

**FECUNDITY AND OVIPOSITION  
IN *MORTONAGRION HIROSEI* ASAHINA, *M. SELENION* (RIS),  
*ISCHNURA ASIATICA* (BRAUER) AND *I. SENEGALENSIS*  
(RAMBUR), COEXISTING IN ESTUARINE LANDSCAPES OF  
THE WARM TEMPERATE ZONE OF JAPAN  
(ZYGOPTERA: COENAGRIONIDAE)**

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*Received May 11, 2005 / Revised and Accepted November 5, 2005*

Adults of the 4 spp., inhabiting an estuarine landscape that includes reed communities and rice paddy fields established on water of varying saline concentration in Mie prefecture, Japan, were studied. The fecundity of *Ischnura* spp. was higher than that of *Mortonagrion* spp. *I. senegalensis* contained the largest number of mature, submature, and immature eggs with the smallest mature egg size, whereas *M. selenion* contained the smallest number of immature eggs with the largest mature egg size. During a 3-day laboratory oviposition experiment without food, all ♀♀ developed eggs, resulting in a greater number of mature eggs than was originally contained. Most of the eggs that developed to maturity were laid by *M. selenion* and *I. asiatica*, while *M. hirosei* laid only half of the number laid by either of these. The oviposition process of the 4 spp. is discussed from the viewpoint of their larval habitat selection.

## INTRODUCTION

Estuarine landscapes provide a unique habitat for Odonata in Japan, though they are increasingly likely to contain brackish water. Ponds, rice paddy fields, channels and sewage drains are the major components of these landscapes and contain varying degrees of saline water, which seems to be disadvantageous for the survival of odonate larvae (CORBET, 1999). In Mie prefecture, *Morton-*

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*agrion hirosei*, *M. selenion*, *Ischnura asiatica* and *I. senegalensis* are common in estuaries in which reed communities are dominant (MATSU'URA & WATANABE, 2004). Although they were found in the same estuarine landscape, *M. hirosei* is restricted to closed, dense reed communities established on brackish water (WATANABE & MIMURA, 2003), suggesting that the eggs and larvae are saline tolerant. INOUE & TANI (1999) reported that in addition to *M. hirosei*, *Orthetrum poecilops miyajimaensis* and *Tholymis tillarga* inhabit seashore with low salinity, but that their distribution is fairly limited. IWATA & WATANABE (2004) showed that young *I. senegalensis* larvae have saline tolerance similar to that of *M. hirosei*. Because the two *Ischnura* species are regarded as being predators of *M. hirosei* (HIROSE & KOSUGE, 1973), the behaviour and the microhabitat of *M. hirosei* must be somewhat different from that of *Ischnura* species. In fact, the two *Ischnura* species and *M. selenion* prefer open habitats, though NISHU (1997) observed that *I. senegalensis* adults enter the reed community in order to prey on *M. hirosei*. In this estuarine landscape, many adults of the three species fly along the margins of the dense reed vegetation inhabited by *M. hirosei*. Therefore, as a strategy for conserving *M. hirosei*, which is classified as an endangered species on the IUCN red list, it would be useful to compare the ecologies of these four coexisting species.

In general, odonate females are likely to develop eggs continuously in the ovaries and to lay eggs throughout their lifetimes following sexual maturation (BICK et al., 1976; WAAGE, 1978). BANKS & THOMPSON (1987) showed that in periods of continuous sunny weather, adult females *Coenagrion puella* came to lay a clutch of eggs every day. By dissection of wild females, WATANABE & ADACHI (1987) clarified the fecundity of *Lestes temporalis*, *L. sponsa* and *L. japonicus*, all of which showed oviposition behaviour in tandem, and it was difficult to get females to lay eggs under laboratory conditions. However, in the present study, females in all of the four species examined oviposited alone in plant tissues on the water, suggesting that with little stress they could lay eggs onto a wet filter paper in a small petri dish. The clutch size, defined as the number of eggs actually laid, has been investigated as an index for lifetime reproductive success in many species (e.g., THOMPSON, 1990; GRIBBIN & THOMPSON, 1990).

WATANABE & ADACHI (1987) classified eggs in ovaries using three categories; mature, submature and immature eggs, but they did not count immature eggs directly under the microscope due to their small size and the numerous ovarioles. In this paper, we attempted to count the number of eggs in all three categories as an index of the fecundity of the four coexisting species by dissecting females, and to count the number of eggs artificially laid in order to assess the oviposition process. This information is an essential component for reproductive strategy of each species and will further advance the development of a detailed conservation strategy for the endangered *M. hirosei*.

## STUDY AREA AND METHODS

We studied a small, dense reed community in an estuarine landscape in Mie prefecture, Japan, that was inhabited by *M. hirosei*. The community was established on brackish water and the growth process of reed was described by MATSU'URA & WATANABE (2004). Surrounding the reed community, there were abandoned rice paddy fields that were supplied by fresh water, resulting in shallow ponds with short emergent plants such as *Schoenoplectus* spp. and also small reeds. *M. selenion* and the two *Ischnura* species flew mainly above the abandoned rice paddy fields and along the margins of the dense reed community.

Just after sexual maturity, females of the four species, which were identified mainly by body colour, were collected during their flying season, from late May to early August, in 2003 and 2004. All females were captured during roosting in early morning, prior to the commencement of their daily oviposition. *I. senegalensis* females are dimorphic. We captured the heteromorphs, because they were prevalent in this population. Twenty-five *M. hirosei* females were collected in the reed community, and 17, 28 and 46 *M. selenion*, *I. asiatica* and *I. senegalensis* females respectively, were collected along the margins of the reed community and in the abandoned rice paddy fields.

Some females were put into 50% ethyl alcohol as soon as they were netted. The abdomen length of each female was measured by using a micrometer. All dissected females were examined under a stereomicroscope to determine the number of eggs of each type remaining in the ovaries (WATANABE & ADACHI, 1987). The number of mature and submature eggs was counted directly. Since the immature eggs were in ovarioles, the number of total ovarioles was counted. The number of immature eggs in an ovariole was counted based on five ovarioles randomly sampled, and then the total number of immature eggs was calculated. The volume of a mature egg was calculated as an oval.

Artificial (laboratory) oviposition was conducted at room temperature in petri dishes (diameter 9 cm) that contained a piece of filter paper soaked in distilled water. The insect inserted eggs into the filter paper. After three days, the number of eggs laid was counted. The females were then dissected to count the number of mature eggs remaining in the ovaries. The results are reported as the mean  $\pm$  standard error.

## RESULTS

As shown in Figure 1, the number of ovarioles containing immature eggs in the four species varied widely. Around 150 ovarioles were found in *M. hirosei* and *I. asiatica*, while *M. selenion* had the lowest (ca 100) and *I. senegalensis* had the highest (ca 270) number, partly due to the abdomen size (cf. Tab. I). In contrast, the number of immature eggs per ovariole for each species was relatively stable, approximately 15 for all species except *I. senegalensis* (ca 18). For *M. hirosei*, ap-

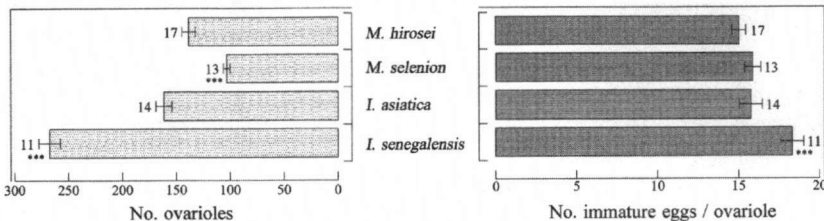


Fig. 1. The mean numbers of ovarioles and of immature eggs per ovariole ( $\pm$ S.E.). The numbers beside each column show the sample size. \*\*\* indicates a significant difference from *M. hirosei* at a probability of less than 0.001 (ANOVA Dunnett T3-test).

Table I  
Female body size, the number of eggs contained and the mature egg size in the four species studied  
( $\pm$ S.E.)

	<i>M. hirosei</i>	<i>M. selenion</i>	<i>I. asiatica</i>	<i>I. senegalensis</i>
Abdomen length (mm)	24.7 $\pm$ 0.4 (17)	21.1 $\pm$ 0.2*** (13)	23.0 $\pm$ 1.3 (16)	26.9 $\pm$ 0.4** (10)
No. immature eggs	2074.3 $\pm$ 114.2 (17)	1629.3 $\pm$ 76.1* (13)	2552.9 $\pm$ 186.4 (14)	4940.9 $\pm$ 301.3*** (11)
No. submature eggs	17.1 $\pm$ 2.8 (14)	17.4 $\pm$ 2.4 (17)	37.6 $\pm$ 4.3** (28)	47.2 $\pm$ 5.5*** (46)
No. mature eggs	83.4 $\pm$ 10.0 (14)	81.3 $\pm$ 10.0 (17)	123.4 $\pm$ 10.0** (28)	239.0 $\pm$ 16.4*** (46)
Mature egg size ( $\times 10^3$ mm <sup>3</sup> )	12.7 $\pm$ 0.3 (17)	14.4 $\pm$ 0.4* (13)	13.1 $\pm$ 0.5 (16)	11.5 $\pm$ 0.2* (11)

\*, \*\*, \*\*\*: significantly difference from those of *M. hirosei* with probability at 0.05, 0.01 and 0.001 (ANOVA Tukey b-test), respectively.

( ): number of samples.

proximately 2,000 immature eggs were contained in a female. The lowest number of immature eggs was found in *M. selenion* (ca 1,600) and the highest number was found in *I. senegalensis* (ca 4,900).

Table I shows the abdomen size in each female, *M. selenion* had the smallest and *I. senegalensis* the largest abdomen. The size difference probably accounts for the number of immature eggs contained. However, there were no significant differences in the number of submature or mature eggs between *M. hirosei* and *M. selenion* (ca 17 and ca 80 for submature and mature eggs, respectively). In spite of the similar size of *I. asiatica* and *M. hirosei*, both the numbers of submature and mature eggs in *I. asiatica* were significantly greater than those in *M. hirosei*. Furthermore, *I. senegalensis* contained the largest number of submature and mature eggs among the four species, indicating the highest fecundity.

Table I also shows the mature egg size for females of each of the four species. *M. selenion* had the largest eggs with the lowest fecundity, while *I. senegalensis* had the smallest eggs with the highest fecundity. The fecundity and egg size of *I. asiatica* were intermediate between those of *M. selenion* and *I. senegalensis*, but *M. hirosei* had relatively low fecundity with relatively small mature eggs.

During the 3 days spent in the petri dishes for the laboratory oviposition experiment, the females were not given access to food, but no females died and there were no fat bodies in the abdomens, suggesting that they exploited their energy to develop mature eggs for oviposition as well as to maintain their own body. There were few submature eggs in any females of any of the species. Most of the mature egg masses were laid, and others remained in the ovaries. Figure 2 shows that each *M. hirosei*, *M. selenion* and *I. asiatica* female had contained total of 200 mature eggs, indicating that, for each female, the number of eggs developed to maturity was the same as the number of mature eggs already contained. The same egg developmental process was observed in *I. senegalensis*, where the total number of mature eggs (ca 450) was twice the number of mature eggs already contained.

Although each female developed during the 3 days a large number of mature eggs, *M. Hirosei* actually laid about 65% of the total number of mature eggs (Fig. 2). Then, a female laid 40 eggs daily, assuming that there was no peculiar egg-laying rhythm during the 3 days. On the other hand, *M. selenion* and *I. asiatica* actually laid more than 90% of the total number of mature eggs, or approximately 60 eggs daily. *I. senegalensis* also laid

more than 80% of the total number of mature eggs (120 eggs daily), suggesting that the three species might have similar egg-developing and oviposition processes. Initially, artificially laid eggs in the filter paper for each species were glistening white; they turned greyish brown within one day and eventually, became dark brown. Most eggs were hatched in distilled water within a month.

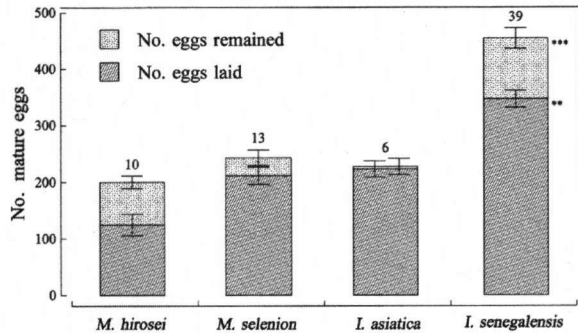


Fig. 2. The number of eggs laid and the number of mature eggs in ovaries immediately after artificially oviposition of three days ( $\pm$ S.E.). The dark striped area and light dotted area indicate respectively the number of eggs laid over three days and the number remaining to be laid after this period. The numbers above the columns show the sample size. \*\* and \*\*\* indicate significant differences from *M. Hirosei* at a probability of less than 0.01 and 0.001, respectively (ANOVA Dunnett T3-test).

## DISCUSSION

The sample date of females may have affected the number of eggs laid because of the pattern of sunny, overcast and rainy days over the period of reproductive activity, as well as the time since they last oviposited or the inter-clutch interval. BANKS & THOMPSON (1987) found that the most important determinant of clutch size in *Coenagrion puella* was the time since the previous clutch was laid. GRIBBIN & THOMPSON (1990) also reported that *Pyrrhosoma nymphula* females did not visit the ponds daily to breed. In the present study, however, no peculiar roosting sites away from the water were observed for any of the four species, suggesting that the females stayed by the water and were able to oviposit daily if they wished. *M. Hirosei* stayed in the understory of the dense reed vegetation during its lifespan and oviposited alone, as has been described by WATANABE & MIMURA (2003, 2004). Females of the other three species also laid in the plant tissues of floating leaves and small emergent plants, under direct sunlight, along the margins of the reed community (IWATA & WATANABE, 2004). Furthermore, all the collected females were identified as recently matured individuals

that were at the very beginning of their reproductive activity.

Although MIZUTA (1985) counted more than 1,000 ovarioles in *Orthetrum albistylum*, nothing was known on the number of ovarioles in any Zygoptera species. If there are no additional ovarioles and if there are no additional immature eggs in the ovariole during the lifespan, the number of immature eggs must reflect the lifetime fecundity of the female, and thus should be comparable among species. In the present study, despite the relatively small variation in the number of immature eggs per ovariole (ca 15 in three species), there was a variation in the number of ovarioles among the four species. Thus, *I. senegalensis*, which is the largest of our species, had the highest fecundity, while *M. selenion* is the smallest and had the lowest fecundity. This relationship was also found in the number of mature eggs contained, suggesting that the clutch size may be large and small for *I. senegalensis* and *M. selenion*, respectively. *I. asiatica* must be intermediate between *I. senegalensis* and *M. selenion* in viewpoint of fecundity and the number of mature eggs contained.

In general, there is a trade-off relationship between egg size and the number of mature eggs contained which is conditioned by the physical space inside a female's abdomen. Species that perform high (low) fecundity with small (large) eggs are regarded as r-selected (K-selected) species (PIANKA, 1970). Although female abdomen sizes in *M. selenion* and in the two *Ischnura* species were somewhat different, it is likely that *M. selenion* is a K-selected and *I. senegalensis* an r-selected species, because of their egg size. *I. asiatica* seems to be intermediate between these. However, *M. Hirosei* females had a peculiar trait for their reproductive strategy among the four species. Although the female abdomen size was not very different from that of *I. asiatica*, it had a relatively lower fecundity.

During the 3 days of the laboratory oviposition experiment, we did not know when the female oviposited, the number of clutches, or the oviposition duration of each bout. In *Coenagrion puella*, initial clutch sizes ranged between 110 and 250 eggs, whereafter the female had 13 mature eggs left (BANKS & THOMPSON, 1987). GRIBBIN & THOMPSON (1990) reported that at the beginning of a day's reproductive activity *P. nymphula* laid a single clutch of 350 eggs on average. In a study of *Chromagrion conditum*, BICK et al. (1976) found that eggs were deposited at a rate of 5.5 per minute, resulting in an average of 200 eggs per oviposition sequence. BENNETT & MILL (1995) also reported that the average clutch size of *P. nymphula* was 245 eggs and that the mean lifetime clutch production was approximately 6. In the present study, it is likely that even when the egg maturation occurred gradually over the 3 days, almost all mature eggs could be laid during a day's oviposition by females of the four species. The largest number of eggs was laid in *I. senegalensis*, though the clutch size was significantly correlated with body size as in *I. graellsii* (CORDERO, 1991).

For *M. Hirosei*, slow egg development was found and a small number of eggs was laid over the 3 days. Since a mature egg in the ovaries is defined as an egg

ready to be laid, there are several possible explanations for the remaining mature eggs. If the energy was exhausted to develop egg maturation, the female would not have had the power required to lay eggs. Alternatively, the oviposition rate may have been too slow to allow the female to lay all of her eggs in 3 days. In addition, the sperm accumulation prior to capture may not have been sufficient, due to, e.g., the female mating behaviour or to the size of bursa copulatrix, though in the field most females that lay eggs in the absence of males have sufficient sperm mass stored from previous copulations (SIVA-JOTHY et al., 1995).

Oviposition sites are among the most important factors in habitat selection (CORBET, 1999). The oviposition preference is influenced by water depth in *Enallagma hageni* (FINCKE, 1986), which prefers the oviposition in deep water, in order to decrease the risk the eggs would dry up in the pools. In contrast, in our four species, water depth did not seem to be a critical factor in the choice of the oviposition site, since the habitat had a constant water supply (e.g., MATSU'URA & WATANABE, 2004). Females of the species with the highest fecundity, *I. senegalensis*, oviposit in various sites irrespective of saline concentration, such as ponds, drains and rice paddy fields, but this is not the case in the other 3 species coexisting in the same estuarine landscape. Although the egg size of *I. senegalensis* was the smallest, the hatchability was not so low, and the young larvae survived even in saline water, as in the case of *M. hirosei* (IWATA & WATANABE, 2004). It was observed that *I. senegalensis* preyed on *M. hirosei* at both the larval and adult stages (HIROSE & KOSUGE, 1973; NISHU, 1997; INOUE & TANI, 1999). Predation on female coenagrionids by odonate larvae has been reported previously (CORBET, 1999). On the other hand, *M. selenion* and *I. asiatica* might be competitors for the habitat, though their saline tolerance in young larval stages was not as high as in *M. hirosei* (IWATA & WATANABE, 2004). WATANABE & MIMURA (2004) studied the diurnal behaviour in *M. hirosei* by observing individuals continuously from sunrise to sunset, and pointed out that in shady perching sites in the dense reed vegetation their body colour seems to be cryptic. Therefore, the female oviposition site selection and the larval habitat selection in *M. hirosei* are likely to maintain the populations with low predation risk. The other three species, in open habitats, compensate for the probably higher predation risk (from large dragonflies and small fish) by their high fecundity. Even though it is a species with low fecundity, *M. selenion* is multivoltine. Because females in tandem oviposition are vulnerable to predation, laying eggs alone, as in our four species, might decrease the risk of mortality. In a comprehensive study on the mortality during oviposition in Zygoptera, FINCKE (1986) provided a maximum estimate of mortality in *Enallagma hageni* at 13% per oviposition bout in an open habitat. Females are certainly be under a strong selection pressure to oviposit in sites which are unlikely to be subjected to adverse conditions during the period of egg development and hatching. Further studies regarding the lifetime oviposition pattern of the four coexisting species are needed.

## ACKNOWLEDGEMENTS

We thank Y. MIMURA and S. IWATA for their field assistance. This study was supported in part by the Pro Natura Foundation.

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