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Investigation of the predation potential of different fish species on brown planthopper (*Nilaparvata lugens* (Stål)) in experimental rice-fish aquariums and tanks

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ABSTRACT

A series of experiments was conducted in rice (*Oryza sativa* L.)-fish aquariums and concrete tanks at Can Tho University, Vietnam, to investigate the feeding behavior and ability to control brown planthopper (BPH) (*Nilaparvata lugens* (Stål)) by fish. In the aquarium studies, fish species introduced were: (1) no fish (control), (2) common carp (*Cyprinus carpio carpio* L.), (3) Nile tilapia (*Oreochromis niloticus niloticus* L.), (4) climbing perch (*Anabas testudineus* Bloch), and (5) clown knife (*Chitala chitala* Hamilton). We found that *C. carpio* and *O. niloticus* mostly grazed on filamentous algae as well as periphyton on aquarium walls, bottom and rice tillers in water. They hardly grazed on BPH on the surface. On the contrary, *A. testudineus* mostly grazed on BPH on rice tillers above the water surface. *C. chitala* spent a lot of time at the bottom corners of the aquariums without grazing on BPH. *A. testudineus* spent more time attempting to graze BPH, especially on rice tillers above the water surface as compared to *C. carpio* and *O. niloticus*. This resulted in a higher number of BPH eaten by *A. testudineus* than by *C. carpio* and *O. niloticus*. As *A. testudineus* was the most promising predator of late instar nymphs and adult BPH in the aquarium studies, different sizes of this species were further tested in rice-fish concrete tank conditions at three development stages of the rice crop. All sizes of *A. testudineus* reduced the number of BPH. The small and medium *A. testudineus* were more efficient in reducing BPH than the larger ones. The present study suggests that *A. testudineus* can be incorporated in rice-fish fields as a part of biological control of BPH.

1. Introduction

The current trend in rice (*Oryza sativa* L.) production in the Mekong Delta, Vietnam, is toward intensification, which may result in short-term gains followed by increased risks linked with large-scale environmental impacts such as pest and disease outbreaks (Berg, 2002). The brown planthopper (BPH), *Nilaparvata lugens* (Stål) (Homoptera: Delphacidae), has been a substantial problem in rice cultivation in Vietnam in recent years (Escalada et al., 2009; Catingdig et al., 2009). Despite environmental, health and sustainability costs, most farmers in the Mekong Delta continue to use insecticides to control BPH and other rice pests (Berg, 2002; Du et al., 2007; Escalada et al., 2009).

Previous studies showed that major stocked fish species cannot be regarded as a fully-fledged pest control measure in rice-fish fields. While they are able to feed on insects falling in water, many of the pests conceal themselves within the rice plant above the water line, which makes it unlikely that fish can feed on them (Gregory, 1997; Gupta et al., 1998; Vromant et al., 2002). Therefore, regular use of pesticides is still practiced in rice-fish systems and is a major constraint to integrated rice-fish farming (WES, 1997; Berg, 2002). Whereas previous rice-fish research and development efforts in the Mekong Delta focused on silver barb (*Barbonymus gonionotus* Bleeker), common carp (*Cyprinus carpio carpio* L.) and Nile tilapia (*Oreochromis niloticus niloticus* L.) (Rothuis et al., 1998; Long, 2002), research on the role of other indigenous species in the rice field ecology is scarce (Halwart, 2005).

Recently, climbing perch (*Anabas testudineus* Bloch) and clown knife (*Chitala chitala* Hamilton) have been cultured in intensive fish ponds. Both *A. testudineus* and *C. chitala* appear to be predators, and are known to feed on insects (Patra, 1993; Rahman, 1989; Rainboth,

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1996). Therefore, these species could be incorporated in rice-fish systems not only to exploit more efficiency the natural resources and to increase the income (high marketable price of these fish species), but also by acting as part of integrated pest management strategies. In order to study the feasibility of such an approach, the feeding behavior and ability to control rice pests by these fish species was investigated. The objectives of this experiment were to find out: (1) whether *A. testudineus* and *C. chitala* feed on BPH that reside above the water line, (2) whether the feeding capacity of *A. testudineus* and *C. chitala* on BPH is higher than that of *C. carpio* and *O. niloticus* and (3) whether fish size, water levels and rice growth stages affect the predation potential of these fish on BPH in rice fields.

2. Materials and methods

A series of experiments was conducted in aquariums and concrete tanks at the Mekong Delta Development Research Institute (MDI), Can Tho University, Vietnam from February to September 2010.

2.1. Aquarium studies to determine the feeding behavior on BPH (experimental setup A)

2.1.1. Experimental setup

The fish species used in this study included *C. carpio* and *O. niloticus* (both phytoplanktivorous and omnivorous species, and the major species stocked in rice fields), *A. testudineus* and *C. chitala* (both predators and indigenous species). The experiment was conducted from February to March 2010 as a one factor experiment with five treatments: (1) no fish (control), (2) *C. carpio*, (3) *O. niloticus*, (4) *A. testudineus*, and (5) *C. chitala*. The five treatments were assigned randomly to five rice-fish aquariums. The experiment was repeated three times, with the variable time as blocking factor in the analysis of variance. Between replications, fish, rice and BPH were removed from the aquariums. The walls and bottom of aquariums were cleaned to remove green filamentous algae as well as periphyton (algae). Each observation started after 3 days of acclimatization. The date of the first, second and third observation was 25th, 28th of February, and 3rd of March 2010, respectively. At these dates, the sunrise and sunset were at 6:15 and 6:04.

2.1.2. Rice-fish aquarium preparation

Fingerlings (3–5 g fish^{-1}) were obtained from local commercial nurseries and further cultured per species in four concrete tanks (5 m × 2 m × 1 m). Fish were fed with commercial feed (18% protein) at a level of 3% body weight. The rice cultivar Jasmine 85 (IR 841-85, cropping duration 100–105 days and susceptible to BPH (Cuong et al., 1997; Du et al., 2007)) was transplanted (17 days after seeding, DAS) in plastic pots (diameter = 12 cm) with rice field soil, at a density of three seedlings per pot. The soil had previously been fertilized with di-ammonium phosphate (DAP, 130 kg ha^{-1}), urea (20 kg ha^{-1}) and potassium chloride (KCl, 30 kg ha^{-1}). The pots with rice were placed in a rice field and the water level was kept at 5 cm to obtain healthy tillering rice.

Five identical recirculation systems for ornamental fish were placed outdoors. Each recirculation system contained an aquarium (60 cm × 40 cm × 50 cm) and a trickling filter. A continuous water flow was obtained by a submerged pump. Each aquarium was divided in two by a mosquito net. The first compartment (40 cm × 40 cm × 50 cm) was used for the fish-rice BPH experiment, and the submerged pump was installed in the second compartment.

In each aquarium, rice-fish field conditions were simulated. Two bowls with rice (30–35 DAS) were placed at the bottom of the aquarium. Sand was added as high as the bowls (7 cm). A sheet covered the sand and rice bowls. The aquariums were then filled with 70% tap water and 30% water collected from the fish tanks, up to 10 cm above the sheet. Before the start of the experiment, the water in the aquariums was partially replaced daily. Each aquarium was covered by a mosquito net to prevent BPH from escaping and to allow light and air to penetrate. To prevent too high water temperatures, a foam sheet was put on top of each aquarium from 9:30 am to 3:00 pm. Every two hours, this foam sheet was removed for 15 min in order to record fish behavior. In addition, each of the recirculation systems was connected to separate water storage and cooling 200 l tanks (SCT) containing similar water as the aquariums and continuously aerated. The flow back entered the first compartment of the aquariums. If the water temperature in aquariums reached 32 °C at noon, some ice was added to the SCT to keep the water temperature about 30–32 °C. The solubility of dissolved oxygen in fresh water at standard sea level pressure and 32 °C is about 7.32 mg/l (Michael et al., 1993).

The BPH used in the experiment were obtained from a stocked population maintained in a net house at MDI, Can Tho University. The gravid females of BPH were collected from infested rice fields and then released into the net house with several fresh rice pots (Jasmine 85, 40–50 DAS) under natural temperature and photoperiod conditions. The BPH rearing method of Heinrichs et al. (1985) was followed. After hatching, the nymphs of BPH molt five times. The young nymphs (1st to 2nd instar) are white and late instar nymphs (4th to 5th instar) are brown. The BPH adult is brownish black with yellowish brown body and transparent wings (short-wing) (Heinrichs et al., 1985; Reissig et al., 1986). Since our collection method (see the following paragraphs) could not select 100 percent late instar nymphs or adult BPH, a mixture of late instar nymphs and adult BPH were used in all experiments.

2.1.3. Video recording and analysis

Each experiment included a preliminary and an experimental period. At the start of the preliminary period, two bowls with rice (30–35 DAS, 4–5 tillers bowl^{-1}) were put in place in each aquarium. Two fish were released into each rice-fish aquarium at 7:00 pm and allowed to acclimate for 60 h (2.5 days). The fish stocking weight of each of the three replications is presented in Table 1. During the acclimatization period no extra food was presented to the fish. Therefore, it was assumed that the fish were hungry at the start of the experiment.

At the start of the experimental period, BPH were collected from the net house in the morning (6:00–7:00 am). Hereto bowls with rice plants containing late instar nymphs and adult BPH were removed and shaken to collect the BPH into a sweepnet. The BPH were then gently transferred into a container, counted and transferred to the rice-fish aquariums. The total number of BPH per rice-fish aquarium was about 100 individuals (Table 1).

After releasing the BPH into the rice-fish aquarium, two video cameras (Panasonic, AG-DP200 and NV-M3000) were used to

Table 1
Fish stocking weight (g fish^{-1}) and number of BPH (individuals aquarium^{-1}) in three replications of the rice-fish aquarium experiment.

Treatment	1st replication			2nd replication			3rd replication		
	Fish weight		BPH	Fish weight		BPH	Fish weight		BPH
	1st	2nd		1st	2nd		1st	2nd	
1. Control			100			103			100
2. *C. carpio*	11.0	11.5	111	10.1	13.2	105	20.3	13.2	98
3. *O. niloticus*	21.5	19.0	100	26.0	28.6	105	32.2	29.1	100
4. *A. testudineus*	10.4	14.0	98	10.3	9.9	100	12.4	12.8	109
5. *C. chitala*	6.1	5.4	102	10.3	9.8	125	9.3	8.5	105

capture the feeding behavior in the four rice-fish aquariums. The first and second rice-fish aquarium were recorded (one camera for each aquarium) at 6:30 am (when the BPH were released in the aquariums 1 and 2), and at 8:30 am, 10:30 am, 12:30 pm, 2:30 pm, 4:30 pm and 5:20 pm. The third and fourth rice-fish aquarium were recorded at 7:10 am (when the BPH were released in the aquariums 3 and 4) and at 9:10 am, 11:10 am, 1:10 pm, 3:10 pm, 5:00 pm and 5:45 pm. Each recording lasted 15 min, except for the first recording which was continued for 30 min. Feeding behavior was quantified on the basis of time percentage engaged. The types of fish feeding behavior were related to grazing on the bottom (algae), at the water surface (BPH), the tank wall under water (algae) and above water (BPH), and on rice tillers under water (algae) and above water (BPH). To measure the distance between the water surface and the BPH above water consumed by fish, a transparent ruler (millimetre scale) was attached to the front of each aquarium. Capture attempts on BPH represent the total number over the observation time (also including unsuccessful attempts). After the video recordings, the water flow in the rice-fish aquarium was stopped and all fish killed by electronic gear. The surviving BPH were collected with an aspirator and counted. The naturally dead BPH, which floated on the water surface or descended to the bottom, were also counted. The number of BPH that were consumed by fish were then calculated by subtracting the remaining ones (both dead and alive BPH) from the initial number released into the aquarium. The percentage of BPH eaten by fish was calculated as: $100 \times (N_{\mathrm{BPH\ eaten}}/N_{\mathrm{BPH\ introduced}})$, where $N_{\mathrm{BPH\ eaten}}$ is the number of BPH eaten by fish and $N_{\mathrm{BPH\ introduced}}$ represents the number of BPH introduced at the start in the same aquarium.

2.1.4. Sampling of water quality

At end of each recording, the water temperature in the aquarium was recorded using a mercury thermometer attached on the wall 5 cm under the water surface. Dissolved oxygen (DO) concentration and pH were measured at the start and at the end of the observation at the center of each fish aquarium (5 cm below the water surface) using portable electronic probes (370 pH Meter Jenway and 55 Dissolved Oxygen YSI).

2.2. Evaluation of BPH predation potential of fish at different size classes, at different rice growth stages and water levels under rice-fish tanks conditions (experimental setup B)

As the aquarium experiments showed that *A. testudineus* was the most promising predator on late instar nymphs and adult BPH (see results of experimental setup A), only this species was used in the subsequent study. A one factor experiment was conducted from July to September 2010 with 4 treatments and 4 replications in a randomized complete block design. Different fish size classes of *A. testudineus* were tested: (1) no fish (control), (2) small size (fingerlings), (3) medium size, and (4) larger fish (market size) (Table 2). Water levels in the rice-fish tank were used as blocking variable: (1) shallow water level (5 cm), (2) medium water level (10 cm), high water level (15 cm), and (4) highest water level (20 cm). The effects of different *A. testudineus* size classes on BPH were investigated during two days at three different rice (Jasmine 85) crop developments: (1) 32 DAS or 14 days after transplanting (DAT), (2) 55 DAS or 37 DAT, and (3) 85 DAS or 67 DAT. For each of these DATs, a different experiment was set up corresponding to the three rice growth stages of Jasmine 85: (1) vegetative phase-from germination to panicle initiation: 0–40 DAS, (2) reproductive phase-from initiation to flowering: 41–70 DAS, and (3) ripening phase-from flowering to maturity: 71–105 DAS. After finishing the first experiment (for rice at 85 DAS), fish, rice and BPH were removed from the tanks. The tanks were then prepared for the next experiment with rice at 55 DAS. After this experiment, a final experiment was set up with rice at 32 DAS. Sixteen experimental rice-fish tanks (plots), each with an area of 0.8 m^2 (1.0 m × 0.8 m), were used for these three experiments. Each plot was filled up with 15 cm soil and covered by a mosquito screen (0.8 m high) to prevent the entrance of insects or the escape of stocked BPH.

Three weeks before each experiment, *A. testudineus* (5, 20 and 60 g fish^{-1}) was obtained from local commercial nurseries and fish farms, and cultured in three tanks (5 m × 2 m × 1 m). Fish were fed with commercial feed (18% protein) at 3% body weight. Prior to each experiment, pre-germinated rice seed was broadcasted at 300 kg ha^{-1} in nursery beds. At 10 DAS, urea fertilizer was applied at a dose of 50 kg ha^{-1}. At 18 DAS, the rice seedlings were transplanted into the rice-fish tanks at a density of 30 hills per rice-fish tank (according to local practice, three seedlings per hill, spacing between hills 15 × 15 cm). Although rice in the Mekong Delta, Vietnam, is cultivated mostly by direct seeding, transplanting was used in this study because it minimizes differences in numbers of rice tillers between rice-fish tanks. Fertilizer doses for this experiment were: urea (100 kg ha^{-1}), DAP (130 kg ha^{-1}) and KCl (60 kg ha^{-1}). This amount was split over 4 days: one day before rice transplanting 100% of the DAP, 20% of the urea and 50% of KCl; at 15, 30 and 55 DAT 40%, 20% and 20% urea were applied, respectively; the 50% remaining KCl was applied at 30 DAT. No insecticide was applied during the cultivation period. This procedure was applied for all three experiments.

Thirty minutes before transplanting rice and seven days before releasing the BPH into the rice tanks, a mosquito killer (Falcon: Transfluthrin (0.065% w/w), Imiprothrin (0.050%), Pemethrin (0.150% w/w); solvent, gas and condiment (99.735% w/w)) was sprayed into the rice tanks to eradicate all arthropods, including natural BPH and their enemies (e.g., ants and spiders). Three days before releasing BPH into the rice tanks, fish were collected from the fish tanks and released in the evening into the rice tanks (2 fish tank^{-1}) for acclimatization. During that period no extra food was provided. In addition, ant killer was scattered around the rice tanks (0.5 m) to keep ants away from the experimental tanks. The BPH were collected from a net house by an aspirator and gently released into the rice-fish tanks in the morning (6:00–9:00 am) at a density of 3 BPH tiller^{-1} or about 15 BPH hill^{-1}, the economic threshold for BPH being 2 late instar nymphs per tiller (Reissig et al., 1986).

After two days of rice-fish tank observation, both fish and BPH were respectively killed by electronic gear and mosquito killer. After all dead BPH fell and floated on the water, rice leaves were cut and moved out of the tanks. The remaining numbers of BPH were counted and the percentage of BPH reduction by fish was calculated as: $100 \times (1-(M_{\mathrm{fish}}/M_{\mathrm{no\ fish}}))$, where M_{fish} is the remaining number of BPH in the rice tank with fish and $M_{\mathrm{no\ fish}}$ represents the mean remaining number of BPH in the control treatment without fish.

2.3. Data analysis

The treatment effects were analyzed as a one-way ANOVA in a randomized complete block design. As *C. chitala* was quiet and spent most of its time without feeding in the bottom corners of the aquarium, the grazing and BPH-feeding success of the different fish species was only compared between *C. carpio*, *O. niloticus* and *A. testudineus*. When ANOVA assumptions were violated, $\log_{10}$, square root and arcsine transformations were used. When an effect was significant, means were compared with the Duncan test. If transformations were not successful, the non-parametric Rank *F*-test was used. In this case, means were compared with the multiple Mann-Witney *U* test. All significance testing was done at the

Table 2
Fish stocking weight (g fish^{-1}) and total length (cm) in the rice-fish tanks at three rice growth stages. Values are means ± SD ($n = 4$).

Treatment (size class)	32 DAS		55 DAS		85 DAS	
	Weight	Length	Weight	Length	Weight	Length
Control	–	–	–	–	–	–
Small	7.8 ± 1.4	8.0 ± 0.5	9.5 ± 2.3	8.5 ± 0.5	5.9 ± 1.1	6.9 ± 0.6
Medium	24.1 ± 3.0	11.3 ± 0.3	26.9 ± 3.0	11.6 ± 0.3	23.7 ± 3.1	11.3 ± 0.2
Larger	70.3 ± 14.9	15.4 ± 0.9	81.2 ± 1.6	16.2 ± 0.4	63.5 ± 6.3	14.9 ± 0.5

$p \leq 0.05$ level. The program used for statistical analysis was STATISTICA, version 9.0.

3. Results

3.1. *Aquarium studies to determine the feeding behavior on BPH (experimental setup A)*

Aquarium water temperature at each video recording (VR) period, as well as DO concentrations and saturation, and pH values at the beginning and the end of the observation time were not significantly different between treatments. The water temperature averaged 27.4 ± 0.9 °C (SD) ($F = 1.00$; d.f. = 4, 8; $P = 0.461$) at first VR (early morning), 27.3 ± 0.8 °C ($F = 0.417$; d.f. = 4, 8; $P = 0.793$) at second VR, 29.8 ± 0.8 °C ($F = 1.00$; d.f. = 4, 8; $P = 0.461$) at third VR, 31.8 ± 1.1 °C ($F = 0.35$; d.f. = 4, 8; $P = 0.839$) at fourth VR, 32.6 ± 0.5 °C ($F = 0.26$; d.f. = 4, 8; $P = 0.893$) at fifth VR, and 31.2 ± 1.1 °C ($F = 0.81$; d.f. = 4, 8; $P = 0.551$) at sixth VR (late afternoon). In the early morning, DO concentrations averaged 6.7 ± 0.3 mg/l ($F = 0.70$; d.f. = 4, 8; $P = 0.615$) and pH values averaged 6.9 ± 0.1 ($F = 0.65$; d.f. = 4, 8; $P = 0.642$). In late afternoon, DO concentrations averaged 7.0 ± 0.1 mg/l ($F = 0.30$; d.f. = 4, 8; $P = 880$) and pH values averaged 7.0 ± 0.1($F = 2.85$; d.f. = 4, 8; $P = 0.097$).

3.1.1. *Grazing behavior*

C. carpio and *O. niloticus* mostly grazed on green filamentous algae as well as periphyton attaching to the wall, bottom and rice tillers (stem) in water. They hardly grazed on BPH on the water surface (Table 3). *O. niloticus* grazed sometimes on BPH on rice tillers above the water surface (0.68 cm). *A. testudineus* mostly grazed on BPH on rice tillers above the water surface (1.14 cm) but they sometimes grazed on BPH on the wall above the water and on the water surface. The time spent grazing on BPH on rice tillers above the water surface was significantly longer for *A. testudineus* than for *O. niloticus*. *C. chitala* spent a lot of time without grazing in the bottom corners of the aquariums. The capture attempts by *A. testudineus* on BPH were 69 times higher as compared to *C. carpio* ($P < 0.05$).

3.1.2. *Effects of fish on BPH in rice-fish aquarium condition*

Table 4 shows the number of remaining BPH (dead as well as alive ones). In the aquariums with *C. carpio*, *O. niloticus* and *A. testudineus*, no dead BPH were found at the end of the experiment. The number of dead BPH was higher in the control aquarium than in the *C. chitala* aquarium. The number of surviving BPH was not significantly different among the control, *C. chitala* and *C. carpio* treatments, but all aquariums had a significantly higher amount of BPH in their aquarium compared with the *A. testudineus* treatment. The number of surviving BPH in the aquarium with *O. niloticus* was significantly lower than in the *C. chitala* aquarium, but higher than in the aquarium with *A. testudineus*. The number of BPH eaten as well as the percentage of BPH eaten was significantly higher in the aquarium with *A. testudineus* than in the aquariums with *O. niloticus* and *C. carpio*.

3.2. *Effect of A. testudineus on BPH in the rice-fish tanks (experimental setup B)*

At 32 DAS, the number of remaining BPH between small and medium *A. testudineus* treatments was not significantly different, but for both classes they are significantly lower, by 48 and 37% respectively, than in the control treatment (Table 5). The number of remaining BPH in the larger *A. testudineus* treatment was not significantly lower as compared to the control and medium *A. testudineus* treatments, but higher than in the small *A. testudineus* treatment. At 55 DAS, the number of remaining BPH in the small, medium and larger *A. testudineus* treatments was

Table 3
The grazing behavior and BPH-feeding success of different fish species in rice-fish aquariums. Values are means ± SD ($n = 3$). Means with the same letter in a row do not differ significantly at $p \leq 0.05$ level.

	Fish			ANOVA		Non-parametrics	
	C. carpio	*O. niloticus*	*A. testudineus*	*F*	*p*-value	Chi-square	*p*-value
Grazing behavior[a]							
Grazing on bottom (algae)	0.58 ± 0.78a	0.83 ± 1.10a	0.00 ± 0.00a	3.586	0.128[b]		
Grazing on surface (BPH)	0.02 ± 0.04a	0.06 ± 0.07a	0.25 ± 0.25a	2.21	0.225[b]		
Grazing on wall under water surface (algae)	0.93 ± 1.59a	2.76 ± 0.81a	0.00 ± 0.00a			5.842	0.054[d]
Grazing on wall above water surface (BPH)	0.00 ± 0.00a	0.02 ± 0.02a	0.47 ± 0.75a			3.307	0.191[d]
Grazing on rice tillers under water surface (algae)	0.21 ± 0.36a	0.57 ± 0.78a	0.00 ± 0.00a			4.587	0.101[d]
Grazing on rice tillers above water surface (BPH)	0.00 ± 0.00a	0.33 ± 0.50a	2.66 ± 1.83b	8.814	0.034[b]		
Total grazing on BPH	0.02 ± 0.04a	0.41 ± 0.58a	3.38 ± 2.0b	13.466	0.017[b]		
Total grazing	1.74	4.57	3.38				
Swimming and resting	98.26	95.43	96.62				
BPH-feeding							
Attempts (number/hour)	0.35 ± 0.61a	6.98 ± 9.67ab	24.30 ± 14.10b	7.323	0.046[b]		
Distance of jumps out of water (cm)	–	0.68 ± 0.60a	1.14 ± 0.23a	2.013	0.292[c]		

[a] Values are percentage of observation time.
[b] Numerator and denominator degrees of freedom are respectively 2 and 4.
[c] Numerator and denominator degrees of freedom are respectively 1 and 2.
[d] Degree of freedom is 2.

Table 4
Effect of fish species on number of BPH in rice-fish aquarium. Values are means ± SD ($n = 3$). Means with the same letter in a column do not differ significantly at $p \le 0.05$ level.

Fish	Introduced BPH (No.)	Remaining dead BPH (No.)	Remaining alive BPH (No.)	BPH eaten by fish (No.)	BPH reduction by fish (%)
Control	101.0 ± 1.7a	11.7 ± 2.5c	89.3 ± 1.5bc	–	–
C. carpio	104.7 ± 6.5a	0.0 ± 0.0a	91.0 ± 5.3bc	13.7 ± 4.0ab	13.0 ± 3.4a
O. niloticus	101.7 ± 2.9a	0.0 ± 0.0a	79.0 ± 16.5b	22.7 ± 16.6b	22.3 ± 16.6a
A. testudineus	102.3 ± 5.9a	0.0 ± 0.0a	53.0 ± 11.5a	49.3 ± 10.3c	48.3 ± 10.6b
C. chitala	110.7 ± 12.5a	6.3 ± 1.2b	104.3 ± 13.7c	0.0 ± 0.0a	0.0 ± 0.0a
ANOVA					
F			9.518	11.547	10.790
p-value			0.004[a]	0.007[b]	0.008[b]
Non-parametrics					
Chi-square	2.80	13.78			
p-value	0.592[c]	0.008[c]			

[a] Numerator and denominator degrees of freedom are respectively 4 and 8.
[b] Numerator and denominator degrees of freedom are respectively 3 and 6.
[c] Degrees of freedom is 4.

significantly lower by 40, 42, and 29%, respectively, than in the control treatment. At 85 DAS, the number of remaining BPH in small and medium *A. testudineus* treatments was significantly lower by 37 and 51%, respectively, than in the control treatment. The remaining BPH in the larger *A. testudineus* treatment was not significantly different as compared to other treatments. An effect of water levels in rice-fish tank on the remaining BPH was only observed at 85 DAS. The number of remaining BPH was significantly higher at the lowest water level (5 cm) as compared to the higher levels.

4. Discussion

The water quality parameters are important for fish feeding and growing. According to Boyd (1990), the optimal water temperature, DO concentration and pH for growth of most warm water fish species are about 25–32 °C, >3 mg/l, and 6.5–8.5, respectively. In the rice-fish aquariums in this study, the water temperature at each video recording period, as well as the DO concentrations and pH values at the beginning and the end of the observation times were always within acceptable ranges.

C. carpio, *O. niloticus* and *A. testudineus* spent about 98, 95 and 97% of their total time swimming and resting, and respectively 2, 5 and 3% of their total time grazing (Table 3). According to Rahman (1989) and Rainboth (1996), *C. chitala* is a predator of surface feeding fish, crustaceans and insects. During the video recording in this study, *C. chitala* did not graze on BPH. In addition, in aquariums with *C. chitala* the total number of remaining BPH (dead and alive ones) was equal to their introduced number (Table 4). All this indicates that in our experimental conditions *C. chitala* did not feed on BPH. The reasons for this were possibly related to the fish size, feeding behavior and the time of observation. The weight of *C. chitala* in this study ranged from 5.4 to 10.3 g fish^{-1} (Table 1) and the length from 10.5 to 12.8 cm. However, we also tried to run the same experiment with two larger *C. chitala* (36.3 and 46.0 g fish^{-1} or 18.6 and 19.0 cm, respectively) and got similar results as with the smaller fish. According to Alikunhi (1957), the adult *C. chitala* is a predatory fish which subsists mainly on small fish. In the early fry stage, it feeds voraciously on carp fry and aquatic insect larvae. Sarkar and Deepak (2009) found that in riverine habitats the preferred diets of *C. chitala* (ranging from 50 to 90 cm in length) are crustaceans, insects, molluscs, minnows and other fish. The

Table 5
Effect of different *A. testudineus* sizes (treatment) on number of BPH in rice-fish tanks at three rice growth stages and four water levels (block). Sub-table A and B show the means ± SD of treatment and block, respectively ($n = 4$). Means with the same letter in a column per effect do not differ significantly at $p \le 0.05$ level (DAS: days after seeding).

A

Fish size	32 DAS			55 DAS			85 DAS		
	Introduced BPH (No.)	Remaining BPH (No.)	BPH reduction by fish (%)	Introduced BPH (No.)	Remaining BPH (No.)	BPH reduction by fish (%)	Introduced BPH (No.)	Remaining BPH (No.)	BPH reduction by fish (%)
Control	450 ± 0a	240 ± 26c	0.0	451 ± 1.0a	249 ± 38b	0.0	450 ± 0.0a	167 ± 26b	0.0
Small	450 ± 0a	126 ± 51a	47.7 ± 21.3	451 ± 1.0a	149 ± 54a	40.0 ± 21.5	450 ± 0.0a	105 ± 46a	37.0 ± 27.4
Medium	454 ± 5a	151 ± 52ab	37.0 ± 21.8	450 ± 0.0a	144 ± 17a	42.2 ± 6.8	450 ± 0.0a	82 ± 50a	50.8 ± 30.2
Larger	453 ± 5a	218 ± 58bc	9.1 ± 24.0	451 ± 0.0a	177 ± 28a	29.0 ± 11.0	449 ± 3.5a	126 ± 77ab	24.8 ± 46.0
ANOVA									
F	1.05	5.19		0.58	7.36		0.33	4.23	
p-value[a]	0.417	0.024		0.644	0.009		0.802	0.040	

B

Water level	32 DAS		55 DAS		85 DAS	
	Introduced BPH (No.)	Remaining BPH (No.)	Introduced BPH (No.)	Remaining BPH (No.)	Introduced BPH (No.)	Remaining BPH (No.)
5 cm	453 ± 5a	199 ± 38a	450 ± 0a	175 ± 33a	449 ± 3a	183 ± 30b
10 cm	452 ± 3a	136 ± 56a	450 ± 0a	179 ± 30a	450 ± 0a	94 ± 32a
15 cm	450 ± 0a	214 ± 42a	451 ± 1a	159 ± 80a	450 ± 0a	90 ± 46a
20 cm	452 ± 5a	186 ± 101a	452 ± 2a	206 ± 68a	451 ± 1a	112 ± 70a
ANOVA						
F	0.38	2.41	1.48	1.20	1.00	6.099
p-value[a]	0.771	0.134	0.284	0.364	0.436	0.015

[a] Numerator and denominator degrees of freedom are respectively 3 and 9.

percentage of insects in their stomach was about 15%, represented by aquatic insects such as back swimmer (*Notonecta* sp.), dragonfly nymphs (Anisoptera) and water boatman (*Sigara atropodonta* Hungerford). As *C. chitala* feeds on insects that live in the water column, in this study BPH escaped predation from *C. chitala* because they mainly stay on the rice tillers above the water level. Rainboth (1996) reported that *C. chitala* feeds in a crepuscular or nocturnal activity pattern. Meanwhile, in the present study *C. chitala* was observed during daytimes (from 6:30 am to 6:00 pm). Therefore, the effects of *C. chitala* on BPH may require further study. However, separate exploratory observation of this fish at night in rice-fish aquariums indicated that BPH were not eaten by *C. chitala* during nocturnal activity.

A similar type of experiments was conducted by Rahman (2006) with *C. carpio* and rohu (*Labeo rohita* Hamilton) in aquariums under fed and unfed condition. He found that *C. carpio* spent 75% of its time swimming and 25% grazing. The shorter grazing time in our study could be explained by the limited natural food in the aquariums as compared to the fish tanks in the study of Rahman (2006). In our study, the natural food in the clear water was likely either absent or extremely limited. Rahman (2006) on the contrary conducted his experiments in larger tanks, treated with decomposed cow manure and chemical fertilizers.

C. carpio and *O. niloticus* are considered as bottom and filter feeders, respectively (Kestemont, 1995; Popma and Masser, 1999). In rice-fish fields, both *C. carpio* and *O. niloticus* feed mainly on detritus, and can also feed on aquatic plants, phytoplankton and zooplankton (Rothuis et al., 1998; Faraponova, 2005). In addition, Saikia and Das (2009) found that, in flooded rice fields, periphyton attached to rice-stems, such as Chlorophyceae, Cyanobacteria and Bacillariophyceae, is the most important food resource for common carp. Common carp and Nile tilapia can consume planthoppers that fall in the water (Yu et al., 1995; Halwart, 2005). Also, in our study, *C. carpio* and *O. niloticus* not only grazed phytoplankton and periphyton in the water (bottom and wall of the aquarium, and on rice tillers), but they also fed on BPH at the water surface (Table 3). This result confirms the suggestion made by Vromant et al. (1998) that *O. niloticus* and *C. carpio* are able to feed on insects floating on the water. In addition, in the present study, *O. niloticus* could graze (capture attempts) on BPH on rice tillers above the water surface (0.68 cm; Table 3). *A. testudineus* was not only more active in the water but jumped out of the water when surfacing for air. Besides this, the fish also grazed on BPH not only on the water surface but also on both aquarium wall and rice tillers above the water surface (1.14 cm; Table 3). These results are in agreement with Patra (1993), Little et al. (1996), and Wijeyaratne and Perera (2001), who considered *A. testudineus* to be a visual feeder, predator and insectivore. Wijeyaratne and Perera (2001) investigated feeding habits of *A. testudineus* within a length range of 3.4–9.0 cm in reservoirs in Sri Lanka. Using the relative volume of each food item, they found that the main food was animal matter (including arthropods) contributing to more than 70% of the diet. *A. testudineus* has specialized labyrinth organs under their operculum which enable them to breathe atmospheric air. They are renowned for their ability to migrate between ponds over land (Yakupitiyage et al., 1998; Binoy and Thomas, 2004). However, none of these authors mentions their ability to capture prey above the water surface, as observed in this study.

The observations made indicate that both *A. testudineus* and *O. niloticus* are able to capture BPH which stay on the lower parts of rice plants above water (around 1 cm; Table 3). BPH are found near the plant base, less than 10 cm above the water level, and move upwards the mid-stem as density (Rubia and Heong, 1989) and relative humidity increase (Isichaikul and Ichikawa, 1993). Both nymphs and adults move laterally like crabs to the opposite side of tillers (Reissig et al., 1986); when disturbed, they fall from time to time into the water but climb again on a rice tiller (personal observation). At the end of the experiment, no dead BPH were observed in aquariums with *C. carpio*, *O. niloticus* and *A. testudineus* while the number of remaining surviving BPH was significantly lower in the aquariums with *A. testudineus* than in those with other fish (Table 4). *A. testudineus* also spent more time attempting to graze BPH, especially on rice tillers above the water surface, as compared to *C. carpio* and *O. niloticus* (Table 3). This means that BPH taken in by *C. carpio* and *O. niloticus* were mainly dead and alive ones, which fell in the water, whereas *A. testudineus* fed also on BPH which did not only fall in the water but stayed above the water surface. This resulted in a higher number of BPH eaten by *A. testudineus* than by *C. carpio* and *O. niloticus* (Table 4). The above results indicate that under the experimental conditions, usually during the daytime, *A. testudineus* showed a better predation potential on late instar nymphs and adult BPH than C. *carpio*, *O. niloticus* and *C. chitala*.

The results of the rice-fish tanks support the *A. testudineus* findings of the aquarium experiments. *A. testudineus* reduced the introduced BPH and these effects were dependent on the fish size (Table 5). Small and medium *A. testudineus* (6–10 g fish^{-1} and 24–27 g fish^{-1}) reduced the BPH better at all three rice growth phases. In addition, at 32 DAS the negative effect of the small size class of *A. testudineus* on BPH was stronger than that of the largest ones (64–81 g fish^{-1}). This can perhaps be explained by size class specific feeding habits. Previous studies on the predator–prey relationships of carnivorous fish species and vice versa (summarized by Weatherley et al., 1987) reported that in general the average size of prey eaten tends to increase with predator size. For this reason, in this study the small and medium *A. testudineus* might have a higher preference for BPH than larger ones.

In intensive fish ponds, stocking size of *A. testudineus* is about 3–5 g fish^{-1}. After 4–6 months culturing, those fish reach market sizes of 60–100 g fish^{-1} (Phu et al., 2006; Long et al., 2006). In rice-fish systems in the middle part of the Mekong Delta, fish can be stocked in the rice-fish fields one month after the seeding of the Summer-Autumn rice crop (April–May to July–August) and can be harvested at the beginning or at the end of the Winter-Spring crop (November–December to February–March). Therefore, if *A. testudineus* is incorporated in rice-fish systems, it may control BPH better in the Summer-Autumn rice crop, when the fish size is still smaller than its market size. For this reason, it is advised that farmers add some small *A. testudineus*, which can be easily found in natural water bodies, into rice-fish fields in the early Winter-Spring rice crop in order to obtain a better biological control of BPH.

On the other hand, the remaining BPH in rice-fish tanks was also significantly affected by water level at rice ripening phase (85 DAS). The remaining BPH was lower in rice-fish tanks with higher water levels (10, 15 and 20 cm) as compared to tanks with the lowest water level (5 cm). This result could be related to the behavior of BPH. In general, the BPH (adults and nymphs) stay on the lower parts of the rice plants (Reissig et al., 1986; Rubia and Heong, 1989). However, when the population is very high (e.g., more than 500 individuals per hill), they are observed to swarm even on flag leaves, the uppermost internodes of panicles, and panicle axes (Mochida and Okada, 1979). During the vegetative and reproductive crop stages, BPH suck the plant sap from the lower parts of the rice plants. In the ripening phase, BPH are concentrated at the slightly higher, younger and softer parts of the stems to feed (personal observation). This could explain why fish had less impact on BPH in rice-fish tanks with the lowest water level (5 cm) at 85 DAS. For the short cropping duration (100 days) rice varieties, the field water level is recommended to raise gradually from 3 to 5 cm initially to about 5–10 cm as the rice plants develop from 30 DAS to 90 DAS

(Hoan, 1998). According to Sogawa and Cheng (1979), a planthopper attack after the heading stage (in the ripening phase) affects the percentage of ripened grains and grain weight. BPH severely damage rice plants in the post flowering stage. For this reason, it is advantageous to keep the water level in the rice fields from after flowering to one week before harvest about 10–15 cm to improve the effects of *A. testudineu*s on BPH. In this period, rice plants are fully developed and around 90–110 cm in height (Thai et al., 2009; Thai, 2010). With these water levels, *A. testudineu*s, which have been observed to jump up to eat developing or ripe rice seeds on panicles when the water depth in rice field is very high, cannot reach the panicles (Nam et al., unpublished). Indeed, on-farm and experimental conditions of rice-fish systems (Ha et al., 2004) indicated that the field water levels between 10 and 15 cm were most appropriate for rice and fish in terms of yield and profit considerations. At the end and beginning of each rice crop, when farmers prepare their land, seed or harvest rice, fish are kept in the trench. To avoid that fish damage the young rice plants, fish are only allowed to enter the rice field when rice plants are already well developed (usually 20 to 25 DAS).

BPH also transmits the ragged stunt and grassy stunt viruses. Rice yield losses are greater when plants are infected at an early growth stage before flowering (Reissig et al., 1986). Ragged stunt and grassy stunt viruses cannot be controlled using pesticides (Bong et al., 2006; Du et al., 2007; Cabauatan et al., 2009). Therefore, when *A. testudineus* is present in rice fields, it may also indirectly assist to control those viral diseases by consuming infected BPH vectors. *A. testudineus* can breathe atmospheric air, therefore it can survive and even enter rice fields where the dissolved oxygen concentrations are usually low (Rothuis et al., 1999; Vromant et al., 2001). With the ability to capture BPH above the water surface, *A. testudineus* could assist the control of other rice insects (e.g. rice white-backed planthopper, *Sogatella furcifera* (Horváth) (Homoptera: Delphacidae); rice black bug, *Scotinophora lurida* (Burmeister) (Hemiptera: Pentatomidae)) which rest on the tillers at the base of the rice plants. As an additional benefit, *A. testudineus* is also considered to be a good biological control agent of mosquitoes in rice fields (Bhattacharjee et al., 2009).

As observed in this study *A. testudineus* is able to capture BPH on rice tillers above the water surface (around 1 cm), whereas under open conditions, BPH are usually found near the plant base, less than 10 cm above the water level. After two days, fewer than 50% of BPH were eaten by *A. testudineus* (Table 5). Therefore, in order to improve the predation potential of *A. testudineus* on insect pests, especially when their density increases (but still lower than the economic threshold), we suggest that the water level in the rice field should be increased to allow fish to reach more insects. The insects on the lower part of rice tillers will then climb higher, and many will concentrate just above the water level. In addition, *A. testudineus* can continuously feed on BPH during the rice crop. For these reasons, *A. testudineus* could be effective in controlling BPH in rice fields. Besides stocked fish in the rice fields, rice-fish farmers should adopt Integrated Pest Management (IPM) strategies. Previous rice-fish studies in the Mekong Delta, Vietnam, suggested that integrated rice-fish farming systems in combination with IPM is an appropriate strategy in order to provide an economically as well as ecologically sustainable alternative to rice-mono cropping (WES, 1997; Berg, 2001, 2002). In cases where the pest pressure is so severe that the use of pesticides may be the only option left, farmers have two choices: drive the fish into the trench (draining the field slowly before spraying and keeping the fish in the trench until the toxicity in the sprayed field is gone), or increase the water depth (before spraying) to dilute the pesticide concentration in the water. In both cases, only pesticides that have low toxicity to fish (green label) can be used (Sollows and Cruz, 2001).

Under rice-fish tank conditions, we limited the insect community by the introduction of only BPH. This indicates that the results presented for the treatment "rice - *A. testudineus*" do not correspond to a natural open rice-fish environment found in experimental fields and farmers' fields. Many parasitoids (e.g. dryinid, *Pseudogonatopus* spp. (Hymenoptera: Dryinidae); strepsipteran, *Elenchus yasumatsui* (Kefune and Hirashima) (Hymenoptera: Strepsiptera)) and predators (e.g. mirid, *Cyrtorhinus lividipennis* (Reuter) (Hemiptera: Miridae); wolf spider, *Lycosa pseudoannulata* (Boesenberg and Strand) (Araneae: Lycosidae); water bug, *Microvelia douglasi atrolineata* (Bergroth) (Hemiptera: Veliidae)) attack all stages of BPH and effectively control this pest under open conditions (Ooi and Shepard, 1994). Therefore, when *A. testudineus* is present in rice-fish fields, it can also prey on natural enemies. Before incorporating this fish species more systematically in rice-fish systems, the optimum density of *A. testudineus* in polyculture with major stocked fish species under open conditions should be investigated.

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