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Survival Rates of *Culex tritaeniorhynchus* (Diptera, Culicidae) Larvae in Fallow Rice Fields before Summer Cultivation

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Abstract: Survival rates and mortality factors were studied with larvae of *Culex tritaeniorhynchus*, a vector mosquito of Japanese encephalitis (JE) virus, by field experiments in fallow rice fields before summer cultivation in Nagasaki, Japan. Adult emergence rates were very low in experimental quadrats with a natural complex of predators including aquatic insects and fishes, the average being 0.02. Higher the predator density, lower the emergence rate. Emergence rates increased notably in enemy-free cages where partial drying and unsuitable quality of water were main mortality factors. The mortality rate due to predation was considered to be density-independent and less influenced by the yearly fluctuation of weather conditions than the mortality rate due to drying or unsuitable quality of water. It was also considered that chemical control in spring or early summer is ineffective to suppress the peak abundance in midsummer and consequently to prevent the JE epidemic. Chemical control in this early season may even be followed by population explosion in midsummer through the elimination of predators. It is desirable that control in this early season depends on methods highly specific to mosquitoes including *tritaeniorhynchus* such as the application of mass-produced parasites to breeding places. Adult control by light traps operated at animal houses may be most practical at the present time of Japan.

Evaluation of various mortality factors in immature stages as well as in adults is indispensable for the success in the integrated control of mosquitoes. This, of course, is true for *Culex tritaeniorhynchus* which is a main vector of Japanese encephalitis (JE) virus in Japan and some other countries. The purpose of this study was to know survival rates and mortality factors with larvae of this mosquito in fallow rice fields before summer cultivation and, based on the results, to discuss methods to control the species.

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PRACE AND METHODS

The study was done in a rice field area in the suburbs of Nagasaki City, Japan. This was a main field for our ecological study on rice field mosquitoes. Fallow rice fields include wet fields and dry ones, and the former is the main breeding source in spring and early summer of *tritaeniorhynchus* and other mosquitoes which prefer open ground pools as larval habitats. Dry fields have water only for a short period of days after heavy rains and their contribution to the reproduction of rice field mosquitoes is relatively small in usual circumstances. Therefore, all the experiments were done in wet fields. For further description of the study area and of fallow rice fields, see Mogi (1978, Section 2).

Survival rates under the presence of predators

A bottomless metal frame of 1 m^2 was pushed into the mud of rice fields quickly not to disperse predators (Fig. 1A). Three frames were set in each of four rice fields. Weeds in the frame were dense in general and water depth did not exceed 10 cm during the experiment. Egg rafts of *tritaeniorhynchus*, which had been laid in the previous night, were released into frames on 26 April 1972 after counting the total number of eggs. Larvae and pupae in each frame were dipped, counted by instars and returned into the frame every two days until extinction of larvae or completion of adult emergence. Then, predators in the frame were collected by filtering all the water through

Table 1. Coefficients to convert relative density per dip into absolute density per m^2 (after Wada and Mogi, 1974)

Developmental stage	Expected no. per m^2 when no. per dip is one
1st instar	256
2nd instar	235
3rd instar	167
4th instar	177
Pupa	141

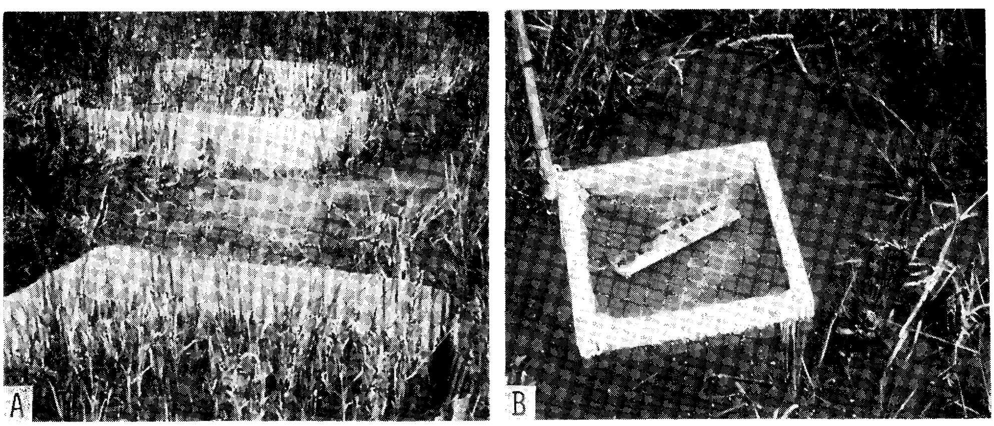

Fig. 1. Experimental devices. A. Metal frame. B. Floating cage.

nets of fine meshes. Predators found on the mud after drainage were collected, too. They were preserved in formalin solution for the later identification and count. Ten or 20 dips were taken per frame per day. Relative density of *tritaeniorhynchus* per dip was converted into absolute density per m^2 (= the absolute number per frame) with coefficients reported already (Table 1). Contamination with wild larvae was unlikely since density of *tritaeniorhynchus* larvae in rice fields was very low in this early season. In fact, neither larvae nor pupae were encountered in the frame before and after the experiment.

Survival rates in enemy-free cages

Newly hatched first instar larvae of a known number were released into cloth nets suspended into water from the floating frame of 30×30 cm tied with a string to the post put in rice fields (Fig. 1B). The number of suvivors was counted every two days. To keep the water in nets as natural as possible, nets of larger meshes were used following the larval growth. Five nets were distributed to five rice fields on 26 April 1973, and 10 nets to five fields on 27 April 1974.

RESULTS

Survival rates under the presence of predators

Absolute numbers of immature *tritaeniorhynchus* per 3 m^2 (sums of absolute numbers in three frames set in respective rice fields) are presented in Table 2. The egg number was based on the direct count before release, but larval and pupal numbers were estimated from the average number per dip. In general, the total number decreased with days after release, but the reversal in numbers occurred three times. The estimated number on the 4th day was smaller than that on the 6th day in both Rice-fields II and IV. The census on the 4th day was done in the rain, therefore dipping efficiency may have been lowered through the reduced proportion of larvae staying at the water surface. Also, the estimated number on the 10th day was smaller than that on the 12th day in Rice-field IV. This reversal may be attributable to the sampling error.

Pupae were collected from three rice fields, but neither fourth instar larvae nor pupae were recovered from Rice-field I despite careful examinations. For the calculation of adult emergence rates, numbers of both hatched larvae and emerging adults must be known. One estimate of the former is obtainable by multiplying the number of released eggs by 0.85, the hatch rate observed in the outdoor insectary. However, egg mortality may not be negligible in the field. Also, the number of emerging adults is unknown since pupal exuviae were rarely discovered even from frames where pupal density was high. Emergence traps were not employed. Therefore, a regression method was applied.

If the daily survival rate (p) is constant throughout the period from hatch to adult emergence, the number of survivors (N_t) on the t-th day after hatch is expressed as follows :

Table 2. Survival and development of *Culex tritaeniorhynchus* in fallow fields before summer cultivation

Rice field	Developmental stage	Days after release											
		0*	2	4	6	8	10	12	14	16	18	20	22
I	Egg	3429**											
	1st instar		1898	820	257								
	2nd instar				557	210	22						
	3rd instar					50	49						
	4th instar												
	Pupa												
	Total	3429	1898	820	814	260	71	0	0	0	0	0	0
II	Egg	3424											
	1st instar		2837	897	616								
	2nd instar				1697	744							
	3rd instar					250	758	84	8				
	4th instar						18	544	322	54	18		
	Pupa									126	148	14	
	Total	3424	2837	897	2313	994	776	628	330	180	166	14	0
III	Egg	3532											
	1st instar		2616	2615									
	2nd instar			93	349	82							
	3rd instar				17	250	92						
	4th instar						81	80	45				
	Pupa									27			
	Total	3532	2616	2708	366	332	173	80	45	27	0	0	0
IV	Egg	3261											
	1st instar		2128	462	39								
	2nd instar				639	140							
	3rd instar					175	79						
	4th instar						68	169	152				
	Pupa									63	7		
	Total	3261	2128	462	678	315	147	169	152	63	7	0	0

* Egg rafts were released into fallow fields.
** No. released.

$$N_t = N_o p^t$$

where N_o is the initial number of hatched larvae. Hence,

$$\log N_t = \log N_o + t \log p$$

which expects the linear regression of the logarithmic number of survivors on t. The observed tendencies are shown in Fig. 1, where the number of survivors is expressed as the total number in Table 2 plus 1. In three rice fields where pupae were recovered, the number of survivors dropped rapidly with the expected start of adult emergence. Also, larvae disappeared from Rice-field I suddenly 12 days after release. Therefore, regression equations were calculated for the points before these sharp declines, and all equations proved to be significant by F-test ($P<0.01$). Numbers of hatched larvae and emerging adults were obtained by giving the equation the value of t corresponding to the mean starting time of each stage (the time when half the population entered each stage) indicated by arrows in Fig. 1. The mean hatch time, 1.53, was observed in the outdoor insectary with egg rafts laid with released ones. The mean emergence time was obtained

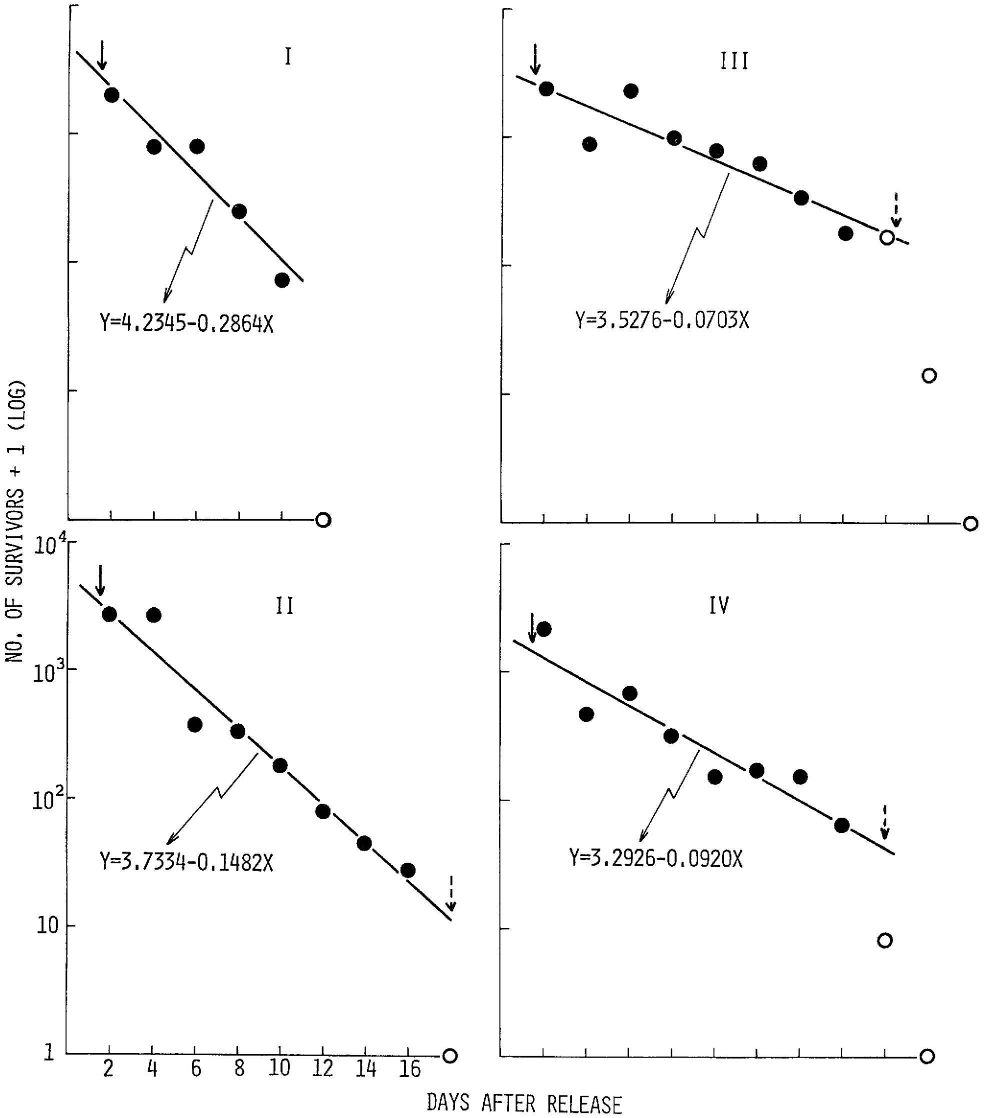

Fig. 2. Survival of *Culex tritaeniorhynchus* larvae under the presence of predators. Hollow circles were not included in the calculation of regression lines because of expected start of adult emergence or sudden increase in the mortality rate (Rice-field I). Solid arrow: Hatch time of half eggs. Broken arrow : Emergence time of half adults.

by adding two days to the mean pupation time calculated from Table 2. Also, p was calculated from the slope of regression lines. The results are presented in Table 3.

The estimated hatch rate was markedly small in Rice-field IV. However, the actual number of first instar larvae recovered from this rice field 2 days after release was not very small compared with those collected from the other rice fields (Table 2), therefore the reliability of this estimate is somewhat doubtful. As mentioned earlier, the very small number of larvae collected at the next census was probably due to the dipping efficiency lowered by the rain. Excepting this point, we can obtain a regression equation $y=3.4083+0.1001x$, which gives 1780 as another estimate for the number of hatched larvae. This may be closer to the actual one. The egg numbers multiplied by 0.85 (hatch rate in the insectary ≒ insemination rate) make 2915, 2910, 3002 and 2772, from which the estimated numbers of hatched larvae were not remarkably different (about 0.9 of or a little larger than the former) except Rice-field IV. This indicates that egg mortality was small. A possible mortality factor in the egg stage is predation by fishes, but the activity of fishes is considered to be restricted in fallow rice fields where weeds are dense and pools are often isolated each other.

Table 3. Estimation of hatch, emergence and daily survival rates of *Culex tritaeniorhynchus* in fallow rice fields before summer cultivation

Rice field	No. of eggs released (A)	No. of 1st instar larvae hatched (B)	Hatch rate (B/A)	No. of emerging adults (C)	Emergence rate (C/B)	Daily survival rate
I	3429	2633	0.7679	0*	0.0000*	0.6755**
II	3424	2629	0.7678	170	0.0647	0.8431
III	3532	3210	0.9088	11	0.0034	0.7093
IV	3261	1417	0.4345	42	0.0296	0.8113
Total	13646	9889	0.7247	223	0.0225	

* From the direct observation.
** For the period from 2 to 10 days after release.

Table 4. Numbers of predators per 3 m^2 in fallow rice fields before summer cultivation

Rice field	Insecta					Osteichthyes	
	Odonata		Hemiptera	Coleoptera	Others	Cyprinida	Cyprinodontida
	Libellulidae Larva	Agrionidae Larva	Notonectidae Larva, Adult	Dytiscidae Larva, Adult		Cobitidae	Cyprinodontidae
I	566	17	40	17	1*		
II	108			14		3	
III	216	18	35		1**	2	2
IV	+			+			+

* Hemiptera : Nepidae (Adult).
** Neuroptera : Corydalidae (Larva).

On the other hand, adult emergence rates were very low in general, the maximum and the minimum being 0.06 and zero, respectively. As the developmental period was rather constant from rice field to rice field, the difference in emergence rates depended largely on the difference in daily survival rates, which closely correlated with the number of predators collected from experimental frames after the completion of adult emergence (Table 4). Frames set in Rice-field IV had to be removed before the predator collection not to disturb agricultural work, therefore predator groups observed during the routine census were indicated. Daily survival rates were clearly lower in rice fields with higher density of Libellulidae (dragonfly : larva), Agrionidae (damselfly : larva) and Notonectidae (backswimmer : larva and adult), all of which are voracious predators against mosquito larvae. Although identification was not done at the species level, some groups of predators were certainly composed of multiple species. For instance, Libellulidae included at least two species, each belonging to the genus *Sympetrum* and *Orthetrum*. Also, at least five species representing multiple genera were recognized in Dytiscidae. Therefore, it can be concluded that not a single species but a complex of predators was responsible for mortality in *tritaeniorhynchus* larvae in fallow rice fields.

Adult backswimmers can fly in and from the frame, but they were found constantly during the experiments in the frame set in Rice-field I. Therefore, the sudden disappearance of larvae released there could not be attributed to the mass invasion of adult backswimmers. A more likely explanation is that the lowering of water depth made it easier for bottom-inhabiting dragonfly larvae to prey on planktonic mosquito larvae. The effect of fishes on survival rates was not clear. The dense vegetation may have offered good refuges for mosquito larvae as well as for eggs.

Survival rates in enemy-free cages

Results are illustrated in Fig. 3. In 1973, no adults emerged from cages. Mortality was high in all cages immediately after release. This mortality may have resulted at least partly from transportation of newly hatched larvae and subsequent sudden changes in living conditions. After this initial crisis, survival rates increased in general, but large mortality was often caused by unwelcome visits of adult and larval Dytiscidae (predacious water beetle). This happening occurred at three of five cages and demonstrated this predator's excellence as a natural enemy against *tritaeniorhynchus* larvae. Probably, the beetle larvae invaded into cages from overlooked gaps between floats and nets, and the adults did from the air. White of floats might have been attractive to flying water beetles. From Cage II, no predators were found despite sharp declines leading to extinction as early as Cage III where predators invaded twice. It is possible that adult water beetles flew away from Cage II after devouring mosquito larvae. Lastly, strong winds with heavy rains on 8 May upset or stranded all cages. Thus, the experiment in 1973 ended in quite incomplete results, although high survival rates under the absence of predators were seen intermittently.

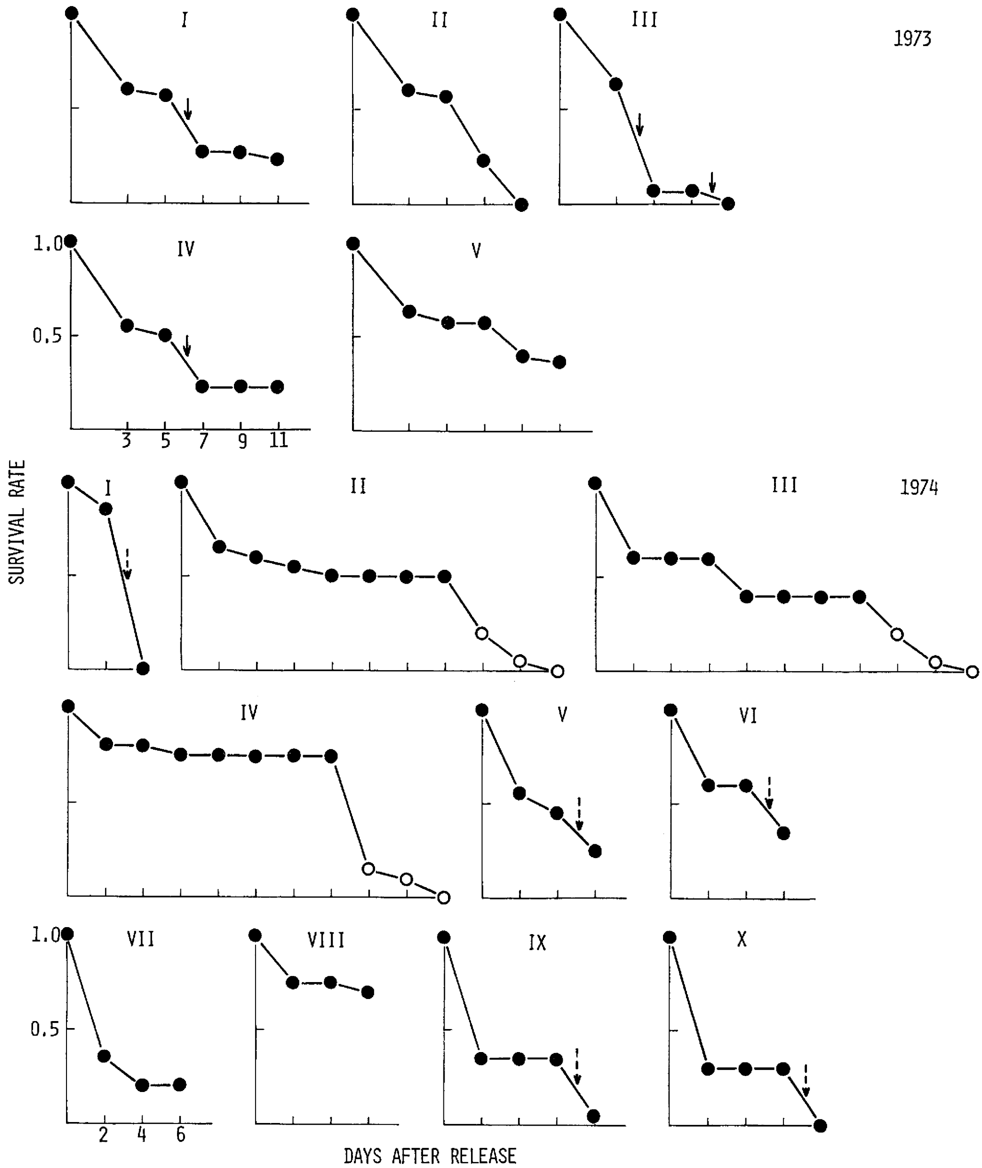

Fig. 3. Survival of *Culex tritaeniorhynchus* larvae in enemy-free cages. Hollow circle : Period of adult emergence. Solid arrow : Invasion of predators. Broken arrow : Partial drying. Curves ending before reaching zero indicate that experiments were stopped owing to bad weather or the start of agricultural work.

In the next year, the experimental device was inspected more closely before use and covered with wire gauze to prevent visitors from the air. Ten cages were distributed among five rice fields ; Cages I and II in one rice field, III and IV in another and the rest similarly. However, the water depth soon levelled down in the first rice field and Cage I was dried up 4 days after release. Therefore, Cage II, which had barely been saved from this accident, was moved to the rice field where Cages III and IV were. General tendencies in survivorship curves agreed with those in 1973 : initial large mortality was followed by much smaller one in general.

It should be remarked that the extents of the initial drop were different among cages, ranging from the minimun 0.15 in Cage I to the Maximum 0.70 in Cage X. It seems difficult to explain all this variation by the experimental error, since the reduction rates were often very similar in two cages set together in one rice field (Cages V, VI and IX, X). This strongly suggests that the extent of initial mortality was influenced by the water quality in respective rice fields. Here "water quality" is used in the broadest sense meaning physical, chemical and a part of biological aspects of water, for instance, temperature, the kind and concentration of inorganic and organic matter, the kind and density of micro-organisms and so on.

Invasion of predators was checked successfully in 1974, but partial drying often caused large mortality. Further, Cages V-X were obliged to be removed according to the start of agricultural work preparing for summer cultivation of rice plants. Consequently, the whole process from the release to the completion of adult emergence was recorded only for Cages II, III and IV, where survival rates just before the start of emergence were 0.50, 0.40 and 0.75, respectively. Ratios of survivors to larval numbers after the initial crisis were 0.77, 0.67 and 0.94. These figures well demonstrate large contribution of predators to high mortality rates of *tritaeniorhynchus* larvae in fallow rice fields. As it took about 16 days for released larvae to emerge as adults, the survival rate of 0.4 is equivalent to the constant daily survival rate over 0.94. Such high daily survival rates were not observed under the presence of predators (Table 3).

However, except Cage III (1973) and VI, IX, X (1974), mortality which was attributable to neither predation nor drying occurred among larvae having survived the initial crisis. This indicates that death due to unsuitable quality of water was not restricted to the period shortly after release.

DISCUSSION

Mortality factors in tritaeniorhynchus *larvae in fallow rice fields*

There were evidenced or suggested three major mortality factors, predation, unsuitable quality of water and drying. There may be additional mortality factors, for instance, parasitization, overcrowding and so on, but they are minor in usual circumstances.

Death due to drying occurred frequently in floating cages in 1974, but did not in 1973. This resulted from the difference in precipitation between the two years : precipitation during late April and early May was very small in 1974 (Mogi, 1978, Fig. 6). This mortality was intensified in the experimental condition where larvae were confined within a small cage which often produced smaller subdivisions in itself by creases of the cloth net when water became too shallow to hang the net. Under the natural condition, larvae can follow the decreasing size of pools more freely by active movement, therefore the probability to be dried up would be much smaller except when all the water evaporates.

Unsuitable quality of water includes two aspects. One is lack or shortage of necessary substances, for instance, shortage of food, and the other is the presence of harmful substances at the concentration sufficiently high to influence survival and development of mosquito larvae. The latter refers to substances of natural origin since insecticides and other man-made chemicals are not applied to fallow rice fields. Probably, this mortality was also enlarged in the present experiment by the following causes. First, as mentioned earlier, larvae which may have been weakened through transportation just after hatch were exposed to the sudden change in environmental conditions. Secondly, we may have been worse in the site selection for putting eggs than gravid females of *tritaeniorhynchus* since females would select water rich with larval food and refuse water which contains substances lethal to larvae.

Predation proved to be a very important mortality factor for the larval population of *tritaeniorhynchus* in fallow rice fileds. One notable feature of this predation is, as mentioned earlier, that not a single species but a complex of predators belonging to various taxa is involved. In this study, predators were collected from very small area of 9 m^2 in three rice fields. The list of predators will easily be enlarged by more extensive surveys. Another feature of this predation is that *tritaeniorhynchus* larvae occupy a very small portion of the whole food consumed by predators. In the first experiment, about 3,000 eggs were released per 3 m^2 and hatched larvae were consumed by, for instance in Rice-field II, about 100 dragonfly larvae. In this example, the number of *tritaeniorhynchus* larvae per dragonfly larva was at most 30, which was too small for a naiad to become an adult. Natural density of *tritaeniorhynchus* larvae is much lower than the level of this experiment. Average density per m^2 of *tritaeniorhynchus* larvae in fallow rice fields of the study area was 156 at the maximum during four years' observation and did exceed 100 only this time (Mogi, 1978, Fig. 14). Further, most larvae of *tritaeniorhynchus* are consumed in young stages. The life of predators certainly depends on organisms larger than *tritaeniorhynchus* larvae in individual size and/or population size.

These two features determine the nature of mortality due to this predation. Involvement of multiple species with different characters increases the stability of this mortality since the decreased number or activity of one species would be compensated by other species. Therefore, this mortality is considered to be less influenced by the yearly fluctuation of weather conditions than mortality due to drying or unsuitable quality of water. Independence of predators' lives from a prey *tritaeniorhynchus*, on the other hand, means that this mortality is density-independent for *tritaeniorhynchus* populations. These two, relative stability and density-independence, are considered to characterize the mortality due to predation in *tritaeniorhynchus* larvae in fallow rice fields. For more comprehensive disccussion on the role of various mortality factors in the population dynamics of *tritaenio-rhynchus*, see Mogi (1978, pp.233−236).

Consideration on the control methods against tritaeniorhynchus *in spring and early summer*

The total mortality in *tritaeniorhynchus* larvae was very large in fallow rice fields before summer cultivation of rice plants, and only about 0.02 of hatched larvae survived to adults under the presence of predators. If one gravid female produces 200 viable eggs, four adults emerge under the absence of egg mortality, which means that one of the two females (the sex ratio is 1:1) must take blood and lay eggs successfully for the population to be kept at a constant level. However, this requirement appears difficult to be satisfied in the field since the apparent oviposition rate of emerging females in the study area was about 0.25 on the average (one out of four emerging females) even when the peak oviposition rate in early July was included (Mogi, 1978, Table 12). Therefore, the growth of *tritaeniorhynchus* populations in late spring and early summer is hardly expected in usual conditions, or the population may even decrease from the level of overwintered ones. In fact, no distinct increase was observed for the *tritaeniorhynchus* population in the Nagasaki district until mid-June when adults of the third generation are expected to start emergence (as for the generation number, see Mogi, 1978, p. 203 ; as for the seasonal prevalence, see Wada et al., 1975, Figs. 1 and 2). On the other hand, JE virus is not introduced into the pig-mosquito infection cycle until late June in Nagasaki at the earliest as evidenced by the yearly first appearance date of pigs with 2-ME sensitive antibody (pigs newly infected with JE virus) (Wada et al., 1975, Table 1). Therefore, chemical controls of *tritaeniorhynchus* in fallow rice fields can be recommended only when they are effective in reducing the midsummer population of adult mosquitoes which is proportional to the yearly total number of human JE cases (Wada et al., 1975, Fig. 6). However, this preventive effect can hardly be expected. As conditions during the midsummer growth of *tritaeniorhynchus* populations, for instance, weather, are variable from year to year, small differences in population levels at the start of the growth would rather be cancelled than be amplified toward the peak abundance. Chemical controls done in a limited area are even dangerous since they may lead to the population explosion in summer under the absence of predators through the rapid reproduction of survivors reinforced by immigrants as exemplified by the small island experiment of Nishigaki (1970). This reaffirms the view presented in an earlier paper (Mogi, 1978, p. 250).

Therefore, the control of *tritaeniorhynchus* in fallow rice fields, if planned, should depend on methods strictly specific to mosquitoes including *tritaeniorhynchus* and with no or negligible risk of environmental pollution. The application of mass-produced parasites specific to mosquito larvae meets this need, therefore basic studies along this line is worth promoting. Another good method is adult control by light traps. Light traps operated at animal houses little influence predator populations. This and the other merits of light traps and their practicability in present-day Japan were emphasized in an earlier paper, too (Mogi, 1978, pp. 251−252).

The above consideration may be applicable to most other region in temperate Japan where rice plants are cutivated once a year in summer, because the complex of predators is expected to exist throughout the wet fallow fields although the species composition would be variable.

Situations in the Ryukyu Islands

In the Ryukyu Islands, situations are quite different from those in Japan proper. In this subtropical region, temperatures in winter and spring are much higher and rice plants are cultivated biannually. It was reported in Okinawa, a main island in the Ryukyus, that the feeding activity of *tritaeniorhynchus* females continues throughout the year and the population often reaches considerable levels in spring or early summer (Iha, 1971). This results in the earlier appearance of pigs with 2-ME sensitive antibody (pigs newly infected with JE virus) and the subsequent occurrence of human JE cases in early summer (Iha, 1971 ; Ura, 1976). In this circumstance, application of chemicals to rice fields in spring or early summer may be effective in reducing the number of human JE cases.

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休閑田におけるコガタアカイエカ幼虫の生存率と死亡要因

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長崎では，コガタアカイエカの越冬からさめた雌成虫は3月下旬に出現し，それ以後，6月末の田植までの期間は，主に休閑中の湿田から発生する．そこで，休閑田における本種幼虫の生存率と死亡要因を明らかにするために野外実験を行った．捕食性天敵が自然状態で存在する枠内に放された幼虫が成虫として羽化する率はきわめて低く，平均約2％であった．枠内の天敵密度が高いほど羽化率は低かった．天敵を除去した網内での生存率は顕著に高く，ここでは水の干上がりと水質不良が主な死亡要因であった．捕食による死亡率は密度非依存的で，また干上がりや水質不良による死亡率に比べて気象条件の年次的変動に影響されにくいと考えられた．殺虫剤による休閑田の幼虫駆除は盛夏の成虫個体数，ひいては日本脳炎患者数を減少させる効果に乏しいのみでなく，天敵を殺してしまうことにより本種が増殖しやすい条件をつくりだす危険もある．従って，この季節の駆除は，天敵に及ぼす影響のごく小さい方法に限定した方がよい．休閑田の幼虫に対する大量生産した寄生虫の散布，畜舎に集まる成虫のライト・トラップによる捕殺などは好ましい方法であろう．沖縄の条件は本土とはことなるので，上の考えをそのまま適用することはできない．

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