

Intraguild predation of water scorpion *Laccotrephes japonensis* (Nepidae: Heteroptera)

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Abstract

Previous work on community structure of the invertebrate fauna of Japanese wetlands indicates the presence of intraguild predation between the heteropterans *Laccotrephes japonensis* (Nepidae) and *Kirkaldyia deyrolli* (Belostomatidae). We designed a series of experiments to understand the biotic interactions of several species of sympatric heteroptera and their shared prey. Adult *Laccotrephes japonensis* (Nepidae), first instar nymph of *Kirkaldyia* (= *Lethocerus*) *deyrolli* (Belostomatidae), *Hyla japonica* tadpole, and 4th instar nymph of *Appasus japonicus* (Belostomatidae) have been shown to be intraguild predator, intraguild prey, common prey, and prey of *L. japonensis*, respectively. To further understand the factors affecting prey preference by *L. japonensis*, we also examined the comparison of swimming speed in the three prey animals (*K. deyrolli* first instar nymph, *A. japonicus* 4th instar nymph, and *H. japonica* tadpole), and effects of prey animals on weight gain of *L. japonensis* adult. Despite there being no significant difference in weight gain or swimming speed of the three prey species, *L. japonensis* exhibited a strong preference for the 1st instar nymph of *K. deyrolli*. We suggested that this may be evidence for one of elimination of a potential of competitor of *K. deyrolli* by *L. japonensis* through intraguild predation.

Introduction

A group of species that exploit the same class of environmental resources in a similar way, regardless of their taxonomic position, is defined as a guild (Root 1967). Intraguild predation (IGP) occurs when a member of the guild preys upon another member of the same guild (Polis et al. 1989; Polis and Holt 1992). IGP is a taxonomically widespread interaction within communities and exists broadly in nature (Polis et al. 1989; Polis and Holt 1992; Rosenheim et al. 1995; Brodeur and Rosenheim 2000; Müller and Brodeur 2002; Arim and Marquet 2004).

In temporary water pools void of large fishes, large heteropterans, such as Belostomatidae, Nepidae, and Notonectidae, are often at the top of the food chain in aquatic communities (Waters 1977; Runck and Blinn 1994). The bugs of Nepoidea, including Belostomatidae and Nepidae, prey upon a variety of aquatic animals such as insects, snails, reptiles, anurans, and small fish in Japanese rice fields (Ohba and Nakasuji 2006). The younger nymphs of the giant water bug *Kirkaldyia* (= *Lethocerus*) *deyrolli* Vuillefroy (Belostomatidae) are considered to be predators that prey mainly upon tadpoles (Ohba and Nakasuji 2006; Ohba et al 2008). In addition, the water scorpion *Laccotrephes japonensis* Scott adult (Nepidae) prey upon *K. deyrolli* younger nymph (Ohba 2007) and tadpole in rice

fields (Ohba and Nakasuji 2006). Under such a situation, adult *L. japonensis*, younger nymph of *K. deyrolli*, and anuran larva (tadpole) are regarded as intraguild predator, intraguild prey, and common prey, respectively (Ohba and Nakasuji 2007). Commonly sympatric in Japanese wetlands adult *L. japonensis* and younger nymph of *K. deyrolli* meet the definition of same guild members, competing with each other for tadpoles and other aquatic invertebrates with younger nymphs of *K. deyrolli* serving as intraguild prey for *L. japonensis*. The sympatric giant water bug *Appasus japonicus* Vuillefroy (Belostomatidae) is not a member of this guild however it mainly preys upon snails and aquatic insects (Ohba and Nakasuji 2006) and therefore has less niche overlap in comparison to the other guild members.

To reveal the characteristics of intraguild predation of *L. japonensis* adults, we examined the selectivity of *L. japonensis* adults when *A. japonicus* fourth instar nymphs (not competitor but prey animal of *L. japonensis*, and similar body-size of first instar nymph of *K. deyrolli*) was added to the *L. japonensis* adult – *K. deyrolli* first instar nymph – *Hyla japonica* Günther (Hylidae) tadpole system (Fig. 1). As a test of the hypothesis that IGP serves as a means for elimination of a potential of competitor by *L. japonensis*, we examined the swimming-speed of the

three prey animals (*K. deyrolli* younger nymph, *A. japonicus* nymph, and *H. japonica* tadpole), and effects of prey preference on weight gain of *L. japonensis* adults.

Methods

Study animals

All study animals, excluding 1st instar nymph of *K. deyrolli*, were collected from Misaki town, north Okayama, central Japan in June 2006. Prey tadpoles at Gosner stage 35–40 (Gosner 1960) were *Hyla japonica* which was dominant species in their field. Adults of *K. deyrolli* were collected from rice fields in the above mentioned site, and their hatchlings (as first instars) were used in the experiment. All animals were returned to the laboratory and maintained individually in plastic cups until testing. All experiments were completed within 2 weeks of capture.

Selectivity of water scorpion

To examine the selectivity of water scorpion, *Laccotrephes japonensis*, adults when three prey species were supplied, an experiment was conducted in an aquarium (200 × 270 mm mouth opening, 200 mm height) maintained at 25.0 °C

water temperature and 16L:8D light cycle. The experiment was conducted in June 2006. River sand was laid on the bottom of the aquarium in a 10-mm thick layer, and dechlorinated tap water was added over the sand surface to a depth of 50 mm. Predation trials on the three preys, tadpoles of *Hyla japonica*, first instar nymph of *K. deyrolli* (competitor and prey animal of *L. japonensis*), and fourth instar nymphs of *A. japonicus* (not competitor but prey animal of *L. japonensis*, and similar body-size of first instar nymph of *K. deyrolli*) were conducted (Ohba and Nakasuji 2006). Until the beginning of experiment, *K. deyrolli* 1st instar nymphs and *A. japonicus* 4th instar nymphs were fed *ad libitum* daily with their prey animals (*Hyla japonica* tadpoles for *K. deyrolli*, and snails, Lymnaeidae for *A. japonicus*) in order to prevent intra- and interspecific predation during the experiment. Each prey was housed individually in a plastic cup (50 mm diameter, 30 mm height) until the beginning of the experiment. *Laccotrephes japonensis* adults were fasted for 24 h before the experiment. Five *Hyla japonica* tadpoles, 5 first instar nymphs of *K. deyrolli* (11.5 ± 0.06 mm, mean body length \pm SE, $n = 40$), 5 fourth instar nymphs of *A. japonicus* (11.9 ± 0.11 mm, $n = 40$) and a *L. japonensis* male (31.6 ± 0.51 mm mean body length \pm SE, $n = 8$) and a female adult (35.7 ± 0.44 mm, $n = 8$) were introduced into the aquarium. Half an hour

before the beginning of the experiment, the prey animals were added to the aquarium for acclimation. The number of carcasses (= fed on by *L. japonensis*) of each prey animals were counted at 2, 4, 6, 12, 24 hours after the beginning of the experiment. Eight replicates were performed.

Effect of prey animal on weight gain of *L. japonensis* adult

To examine the effects of different prey on weight gain of *L. japonensis* adult, a rearing experiment was conducted at 25.0°C water temperature and 16L: 8D light cycle. The experiment was conducted in June 2006. First, *L. japonensis* adults were collected from the field, and were kept without food during three days.

Laccotrephes japonensis adult were maintained individually in cups (80 mm diameter, 80 mm height) filled with water to a depth of 20 mm. One *H. japonica* tadpole, a 1st instar nymph of *K. deyrolli*, and a 4th instar nymph of *A. japonicus* were used as prey animals of *L. japonensis* adults. The wet weight of blotted *L. japonensis* was recorded before and after each replicate. The *H. japonica* tadpole, 1st instar nymph of *K. deyrolli*, and 4th instar nymph of *A. japonicus* were replicated 26, 30 and 15 times, respectively. Wet weight was used as a standard measure for consistency even though water content of ingested material may vary

across prey taxa thus affecting nutrient content. The advantage of obtaining more accurate information about nutrient intake by preparing the predators for dry weight analysis or attempting to time weight measurements is offset by the time requirement and uncertainty of gut passage time. While there is significant research on this topic among fishes and terrestrial invertebrates, data is lacking for aquatic invertebrate communities especially predators (Mayntz et al. 2005).

Comparison of swimming-speed in three prey animals

In general, aquatic animals quickly escape from a predator by swimming right before a predation attempt. To compare escape capacity, swimming-speed of the three prey animals was examined. The experiment was conducted in an elongated container (200 mm long, 25 mm wide, 20 mm height, 10 mm water depth) maintained at 25.0 °C water temperature and 16L:8D light. Swimming-speed per 100 mm when each prey animal was pecked by chopstick was recorded with a stopwatch. The *H. japonica* tadpole, 1st instar nymph of *K. deyrolli*, and 4th instar nymph of *A. japonicus* were replicated 19, 17 and 15 times, respectively.

Statistical analyses

The mortality of the three prey animals were compared using a repeated-measures one-way ANOVA, with prey animal (*H. japonica* tadpole, 1st instar nymph of *K. deyrolli*, or 4th instar nymph of *A. japonicus*) as the between-subject, and lapsed times as the within-subject factor. Mauchly's test indicated a significant violation of the assumption of sphericity ($P < 0.001$). Therefore, significance levels for within-subject effects were calculated using Greenhouse-Geisser for the degrees of freedom (Quinn and Keough 2002). Separate one-way ANOVA tests were applied to the differences among prey in each nymphal stage when a significant interaction effect was encountered in the between-subject and within-subject tests. Scheffe's tests were performed to assess differences among prey when significant effects were detected in the separate one-way ANOVA. \log_{10} transformations or arcsine-square-root transformations for exact values were made in order to standardize variances and improve normality, if necessary to satisfy the assumptions of the ANOVA model.

For the swimming-speed and the effects of different prey on weight gain of *L. japonensis* adults, a Kruskal-Wallis test was applied to the differences among the three prey animals. Statistical significance was set at $\alpha = 0.05$. All

statistical tests were conducted using computer software (SPSS for Windows ver. 11.0; SPSS Inc.).

Results

Selectivity of water scorpion

A repeated-measures one-way ANOVA revealed that prey animal in between-subject, and lapsed time and prey animal-by-lapsed time interaction effects were all significant factors (Prey animal: $F_{2,21} = 10.2$, $P = 0.001$; Lapsed time: $F_{1,2,26.0} = 18.7$, $P < 0.001$ (after Greenhouse-Geisser correction); Prey animal-by-Lapsed time: $F_{2.5,26.0} = 2.5$, $P = 0.035$ (after Greenhouse-Geisser correction), Greenhouse-Geisser $\varepsilon = 0.310$, for arcsine-square-root transformed data).

The mortality differed significantly among prey animals after 2h (one-way ANOVA, $F_{2,21} = 6.03$, $P = 0.009$, Fig. 2), 4h ($F_{2,21} = 5.70$, $P = 0.011$), 6h ($F_{2,21} = 7.17$, $P = 0.004$), 12h ($F_{2,21} = 15.08$, $P < 0.001$) and 24h ($F_{2,21} = 18.07$, $P < 0.001$). After 2h, the mortality of *K. deyrolli* nymphs was significantly higher than that for either the *A. japonicus* nymph or tadpole trials (Scheffe's test, $P < 0.05$), but it did not differ significantly between the *A. japonicus* nymph and tadpole trials ($P =$

0.902). Similarly, after 12-24h, the mortality of *K. deyrolli* nymphs was significantly greater than that of the *A. japonicus* nymphs or tadpoles (Scheffe's test, $P = 0.032$). From 4-6h, it was not significantly different between the *K. deyrolli* nymph and *A. japonicus* nymph trials ($P = 0.099$). After the 2h and 12-24h periods, mortality of *K. deyrolli* nymphs was demonstrably higher than that of the other prey animals. The mortality of tadpoles was significantly the lowest of the three prey animals in all experimental periods (Fig. 2).

Effect of prey animal on weight gain of *L. japonensis* adult

There was no significant difference among the three prey animals in weight gain (mean \pm SE in weight gain, *Hyla japonica* tadpole = 44.62 ± 5.45 mg, *K. deyrolli* nymph = 33.85 ± 5.00 mg, *A. japonicus* nymph = 42.80 ± 6.23 mg, Kruskal-Wallis test, $H = 1.94$, $P = 0.379$).

Comparison of swimming-speed in three prey animals

There was no significant difference among the three prey animals in swimming-speed (mean \pm SE time in seconds to swim 100mm, *Hyla japonica* tadpole = 11.15 ± 1.31 , *K. deyrolli* nymph = 9.11 ± 0.63 , *A. japonicus* nymph =

9.80 ± 0.82 , Kruskal-Wallis test, $H = 1.94$, $P = 0.379$). Thus, the swimming-speed of three prey animals was almost the same.

Discussion

Understanding the biotic-interactions of sympatric aquatic Heteroptera and their prey in temporary pools is necessary to reveal intraguild predation (IGP) and its role in community dynamics. Of the prey tested *K. deyrolli* nymphs were significantly preferred by *L. japonensis*. However, there was no significant effect of prey identity on weight gain to *L. japonensis* adult nor were there significant differences in predator avoidance abilities of three prey animals.

Some studies refer to the meaning of IGP: (1) elimination of a potential predator (mosquito larva; Corbet and Griffiths 1963), (2) elimination of a potential of competitor (blow fly; Blackith and Blackith 1984), (3) obtainment of a good quality meal (predatory bug; Lucas and Alomar 2001), (4) encounter rate dependency (lacewing, Lucas et al. 1998) and (5) risk reduction for the shared prey (Crumrine 2005). In present study, considering the five potential roles of IGP in community structure we tentatively eliminate three of them and provide support for the role of competitive exclusion. Although *K. deyrolli* adults are larger than *L.*

japonensis adults, previous field work never recorded *L. japonensis* adults as the prey item of *K. deyrolli* adults (Ohba and Nakasuji 2006; S. Ohba, unpubl.). Thus, we do not regard the IGP of *L. japonensis* adult as the elimination of a potential predator (Corbet and Griffiths 1963). The effect of weight gain on *L. japonensis* adults was not different among the three prey animals so IGP of *K. deyrolli* nymphs cannot be regarded as an especially high quality meal (Lucas and Alomar 2001). Water content of the three species may differ thereby affecting the quality of each prey type. Further work on this aspect of prey quality would require an experiment to differentiate the three prey species based on wet weight versus dry weight and nutrient content. Experimental design ruled out encounter rate as an explanation for prey choice by *L. japonensis* (Lucas et al. 1998). We did not address whether or not IGP results in risk reduction for the anuran larvae, however this does not discount a role for competitive exclusion between *L. japonensis* and *K. deyrolli*. It is likely that both roles are part of this interaction.

Hyla japonica tadpoles may adopt a motionlessness or crypsis tactic to reduce detection by *L. japonensis* adults, as in other anuran larvae accounting for their low mortality rate in this experiment (Skelly and Kiesecker 2001; Relyea 2001; Van Buskirk 2002; McIntyre et al. 2004; Swart and Taylor 2004). But there

is no reason to believe this is more effective in the laboratory than in the field, although *L. japonensis* adults may include more anuran larvae in their diet during seasonally abundant periods (Ohba and Nakasuji 2007; Ohba et al. 2008). In addition, the encounter rate of tadpole with *L. japonensis* adult might be lower than that of 1st instar *K. deyrolli* and 4th instar *A. japonicus*.

The present experiment to examine prey selectivity of *L. japonensis* adults was not designed to make microhabitat and refuge for prey. However, field experiment including microhabitat and refuge structure for prey need to be done in order to consider the escape ability of each prey species. This problem may affect the prey selectivity of *L. japonensis* adults in the experiment and cause some difference between the laboratory and rice fields. It should be dealt in a future study.

In present study, we revealed that *L. japonensis* adult selectively preys upon *K. deyrolli* nymphs. Furthermore we provide support for the hypothesis that IGP between these two predators serves as one of elimination of a potential of competitor. While an ontogenetic shift with *K. deyrolli* adults feeding on *L. japonensis* nymphs is not likely (Ohba and Nakasuji 2006), interactions between several instars of these two species might be an interesting subject for future

study.

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Figure Legends

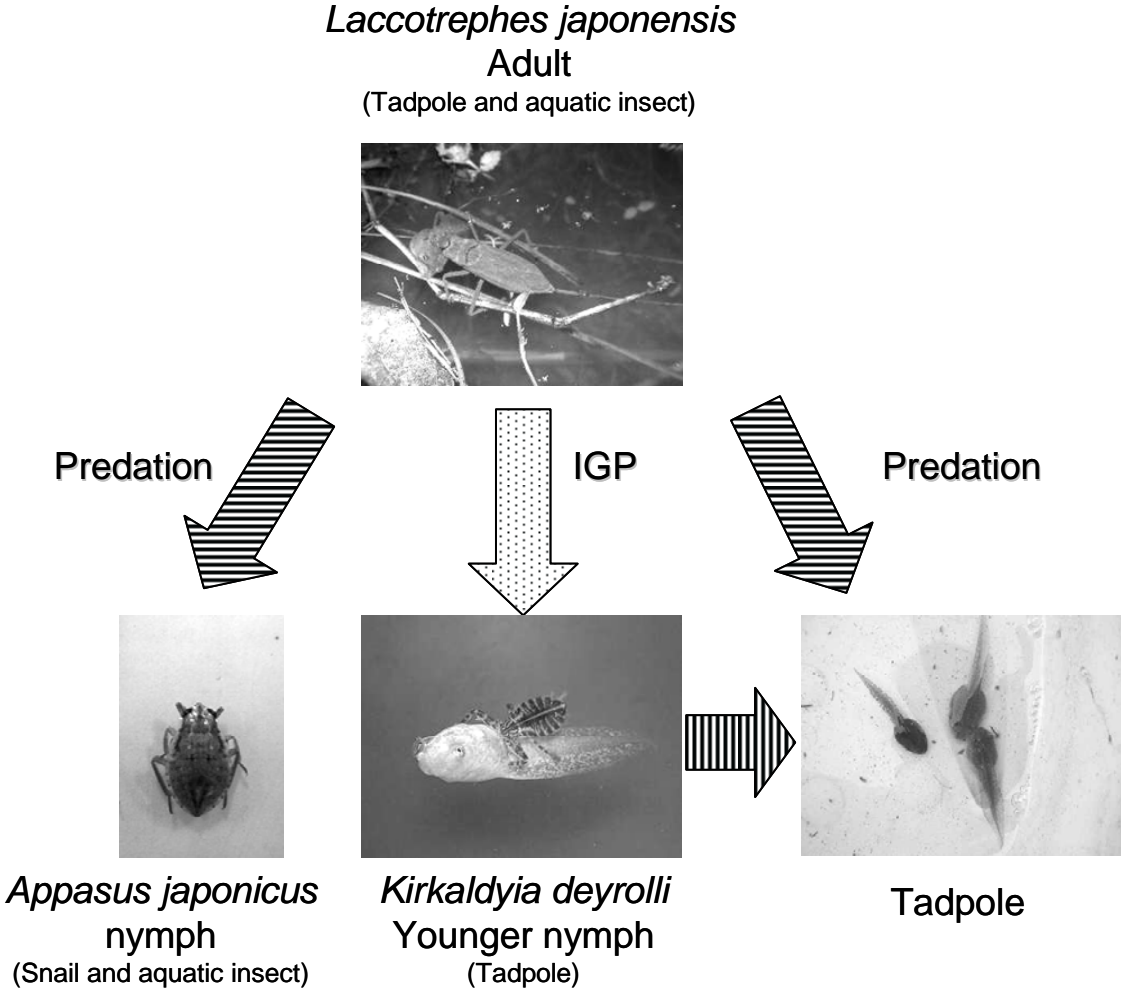


Fig. 1. Schematic representation of interactions among *L. japonensis* adult, *K. deyrolli* younger nymph, *A. japonicus* nymph, and tadpoles. *IGP*, intraguild predation. Words in parenthesis of each predatory bug showed main dietary item in the field (Ohba and Nakasuji, 2006).

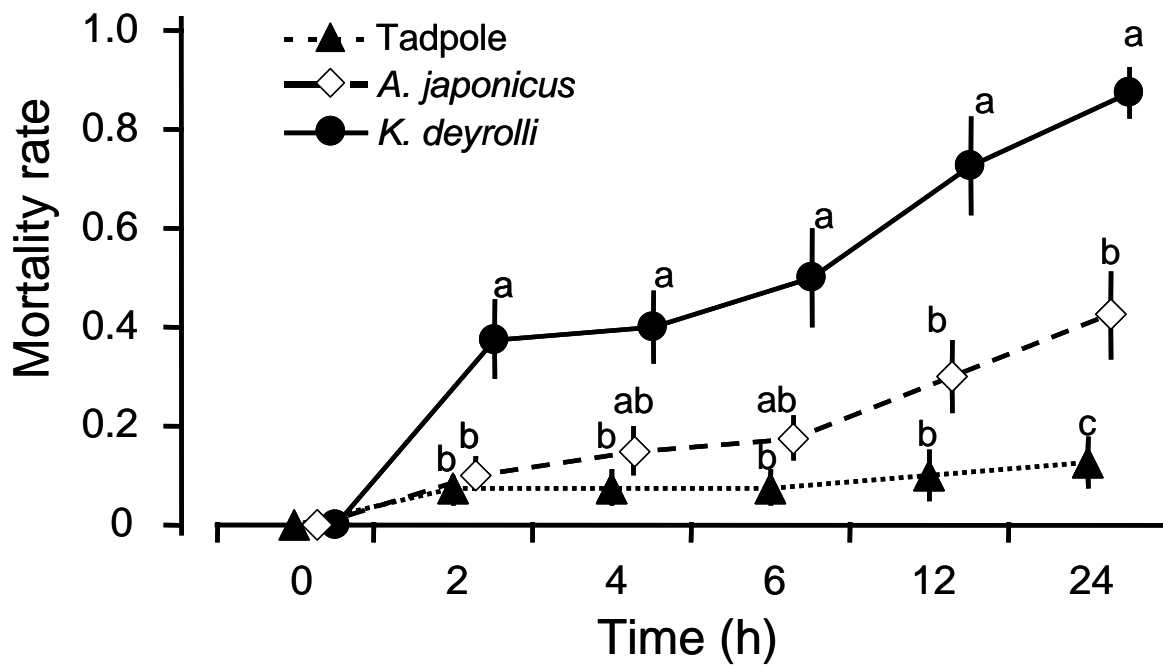


Fig. 2. Comparison of the mortality rate (mean \pm SE) among three prey animals.

Different letters among each time denote significant differences ($P < 0.05$, Scheffe's test).