Formosan Entomologist
Journal Homepage: entsocjournal.yabee.com.tw

The Effect of Trapa natans on the Number of Water Striders Gerris nepalensis and the Consequent Foraging Pressure on the Leaf Beetle Galerucella nipponensis 【Research report】

菱角Trapa natans對於水黽Gerris nepalensis數量之影響暨水黽對於葉甲Galerucella niponensis之捕食壓力【研究報告】

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Received: 2010/10/31 Accepted: 2010/12/26 Available online: 2010/03/01

Abstract

It is hypothesized that the presence of water caltrop, Trapa natans leaves increases the number of Gerris nepalensis in its habitat and at the same time increases the foraging pressure on the leaf beetle, Galerucella nipponensis. This tends to support a possible symbiotic relationship of G. nepalensis to T. natans. The four questions in this study and their answers are as follows. 1) Can leaves of T. natans attract G. nepalensis? Based on the four experiments in this study, G. nepalensis was attracted to leaves of T. natans, especially those injured by the beetle, Gl. nipponensis. Even filter paper treated with liquids extracted from the leaves of T. natans injured by the beetle, Gl. nipponensis was preferred by G. nepalensis. 2) Do females of G. nepalensis prefer leaves from T. natans for their oviposition substrates over substrates made from other floating plants? When presented with artificial leaves made from polystyrene mimicking leaves of T. natans and other species of water floating plants such as Hydrocharis dubia and the fallen leaves of the camphor tree, Cinnamomum camphora, the females of G. nepalensis chose the artificial T. natans leaves as their oviposition substrates. Moreover when presented with real T. natans leaves and the artificial ones, they chose the real ones as their substrates. 3) Can G. nepalensis kill Gl. nipponensis by eating their embryos and the first, second and third instar larvae? Based on the survival rate, it is evident that adult G. nepalensis kill the embryos of Gl. nipponensis, and the later nymph stages of G. nepalensis can kill the younger stages of Gl. nipponensis very efficiently. 4) Is the success rate of overwintering and the reproductive activity that follows in the next spring enhanced by the accompanying of G. nepalensis with T. natans prior to overwintering? In the experimental group, in which G. nepalensis were accompanied with leaves of T. natans for 2 weeks before overwintering, 90.7% of the adult G. nepalensis survived and overwintered for 3 months. This was a little bit higher than the 81.5% survival rate by the control group, in which G. nepalensis were accompanied with artificial T. natans leaves made from polystyrene. Overwintered adults in the experimental group laid eggs in the following spring for 52 days on average, a significantly longer period than the 38 days for those in the control group. This led to a fecundity of 217 eggs on average compared to the 132 eggs laid by the control group. A symbiotic relationship between G. nepalensis and T. natans is possible against Gl. nipponensis especially based on the increasing foraging pressure from the water striders.

摘要

本試驗假設菱角Trapa natans葉子能增加水黽數量，進而增加葉甲Galerucella niponensis之被捕食壓力，故此現象顯示水黽與菱角間可能具共生關係；因此本試驗由下列四方面進行研究：(1) 菱角葉是否能吸引水黽？(2) 雌水黽是否偏好菱角葉做為產卵處，即使是以聚苯乙烯製作之假菱角葉？(3) 水黽是否能取食葉甲之胚胎及第一、二、三齡之幼蟲？(4) 菱角是否能增加水黽越冬之存活率及次年春季之繁殖活動？試驗結果顯示，菱角葉能吸引水黽，特別是被葉甲傷害之菱角葉；即便有其他浮水植物，如水鱉Hydrocharis dubia或樟樹Cinnamomum camphora落葉，雌蟲仍偏好假菱角葉作為產卵處；水黽成蟲能取食葉甲胚胎，若蟲亦能有效率的捕食葉甲幼蟲；當試驗組之水黽與菱角葉共存，越冬存活率達90.7% 且存活時間達三個月；然對照組水黽與假菱角葉共存，其存活率僅81.5%；試驗組水黽在次年春季來臨後之52日內，共產217顆卵；而對照組水黽在春季來臨後之38日內，產132顆卵；故可推論水黽Gerris nepalensis藉由捕食葉甲Galerucella niponensis之方式與菱角Trapa natans達成共生關係。

Key words: chemical attraction, diapause posture, spring reproduction, oviposition substrates, feeding pressure

關鍵詞: 化學吸引、滯育狀態、春季繁殖、產卵介質、捕食壓力。

Full Text: PDF(0.64 MB)

台灣昆蟲 31: 51-65 (2011)
Formosan Entomol. 31: 51-65 (2011)

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ABSTRACT

It is hypothesized that the presence of water caltrop, *Trapa natans* leaves increases the number of *Gerris nepalensis* in its habitat and at the same time increases the foraging pressure on the leaf beetle, *Galerucella nipponensis*. This tends to support a possible symbiotic relationship of *G. nepalensis* to *T. natans*. The four questions in this study and their answers are as follows. 1) Can leaves of *T. natans* attract *G. nepalensis*? Based on the four experiments in this study, *G. nepalensis* was attracted to leaves of *T. natans*, especially those injured by the beetle, *Gl. nipponensis*. Even filter paper treated with liquids extracted from the leaves of *T. natans* injured by the beetle, *Gl. nipponensis* was preferred by *G. nepalensis*. 2) Do females of *G. nepalensis* prefer leaves from *T. natans* for their oviposition substrates over substrates made from other floating plants? When presented with artificial leaves made from polystyrene mimicking leaves of *T. natans* and other species of water floating plants such as *Hydrocharis dubia* and the fallen leaves of the camphor tree, *Cinnamomum camphora*, the females of *G. nepalensis* chose the artificial *T. natans* leaves as their oviposition substrates. Moreover when presented with real *T. natans* leaves and the artificial ones, they chose the real ones as their substrates. 3) Can *G. nepalensis* kill *Gl. nipponensis* by eating their embryos and the first, second and third instar larvae? Based on the survival rate, it is evident that adult *G. nepalensis* kill the embryos of *Gl. nipponensis*, and the later nymph stages of *G. nepalensis* can kill the younger stages of *Gl. nipponensis* very efficiently. 4) Is the success rate of overwintering and the reproductive activity that follows in the next spring enhanced by the accompanying of *G. nepalensis* with *T. natans* prior to overwintering? In the experimental group, in which *G. nepalensis* were accompanied with leaves of *T. natans* for 2 weeks before overwintering, 90.7% of the adult *G. nepalensis* survived and overwintered for 3 months. This was a little bit higher than the 81.5% survival rate by the control group, in which *G. nepalensis* were accompanied with artificial *T. natans*

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leaves made from polystyrene. Overwintered adults in the experimental group laid eggs in the following spring for 52 days on average, a significantly longer period than the 38 days for those in the control group. This led to a fecundity of 217 eggs on average compared to the 132 eggs laid by the control group. A symbiotic relationship between *G. nepalensis* and *T. natans* is possible against *Gl. nipponensis* especially based on the increasing foraging pressure from the water striders.

Key words: chemical attraction, diapause posture, spring reproduction, oviposition substrates, feeding pressure

Introduction

Several reports have been written on chemically mediated symbiotic relationships between plants and carnivores (insects or mites) that eat the herbivores feeding on these plants (Takabayashi *et al.*, 1995; Takabayashi and Dicke, 1996; Shiojiri *et al.*, 2000; Nikovic *et al.*, 2001). Such plants release "SOS chemicals" into the air that attract carnivores when attacked by herbivores. For example, the herbivorous mite, *Tetranychus urticae* attacks the Lima bean, *Phaseolus limensis.* When the mite damages the leaves of the bean, the Lima bean synthesizes and releases special chemical attractants (4,8-dimethyl-1,3 (_E), 7-nonatriene; Linalool; Methyl salicylate; (E)-E)-β-Ocimene etc.) into the air. The carnivorous mite, *Phytoseiulus persimilus* is attracted to these chemicals and is very efficient in eating the herbivorous mites on the leaves (Takabayashi and Dicke, 1996). Besides volatiles, plants have other ways of attracting predators, e.g., production of extra-floral nectar or by providing leaf domatia as shelter for the predators. In addition, the plant will provide extra-floral nectories to the parasitoids of the plant's herbivores. The nectar producing epidermal glands located on vegetative plants produce the extra-floral nectories (Elias, 1983). For example, the extra-floral nectar of cotton, *Gossypium hirsutum* is a food source that is available early in the season when floral nectar is not yet available in the cotton and can be used by the parasitoid wasp, *Microplitis croceipes* (Röse *et al.*, 2006). Beneficial mites (predators or fungivores) have been postulated to potentially benefit from leaf domatia on the underside of leaves by having a secure structure in a humid environment in which to molt, lay eggs and take refuge from predaters. In return the plant benefits from the mites because they remove the herbivorous mites, other damaging arthropods and fungal pathogens from the leaves (Agrawal, 1997, Agrawal and Karban, 1997).

Gerris nepalensis is a relatively common water strider that is bi- and/or tri-voltine in Kochi, Japan (33°N) (Furukawa and Harada, 1998; Harada, 2003). It can be commonly observed on the leaves of the floating water plant, *Trapa natans* (Ikeda and Nakasuji, 2002; Saiki and Harada, 2003). The leaves of *T. natans* are present from May to November when *G. nepalensis* is reproducing and/or growing. At the same time, the leaf beetle (*Galerucella nipponensis*) seriously damages the leaves of this water plant (Saiki and Harada, 2002). However, the adults of *G. nepalensis* eat the eggs, larvae and pupae of *Gl. nipponensis* (Ikeda and Nakasuji, 2002; Tanaka and Nakasuji, 2002; Ikeda, 2004). Thus, a symbiotic relationship between *T. natans* and *G. nepalensis* against the herbivorous *Gl. nipponensis* can be hypothesized. Harada *et al.* (2008) reported on an experimental result of this symbiotic relationship. Exposure of the water strider, *G. nepalensis* to the leaves of the water lily, *T. natans*, during either the nymphal or adult stages causes a higher proportion of reproductive females (60.0%) and increase the number of eggs laid by *G. nepalensis* (25.1 ± 8.1) compared to having exposure to the leaves of another floating plant, *Hydrocharis dubia* (20.2%, 6.7 ± 17.8) or artificial *T. natans* leaves made of polystyrene (24.2%, 20.0 ± 16.9). A significantly higher percentage (76.4) of eggs laid by females that were reared on the leaves of *T. natans* in the nymphal and adult stages, successfully developed to the first stage of nymph compared to those reared on artificial *T. natans* leaves (Harada *et al.*, 2008).

Association with *T. natans* leaves can increase the number of *G. nepalensis* in their habitat and is likely to increase the foraging pressure on the leaf beetle, *Gl. nipponensis.* This in turn leads to a possible symbiotic relationship of *G. nepalensis* to *T. natans*. However, there has been no evidence of such foraging pressure by the water striders on the water caltrop. At present, the four questions relating to the symbiosis that remain to be answered are:

1. Can leaves of *T. natans* injured by the beetle *Gl. nipponensis* attract substantially more *G. nepalensis* than intact leaves?
2. Do females of *G. nepalensis* prefer *T. natans* leaves as a substrate to lay eggs over leaves from other kinds of floating plants?
3. Can the larvae of *G. nepalensis* at different ages eat and kill the first, second and third stages of larvae of *Gl. nipponensis*?
4. Can the success rate of overwintering and the reproductive activity that follows the next spring be promoted by the association of *G. nepalensis* with *T. natans?*

Materials and Methods

Experiment 1 (Question 1): Orientation experiments 1-4

More than 50 female-male pairs of apterous adults of *G. nepalensis* were collected from a lotus pond (Ishizuchi Pond: 0.24 km^2) located in Kochi City, Japan (33˚30'N, 133˚30'E), from August to October, 2003 for orientation experiments 1-4. This pond contained several floating water plants, *T. natans* L. some intact and some injured by the plant leaf beetle, *Gl. nipponensis.* In addition it contained several other (intact) floating plants, *H. dubia*. The adult *G. nepalensis* were reared as a mass culture under 15.5 h light-8.5 h dark (15.5L-8.5D) at 25 ± 2°C to promote active reproduction (Harada, 2003) under 2,000 Lux in an incubating room. The adult *G. nepalensis* were fed on adult flies, *Lucilia illustris,* which were provided freshly each day at a rate of one fly per 2 adults. The intact leaves and the injured leaves of *T. natans* and the intact leaves of *H. dubia* were kept in separate aquaria in a sun-room which received with more than 5,000 Lux of sunshine during the daytime. No lighting equipment was used at night, and the day-night air temperature fluctuated between 25°C and 35°C. The adult *G. nepalensis* and the water plants were used in the behavioral experiments within 3 months after having been collected.

Orientation experiment 1: Can *G. nepalensis* distinguish *T. netans* leaves damaged by *Gl. nipponensis* from intact ones?

The orientation experiment was conducted in an experimental aquarium (70 x 100 x 25 cm) with green colored glass in a climate-controlled room at 25 ± 2°C which was completely isolated from the outside, both visually and auditory. The experimental aquarium was filled with fresh water up to a height of 10 cm. An amount of leaves approx. 25 cm^2 was located at one side of the longitudinal axis, and another amount of leaves approx. 25 cm^2, but of a different type of leaves, was then located at the other side of the longitudinal axis. Then, ten female-male pairs of adult *G. nepalensis* were released at the center of the water area. Twenty minutes later, the number of individuals that were touching each set of leaves was counted. The trials were replicated forty or eighty times in total using the same individuals, although individual water striders were used repeatedly in order to generate a larger sample size due to the fact that the number of specimens was limited.

The locations of these two types of leaves were exchanged with each other every 10 trials. Forty or eighty replications were performed for each of the three kinds of experimental combinations.

A. Intact leaves of *T. natans* versus *T. natans* leaves damaged by *Gl. nipponensis*
B. *T. natans* leaves damaged by *Gl. nipponensis* versus intact leaves of *H. dubia*
C. Intact leaves of *T. natans* versus intact leaves of *H. dubia*

Orientation experiment 2: Can adult *G. nepalensis* detect hidden (visually undetectable) *T. natans* leaves damaged by *Gl. nipponensis* from intact ones?

The same ten female-male pairs of adult *G. nepalensis* that were used in orientation experiment 1 were again used several days later. The same 80 trials performed in experiment 1 were carried out for three different arrangements. However, the leaves that were located on either side of the axis were placed on a circular bottom plate measuring 12 cm in diameter, made of white paper pasted onto a 1 mm thick styrene plate to allow it to float on the water, and the leaves themselves were covered by a white paper cylinder dome (8 cm diameter and 2 cm height) and were visually completely undetectable by the adult *G. nepalensis*. Intact or damaged leaves of *T. natans* were put on the bottom plate of the cylinder dome made of white paper to shut out visual access from the water striders, and the top-roof of the cylinders were open for waters striders to sense the odors from the leaves. In addition, the side of the dome contained more than a hundred 1 mm diameter holes and the water strider still could not see anything so that the volatile gases from the leaves could escape.

Orientation experiment 3: Can adult *G. nepalensis* differentiate between the liquid extracted from *T. natans* damaged by *Gl. nipponensis* and those from intact *T. natans* leaves?

The same ten female-male pairs of adult *G. nepalensis* that were used in orientation experiments 1 & 2 were again used several days later. The same 80 trials as performed at experiments 1 and 2 were carried out for 3 different combinations. We then placed white filter paper (2 x 2 cm) on the 1 mm thick 12 cm diameter plates of white paper pasted onto styrene foam located on both sides of the experimental arena. Then the filter paper including 20 mL of liquid extracted from leaves which had been mashed in a mortar and a wooden pestle was attached to the edge of the plates. The liquid was soluble to the water of the experimental aquarium.

Orientation experiment 4: Can the ability of the adult *G. nepalensis* to differentiate leaves of *T. natans* damaged by *Gl. nipponensis* from intact ones be enhanced by creating a food shortage for the water strider?

The same ten female-male pairs of adult *G. nepalensis* that were used in the orientation experiments 1, 2 & 3 were fed one fly of *L. illustris* per one adult *G. nepalensis* only every 3 days for 2 weeks. Then the starved female-male pairs were used again for experiment 4. The same trials as carried out in experiment 1 were repeated for the two combinations of the two sets of leaves:

A. Leaves of *T. natans* damaged by *Gl. nipponensis* versus intact *H. dubia* leaves,
B. Intact leaves of *T. natans* versus intact *H. dubia* leaves.

Experiment 2 (Question 2): Experiment to determine the choice made by *G. nepalensis* regarding the substrates for laying eggs.

In mid-August 2005, nine female and male pairs of apterous *G. nepalensis*, one unit of the water caltrop, *T. natans* without damage from *Gl. niponensis* and one unit of another species of water floating plant, *H. dubia* were collected from the lotus pond located in Kochi. Fallen leaves of the camphor tree, *Cinnamomum camphora* were also collected from a garden on the campus of Kochi University located in Kochi City. For the experiments on choice for oviposition sites, artificial leaves made of polystyrene foam were also used.

A transparent aquarium, measuring 30 cm in diameter x 15 cm in height was filled with fresh water up to a height of 10 cm. Leaves from 2 different types of plants as well as artificial leaves each covered 15 cm^2 of the water surface. In addition, three pairs of apterous females and males of *G. nepalensis* were released on the water in the aquarium. The choices of the two kinds of leaves available to the three females were as follows:

A. Actual leaves of *T. natans* and artificial *T. natans* leaves.
B. Leaves of the camphor tree, *C. camphora* and artificial *T. natans* leaves. Camphor is believed to be toxic to *G. nepalensis* and is sometimes used as a repellent (Nerio *et al.*, 2010). *G. nepalensis* can be assumed to avoid laying eggs on camphor tree leaves probably due to their aromatic effect.
C. Leaves of another floating water plant, *H. dubia* and artificial *T. natans* leaves. In each type of combination, the number of eggs laid on the *T. natans* leaves, the articificial *T. natans* leaves and leaves of *C. camphora* or *H. dubia* were counted each day for a total of 25 days. Artificial leaves were made of 1 mm thick compressed type of polystyrene foam, light-blue in color and with an identical size and shape of the leaves of *T. natans*. Because *H. dubia* are common in the habitat of *G. nepalensis,* it is possible that this insect can differentiate between real leaves of *H. dubia* and artificial *T. natans* leaves.

Experiment 3 (Question 3): Feeding experiments

More than 25 female-male pairs of overwintering adults of apterous *G. nepalensis* were collected from the lotus pond on December 15, 2004. They were reared in an incubating room under 15.5L-8.5D with lighting from fluorescent lamps that emitted more than 2,000 lux at 25.0 ± 2°C over the next 2 generations into the first to the fifth stage of nymph of the F3 generation. These F3 generation nymphs were used for the foraging experiment to answer Question 3. All nymphs and adults of *G. nepalensis* from the P to F3 generations reared in the laboratory were fed on adult flies, *Lucillia illustris* at a rate of 5 bugs of the 1st and/or 2nd stages per 1 fly, 3 bugs of the 3rd and/or 4th stages per 1 fly and 2 bugs of 5th stage of larvae and/or adult stage per 1 fly.

More than 25 female-male pairs of the floating plant beetles, *Gl. nipponensis* and 10 units of the floating plant, *T. natans* were collected from the lotus pond from August to November, 2005. All beetles and plants collected were kept in the incubating room under the conditions of 2,000 Lux of lighting, and 15.5L-8.5D at 25.0 ± 2°C. The beetles were fed on leaves of *T. natans* collected from the pond. Eggs laid by the beetles were collected as well as the larvae of the first to third stages of *Gl. nipponensis*, and were used for the foraging experiments.

Feeding experiment on eggs

Eggs laid on the artificial leaves made of polystyrene foam under the condition of 15.5L-8.5D at 25 ± 2°C by adult *Gl. nipponensis* (on average 8.1 or 11.2 eggs per dish) were released within 24 hrs after oviposition, on each dish (plastic, transparent, 15 cm in diameter and 5 cm high) which was filled with fresh water, 2 cm high. This procedure was replicated 90 times. One female-male pair of adult apterous *G. nepalensis* was released in each of the 36 dishes (Experimental group). The remaining 54 dishes did not contain any water striders (control group). The incubation of the 90 dishes was performed under the conditions of 15.5L-8.5D at 25 ± 2°C. The hatching process of the eggs and the duration of the embryonic development were recorded.

Feeding experiment on 1^{st} stage larvae of *Gl. nipponensis*

More than 15 intact-leaves of *T. natans* and 30 larvae of *Gl. nipponensis* at the 1^{st} stage, and within 24 hrs after hatching, were placed in white plastic dishes, measuring 35 cm long x 25.5 cm wide x 17.5 cm high. They were filled with fresh water to a height of 5 cm. This procedure was replicated 31 times. Thirty 1^{st} instar nymphs of *G. nepalensis* , within 24 hrs after hatching, were then placed in each of the 8 plastic dishes containing leaves and 1^{st} stage beetle larvae (1^{st}-1^{st} group: 1^{st} stage of larvae *Gl. nipponensis* versus the 1^{st} stage of nymphs *G. nepalensis*). Thirty nymphs at the 2^{nd} stage of *G. nepalensis,* within 24 hrs after molting, were then placed in each of the other 8 plastic dishes (1^{st}-2^{nd} group). Thirty nymphs, 3^{rd} stage of *G. nepalensis* within 24 hrs after molting, were then placed in each of another 8 plastic dishes (1^{st}-3^{rd} group). No nymphs of *G. nepalensis* at all were placed in the other 8 plastic dishes (1^{st}-nothing group: 1^{st} stage of larvae *Gl. nipponensis* versus no *G. nepalensis*). The number of nymphs of *G. nepalensis* was kept at 30 throughout the observation period. Survival of *Gl. nipponensis* 24hrs and 48hrs later was recorded in all four experimental groups. All the incubations were performed under the conditions of 15.5L-8.5D at 25 ± 2°C.

Feeding experiment on 2^{nd} instar larvae of *Gl. nipponensis*

Feeding experiments on 2^{nd} instar larvae of *Gl. nipponensis* were performed in the same manner as performed on the 1^{st} instars, making four experimental groups: Group 1, 2^{nd} stage larvae of beetles versus 1^{st} stage of nymphs of water striders; Group 2, 2^{nd} stage larvae of beetles versus 2^{nd} stage of nymphal water striders; Group 3, 2^{nd} stage larvae of beetles versus 3^{rd} stage of nymphal water striders; Group 4, 2^{nd} stage larvae of beetles versus no water striders.

Feeding experiment on 3^{rd} instars larvae of *Gl. nipponensis*

Feeding experiments on 3^{rd} instars larvae of *Gl. nipponensis* were performed in the same manner as performed on the 1^{st} and 2^{nd} stages of larvae except for making the following three experimental groups: Group 1, 3^{rd} stage larvae of beetles versus 3^{rd} stage of nymphs of water striders; Group 2, 3^{rd} stage larvae of beetles versus 5^{th} stage of nymphal water striders; Group 3, 3^{rd} stage larvae of beetles versus no water striders.

Experiment 4 (Question4): Overwintering experiment

More than 20 female-male pairs of overwintering and apterous adults of *G. nepalensis* and three units of *T. natans* without damage by *Gl. nipponensis* were collected in the fall of 2004. They were used for an overwintering experiment under natural conditions in Kochi City.

The effect of associating with *T. natans*: overwintering success and reproductive activity the next spring

On 15^{th} Dec, 2009, ten male-female pairs of apterous *G. nepalensis* were placed in a white plastic dish measuring 35 cm long x 25.5 cm wide x 17.5 cm high, and filled with fresh water to a height of 5 cm. This procedure was replicated 5 times. One unit (15-20 leaves included) of *T.*

natans was then placed on the water surface of three of the prepared dishes, while on the water surface of each of the other three dishes 15 artificial *T. natans* leaves made from polystyrene foam were placed instead. Consequently, a total of six dishes were then kept under natural conditions: 3 dishes with *T. natans* leaves (experimental group) and 3 dishes with artificial *T. natans* leaves (control group) for two weeks till the end of December, 2004.

The ten female-male pairs of *G. nepalensis* in each dish were then transferred to other same-sized dishes in which half the surface of the bottom was filled with litter and the other half with water. A large fallen leave of southern magnolia, *Magnolia grandiflora* (15 x 8 cm) was put on the litter in each dish. Each individual was marked with a small piece of colored tape for identification. The following were then recorded each day until the next spring: survivorship during the overwintering period, where was each individual: on land or on the water's surface, and did they adopt the "diapauses-posture" (Harada & Gon, 2006) or not. When individuals began to stride on the water the next spring, they were transferred to a transparent dish, measuring 15 cm in diameter x 5 cm in height, and filled with fresh water to approx. 5-10 mm high. Each dish included one female-male pair of *G. nepalensis* and one artificial *T. natans* leave made of polystyrene foam. Each pair was fed each day on one fly of *L. illustris*. The number of eggs laid was recorded every day for each dish. All incubations took place under natural conditions.

Statistical analysis

The χ^2-test between data to 50-50% theoretical value was used for Experiment 1 (Question 1) as the orientation experiment. The Mann-Whitney U-test was used to analyze the number of eggs laid each day on the two substrates for Experiment 2 (Question 2). The Mann-Whitney U-test was also used to analyze the effect of the adult *G. nepalensis* on the survival of the embryos of *Gl. nipponensis* and to determine the difference between the experimental (with water striders: 36 replicates) and the control (without water striders: 54 replicates). The χ^2-test was used for the analysis of the effects of feeding on 1^{st}-3^{rd} instars larvae of *Gl. nipponensis* in Experiment 3 (Question 3). The χ^2-test was also used to analyze the success ratio of the overwintering adult *G. nepalensis*. The Mann-Whitney U-test was again used to analyze the number of days that overwintering adults did not adopt the "diapauses-posture" and the character-ristics of reproduction (number of eggs, preoviposition period, oviposition period) the following spring in Experiment 4 (Question 4).

Results

Experiment 1 (Question 1): Orientation experiments 1-4

Orientation experiment 1: Can *G. nepalensis* differentiate *T. natans* leaves damaged by *Gl. nipponensis* from intact *T. natans* leaves?

Based on the results shown in Table 1, adult *G. nepalensis* is only slightly (but statistically significantly) more attracted to leaves of *T. natans* than leaves of *H. dubia*. However, *T. natans* leaves damaged by *Gl. nipponensis* tend to attract more adults of *G. nepalensis* than intact leaves of *T. natans*, although the difference was not statistically significant.

Orientation experiment 2: Can adult *G. nepalensis* differentiate between hidden (visually undetectable) leaves damaged by *Gl. nipponensis* and intact ones of *T. natans*?

Based on the results shown in Table 1, it is evident that leaves damaged by *Gl. nipponensis* can statistically significantly but slightly attract more adult *G. nepalensis* in comparison with intact *T. natans* leaves and intact *H. dubia* leaves, even when the

Table 1. Result of orientation experiments 1-3. Total number (%) of individuals oriented to the leaves or filter paper including the liquid extracted from leaves on each side of the experimental area, and the χ^2- test between data and 50-50% theoretical value. There were no significant differences between sexes in experiments A-C.

A. Intact versus damaged *T. natans*					
	Intact *T. natans*	Damaged *T. natans*	χ^2-value	*df*	*p*
Visible leaves	645 (45.6)	771 (54.4)	11.21	1	< 0.001
Invisible leaves	517 (47.0)	584 (53.0)	4.08	1	0.043
Liquid extracted from leaves	653 (46.6)	748 (53.4)	6.441	1	0.011
B. Damaged *T. natans* vs intact *H. dubia*					
	Damaged *T. natans*	Intact *H. dubia*	χ^2-value	*df*	*p*
Visible leaves	436 (56.4)	337 (43.6)	12.68	1	< 0.001
Invisible leaves	621 (58.6)	439 (41.4)	31.25	1	< 0.001
Liquid extracted from leaves	841 (63.0)	493 (37.0)	89.61	1	< 0.001
C. Intact *T. natans* vs intact *H. dubia*					
	Intact *T. natans*	Intact *H. dubia*	χ^2-value	*df*	*p*
Visible leaves	744 (55.8)	590 (44.2)	17.78	1	< 0.001
Invisible leaves	649 (52.3)	591 (47.7)	2.71	1	0.099
Liquid extracted from leaves	715 (50.1)	712 (49.9)	0.006	1	0.936

leaves are not visible. However, adult *G. nepalensis* can not differentiate intact leaves of *T. natans* from intact leaves of *H. dubia* if they can not see the leaves side by side.

Orientation experiment 3: Can adult *G. nepalensis* differentiate liquid extracted from *T. natans* damaged by *Gl. nipponensis* from liquid extracted from intact *T. natans* leaves?

Based on the results shown in Table 1, liquid extracted from *T. natans* leaves damaged by *Gl. nipponensis* attracted significantly more adult *G. nepalensis* in comparison with liquid extracted from intact leaves of *T. natans or H. dibua,* whereas liquid extracted from intact leaves of *T. natans* could not attract more adult water striders than liquid extracted from intact leaves of *H. dubia*.

Orientation experiment 4: Can the ability of adult *G. nepalensis* to differentiate the leaves damaged by *Gl. nipponensis* from intact leaves of *T. natans* be enhanced through a shortage of food for the water strider?

Based on the results shown in Table 2, a higher proportion of individuals (61%) homed in intact *T. natans* leaves rather then on intact *H. dubia* leaves. In addition about 65% of the individuals homed in on *T. natans* leaves damaged by *Gl. nipponensis*. These percentages are significantly higher than the percentages when the adult *G. nepalensis* were supplied with sufficient food (χ^2 test, *T. natans* leaves damaged versus intact leaves of *H. dubia,* 56.4% to leaves damaged when not starved, χ^2 value = 14.68, $df = 1$, $p < 0.001$; intact *T. natans* leaves versus intact *H. dubia* leaves, 55.8% to *T. natans* leaves when not starved, χ^2 value = 7.19, $df = 1$, $p = 0.007$).

Experiment 2 (Question 2): Experiment on choice of substrate to lay eggs

A. Leaves of *T. natans* versus artificial *T.*

Table 2. Results of the orientation experiment 4. Total number (%) of "starved" individuals oriented to the leaves located on each side of the experimental area and the χ^2- test between sexes and also between total data and 50-50% theoretical value.

A. Damaged *T. natans* vs intact *H. dubia*					
	Damaged leaves	Intact leaves	χ^2-value	*df*	*p*
Females	273 (62.3)	165 (37.7)	-	-	-
Males	310 (68.7)	141 (31.3)	4.041	1	0.044
Total	583 (65.6)	306 (34.4)	86.31	1	< 0.001
B. Intact *T. natans* vs intact *H. dubia*					
	T. natans leaves	*H. dubia* leaves	χ^2-value	*df*	*p*
Females	352 (57.4)	261 (42.6)	-	-	-
Males	409 (64.4)	226 (35.6)	6.40	1	0.011
Total	761 (61.0)	487 (39.0)	60.16	1	< 0.001

natans leaves throughout the 25 day observation period, most eggs were laid on the leaves of *T. natans* (Mann-Whitney U-test: z = -5.46, p < 0.001) (Fig. 1), and not on the artificial *T. natans* leaves.

B. Leaves of the camphor tree, *C. camphora* versus artificial *T. natans* leaves throughout the 25 day observation period, more eggs were laid on artificial *T. natans* leaves than on the leaves of the camphor tree (Mann-Whitney U-test: z = -3.702, p < 0.001) (Fig. 2A).

C. Leaves of the floating water plant, *H. dubia* versus artificial *T. natans* leaves in the periods of the 5th to the 9th day, the 15th to the19th day and the 22nd day, more eggs tended to be laid on the artificial *T. natans* leaves than on the leaves of *H. dubia* (Mann-Whitney U-test: z = -2.121, p = 0.034) (Fig. 2B).

Experiment 3 (Question 3): Feeding experiment on eggs of *Gl. nipponensis*

A lower percentage of first stage nymphs (50.0%, 201 hatching/402 eggs, 11.2 eggs on average x 36 replicates) hatched from eggs which had been accompanied with adult *G. nepalensis* as a feeding pressure compared (82.4%, 360/437, 8.1 eggs x 54 replicates) to the rest who were not associated with *G. nepalensis* (no feeding pressure) (Mann-Whitney U-test: z = -4.2788, p < 0.001).

Feeding experiments on 1st-3rd instars larvae of *Gl. nipponensis*

The feeding pressure by *G. nepalensis* of the lower or the same stage as the accompanying larvae of *Gl. nipponensis* was relatively small, and the survival of larvae of the beetle was high at 75-85% and 50-78% after 24 and 48 hours of being together, respectively (Table 3). On the other hand, the feeding pressure was significant and high when the stage of the water strider was higher than that of the beetle larvae. The survival in those dishes was very low at 6-43% and 0-23% after 24 and 48 hours of being together, respectively (Table 3).

Experiment 4 (Question 4): Overwintering experiment

The effect of 2 weeks of being together with *T. natans* in the fall on activity and reproductive activity the next spring

In the experimental group, complete with *T. natans* leaves for 2 weeks prior to overwintering, 90.7% of the adult and apterous *G. nepalensis* (n = 54 before overwintering) survived and overwintered for 3 months, and they tended to have a slightly higher survival rate of 81.5% than the control group who were associated with artificial *T. natans* leaves made of

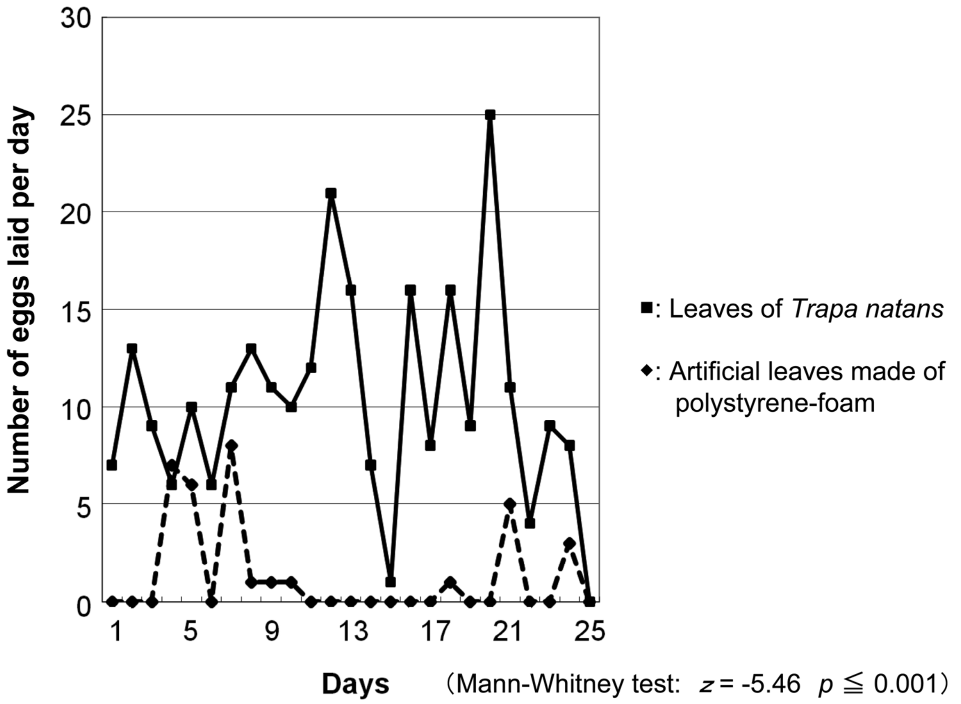

Fig. 1. Which leaves are chosen as the oviposition substrate by the adult *Gerris nepalensis*? : *Trapa natans* leaves without damage from *Galerucella nipponensis* versus the artificial leaves of *Trapa natans* (Question 2).

polystyrene foam, but the difference was not significant (χ^2-test, χ^2-*value* = 1.935, *df* = 1, *p* = 0.164).

Overwintering adults in the experimental group showed a temporal cancelling of their diapauses posture [all six legs folded in line with their body like a stick [Harada & Gon (2006)] for slightly but statistically significant longer days during the first and the third 30 day periods of their overwintering, compared to the adults in the control group (Table 4). Overwintered adults in the experimental group laid eggs for a significantly longer period (52 days on average) the following spring compared to the 38 days of egg laying for those in the control group, which worked out to a fecundity of 217 eggs on average for the experimental group compared to 132 eggs laid by the control group (Table 4).

Discussion

The presence of *T. natans* before the overwintering promotes a lower frequency of adopting the "diapauses posture" during overwintering and encourages a longer reproductive period leading to higher fecundity the following spring in *G. nepalensis*. These effects of *T. natans* lead to an increased number of *G. nepalensis*, especially the newly-hatched first stage of nymphal *G. nepalensis*. This has been found to be especially so in recent years with the global warming and with the temperature in the winter becoming a bit more moderate (Kochi Meteorological Station, 1989-2009).

Leaves of *T. natans* can attract to some extent the adult *G. nepalensis* and this effect is enhanced when the water striders are starved (Tables 1-4). Some

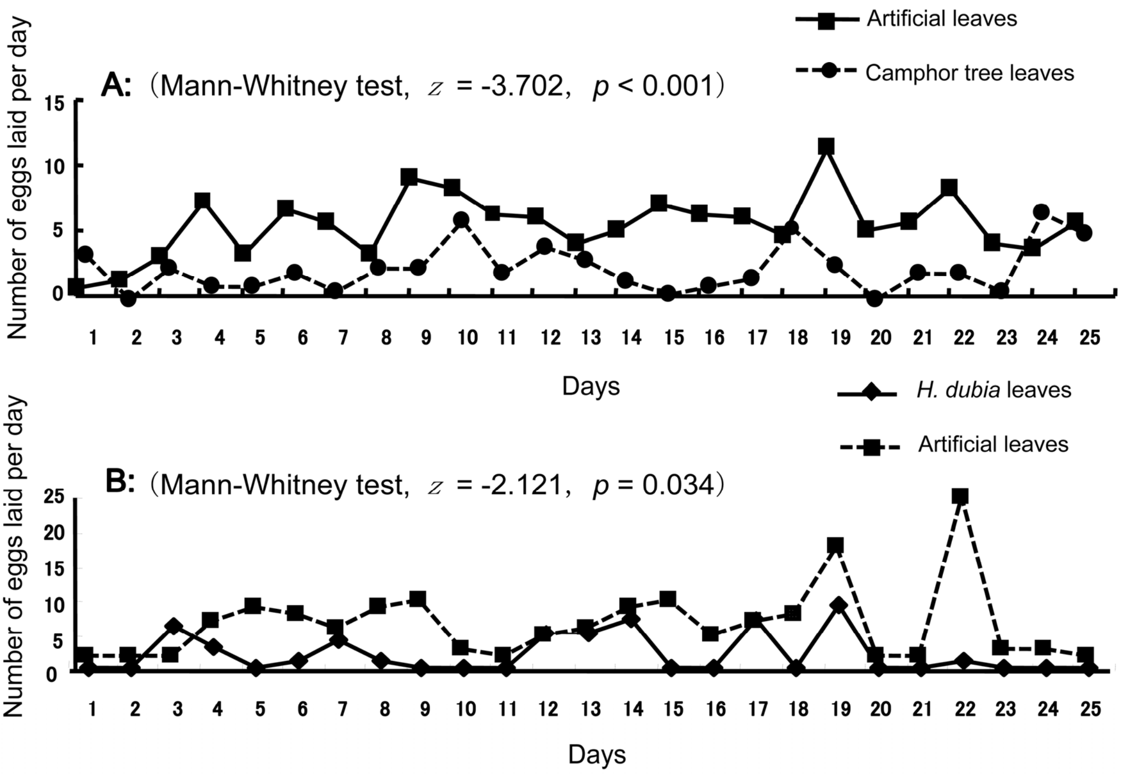

Fig. 2. Which leaves are chosen as the oviposition substrate by the adult *Gerris nepalensis*? : Camphor tree leaves versus the artificial leaves of *Trapa natans* (A); *Hydrocharis dubia* leaves versus the artificial leaves of *Trapa natans* (B) (Question 2).

olfactory key substances or key substances soluble in water might possibly be synthesized in the leaves, especially those damaged by the plant beetle, *Gl. Nipponensis,* and then released from the leaves into the water or the air. Such key substances may attract the adult *G. nepalensis* to the leaves (Tables 2, 3). Moreover, females of *G. nepalensis* can choose the leaves of *T. natans* as the substrate to lay their eggs on (Fig. 1, 2). Being in the presence of *T. natans* can increase the rate of success of the larval growth and the number of eggs laid (Harada *et al.*, 2008). Moreover, adult *G. nepalensis* can kill the eggs of *Gl. nipponensis* (Ikeda and Nakasuji, 2002; Ikeda, 2004) as well as the pupae of the beetles (Tanaka and Nakasuji, Saiki and Harada, 2002). In addition, elder nymphs of *G. nepalensis* effectively feed and kill the younger larvae of *Gl. nipponenisis* (Table 3) (Tanaka & Nakasuji, 2002; Saiki & Harada, 2002). Especially in the last stage of larvae the water striders can put a high feeding pressure on the larvae of the beetles (Table 3). Therefore, these unknown chemicals are key substances to enhance the feeding pressure of *G. nepalensis* on *Gl. nipponensis*, although there is no definite evidence for the existence of such key substances in the field of chemical ecology. This high foraging pressure might be to the benefit of the water caltrop *T. natans* because it reduces leaf damage. In the future, rearing bugs on water that is circulating from a

Table 3. The feeding effect of the nymphs of *Gerris nepalensis* on the percent survival of the larvae of *Galerucella nippnensis* 24 hours and 48 hours after the getting-together of the larvae of the beetles with the nymphs of the water striders [% survival (from number of the larvae of the beetles just before they meet)]

24 hours later	The stages of *G. nepalensis* co-existing with the larvae of the beetles					χ^2-test	
Instar of beetles	No nymphs	1st instar	2nd instar	3rd instar	5th instar	χ^2-value	*p*
1st instar	99.3 (300)	85.0 (300)	6.1 (180)	0.0 (150)	-	718.1	< 0.001
2nd instar	98.2 (56)	98.1 (52)	80.8 (52)	42.9 (56)	-	69.5	< 0.001
3rd instar	98.1 (52)	-	-	76.9 (52)	32.7 (52)	54.3	< 0.001
48 hours later	The instars of *G. nepalensis* which co-existed with the larvae of the beetles					χ^2-test	
Instar of beetles	No nymphs	1st instar	2nd instar	3rd instar	5th instar	χ^2-value	*p*
1st instar	97.9 (300)	77.6 (300)	0.0 (180)	0.0 (150)	-	680.7	< 0.001
2nd instar	89.3 (56)	86.5 (52)	69.2 (52)	23.2 (56)	-	69.2	< 0.001
3rd instar	94.2 (52)	-	-	50.0 (52)	13.5 (52)	68.2	< 0.001

Table 4. Effect of the presence of *Trapa natans* leaves on the overwintering success and reproductive activity the next spring of the water strider, *Gerris nepalensis*

	Number of days when overwintering adults did not adopt the "diapauses-posture"								
	The first 30 days (Jan.)			The second 30 days (Feb.)			The third 30 days (Mar.)		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Exp. Group#	4.28	4.20	46	1.22	3.29	46	1.00	2.39	46
Control Group$	3.05	4.39	38	0.66	1.76	38	0.77	0.27	38
Mann-Whitney	*z*	*p*		*z*	*p*		*z*	*p*	
U-test	-1.974	0.048		-0.702	0.483		-2.323	0.020	
	Reproductive activity after the overwintering								
	Fecundity (total number of eggs)			Preoviposition period			Oviposition period		
	Mean	SD	*n*	Mean	SD	*n*	Mean	SD	*n*
Exp. Group#	217.41	155.87	22	10.43	8.03	21	52.10	20.18	21
Control Group$	132.52	105.29	23	8.05	5.45	21	37.86	17.59	21
Mann-Whitney	*z*	*p*		*z*	*p*		*z*	*p*	
U-test	-1.760	0.078		-0.971	0.332		-2.315	0.021	

Accompanied by leaves of *T. natans* for 2 weeks just before overwintering.

$ Accompanied by artificial *T. natans* leaves made of polystyrene foam just before overwintering.

container that grows only the water caltrop might help to clarify the nature of the effect of this caltrop on the water strider and also the chemical ecological approaches that should be tried to determine the key substance.

Chemical relationships between plants and carnivores (insects or mites) that eat the herbivores that feed on the plants have been reported by Takabayashi & Dicke (1996), Shiojiri *et al.* (2000) and Nikovic *et al.* (2001). However, the relationship between the water caltrop *T. natans* and the carnivorous water strider *G. nepalensis* seems to be closer than those cases. Their symbiosis include not only a chemical attraction, but also possible chemical triggering to lay eggs on the leaves of the plant and thus increasing the number of carnivorous individuals that have contact with the plants (Harada *et al.*, 2008). However, further experiments are needed to certify that some chemical (air borne or water-solved chemical) triggers the ovipositing behavior by the water striders in the future. For example, choice experiments could possibly be conducted between the artificial *T. natans* leaves including liquids extracted from leaves of the water caltrop *T. natans* and those of another floating water plant, *H. dubia*.

When leaves of the water caltrop *T. natans* were damaged by the beetles, *Gl. nipponensis*, some chemical could have been synthesized and extracted into the air or water and this chemical could possibly attract the carnivorous water striders *G. nepalensis* to feed on *Gl. nipponensis*. When the water striders were starved, the responding threshold to the chemicals could have become lower inducing them to reach to the leaves with damage and thus a higher possibility of food.

In conclusion, this study demonstrated that 1) leaves of the water caltrop *T. natans* injured by herbivorous leaf beetle *Gl. nipponensis* attracted more predacious water strider *G. nepalensis* than did the intact leaves; 2) females of *G. nepalensis* preferred laying eggs on *T. natans* leaves to other floating plants or substrates; 3) old nymphs of predacious *G. nepalensis* ate and killed the young larvae of the herbivorous *Gl. nipponensis*, 4) *G. nepalensis* provided with leaves of water caltrop *T. natans* had a higher, although not significantly higher, successful overwintering rate and a significant higher fecundity the next spring compared to the control group.

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Received: October 31, 2010
Accepted: December 26, 2010

菱角 ***Trapa natans*** 對於水黽 ***Gerris nepalensis*** 數量之影響暨水黽對於葉甲 ***Galerucella niponensis*** 之捕食壓力

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摘　　要

本試驗假設菱角 *Trapa natans* 葉子能增加水黽數量，進而增加葉甲 *Galerucella niponensis* 之被捕食壓力，故此現象顯示水黽與菱角間可能具共生關係；因此本試驗由下列四方面進行研究：(1) 菱角葉是否能吸引水黽？(2) 雌水黽是否偏好菱角葉做為產卵處，即使是以聚苯乙烯製作之假菱角葉？(3) 水黽是否能取食葉甲之胚胎及第一、二、三齡之幼蟲？(4) 菱角是否能增加水黽越冬之存活率及次年春季之繁殖活動？試驗結果顯示，菱角葉能吸引水黽，特別是被葉甲傷害之菱角葉；即便有其他浮水植物，如水鱉 *Hydrocharis dubia* 或樟樹 *Cinnamomum camphora* 落葉，雌蟲仍偏好假菱角葉作為產卵處；水黽成蟲能取食葉甲胚胎，若蟲亦能有效率的捕食葉甲幼蟲；當試驗組之水黽與菱角葉共存，越冬存活率達 90.7% 且存活時間達三個月；然對照組水黽與假菱角葉共存，其存活率僅 81.5%；試驗組水黽在次年春季來臨後之 52 日內，共產 217 顆卵；而對照組水黽在春季來臨後之 38 日內，產 132 顆卵；故可推論水黽 *Gerris nepalensis* 藉由捕食葉甲 *Galerucella niponensis* 之方式與菱角 *Trapa natans* 達成共生關係。

關鍵詞：化學吸引、滯育狀態、春季繁殖、產卵介質、捕食壓力。

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