ESTIMATION OF ABSOLUTE NUMBERS OF DAMSELFLY NYMPHS (ODONATA: COENAGRIONIDAE) BY DIPPER SAMPLING IN CALIFORNIA RICE FIELDS WITH SEASONAL, SPATIAL DISTRIBUTIONS AND VEGETATION ASSOCIATION

T. MIURA, R. M. TAKAHASHI AND R. J. STEWART

University of California Mosquito Control Research Laboratory, 9240 S. Riverbend Avenue, Parlier, CA 93648

ABSTRACT. Estimates of relative and absolute density in rice field populations of damselfly nymphs (predominantly *Enallagma civile* with few *Ischnura denticollis*) were compared using the regression method. An equation, $X = Y \div 0.0016$, allows estimation of absolute density (X) from a relative density index (Y, dipper count). In the rice growing area of Fresno, California, nymphal population peaks appeared during June and August approaching 3–5 million per 0.405 ha (1 acre). Spatial distribution was theoretically represented fairly well by a negative-binomial distribution. The degree of clumping is one of overdispersed types; it is especially classified as a model of randomly distributed colonies with mean colony size fixed. Presence or absence of submerged vegetation markedly affected damselfly nymphal density but the biomass of submerged or emerged vegetation was not a significant factor.

INTRODUCTION

Damselfly nymphs are the most abundant aquatic insect inhabiting San Joaquin Valley rice fields in California (Miura et al. 1981). Odonata nymphs are generalized predators and usually prey on small crustacea, annelids and immature insects, including mosquito and chironomid larvae (Sailer and Lienk 1954, Merrill and Johnson 1984, Miura and Takahashi 1988). Although there are many other predators in the Central California rice fields, Miura et al. (1981) predicted that damselflies are a major contributor to mosquito predation in rice fields because damselflies dominate overwhelmingly in numbers and also share the same habitat as mosquito larvae.

Determinations of the total numbers of mosquito larvae and their predators in a given area are essential for the development of an integrated mosquito management program. Absolute densities of *Culex tarsalis* Coquillett larvae in Fresno rice fields have been estimated using dipper counts (Stewart and Schaefer 1983), and population densities and growth rates of the important mosquitofish predator, *Gambusia affinis*, in the same area were determined using capture-recapture methods (Miura et al. 1982, Stewart and Miura 1985).

Cochran (1953), Southwood (1978) and Downing (1986) suggested that regression analysis might be used to predict absolute population densities from relative indices, where a variable such as relative index is correlated with another variable such as absolute density of the population from which the relative index was determined.

The objectives of this study were: 1) to estimate the absolute population of damselfly nymphs from dipper counts, 2) to determine their seasonal and spatial distributions and 3) to define the relationship between damselfly nymphs and vegetational characteristics in the rice field.

MATERIALS AND METHODS

Study area: A series of 8.0 to 11.0 ha fields of commercial rice at the western edge of Fresno County, California, were used for this study. Rice has been grown in this area for many years on a continual basis because the land is poorly drained (Lost Hills clay loam) and easily accessible to canal water. Normally, rice culture starts during mid-April to mid-June. Larvae of *Cx. tarsalis* appear in fields 3–5 weeks after initial flooding. Predominant weeds within the rice fields are barnyard grass (*Echinochloa crusgalli* (Linn) Beauv.), sedge family (*Cyperus* spp., *Scirpus* spp.), water-nymph (*Najas* sp.) and stone wort (*Chara* sp.). The water-nymphs are especially abundant and form mats covering entire basins of rice fields which provide an ideal habitat for damselfly nymphs.

Dipper efficiency for collecting damselfly nymphs: Circular sampling enclosures were made from 30-cm high aluminum sheets (Stewart and Schaefer 1983) to give a sampling area of 1 m^2. At the start of each sampling day, 5 enclosures were placed at random within a rice paddy. A white enameled mosquito larval dipper (1-pint capacity) with a long handle (1.5 m) was used as the sampling device for relative abundance estimates.

Tests were performed 4 times between June and August when rice plant heights ranged from 0.4 to 1 m. Dip samples inside the enclosures were taken between 1000 and 1800 h with the dipper. Each enclosure was divided into quadrants by marks on the outside. The quadrants aided in sampling over the entire enclosed area and reduced bias caused by the individual samplers. Ten dip samples were taken randomly from each enclosure. The contents were counted and returned to the same place where taken after each dip. To reduce disturbances to the samples, dips from the same enclosure were taken at 2- to 3-min intervals.

After the dip samples were completed, absolute population densities of damselfly within enclosures were determined by the removal method (Southwood 1978); 5 sweep collections made by dragnets (20 × 14 cm with 1.5-mm opening mesh net) were pooled into one sample unit (Wada's "super unit," Wada 1962a) and preserved in a jar with 75% ethanol. Four or 5 sample units, depending on the density of the damselfly, were collected from each enclosure and these samples were examined under the stereoscope in the laboratory. Absolute population density of each enclosure was then determined by the regression method (Wada 1962a, Miura 1980).

The relationship between the relative estimate (dipper count) and the absolute estimate (removal method) for the enclosures was examined by the correlation method (Ostle 1954) and a linear regression equation was calculated.

Seasonal and spatial distributions: Concurrent with sampling *Cx. tarsalis* larvae with a dipper, we also conducted a field sampling program to study seasonal population changes and spatial distribution of damselfly nymphs in rice fields. Detailed sampling methods used were reported by Stewart et al. (1983). In brief, the sampling sites (100 sites per 8 ha) were selected evenly over the fields by using aerial photographs and the sites were marked with wooden stakes (2 × 2 × 100 cm) numbered over each field. One dip sample was taken at each stake and examined by eye for nymphs and other nontarget organisms. All captured taxa were recorded separately for each sample site. Samples were taken weekly throughout the rice growing season during 1980, 1981 and 1982.

Absolute abundance was estimated by using the linear regression equation calculated in the dipper efficiency study. The dipper collection data were also examined for goodness of fit to the theoretical distributions of Poisson (Anscombe 1948) and negative-binomial distribution (Bliss and Owen 1958). Iwao's "Patchiness regression indices" (Iwao 1968, Southwood 1978) were also calculated.

Vegetational association: Three vegetational conditions were investigated on newly constructed rice plots at the University of California, Kearney Agricultural Center, Parlier. They were: 1) normal rice stand with bare soil bottom (36 kg rice seed/ha planted), 2) dense rice stand (45 kg rice seed/ha planted) with bare soil bottom and 3) normal size stand with submerged vegetation (36 kg rice seed/ha; ca. 70% of soil surface was covered by mixed growth of *Najas* sp. and *Chara* sp.).

The 3 vegetational conditions were simulated in the rice plots which were arranged in a 3 × 3 Latin square design (Cochran and Cox 1957). Damselfly nymph densities were determined by taking 30 dip samples from each plot. The data obtained were examined by the Latin square method. This study was conducted during the 1985 rice growing season.

Relationship between damselfly densities and biomass of submerged vegetation at the western Fresno County rice fields was studied by taking random area samples within the rice field with a 0.045-m^2 (40-cm high) area sampler. The sampler was thrust as quickly as possible into the water and down into the substrate for about 5 cm, creating a watertight seal from outside the sampler. Damselfly nymphs were collected by scooping out all water contained within the sampler, screening through a 0.5 mm mesh sieve and releasing the contents in a shallow, white pan containing clear filtered water from the field. The nymphs were counted and recorded. All vegetation within the sampler from ground level up was cut and washed free of clinging insects and debris and then placed in plastic bags.

The vegetation samples were taken to the laboratory within a few hours of collection. Once there, they were removed from the bags, further washed of debris and then blotted dry with paper towels. They were then placed in a drying oven at 105°C for 24 h. The vegetation was then cooled in desiccation jars for 1 h before weighing. There were 132 samples collected periodically throughout the 1984 rice growing season. The data were analyzed using correlation methods.

RESULTS AND DISCUSSION

Dipper efficiency and the derivation of absolute estimate: Table 1 summarizes results of the dipper efficiency study. Average number of dipper counts from 20 enclosures was 1.35 nymphs per dip ranging from 0.2 to 2.13. Average predicted absolute population density per enclosure was 813.7 nymphs (range; 230–1,150).

Removal method was used to predict absolute densities within each enclosure (Table 1) because it is relatively simple and able to obtain reasonably precise estimations. We have found this method useful for estimating population densities of mosquito larvae in small confinements such as small artificial ponds, catch basins of storm drain systems, tree holes and cemetery flower vases (Miura 1980). Wada (1962b) used this method to estimate population size of immature stages of mosquitoes breeding in fertilizer pits and tide pools. Mogi et al. (1984) also

Table 1. Relationship between dipper counts, absolute density and removal numbers by net in 1-m^2 enclosures.

Enclosure no.	Mean no. nymphs/dip	Estimated[a] total no. nymphs/ m^2	Actually removed by drag net from 1 m^2	Proportion of population removed
1	2.13	1,150	675	0.59
2	1.73	820	473	0.58
3	1.17	1,090	672	0.62
4	1.73	1,100	682	0.62
5	1.20	940	594	0.63
6	1.13	800	473	0.59
7	1.53	1,130	504	0.45
8	1.50	900	365	0.41
9	1.20	800	473	0.59
10	1.07	530	406	0.77
11	1.30	720	410	0.57
12	1.53	900	547	0.51
13	0.75	500	183	0.37
14	1.90	1,000	330	0.33
15	1.60	800	474	0.59
16	1.55	790	318	0.40
17	0.65	456	372	0.82
18	0.20	230	154	0.67
19	1.15	740	357	0.48
20	2.00	878	463	0.53
Total	27.01	16,274	8,835	11.10
Mean	1.35	813.7	441.75	0.55

[a] By removal method.

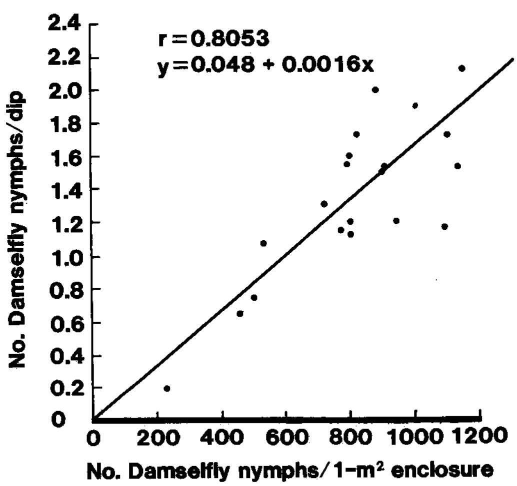

Fig. 1. Scatter diagram showing the relationship between absolute and relative densities with a regression line and correlation coefficient.

used this method to estimate absolute populations of immature stages of *Aedes baisasi* Knight and Hull, *Culex tuberis* Bohart and *Uranotaenia ohamai* Ohama inhabiting crab holes. The conditions that must be satisfied to use this method are summarized by Southwood (1978) and Service (1976). An important factor is having adequate sampling size because a sufficient number of organisms should be removed from the site at each sampling, whereby subsequent catches would be reduced, thus a large portion of the parent population should be removed by collecting (Zippin 1956). In this study, we have removed ca. 55.5% (33–82%) of nymphs from enclosures thus, we have obtained reasonably reliable estimations (Table 1).

Once dipper counts (Y_i) and absolute densities (X_i) from enclosures were determined, a scatter diagram (Fig. 1) was constructed to inspect the approximate form of the relationship between these 2 measurements (Y_i and X_i). The scatter diagram showed a strong tendency of a straight line relationship. The linearity was tested by correlation method; the correlation coefficient (r) was 0.805, highly significant at the 1% critical point. A linear regression equation was then constructed as $Y_i = 0.048 + 0.0016X_i$. If measurements of Y_i and X_i are perfectly proportional, the line must intercept at the origin ($Y = 0$, $X = 0$). Our calculated α was near zero and the hypothesis $\alpha = 0$ was unable to be rejected ($t = 0.538$) statistically. Equation $Y_i = \alpha + \beta X_i$ was reformulated in terms of our model, $Y_i = 0.0016X_i$, then absolute population densities (X_i) were derived from dividing dipper counts (Y_i) by 0.0016.

Seasonal and spatial distribution: Our field observations of adults indicated that *Enallagma civile* (Hagan) was the most abundant (ca. 80%) adult damselfly flying over the rice fields, followed by *Ischnura denticollis* (Burmeister). A few *Ischnura perparva* Selys and *Ischnura cervula* Selys adults were also observed. However, only *E. civile* and *I. denticollis* nymphs were collected from the study area.

Figure 2 shows the seasonal abundance of damselfly nymphs collected from the study fields over the 3-year sampling period. In the Fresno area, nymphal population peaks appeared during July and August each year approaching 3–5 million per 0.405 ha (1 acre). Their population reached to 2–4 million per 0.405 ha even in the fields planted with mosquitofish (predators of many invertebrates).

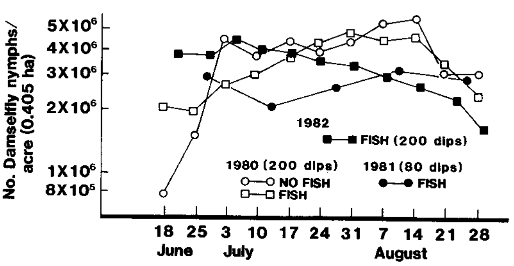

Fig. 2. Seasonal abundance of damselfly nymphs in Fresno rice fields during 1980, 1981 and 1982 seasons.

Table 2 shows typical field collection data by dipper, e.g., the July 2 collection contained 45 dips with no nymphs; 31 dips of 1 nymph, etc. Overall, nymphs were collected from throughout the rice fields indicating near random to clumped distribution patterns. Comparison of goodness of fit tests of nymphal distribution to Poisson and negative-binomial distributions are summarized in Table 3. Near the start of the season (first 2 collections) the population densities and variances were near equal, indicating a near random distribution; however, as the season progressed, the variances to mean ratios increased, indicating increases of clumpings. Later in the season the ratios were reduced again. This clumping pattern was also examined by Iwao's patchiness regression method (Iwao 1968, Southwood 1978) and the results, i.e., the relation of mean crowding ($\mathring{x}$) to mean density ($\bar{x}$) are illustrated in Fig. 3. The index of basic contagion (α) was greater than zero (0.716) and the density contagiousness coefficient (β) was near unity (0.945). Thus the distribution pattern of immature stages of damselfly in Fresno Westside commercial rice fields is one of overdispersed distributions. Iwao classified this type patchiness distribution, $\alpha > 0$ and $\beta = 1$, as a

Table 2. Spatial distribution patterns of damselfly nymph in a Fresno County rice field in 1980.

Frequency class (no. nymphs/ dip)	Observed frequency (expected frequency from best fit distribution)			
	July 2	July 9	July 16	August 27
	NB[a]	NB	Poisson	NB
0	45 (48.21)	41 (44.93)	30 (27.30)	57 (58.60)
1	31 (27.60)	34 (28.24)	33 (34.61)	23 (23.99)
2	16 (13.38)	13 (14.46)	18 (21.94)	13 (9.76)
3+	8 (10.81)	12 (12.37)	16 (13.15)	6 (6.55)
k	1.4401	1.5892		1.0132
x	0.9500	1.0400	1.2680	0.6869
s^2	1.4419	1.5337	1.4482	0.9112

[a] NB = Negative binomial distribution.

Table 3. Comparison of goodness of fit test of damselfly nymphal distributions in one rice field during 11 weeks of sampling to Poisson and negative-binomial (NB) distribution.

Week	$\bar{x}$	S^2	$S^2/\bar{x}$	Poisson S^2	Poisson df	Poisson *P*	NB (K_c = 1.7474) S^2	NB df	NB *P*
1	0.5300	0.7365	1.389	8.9440	3	0.0300	1.5278	2	0.4658
2	0.4900	0.5757	1.175	1.5582	2	0.4588	0.0730	1	0.7870
3	0.9500	1.4419	1.528	2.2655	3	0.5192	1.0988	2	0.5773
4	1.0400	1.5337	1.475	4.2361	3	0.2371	1.3788	2	0.5019
5	1.2680	1.4482	1.142	1.6672	3	0.6443	2.8642	2	0.2388
6	1.9700	3.4637	1.758	7.7820	4	0.0999	3.9663	3	0.2651
7	1.6364	2.3562	1.440	12.8896	5	0.0244	1.6665	4	0.7968
8	1.7000	2.6364	1.551	8.8571	5	0.1149	3.4972	4	0.4703
9	1.3400	2.1863	1.632	16.3934	4	0.0025	6.0154	3	0.1109
10	0.8687	1.1356	1.307	8.7332	3	0.0331	8.9841	2	0.0112
11	0.6869	0.9112	1.326	7.2197	3	0.0652	1.3091	2	0.5197

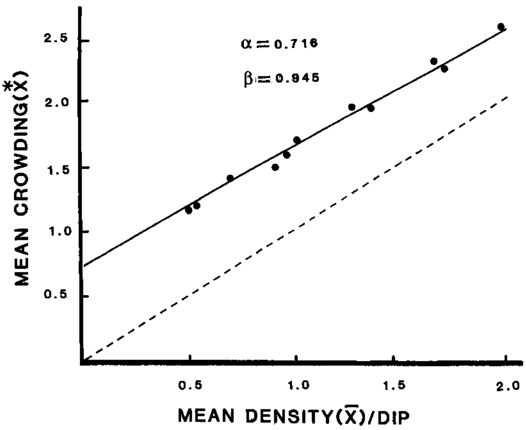

Fig. 3. Iwao's patchiness regression showing the relationship between mean crowding ($\overset{*}{x}$) and mean density ($\bar{x}$) for the distribution of damselfly nymphs in Fresno Westside rice fields.

Table 4. Habitat selection by damselfly nymphs in rice fields. Number of nymphs collected from 9 different rice plots (6.1 × 6.1 m).

	Replicates			
Vegetation	1	2	3	$\bar{x}$[a]
Normal rice stand with submerged vegetation	35	52	37	41.33 B
Normal rice stand with bare bottom	12	2	5	6.33 A
Dense rice stand with bare bottom	9	10	5	8.00 A

[a] Means followed by the same letter are not significantly different (α = 0.05, Duncan's multiple range test).

"model of randomly distributed colonies (mean colony size fixed)."

Vegetational association: Table 4 shows the results obtained from the vegetation association study. The data that were analyzed by a 3 × 3 Latin square method showed that only the treatment effect (i.e., vegetation) was highly significant (F = 20.69, df = 2.2; $P < 0.05$). Position and subsoil of the plots might have influenced vegetational growth, but no significant effect on the density of nymphal population was detected. Results from Duncan's multiple range test showed that more damselfly nymphs were captured in the plots with submergent vegetation than those without. In addition, it was found that the density of rice stands also had little effect on damselfly density. The rice fields with submergent vegetation created an ecological community entirely different from that of the bare bottom rice field. The submerged vegetation can be considered as a carpet on the bottom and it provided ideal resting and hunting places for many organisms, such as chironomid midge larvae and aquatic beetle larvae, as well as for the damselfly nymphs.

The results of analysis to determine the relationship between damselfly nymph density and biomass of submergent vegetation (*Najas* sp. and *Chara* sp.) showed no relationship between biomass of submerged vegetation and density of nymphs (r = 0.11, $P > 0.1$). Based upon these results, we have concluded that the absence and presence of submergent vegetation carpeting the bare bottom may be an important factor for habitat selection by damselfly nymphs but the biomass of the submerged vegetation has little effect on damselfly nymph density.

ACKNOWLEDGMENTS

This study was funded, in part, by USDA Cooperative Agreement No. 82 CRSR2-1010, and by a California State Appropriation for mosquito control research. We thank M. J. Pitcairn, University of California, Davis, for helpful criticism of the manuscript.

REFERENCES CITED

Anscombe, F. J. 1948. The transformation of poisson, binomial and negative-binomial data. Biometrika 35:246–254.

Bliss, C. I. and A. R. G. Owen. 1958. Negative binomial distributions with a common k. Biometrika 45: 37–58.

Cochran, W. G. 1953. Sampling techniques. Wiley, New York.

Cochran, W. G. and G. M. Cox. 1957. Experimental designs. 2nd Ed. Wiley, New York.

Downing, J. A. 1986. A regression technique for the estimation of epiphytic invertebrate populations. Fresh Water Biol. 16:161–173.

Iwao, S. 1968. A new regression method for analyzing pattern of animal populations. Res. Pop. Ecol. 10: 1–20.

Merrill, R. J. and D. M. Johnson. 1984. Dietary niche overlap and mutual predation among coexisting larval Anisoptera. Odonatologia 13:387–406.

Miura, T. 1980. Estimation of mosquito population size in confined natural breeding sites. Proc. Calif. Mosq. Vector Control Assoc. 48:108–112.

Miura, T., R. J. Stewart and R. M. Takahashi. 1982. Application of a capture-recapture method for estimating densities of mosquitofish in rice fields. Proc. Calif. Mosq. Vector Control Assoc. 50:54–57.

Miura, T. and R. M. Takahashi. 1988. A laboratory study of predation by damselfly nymphs, *Enallagma civile* upon mosquito larvae, *Culex tarsalis*. J. Am. Mosq. Control Assoc. 4:129–131.

Miura, T., R. M. Takahashi and W. H. Wilder. 1981. The selected aquatic fauna of a rice field ecosystem with notes on their abundances, seasonal distributions and trophic relationships. Proc. Calif. Mosq. Vector Control Assoc. 49:68–72.

Mogi, M., I. Miyagi and T. Okazawa. 1984. Population estimation by removal methods and observations on population regulating factors of crab-hole mosquito immatures (Diptera: Culicidae) in the Ryukyus, Japan. J. Med. Entomol. 21:720–726.

Ostle, B. 1954. Statistics in research basic concepts and techniques for research workers. Iowa St. Coll. Press. Ames, IA.

Sailer, R. I. and S. E. Lienk. 1954. Insect predators of mosquito larvae and pupae in Alaska. Mosq. News 14:14–16.

Service, M. W. 1976. Mosquito ecology. Appl. Sci. Publ. Ltd., London.

Southwood, T. R. E. 1978. Ecological methods with particular reference to the study of insect populations 2nd ed. Methuen and Co. Ltd., London.

Stewart, R. J. and T. Miura. 1985. Density estimation and population growth of mosquitofish (*Gambusia affinis*) in rice fields. J. Am. Mosq. Control Assoc. 1:8–13.

Stewart, R. J. and C. H. Schaefer. 1983. The relationship between dipper counts and the absolute density of *Culex tarsalis* larvae and pupae in rice fields. Mosq. News 43:129–135.

Stewart, R. J., C. H. Schaefer and T. Miura. 1983. Sampling *Culex tarsalis* (Diptera: Culicidae) immatures on rice fields treated with combinations of mosquitofish and *Bacillus thuringiensis* H-14 toxin. J. Econ. Entomol. 76:91–95.

Wada, T. 1962a. Studies of the population estimation for insects of medical importance. 1. A method of estimating the population size of mosquito larvae in a fertilizer pit. Endemic Dis. Bull. Nagasaki Univ. 4:22–30.

Wada, T. 1962b. Studies of the population estimation for insects of medical importance. 2. A method of estimating the population size of larvae of *Aedes togoi* in the tide-water rock pool. Endemic Dis. Bull. Nagasaki Univ. 4:141–156.

Zippin, C. 1956. An evaluation of the removal method of estimating animal populations. Biometrics 12:163–178.