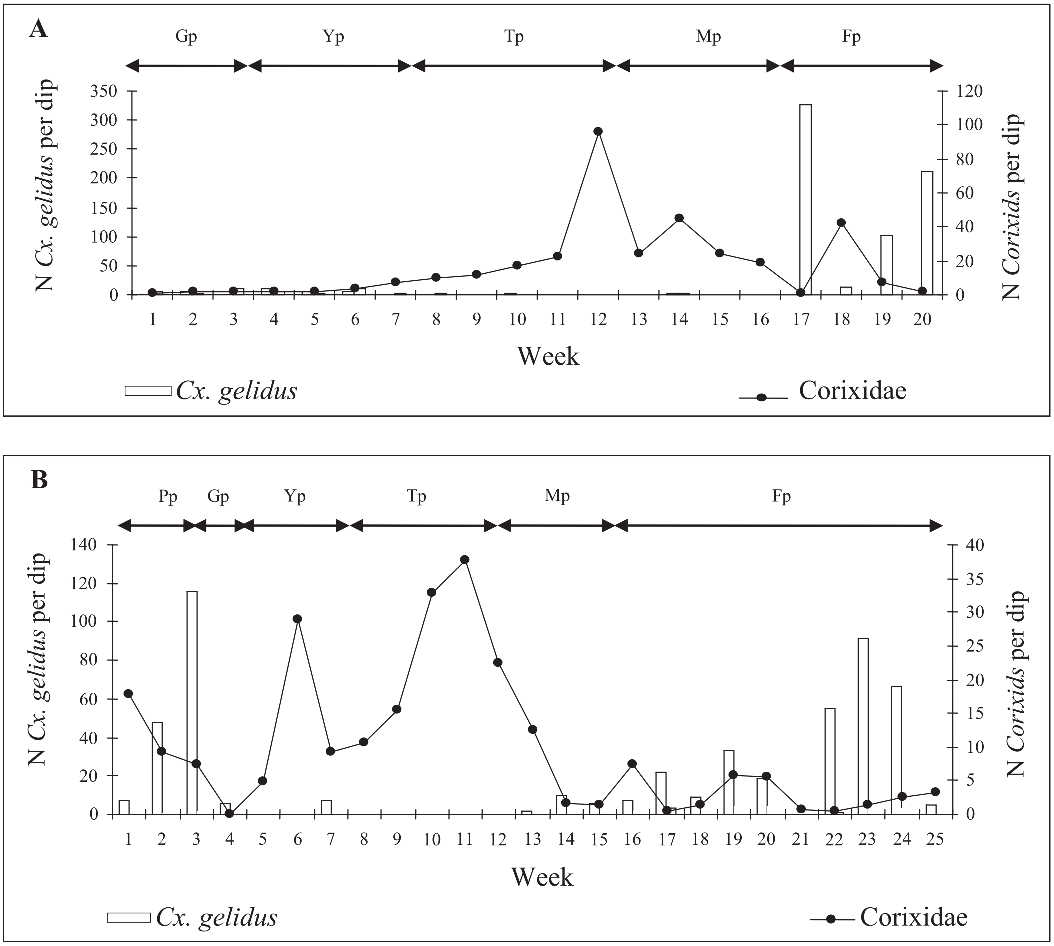

Figure 3. Interactions of *Cx. gelidus* immatures stages with Corixidae in rice fields proper (RFP) during the first (A) and 2nd (B) rice cultivation cycles

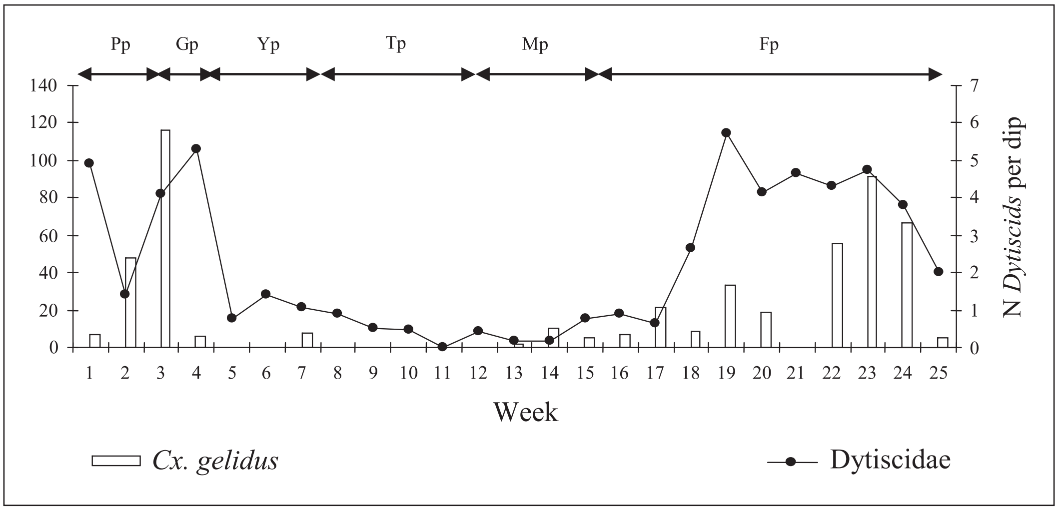

Figure 4. Interactions of *Cx. gelidus* immatures stages with Dytiscidae in rice fields proper (RFP) during the 2nd rice cultivation cycle

coincided with a decrease in that of the predator during both cycles.

Dytiscid population dynamics and interactions with *Cx. gelidus*

The genera encountered were *Canthydrus*, *Laccophilus* and *Hydrovatus*. The density of dytiscids peaked during the first week (4.9 individuals/dip), decreased at the end of Pp and increased again to its maximum (5.3 individuals/dip) at the beginning of Yp. The population decreased gradually during Tp and dytiscids were almost absent during Mp. They rebounded and increased to a major peak at the 19th week (5.74 individuals/dip) during Fp. Their density remained high and nearly constant during the 4 next weeks, but decreased thereafter. Dytiscids exhibited a strong positive correlation with *Cx. gelidus* densities ($r = -0.396$, $P<0.01$). The peak in the mosquito population during Gp and the high and constant densities during Fp corresponded to increased dytiscid density (Figure 4).

DISCUSSION

Despite the close proximity of DCs and RFP, both *Cx. gelidus* and insect predators were present in lower abundance in DCs. This was probably related to how irrigated rice fields function. In general, the water from the main reservoir is supplied to the entire field uniformly through irrigation canals. Aquatic habitat insect productivity has often been associated with its degree of stability (Walker *et al.*, 1991). Flooded paddies have greater stability and the feeding stages of insects may have abundant food resources as the water is stagnant thereby allowing plankton development. After a certain period, the stagnant water is discharged from the rice fields through drainage canals and discarded (Johnson & Miller, 1973). Drainage canals hold water almost all year round, contain more vegetation for longer period, and also provide refuge for aquatic predators when rice fields have been artificially or naturally dried (Abu Hassan, 1994). Drained water has high concentrations of nutrients and soluble salts that can interfere with the uptake of other nutrients. These chemicals may inhibit microbial growth and consequently prevent larval growth in DCs. Water in DCs also has increased turbidity. According to Paaijmans *et al.* (2008), there are many possible ways in which, water turbidity can have effect on mosquitoes. For example, suspended soil particles can increase water temperatures at midday, thereby forcing near-surface dwellers such as *Culex* larvae to remain submerged for long periods of time. There may also be direct interference of soil particles with larval feeding that would slow the larval growth and decrease survival (Ye-Ebiyo *et al.*, 2003). *Culex gelidus* was almost absent from DCs. Thus, a possible explanation of this absence is that this mosquito can discriminate habitats for egg depositions that enhance success for its offspring. The observed low prevalence in DCs is likely an adaptation to prevent unsuccessful development.

In RFP, L1 and L3 larvae of *Cx. gelidus* were highly present during the first cycle. The abundance of young instar larvae has been often correlated with increased oviposition. Mutero *et al.* (2004a) working in Kenyan rice fields observed increased densities of young *An. arabiensis* larvae one week post-rice transplantation. They suggested that the increase in larval population level was the result of increased egg-laying. In fact, mosquito oviposition behaviour is strongly influenced by visual stimuli. Evidence has been produced which demonstrates that synthetic fertilizers applied to RFs induce the disappearance of water turbidity, thus creating a sharp visual contrast that is very attractive to gravid mosquito females (Mutero *et al.*, 2004b). Although fertilizer content was not measured in the current study, in general, fertilizers are applied to nurseries, ploughed and newly transplanted fields in most RFs of tropical Asia (Das *et al.*, 2006). Indeed, the earlier cycle will tend to receive fertilizers first as it starts the rice growing season.

There was clearly a relationship between the cultivation phase and the densities of *Cx. gelidus*. Overall, the plough/germination and fallow phases provided better breeding conditions and the observed temporal abundance patterns could be explained on the basis of RFs evolution, well studied previously (Mogi & Miyagi, 1990; Abu Hassan, 1994; Forattini *et al.*, 1994, Che Salmah *et al.*, 1999). In fact, after the start of flooding, ploughed/germinating fields exhibit gradual changes in appearance with an initial "open/flooded" aspect turning to young fields with rice plants <30 cm, almost entirely exposed to direct sunlight. These changes result in a vigorous vegetative aspect during Tp when the water surface is half-shaded by the plants. This period is the time when most of the prey species have established their populations, thus providing ample food supply for the predator species. Vigorous vegetative growth may cause mechanical obstruction, thereby prevents oviposition (Forattini *et al.*, 1994). The progressive shading of the water surface results in tiller fields. They further develop to fallow fields characterized by a reduced vegetation cover and depressions filled with less clear water. This aspect of RFs water has been reported very attractive to gravid females, which may explain the high prevalence of *Cx. gelidus* during Fp.

In rice fields, predator ability has been often related to the presence of alternative preys, because they can attract predators. Benfield & Minello (1996) and Luzier & Summerfelt (1997) observed that the foraging behaviour of some shrimp reduce predator efficiency to detect and catch mosquito larvae. Chase *et al.* (2002) and Blaustein & Chase (2007) recorded reduced predation responses by insect predators on rice field larval mosquitoes. They suggested that the reduction was a result of the presence of controphic species that served as alternative preys. In the present study, the predators encountered included highly predaceous coenagrionids, which consume large numbers of larvae (McDonald & Buchanan, 1981) and corixids. Although their predation ability was not specifically analysed in the present study, the population level of the two predators declined when *Cx. gelidus* densities increased. This finding corroborates the earlier reports that *Culex* larvae showed positive correlation with predators (Victor & Reuben, 1999). Both insect predators peaked in tiller phase. This period is earlier than the time of mosquito density peak. Due to this time lag, a proportion of the mosquito larval population escapes predation thus allowing them to develop. It is usually assumed that in rice fields, "predator pressure" is the major force other than parasitism and pathogenic infection that reduces the abundance of larval JEV vector populations (Mogi *et al.*, 1980; Miura & Takahashi, 1988; Das *et al.*, 2006). Therefore, the observed high densities of the mosquito during Fp could be partly explained on the basis of feeding preference of the predator that play an important role in prey survival. For example, the ceonagrionid *Pseudagrion salisburyense* prefers chironomid and psychodid to other preys (Chutter, 1961), while corixids are preferred preys for *Anax junius* (Folsom, 1978). In contrast, dytiscids showed a strong positive correlation with *Cx. gelidus* densities. When dysticids reached their peak of abundance during Gp and Fp, the population level of the mosquito declined, but rebounded rapidly during the next cycle.

To address the efficiency of insecticide-based control of mosquito-borne diseases including JEV, Gu & Novak (2005) argued for the need to identify and direct larval control efforts to the most productive habitats. There is a close association between predation level and adult body and population sizes. Indeed, low predation pressure will tend to produce larger bodied adults and higher population density than high predation pressure (Agudelo-Silva & Spielman, 1984; Klowden *et al.*, 1988; Dieng *et al.*, 2002). Large body size has a positive effect on vector capacity (Walker *et al.*, 1998; Schneider *et al.*, 2004). Several parameters come into play when the vectorial capacity of a mosquito for an arbovirus is considered (*Reisen*, 1989). In particular, population density plays a crucial role in the competence of a vector. Indeed the more individual vectors are present, the more likely they will be able to transmit a pathogen. Therefore, it is likely that RFP will produce adult *Cx. gelidus* at great number as it contains small numbers of weak predators during Fp, a period of increased mosquito density. In DCs, partial predation could reduce competition by having fewer individuals surviving to compete for food. These individuals may benefit from a reduction in competition for food and emerge with a large body size. Thus, in this often neglected sub-habitat, an increase in adult body size is possible and this could potentially result in an increase in vector capacity. In addition, due to its open aspect and the permanence of water, DCs can be an attractive habitat for ovipositing females. Therefore, the vigorous vegetative growth and the increased abundance of predators during Tp may contribute to DCs colonization. The turbid aspect of DCs water is also important in mosquito survival. An increased turbidity can decrease the chance of being preyed upon due to a lower visibility (Paaijmans *et al.*, 2008). In this regard, DCs should be considered for the control of JEV vectors. For example, due to its small size, DCs may require only small amounts of insecticides to achieve population control of *Cx. gelidus* and other JEV vectors.

In aquatic habitats such as RFs, predation is a major mortality factor responsible for the maintenance of vector density below a critical threshold where disease transmission could not occur. The results of this study indicated that *Cx. gelidus* is present throughout the rice growing season with greater abundance during the Fp. This period is the time when most predators appeared in small numbers. Clearly, this limited predation pressure will tend to result in outbreaks of *Cx. gelidus*. For successfully controlling this JEV vector, any operational control programme should consider larviciding just before and during the Fp.

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