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EVALUATION OF LINE TRANSECT METHOD FOR ESTIMATING *MORTONAGRION HIROSEI* ASAHINA ABUNDANCE IN A DENSE REED COMMUNITY (ZYGOPTERA: COENAGRIONIDAE)

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The results of the mark and recapture method for estimating the number of *M. hirosei* adults were compared to those of census counts using the line transect method carried out in the same habitat, a dense reed community established in brackish water. The mark and recapture method gave a daily estimate of about 1000 and 800 individuals of each sex at the peak population in early July of 2003 and 2004, respectively. These results did not agree with the estimate from the census counts, giving 600 ♂ at that time in the same habitat. Some limitations of the line transect method were discussed for estimates of adults perching in the understory of the dense reed community. However, a relationship was observed with regard to daily population estimates of the line transect method and the mark and recapture method, indicating that the line transect method can be an effective tool for monitoring populations of the endangered damselflies inhabiting such a dense plant community.

INTRODUCTION

Estimates of population size and knowledge of life history characteristics are important goals in conservation biology and such estimates are also essential for monitoring endangered species. The population ecology of an endangered brackish water damselfly, *Mortonagrion hirosei*, in a small, isolated reed community in Mie prefecture, Japan has been investigated using the mark and recapture method (e.g. WATANABE & MIMURA, 2003). Although this method in-

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volves many assumptions, there was a tremendous amount of effort and skill of researchers who must place individual numbers on this organism's vulnerable thin wings, as well as handle their small body when captured. Anaesthetizing such a small insects immediately after capturing is needed to stay still for a few minutes to write numbers, to measure the body size, and to identify the age, and also for the insects to show their normal behaviour after release. In addition, several days of good weather during the flying season are required; unfortunately, this is the rainy season in Japan. The high density of tough reed stems also inhibits sweeping a particular area with a net in order to capture successfully adults perching in the understory of the community without disturbance of other perching individuals. Furthermore, the impact of repeated recapturing might present an increased risk of injury to the adult insect. Therefore, census counts using the line transect method might provide a good alternative method of obtaining a population estimate, due to the lack of direct physical contact with the organism. The line transect method also requires fewer person-days of effort than does the mark and recapture method, and is appreciably more cost effective in terms of assessing damselfly numbers. However, it is not so easy to determine the relationship between the line transect method and the mark and recapture methods in order to obtain the estimated numbers of odonate species.

Various line transect methods are widely used for estimating animal abundance; these methods have been described in detail by SOUTHWOOD & HENDERSON (2000). Generally, an observer travels along a line, recording any animals detected. In brief, all animals are counted, and animal density is estimated as the number of animals divided by the area of the strip line, assuming a given distribution pattern in the habitat.

The line transect methodology enables estimate of the size of a variety of many animal populations, but the most popular method of studying abundance in many damselfly species remains the mark and recapture method (BENNETT & MILL, 1995). For example, the population parameters of *Calopteryx japonica* in an isolated habitat were assessed by WATANABE et al. (1998), using the mark and recapture method. CORDOBA-AGUILAR (1994) also estimated the population size and survivorship of *Hetaerina cruentata* using this approach and showed the highest daily survival rate among the Calopterygidae. BEUKEMA (2002) used the mark and recapture method to assess the changing distribution pattern of *C. haemorrhoidalis* along a stream. On the other hand, BICK & BICK (1965) used both the mark and recapture method and the line transect method for *Argia apicalis*, but they did not examine any relationships between results obtained by these two different approaches to population estimations. Few studies have assessed the performance of the line transect method in terms of assessing odonate populations of known size.

We compared the estimate of *M. hirosei* abundance obtained by the line transect method and by the mark and recapture method. Since *M hirosei* adults are perchers, and are distributed across the floor of reed communities (WATANABE et al., 2002), the census count was deemed effective for assessing the abundance of this species. The results presented here pave the way for a discussion of approaches to monitoring population for conservation of this species.

MATERIAL AND METHODS

The population of *Mortonagrion hirosei* was studied in a small reed community (ca. 10×50 m) established on an estuary in Mie prefecture, which is known to support a complete population, as described in detail in WATANABE & MIMURA (2003). Because the rice fields at the borders of the original reed community were abandoned in autumn of 2002, the rhizome development of reeds with dense aerial shoots was encouraged in the rice fields. Consequently, in early June of 2003, the area of the reed community available to *M. hirosei* adults increased to 840 m^2, though there was little water on the floor of the new additional community, i.e., the aqueous section amounted to about half of the new area.

The mark and recapture method was carried out in this reed community on 44 mostly clear days in late May to early August of 2003, and 17 days in late June to mid July of 2004. Adults were captured using an insect net; care was taken to avoid damaging the wings, and procedures were performed using CO_2 anaesthesia. Then, each damselfly was carefully marked with a unique number on the undersurface of the left hind wing using a black felt-tipped pen. The sex and age of each marked individual were recorded. The wing marking was considered to have only a minor effect on flight activity (and the probability of mating), since most damselflies began to fly normally after being marked, and perched soon after coming out from the anaesthetic state. During the marking, 16 males and 17 females in 2003, and 22 males and 24 females in 2004 were wounded, and treated as dead in the calculations.

Census counts of *M. hirosei* using the line transect method were carried out weekly in the same reed community from July to August of 2003 (total: 4 counts), and from May to July of 2004 (total: 12 counts). In order to exclude the effect of adult distribution in the community due to disturbance caused by the mark and recapture sampling, each census count in the line transect method was conducted at least three days after the mark and recapture sampling. We counted all perching adults of both sexes (lone individuals and tandem pairs) detected by walking a 89 m line, with 0.5 m on both sides of the line. This line winded large its way across the reed community. Because the walking itself might disturb the perching behaviour of adults resulting in the decline in the numbers of adults along the line, the census count was once for about 1 hour in the reed community around noon.

The probability of detecting a *M. hirosei* adult along the observation line appeared to be low, because perching behaviour is cryptic under a shade dark floor in such a reed community. Individuals, flying in attempt to shift to a perching site due to being disturbed by passing researchers, were easily detected. Therefore, throughout the flying season, individuals still perching along the line were most likely overlooked.

All adults detected along the line were counted, and the sex and the sexual maturity were recorded. The ratio between immature and mature individuals in each census count differed from that obtained by the mark and recapture sampling, that is, both a high and a low proportion of immature adults were observed in June and July, respectively. Therefore, the number of adults estimated by the census counts using the line transect method included immature and mature individuals for each sex.

RESULTS

Observations made during the mark and recapture samplings showed that both sexually immature individuals and mature male and female adults tended to stay

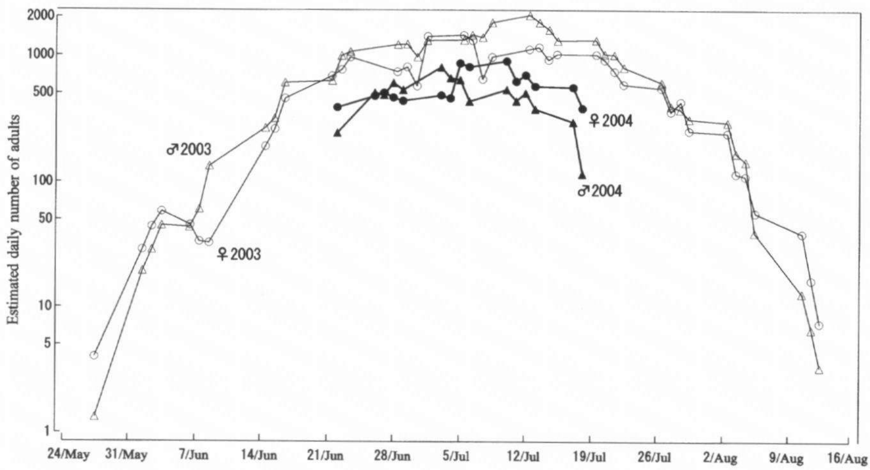

Fig. 1. Seasonal changes in the estimated daily number of male and female *Mortonagrion hirosei* in 2003 (open circle and open triangle) and 2004 (solid circle and solid triangle), using the Manly and Parr model. The curve shows three-point moving average.

on the understory of the reed community; moreover, the spatial distribution of the perching sites was roughly uniform, and all individuals perched at levels of approximately 20 cm in height. There was little difference in the observed perching height among the adults, located in the original reed community and in the additional reed community lacking water.

We marked 2,961 males (740 recaptured at least once) and 2,212 females (465 recaptured) throughout the flying season of 2003, indicating that recapture rate was more than 25% within several sampling days. Using both Jolly's model and the Manly and Parr model (as described in SOUTHWOOD & HENDERSON, 2000), the daily change in the estimated number was similar between the models, including the results for immature as well as mature individuals, as both co-existed in the same reed community. Although the SD for each estimate in males was smaller than that for females using either model, the same population change was observed in both sexes. On the other hand, 1,560 males and 1,845 females were marked from late June to mid July of 2004.

Figure 1 shows that a single peak

Table I

Total number of *M. hirosei* adults counted in 12 days of line census method from late May to early August in 2004 for 4 sections from start to end of the line (89 m), divided in accordance with the reed community structure

Section (m)	Number of males	Number of females	Total
0-20	35	31	*66*
20-40	95	108	*203*
40-69	93	77	*170*
69-89	74	73	*150*
Total	*297*	*289*	*589*

was apparent throughout the flying season for both sexes: in 2003 about 1,000 to 2,000 individuals of each sex calculated in late June to early July using Manly and Parr model. Most daily estimated numbers of males were larger than those of females. Assuming that the sex ratio in this reed community was uniform, we calculated the whole population by doubling the number of males, and the daily population density was 3.5 per square meter at the time of the peak population. In 2004, about 800 males and 800 females were calculated in early July using Manly and Parr model, indicating that the daily population density was 1.9 per square meter.

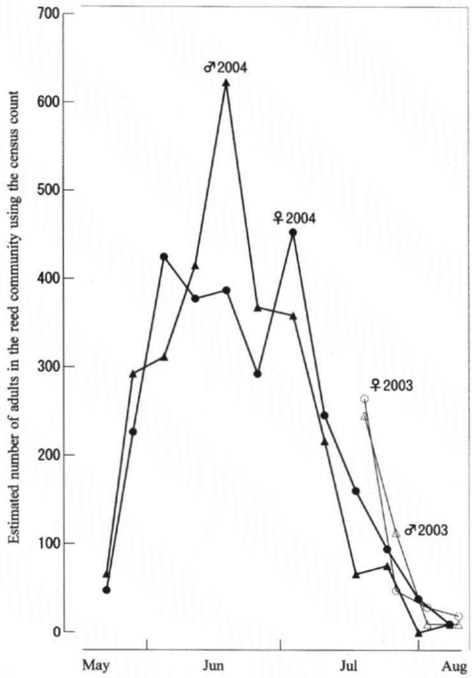

Fig. 2. Seasonal changes in the total density obtained from census counts using the line transect method for male and female *Mortonagrion hirosei* in 2003 (open circle and open triangle) and 2004 (solid circle and solid triangle).

Because the habitat was isolated, the immigration and emigration rates of *M. hirosei* adults was expected to be very low throughout the flying season. The number of immigrants into the population was considered to be the number of new adults that emerged in the habitat. Using Jolly's model, there was a peak emergence in early July, i.e., approximately 400 individuals were estimated as daily immigrants for each sex. Consequently, the total population numbers were estimated at 8,131 males and 7,245 females, indicating that the total population size was about 16,000 in 2003.

The census line was divided into 4 sections according to the structure of the reed community. Along the 20 m from the start site of the line, this section included a patch of the new additional community, which seemed not to be a suitable habitat for breeding of *M. hirosei* due to the water deficiency on the reed bed. Consequently, Table I shows that the total number of adults in the section counted in 2004 was low. All individuals detected was perching alone and no sexually mature females was found. Along the line from 20-40 m, the section was covered by a dense reed community, in which we observed a few tandem pairs and ovipositing females. Although the section of line from 40-69 m included a small open area with water depth more than 10 cm, which was likely to unsuitable habitat of the

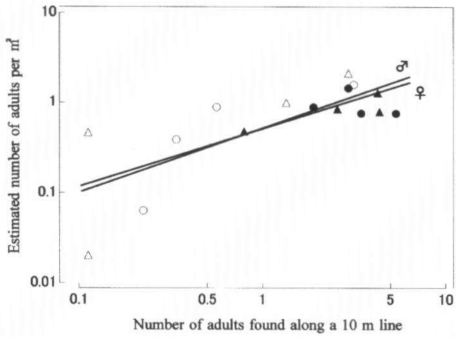

Fig. 3. The relationship between the number of adults detected along a 10 m line (D) and the estimated daily number of adults per square meter as determined by the Manly and Parr model (E). Circles represent for females in 2003 (open) and 2004 (solid), and triangles for males in 2003 (open) and 2004 (solid). – [For females: log E=-0.29+0.64 log D, r^2=0.56, 0.05>P>0.01; – For males: log E=-0.28+0.71 log D, r^2=0.58, 0.05>P>0.01]

larvae, 170 adults were counted. On the othér hand, the reed density of the section along the line from 69-89 m was also high but partly low with the other emergent plants such as *Scirpus* spp. *Ischnura senegalensis* that is a predator of *M. hirosei* was observed in this section, probably due to light environment. However, a number of *M. hirosei* utilized this section. Except a patch of new additional reed community, therefore, adults might distribute the entire reed community, and there was no difference in the section selection between sexes.

The changes in the estimated daily number of adults according to the census counts of both sexes are shown in Figure 2. Although females showed somewhat more delayed maturation than males, the sex ratio did not clearly differ from a 1:1 ratio, and the population change was synchronous between the sexes. A single peak was also observed for both sexes: about 600 males and 450 females were observed in late June to early July of 2004, suggesting the presence of more than 1000 adults daily, a result which did not agree with the daily population estimates using the mark and recapture method.

When the population reached a peak in the community, approximately 5 individuals for each sex per 10 m of the line were observed, indicating that individuals perched at least every 1 m along the line. Each number of adults obtained using the line transect method was compared with the estimated daily number calculated using the Manly and Parr model at the nearest date of the census count (Fig. 3). Clear relationships were observed between methods for both sexes. The regression lines indicated that the number of adults detected by the line transect method determined the daily population density without reliance on any marking procedure.

DISCUSSION

The estimated daily number of this damselfly is of great importance in the ecology and conservation of *M. hirosei*, a so-called percher that inhabits the understory of isolated dense reed communities. Studies of the mark and recapture method were timed to maximise the probability of capturing individuals during the entire emergence period. The estimated daily population density was found to be high (3.5 adults per square meter in 2003), and the *M. hirosei* population appeared to increase starting in 2001, as reported by WATANABE & MIMURA (2003). The estimated total population in 2003 had also increased to 2.5 times of that in 2001. The abundance of reed stems and the enlarged area of the reed community also appeared to support the larger population.

Despite the cryptic perching behaviour of *M. hirosei*, a remarkably high capturing intensity was achieved because the population was restricted to a small area. The sex ratios, based on both the estimated daily numbers and the total population number throughout the flying season, as based on the mark and recapture method, appeared to be roughly uniform. This is not always the case with damselflies, particularly in the species that adopt territorial tactics, where mature males return to water to establish territories, but mature females tend to visit such locations only to oviposit. Consequently, studies of adult damselflies report sex ratios that are highly male-biased, due to male-male competition and mating systems at the oviposition sites (ANHOLT, 1997). The probability of recapture of territorial males is higher than that for females (WATANABE & TAGUCHI, 1988), because females tend to stay in the forest with only short visits to water sources for oviposition. STOKS (2001) also showed that population size estimates for *Lestes sponsa* were about twice as large for males as for females, according to the mark and recapture method. However, counting the exuvia did not show any bias in the sex ratio at emergence in a pond that is a larval habitat, and there were no substantial differences in the timing of male and female emergence in *Somatochlora hineana* (FOSTER & SOLUK, 2004). *M. hirosei* females stay in reed communities together with males without a maiden flight away from the emergence site, as is also the case with *Calopteryx japonica* (WATANABE et al., 1998), in which females move only rarely out of their natal area, as reflected by the observed stabilities in the sex ratio in an observed population (WATANABE & MIMURA, 2003). Therefore, the *M. hirosei* population tends to be fully isolated, and thus serves as a good indicator for comparisons of different population survey methods.

The estimates of daily population size from the census counts using the line transect method and from the Manly and Parr model using the mark and recapture method were not in good agreement, especially considering that the census counts were based only on the number of adults noticed by researchers walking along the line. Although the habitat in this study was entirely isolated, a considerable number of unnoticed individuals must have been present along the line at each census count. Therefore, there are some limitations to census counts obtained using the line transect method, as there are with the mark and recapture method. One of the most difficult problems in this regard is the potential for damaging adult habitats. Searching a regular and confined area of a dense reed community over a period of a week can cause significant localized disturbance of the standing reeds, providing a sunlit area on the community floor along the line, which would be preferred by *Ischnura senegalensis* adults that are a major predator of *M. hirosei* (NISHU, 1997). Small open space in such a dense reed community might also allow predatory birds space for landing. Predation is generally the major cause of mortality throughout the adult life of this species; individual damselflies in shady habitats may generally be less exposed to predation than those in open habitats (UEDA & IWASAKI, 1982). *M. hirosei* adults might avoid the line of transection for perching sites due to high light intensity and due to high predation risk. Furthermore, because of cryptic perching behaviour and cryptic body colour, high tendency to escape in response to the disturbance caused by passing researchers; and poor light intensity at perching sites (WATANABE et al., 2002), the precision of detection for the census counts using the line transect method might be low. Odonata living in shady tropical forests often have very bright points of yellow or blue on the face and towards the tip of the abdomen; these spots make individuals shine like lights in the gloom (BROOKS, 2002). Four conspicuous green spots on the pterothorax of sexually mature males of *M. hirosei* might play the same role in the tropical species, but did not increase the probability of detecting individuals under the conditions used for the line transect method. Care should be taken when basing management decisions on census counts using the line transect method. However, when census counts are obtained using the line transect method, one must take care to minimize the stress associated with the capture and handling of the adult body while reducing sampling efforts, costs, and negative impacts on the habitat. The line transect method is in general easier to use for identification than is the mark and recapture method, which requires highly trained researchers for field survey.

Future studies of the estimated abundance of *M. hirosei* should seek to justify the assumptions of the mark and recapture method, as well as those of the line transect method. In addition, increased emphasis should be placed on obtaining accurate comparisons of the population estimates obtained using the line transect method and the mark and recapture method. Although the relationship between the density of aggressive species and dispersal remains controversial (CORBET, 1980), there was no dispersion of *M. hirosei* with a high density in the present study area (WATANABE & MIMURA, 2004). Therefore, protection and management of reed communities is essential to sustain the existing populations of *M. hirosei* adults. Repeated samplings using the mark and recapture method can result in damaged damselfly bodies and habitat, and avoiding such damage is crucial. Census counts obtained by the line transect method applied to the *M. hirosei* population are thought to be a better method for providing a reliable indirect means of monitoring damselfly populations in a dense reed community over the entire flying season than those obtained by the mark and recapture method.

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