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Dispersal and reproductive responses of the water strider, *Aquarius paludum* (Hemiptera: Gerridae), to changing NaCl concentrations

MANABU KISHI[1], TETSUO HARADA[2] and KENJI FUJISAKI[1]

[1]Laboratory of Insect Ecology, Graduate School of Agriculture, Kyoto University, Kyoto 606-8502, Japan;
e-mail: mkishi@kais.kyoto-u.ac.jp

[2]Laboratory of Environmental Physiology, Faculty of Education, Kochi University, Kochi 780-8520, Japan;
e-mail: haratets@cc.kochi-u.ac.jp

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Abstract. The responses of females of the water strider, *Aquarius paludum*, to changes in NaCl concentration were examined in the laboratory. The insects were sampled seasonally in 2002 and 2003 at two reservoirs in Kochi, Japan, connected by a waterway, one at the mouth of the river with high NaCl concentrations (the range in NaCl concentration during a year: 0.1–1.08%) and one 700 m upstream, where the salinity was lower (0.03–0.23%). Sudden increase in NaCl concentration, from 0.45 to 0.9%, after adult strider emergence suppressed reproduction and promoted flight activity, whereas a decrease in salinity did not affect either trait. In the field, *A. paludum* was univoltine in brackish and multivoltine in freshwater ponds. Thus, the number of breeding periods per year was limited by fluctuations in the NaCl concentration in brackish habitats. Our results suggest that *A. paludum* can breed in brackish waters when the NaCl concentration is below the limitation for reproduction and growth. Abrupt increases in NaCl concentration caused by seawater surges, such as those following typhoons, can trigger the migration of individuals, which move to areas of lower NaCl concentration and so mix the genes of individuals inhabiting brackish and freshwater bodies.

INTRODUCTION

There are more than 560 species of water striders in the family Gerridae. They are found in a range of marine, brackish and freshwater habitats (Andersen, 2000). Gerrids are widespread except in Northern and Southern polar areas. Water striders living in freshwater habitats are able to cope with severe environmental stresses such as drought and major temperature fluctuations (e.g., *Aquarius paludum*; Harada, 2003).

A common Japanese freshwater species, *Aquarius paludum*, in the subfamily Gerrinae, exhibits flexible responses to environmental fluctuations, including changes in photoperiod and temperature, desiccation during overwintering on land and the drying up of surface waters (Harada et al., 2000; Harada, 2003; Kishi et al., 2002). Finnish populations of *Gerris thoracicus* avoid extinction when summer conditions in freshwater habitats become severe by extending their habitat range into brackish conditions, where larval development is prolonged (Vepsäläinen, 1978). Another species, R90% of the *Malesheumatobates rileyi* (L.), responds morphologically to stresses caused by changes in the pH and aluminum ion content of the water by fluctuations in the asymmetry of its legs (Drover et al., 1999).

In brackish water more than 90% of the males of populations of the freshwater species *A. paludum*, exposed to NaCl solutions throughout their larval and adult stages, histolyse their flight muscles and are unable to fly (Kishi et al., 2006). Females exposed to 0.9% NaCl solution immediately following emergence, or 20 days later, show a delay in oviposition and greater flight capabilities than individuals maintained in freshwater throughout their lives. Thus females that are swept into brackish water by streams following heavy rain can be fertilized and then fly away and deposit their eggs later, the so-called "breed there and later" tactic. Thus, *A. paludum* shows a range of tactics in response to variations in salinity, depending on the developmental stage (Kishi et al., 2006). On the other hand, 47 species of marine water striders are adapted to seawater and inhabit coastal areas or the ocean (Spence & Andersen, 1994). In contrast to fresh water, the marine water striders, *Halobates sobrinus* and *H. flaviventris*, show a reduction in fitness when exposed to fresh water (Cheng, 1985).

There are no data on the responses of freshwater striders raised in brackish waters with relatively low NaCl concentrations to changes in osmotic pressure at high and low salinities. Here, the dispersal and reproductive characteristics of freshly emerged *A. paludum* adults, exposed to high and low salt concentrations in the water, were assessed. To give an ecological perspective to this laboratory study wild populations around a river mouth and upstream were also surveyed from spring to autumn.

MATERIALS AND METHODS

Field sampling

Striders were randomly sampled from May to October 2002 and from April to December 2003 at Daizen Pond in Kochi (33°33′N), a reservoir with a high NaCl concentration at the mouth of the Shimoda River and a second reservoir with a low NaCl concentration located about 700 m upstream. These reservoirs were connected by a waterway. Samples were taken at approximately 10-day intervals, beginning in April at Daizen

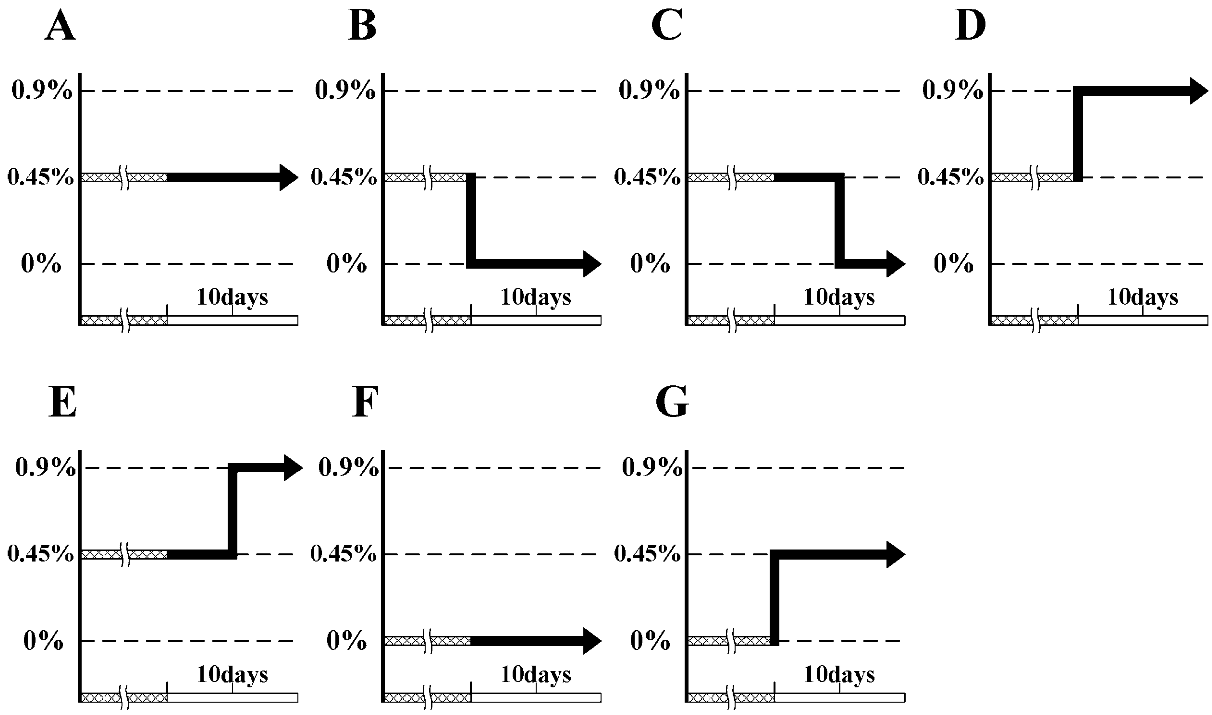

Fig. 1. Schematic representation of the experimental protocols for groups A–G, showing the sequence of exposure of *Aquarius paludum* to three concentrations of NaCl, 0, 0.45, and 0.9%. Black and white bars refer to larval and adult periods, respectively.

Pond and in May at the upstream pond. Sampling ended in late December 2003. Samples were collected from a boat along the south shore of both ponds, by making 50 sweeps of a circular net (diameter, 30 cm) with a 1-m handle, per hour.

The numbers of adults and of larvae at each instar were recorded. The concentrations of NaCl were measured using a portable electric conductivity meter (CM-21P; TOA Electronics, Ltd.). Rain fall in the 5 days after each sample was calculated from the Annual Weather Reports for Japan for 2003 and 2004 (Japan Meteorological Station, 2003, 2004).

Responses to changes in NaCl concentration in the laboratory

Adult striders collected at the Daizen Pond in 2003 were kept in mass culture using a 0.7% NaCl solution (the concentration at the collection site), at 20 ± 2°C and a 15.5L : 8.5D photoperiod. Eggs laid by the females were placed either in a 0.45% NaCl solution or freshwater (0% NaCl) within 24 h of deposition. These eggs were kept in plastic containers (34 × 23.5 × 4.5 cm) from egg hatch to adult emergence under the same photoperiod and temperature conditions as the mass culture.

Long-winged adults exposed to 0.45% NaCl throughout their embryonic and larval development were used to establish the following 5 experimental groups (Fig. 1).

1. Group A: Maintained at the same NaCl concentration after adult emergence (control group);
2. Group B: Transferred to freshwater immediately after adult emergence;
3. Group C: Transferred to freshwater 10 days after adult emergence;
4. Group D: Transferred to a 0.9% NaCl solution immediately after adult emergence;
5. Group E: Transferred to a 0.9% NaCl solution 10 days after adult emergence;

In addition, individuals reared in freshwater conditions were used to establish the following 2 groups:

6. Group F: Developed in freshwater and kept in freshwater following adult emergence; and
7. Group G: Developed in freshwater and transferred to a 045% NaCl solution following adult emergence.

Adults were kept in pairs in circular plastic containers (diameter, 14 cm; height, 5 cm) for the assessment of longevity, reproductive and dispersal traits. After death, all adults, except those that decomposed, were dissected and their reproductive systems examined. The presence or absence of mature oocytes was used to assess the female reproductive activity at death. The flight propensity of adults was evaluated using the method of Harada et al. (1997). The number of eggs laid by each female was counted every 2 days.

Rearing conditions

Adults of the fly *Lucillia illustris* were provided as food each day, at a rate of one fly per five first or second instar individuals, one fly per three third or fourth instar individuals, and one fly per two fifth instar or adult individuals. Wooden sticks floating on the water provided the striders with sites for egg laying and resting. Animals were raised at a 15.5L : 8.5D cycle at 20 ± 2°C (conditions promoting reproduction; Harada, 1992, 1993).

RESULTS

Field samples and NaCl concentration

The NaCl concentrations in Daizen Pond were higher than those upstream (*t*-test: $t = 7.069$, df = 19, $p < 0.001$; Fig. 2a, c) and were especially high at the end of August and beginning of September, following typhoons (Fig. 2b, c). In 2002 and 2003, there was a significant negative correlation between NaCl concentration at this site and precipitation in Kochi City (least square-test: $r = -0.473$, $p = 0.002$ in 2002; $r = -0.413$, $p = 0.045$ in 2003; Fig. 2b, c) (Japan Meteorological Station, 2003, 2004). The density

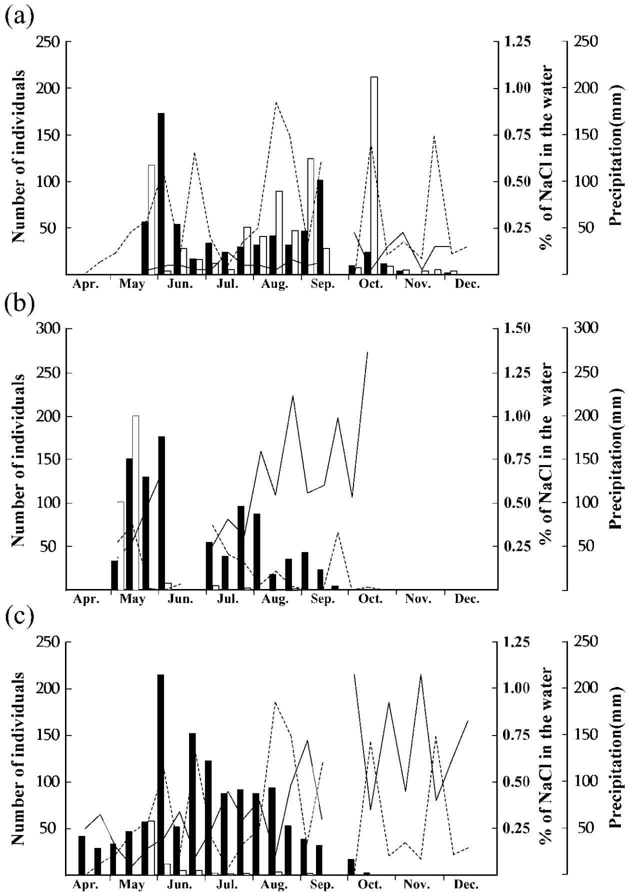

Fig. 2. Seasonal variation in the number of fourth and fifth instar larvae and adults of *Aquarius paludum* collected at the brackish Daizen Pond and another pond, 700 m upstream, with a lower NaCl concentration. (a) Numbers of striders caught at the pond upstream of Daizen Pond in 2003; (b) and (c) Numbers caught by timed sampling at Daizen Pond, in 2002 and 2003, respectively. Open bars: number of fourth and fifth instars; solid bars: number of adults. Solid and dotted lines show seasonal variation in NaCl concentration in the water and in precipitation, respectively, at each pond.

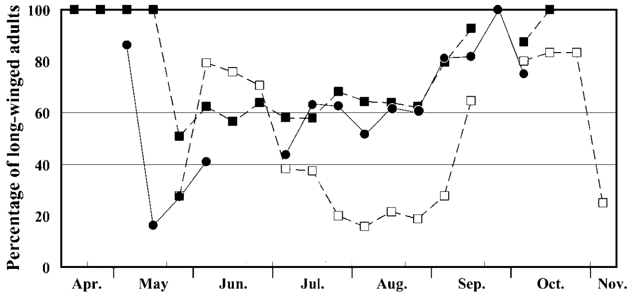

Fig. 3. The percentage of long-winged adults of *Aquarius paludum* at the brackish Daizen Pond and at a second pond 700 m upstream. Open squares and dotted line: percentage at the upstream pond in 2003; solid circles and line: percentage at Daizen Pond in 2002; solid squares and dotted line: percentage at Daizen Pond in 2003.

of adults at Daizen Pond throughout the seasons in 2003 was higher than that at the upstream site (paired *t*-test: $t = 2.321$, df = 13, $p = 0.037$; Fig. 2a, c). However, over the same period, the density of fourth and fifth instar larvae at Daizen Pond was lower than that measured upstream (paired *t*-test: $t = -5.110$, df = 11, $p < 0.001$; Fig. 2a, c).

More than 50 fourth and fifth instar individuals were collected at Daizen Pond at the end of May 2003; shortly before a very large sample of adults was collected (220 at the beginning of June 2003; Fig. 2c). From late June through August, more than 90 adults were collected at Daizen Pond (Fig. 2c). The seasonal change in adult density at the upstream pond tracked that of the larvae (Fig. 2a). However, an extremely high peak in larval density did not result in a corresponding peak in adult density in October (Fig. 2a).

The percentage of long-winged adults at Daizen Pond was significantly higher than at the upstream pond (χ^2-test: $\chi^2 = 19.931$, df = 1, $p < 0.001$; Fig. 3). It is possible that adults from other water bodies, including the upstream site, could have contributed to the population at Daizen Pond.

The concentration of NaCl in the Daizen Pond rose considerably following a typhoon at the end of September 2003, and decreased rapidly in October. From October to December, the average NaCl concentration was about 0.75% and the concentration fluctuated with a cycle of about one month (Fig. 2c). The NaCl concentration ranged from 0.05 to 0.4% in spring and summer and from 0.30 to 1.10% in autumn and winter. In the upstream pond, the concentration was less than 0.25% from May to December (Fig. 2a).

There was no significant correlation between the density of adult striders and the NaCl concentration of the water of Daizen Pond (least square-test: $r = -0.292$, $p = 0.291$ in 2002; $r = -0.075$, $p = 0.667$ in 2003). However, after the NaCl concentration reached more than 0.7%, adults became extremely rare (Fig. 2b, c). There was significant negative correlation between larval density and

TABLE 1. Preoviposition period, number of eggs laid and longevity of *Aquarius paludum* kept in seven different salinity regimes.

	A		B		C		D		E		F		G	
	n	Means (±SE)	n	Means (±SE)	n	Means (±SE)	n	Means (±SE)	n	Means (±SE)	n	Means (±SE)	n	Means (±SE)
Preoviposition period (days)	31	27.06 ±1.31	31	25.42 ±1.38	18	25.28 ±1.78	11	29.09 ±1.71	11	24.91 ±1.87	18	25.00 ±1.42	7	23.71 ±1.66
Total number of laid eggs	31	112.10 ±16.79	31	118.68 ±15.77	17	153.12 ±24.42	11	75.91 ±19.24	9	50.11 ±9.90	17	90.29 ±15.21	7	103.86 ±26.74
Longevity (days)	74	40.99 ±1.58	77	42.52 ±1.56	35	37.46 ±2.36	77	33.04 ±1.57	44	32.00 ±1.71	38	37.21 ±1.87	30	35.83 ±2.53

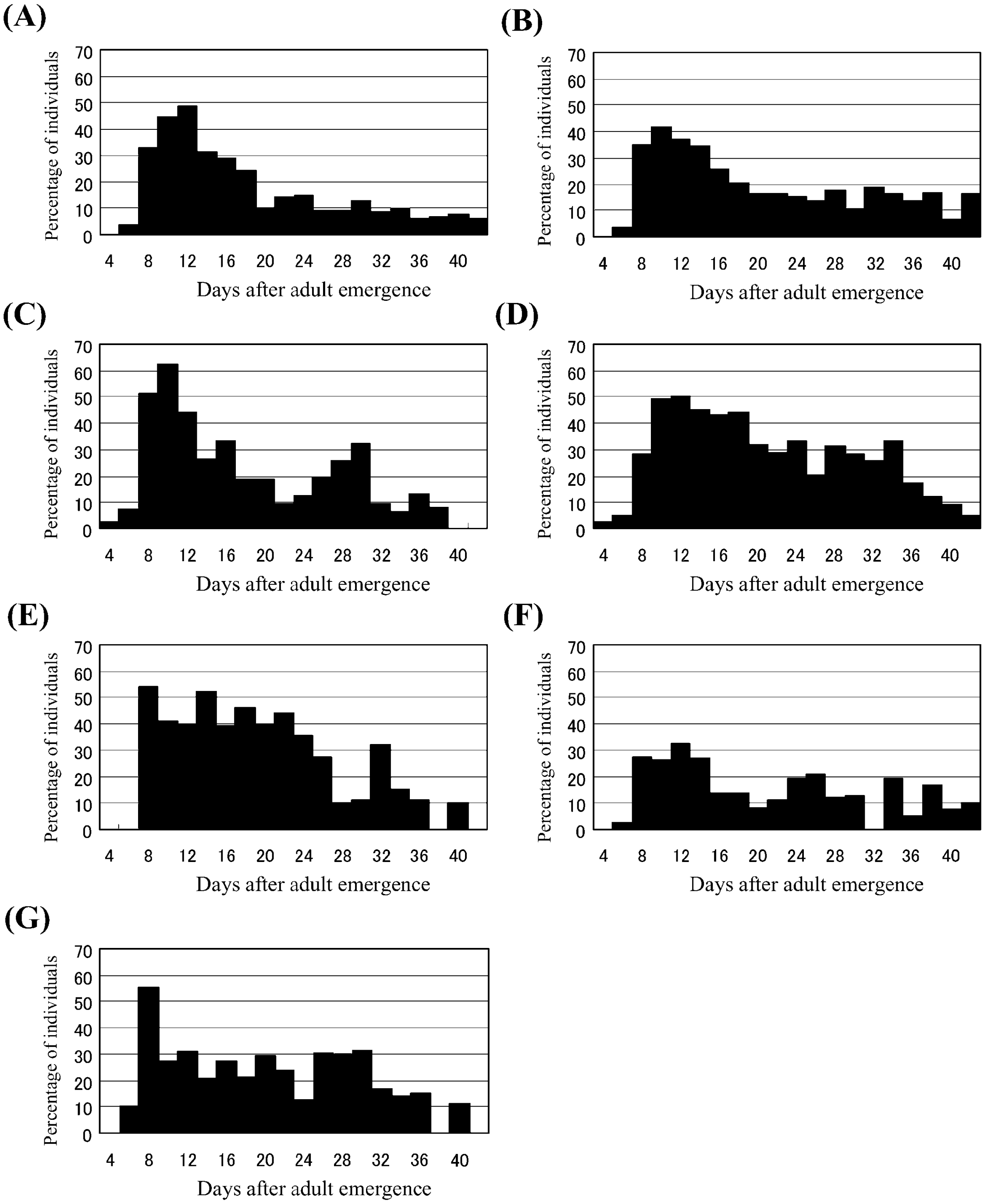

Fig. 4. Effects of increasing or decreasing the NaCl concentration, immediately following adult emergence or 10 days later, on the proportion of *Aquarius paludum* females showing flight activity (cf. Fig. 1).

salinity in 2003 (least square-test: $r = -0.457$, $p = 0.017$). These results suggest that when the NaCl concentration is very high (0.71–1.11%), adults disperse by flight to other water bodies or die. The NaCl concentration at which larval growth ceases in the wild may be approximately 0.45%.

Effects of decreasing NaCl concentration from 0.45% to 0%

There were no significant differences in any of the characteristics of either of the two groups of experimental striders (NaCl concentration decreased from 0.45% to 0% at adult emergence and 10 days later) and those kept in conditions with a 0.45% NaCl solution after adult emergence (Tables 1, 2a). The flight propensity at 4–42 days after adult emergence was not significantly different in these three groups of striders (χ^2–test: χ^2 cal = 3.72, df = 2, $p = 0.156$; Fig. 4A, B, and C).

Effects of increasing the NaCl concentration from 0.45% to 0.9%

There were no significant differences in either pre-oviposition period or number of eggs laid between those animals exposed to 0.9% NaCl and those maintained at

TABLE 2. Statistical analysis of the longevity and reproductive traits of *Aquarius paludum* subjected to changes in NaCl concentration. (a) Decrease in NaCl concentration from 0.45% to 0%; (b) Increase in NaCl concentration from 0.45% to 0.9%; (c) Increase in NaCl concentration from 0% to 0.45%. Asterisks indicate significant differences (*$p < 0.05$; **$p < 0.05$; *** $p < 0.01$).

	Statistic test	n	χ^2-value	df	*p*
A					
Preoviposition period	Kruskal-Wallis	80	1.012	2	0.603
The number of eggs	Kruskal-Wallis	79	2.188	2	0.335
Longevity	Kruskal-Wallis	186	1.967	2	0.374
The percentage of females that laid eggs	Fisher's probability	100	1.563	2	0.495
The percentage of female nett mature oocyte	Fisher's probability	88	1.476	2	0.736
B					
Preoviposition period	Kruskal-Wallis	53	2.212	2	0.331
The number of eggs	Kruskal-Wallis	51	3.658	2	0.161
Longevity	Kruskal-Wallis	195	18.310	2	<0.001***
The percentage of females that laid eggs	Fisher's probability	103	20.173	2	<0.001***
The percentage of female nett mature oocyte	Fisher's probability	87	14.716	2	0.002**
A × D	Bonferroni				<0.001***
A × E	Bonferroni				0.776
E × E	Bonferroni				0.067*
C					
Preoviposition period	Kruskal-Wallis	56	2.106	2	0.349
The number of eggs	Kruskal-Wallis	55	0.121	2	0.941
Longevity	Kruskal-Wallis	152	4.806	2	0.090
The percentage of females that laid eggs	Fisher's probability	74	8.509	2	0.014**
A × F	Bonferroni				0.928
A × G	Bonferroni				0.053*
F × G	Bonferroni				0.013**
The percentage of female nett mature oocyte	Fisher's probability	68	2.368	2	0.306

0.45% (Tables 1, 2b). The longevity of insects exposed to the higher NaCl concentrations after adult emergence was significantly shorter than that of the control group (Table 2b; groups D and E). Moreover, a significantly lower percentage of the female striders exposed to increase to a high-salinity laid eggs and had mature oocytes in their gonad at the end of their life than those in control group A (Table 2b; Fig. 5A, D, and E). The percentage of females with mature oocytes in their gonads when they died was considerably reduced in adults transferred to a higher NaCl solution after adult emergence and significantly lower in group D (transferred soon after adult emergence) than in any other group (Table 2b; Fig. 5A, D, and E). The flight propensity of groups D and E subjected to salinity increase was significantly higher than that of the control group A, kept throughout the experimental period at 0.45% NaCl treatment (χ^2-test: χ^2 cal = 53.95, df = 2, $p < 0.001$; Fig. 4A, D, and E).

Effects of increase in NaCl concentration from 0% to 0.45%

There was no significant effect on reproductive traits when the NaCl concentration was increased from 0% to 0.45% (Table 2c; groups A, F, and G). The percentages of females with mature oocytes in their gonads at death was lower in group G than in the other two groups (Table 2c; Fig. 5A, F, and G). However, the percentage of females that laid eggs before they died did not differ in the three groups (Table 2c; Fig. 5A, F, and G). The flight propensity was significantly higher in adults transferred to a 0.45% NaCl solution after adult emergence (group G) than in those reared and kept in containers with a 0.45% NaCl solution throughout the experimental period (group A) (χ^2-test: χ^2 cal = 4.63, df = 1, $p = 0.031$). There was no significant difference in the flight propensity between adults kept at 0.45% NaCl throughout the experimental period (group A) and those reared and kept in freshwater conditions for the same period (group F) (χ^2-test: χ^2 cal = 2.25, df = 1, $p = 0.133$; Fig. 4A, F, and G).

DISCUSSION AND CONCLUSIONS

Use of brackish water by *Aquarius paludum*

In the period from May to September 2003, many adults may have moved or been carried by water flow to brackish habitats (0.1–1.08% NaCl) at the mouth of the river. Larvae are able to grow for a limited period in NaCl concentrations lower than about 0.45%. When strong stream flow caused by rainfall carries water striders downstream to areas with NaCl concentrations up to 0.9%, flight propensity increases and the striders are able to fly to more suitable areas.

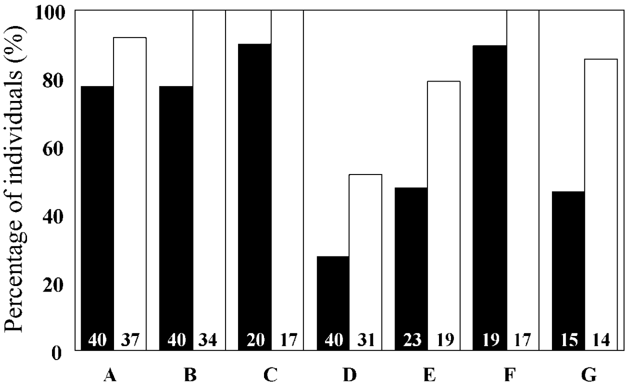

Fig. 5. Reproductive status of *Aquarius paludum* individuals in the seven experimental regimes. Open bars: percentage of females that laid eggs; solid bars: percentage of females with mature oocytes in their gonads when they died. Numbers within each bar are the total number of individuals.

Effects of increased NaCl concentration on dispersal and reproductive status: ecological significance

An abrupt increase in NaCl concentration from 0% to 0.9% just after adult emergence suppresses egg-laying and promotes flight propensity in *A. paludum* (Kishi et al., 2006). Even a relatively moderate increase in the NaCl concentration from 0.45% to 0.9% was found to produce the same effect. Adults of *A. paludum* inhabiting brackish water around the mouths of rivers may be exposed to similar increases in salt concentration during incursions of sea water following typhoons, which occur in August through to October (Fig. 2b, c).

Adults appear to respond to abrupt increases in NaCl by maintaining high flight propensity, thus avoiding areas with high salt concentrations, which are not suitable for reproduction and larval growth (Fig. 2b, c and 4D, E). Heavy rains can increase the amount of water flowing downstream, and many water striders may be transported to the river mouth (Fig. 2). Again, flight triggered by the abrupt increase in NaCl concentration may allow movement to areas with lower salt concentrations, which are more suitable for reproduction and growth.

Aquarius paludum, unlike marine water striders (Cheng, 1985), escapes from brackish water by flight. Marine water striders are physiologically adapted to maintain a constant osmotic pressure even in hyperosmotic condition of seawater and have a low fitness or are unable to skate on fresh water. However, *A. paludum* is a terrestrial species and the osmotic pressure of its haemolymph is much lower than that of sea water. Thus the different osmotic pressure experienced in terrestrial and marine habitats result in the species living there developing different physiological responses to osmotic pressure.

Life-cycle in brackish water

The European water strider, *Gerris thoracicus*, uses brackish water as a refuge (Vepsäläinen, 1978). *Aquarius paludum* uses the brackish habitat not only as a refuge but also a breeding place in May and June when the NaCl concentration is relatively low, less than 0.45%. As *A. paludum* reproduces in brackish water only when the salinity is reduced, the population is univoltine in Daizen Pond, but trivoltine in the upstream pond (Fig. 2a). In freshwater habitats in Kochi, *A. paludum* can be trivoltine or tetravoltine (Harada, 2003). Consequently, it is proposed that *A. paludum* can alter its life cycle according to the salinity of its habitat.

Previous research on *A. paludum* indicate that environmental factors such as day length, temperature, and drying out of water bodies affect not only reproduction but also migratory traits such wing polymorphism, flight muscles and flight propensity (Harada, 1998, 2003; Harada et al., 2000). These responses seem to be mostly associated with the oogenesis-flight syndrome (Johnson, 1969).

Increase in NaCl concentration reduced reproduction, but didn't stop it. Moreover, it resulted in migratory ability being maintained for a long time. It seems that females of *A. paludum* can switch between reproduction and migration depending on the current osmotic pressure of the habitat. This flexible strategy can be adaptive for inhabiting brackish waters in which NaCl concentration often increase and decrease. For such a flexible strategy, they need to develop a physiological plasticity. In contrast, males exposed to 0.5 or 0.9% NaCl salinity show a reduced flight ability and prolonged longevity (Kishi et al., 2006). Thus, the responses of *A. paludum* to changes in NaCl concentration show that this species can adapt to several extreme environments, and this plasticity may account for this species showing wide distribution in the Palearctic.

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