# Impact of Predation by Water Insects on Fish Seed Production in Lao PDR

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#### Abstract

The predation of cultured larval fish by water insects presents a serious issue for aquaculture development in rural areas of the Lao People's Democratic Republic (Lao PDR). In this study, the species composition of predatory water insects was monitored in a fish nursing pond. Laboratory predation experiments were then performed to examine the predation potential of water insects on 3 major cultured fish species, including *Barbonymus gonionotus, Cirrhinus cirrhosus*, and *Cyprinus carpio*. Anisops spp. was considered to have the highest impact on mortality by predation in Lao PDR. It was estimated that *Anisops bouvieri* can prey on 10.4-46.7% of larval fish within 24 h under standard rearing conditions recommended by FAO.

**Discipline:** Aquaculture

Additional key words: Backswimmer, Anisops, prey, larval fish, nursing pond

# Introduction

In developing countries of Southeast Asia, including Lao PDR, the supply of seed fish is one of the factors limiting the development of freshwater aquaculture<sup>8, 10</sup>. It is desirable, when we consider the transportation facilities in Lao PDR, that seed producers in rural areas be able to provide fish seed to rural aquaculture farmers. However, the predation of larval fish in nursing ponds by water insects presents a serious obstacle to seed production in rural areas<sup>2, 4</sup>. Several previous studies have suggested countermeasures for the extermination of predatory water insects<sup>1, 2</sup>, but these have not been used effectively to prevent predation. This is at least partly due to the lack of basic information regarding fish seed predation, including that in which predatory species are involved.

The purpose of the present study was to collect basic information regarding predatory water insects in nursing ponds using field surveys, and estimate the potential impacts of such predatory insects using laboratory experiments.

# Materials and methods

#### 1. Field sampling survey

Field surveys were conducted on 3 occasions (from August 7 to August 13, 2007; September 22 to September 28, 2007; and August 20 to August 26, 2009) at the Namxouang Aquaculture Development Center (NADC), located 40 km north of Vientiane City in Lao PDR. A 600 m<sup>2</sup> nursing pond (20 m  $\times$  30 m, average depth 1 m) was dried up for several days before being refilled with water. The water was filtered with a mosquito net (mesh size, approximately 1.0 mm<sup>2</sup>) set at the water inlet to prevent contamination by aquatic insects during refilling of the pond. Water insects that appeared in the pond were sampled daily for 7 days, starting 3 days after it had been refilled. Each sample was collected at noon. The water insects were captured by dragging a sweep-

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net  $(0.5 \times 0.5 \text{ m}; \text{mesh size}, 750 \text{ }\mu\text{m})$  along a line 1.0 m from the pond wall. The sampled insects were stored in ethanol (90%) for identification to the family level<sup>3, 6</sup>. Predatory water insects were identified based on William and Feltmate (1992)<sup>11</sup> from all sampled insects, and the wet body weight (g) of each individual was measured. The growth stages of the insects were identified as adult or larval respectively.

# 2. Laboratory predation experiment

As Anisops spp. made up the greatest biomass of predatory species in the field surveys, A. bouvieri (Kirkaldy, 1704) was selected as the predator for the predation experiments. The larval fish of 3 species (produced at NADC), namely, Barbonymus gonionotus, Cirrhinus cirrhosus (Bloch, 1795), and Cyprinus carpio, were used as prey. The experiments were conducted twice (in August 2008 and June 2009) at NADC under laboratory conditions. A single predatory A. bouvieri individual was placed in an aquarium ( $25 \times 40 \times 25$  cm) with 10 larval fish of 1 prey species, and the mortality rates of the larval fish within 24 h were recorded. The experiments were conducted every 3 days, with 4 replications per species, for 21 days after hatching. The aquariums were covered with transparent plastic boards and the water was gently aerated. During the experiments, the mean water temperature was 28.9°C (standard deviation, 0.8), the mean pH was 7.5 (0.3), and the mean levels of dissolved oxygen (DO) were 8.47 mg·  $L^{-1}$  (0.47) during the experiments. The photoperiod was controlled with light and dark periods of 12:12 h. A control aquarium containing 10 larval fish with no predator present was prepared for each experiment. Adults of A. bouvieri were collected in nursing ponds in NADC and cultured in a 30-L polycarbonate tank for 3 days before the experiments. One day before each experiment, predators were transferred to the experimental aquarium. At the beginning of the experiment, the larval fish were transferred to the experimental aquarium using a pipette. The total lengths of 10 larval fish, representing a batch of experimental fish species, were measured simultaneously. Dead larval fish were collected from the experimental and control chambers, 12 and 24 h respectively after the onset of the experiment, and the number of dead individuals within 24 h was recorded. The dead larval fish were examined under a microscope (SZ4045, Olympus), and the causes of death (predation by insects) were confirmed. Two-way ANOVA was used to examine marked interactions between fish species and the age of the larval fish. When marked interactions were observed, One-way ANOVA and Tukey's HSD test were performed to examine the significance of differences in larval fish age and mortality between each fish species. Before this analysis, the numbers of dead larval fish were log-transformed to standardize the variances.

#### Results

# 1. Field sampling survey

A total of 1450 predatory water insects representing 8 families were collected during the sampling period. These included Coenagrionidae larvae, Libellulidae larvae, Nepidae adults, Belostomatidae adults, Notonectidae adults and larvae, Gerridae adults and larvae, Dytiscidae larvae, and Hydrophilidae larvae (Table 1). Coenagrionidae larvae made up 49.4% of the total of predatory insects. However, the biomass (wet weight) of Coenagrionidae larvae accounted for only 14.0% of the total biomass of all sampled insects (4.60 g). Although Notonectidae individuals accounted for 18.9% of the to-

Table 1. Mean number of individuals (standard deviation) and wet body weight per type of predatory water insect<br/>collected during sampling (1.0 m², 3 times) (7 days)

Order	Family	Number of individuals		Wet body weight	
		Mean number	Proportion of individuals (%)	Mean weight (g)	Proportion of weights (%)
Odonata	Coenagrionidae (larvae)	$478.0 \pm 203.66$	49.4 ± 36.99	0.644 ± 0.34	$14.0\pm0.12$
	Libellulidae (larvae)	$18.0 \pm 14.73$	$1.9 \pm 2.67$	$0.697 \pm 0.57$	$15.1\pm0.17$
Hemiptera	Nepidae (adult)	$0.7 \pm 0.58$	$0.1 \pm 0.11$	$0.053 \pm 0.05$	$1.1\pm0.01$
	Belostomatidae (adult)	$0.7 \pm 0.58$	$0.1 \pm 0.11$	$0.08 \pm 0.07$	$1.7\pm0.02$
	Notonectidae (adults and larvae)	$183.3 \pm 39.8$	$19.0 \pm  6.43$	$1.975\pm0.31$	$42.9\pm0.17$
	Gerridae (adults and larvae)	$52.7 \pm 36.5$	5.4 ± 7.11	$0.07 \pm 0.03$	$1.5\pm0.02$
Coleoptera	Dytiscidae (larvae)	194.7 ± 168.59	$20.1 \pm 43.56$	$0.913 \pm 0.79$	$19.8 \pm 0.34$
	Hydrophillidae (larvae)	$38.7 \pm 8.08$	$4.0~\pm~~2.22$	$0.17 \pm 0.03$	$3.7\pm0.03$

tal, their biomass accounted for 42.9% of the total biomass, which was the maximum among all families. All Notonectidae species sampled were identified as *Anisops* spp.

The total biomass of Coenagrionidae, Libellulidae, and Dytiscidae larvae collected in 7 days accounted for 48.9% of the total overall biomass (Table 1). Coenagrionidae larvae biomass increased over time after refilling of the pond; however, the biomass of Notonectidae had reached a high level, relative to the other species, after 3 days (Fig. 1). The total number of individuals of *Anisops* spp. was 275, comprising 81 larvae (29.5%) and 194 adults (70.5%). Within the samples, 135 (69.6%) individuals were adult *A. bouvieri*. The mean density of *Anisops* spp. adults in the pond throughout the observation period was 18.5 individuals per m<sup>2</sup>·day<sup>-1</sup>. The biomass of *Anisops* spp. (Notonectidae) was the highest of all the predatory insects sampled during the survey (Table 1).

# 2. Laboratory predation experiment

According to the results of the field survey, *A. bouvieri* was selected as the experimental predator. The mean body length of the predators used in the predation experiment was 6.06 mm (standard deviation, 0.84). The body size of the experimental prey changed over time as follows. The total lengths of larval silver barb were 4.0 mm (standard deviation, 0.74) 3 days after hatching, 5.6 mm (0.56) 12 days after hatching, and 9.4 mm (1.03) 21 days after hatching respectively. The total lengths of the larval Indian carp were 7.6 mm (0.43) 3 days after hatching, 12.5 mm (0.00) 12 days after hatching, and 15.7 mm (0.41) 21 days after hatching respectively. The total lengths of larval common carp were 7.9 mm (0.41) 3 days after hatching, 10.1 mm (0.25) 12 days after

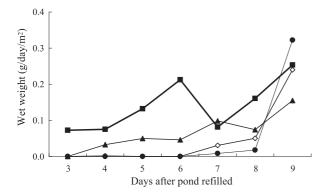
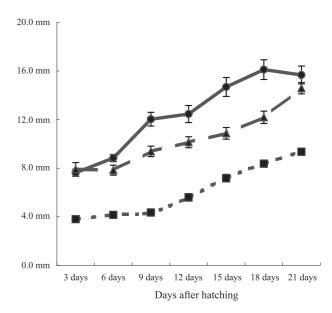


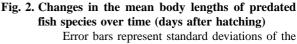
Fig. 1. Mean wet body weight of 4 families of predatory insects collected during the sampling period (7 days) on 3 separate occasions
→-: Coenagrionidae, ---: Libellulidae,
---: Notonectidae, ---: Dytiscidae.

hatching, and 14.6 mm (0.00) 21 days after hatching respectively. The latter 2 species grew faster than the first (Fig. 2). The mean number of larval fish that died per day ranged from 2.5 to 5.5 for silver barb, 0 to 5.5 for Indian carp, and 0 to 5.0 for common carp. Conversely, the mean number of dead larval fish within 24 h in the control group was 0.2 (standard deviation, 0.06; n =28). A two-way ANOVA demonstrated that the mean level of predation differed among fish species and with fish age. The number of dead silver barb larvae was significantly associated with age (one-way ANOVA,  $F_{6, 21}$  = 5.47, p < 0.01); however, no clear trend between mortality and fish age was observed (Fig. 3). Conversely, a clear trend was observed between the number of dead Indian carp and common carp over time from hatching, with mortality declining with increased age (Fig. 3).

#### Discussion

Among the 8 families that were sampled in the pond, the number and biomass of Nepidae, Hydrophilidae, and Belostromatidae were very low, likewise the biomass of Gerridae. Since adults and larvae of the Gerridae family inhabit pond water surfaces, adult Gerridae cannot prey upon fish that swim in the water. The results suggested that larvae of Coenagrionidae, Libellulidae, and Dytiscidae and adults and larvae of Notonecti-





- mean. **— —** : Silver barb (*Barbonymus gonionotus*),

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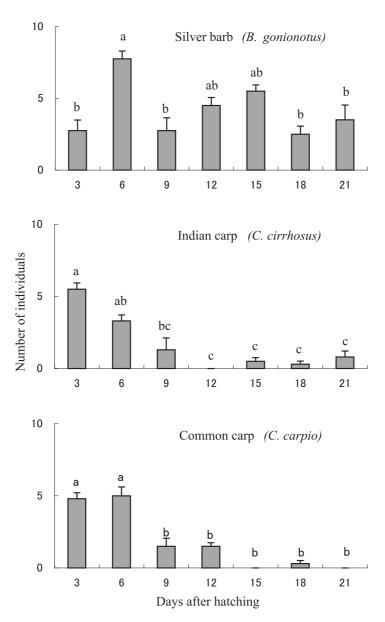


Fig. 3. Changes in the mean numbers of fish predated by Anisops bouvieri at different ages of larval fish Error bars represent standard deviations of the means. Letters (a, b, c) above bars indicate significant differences based on Tukey's HSD test.

dae are potential predators of fish seed in nursing ponds. In this survey, predatory water insects were removed by drying the pond, and then filtering water using fine mesh nets after the pond had been refilled. The biomass of Coenagrionidae, Libellulidae, and Dytiscidae larvae was low 3 days after refilling and increased gradually over time (Fig. 1), which suggests that the drying and filtering methods were effective for the removal of larvae of these 3 families, because they cannot fly. Coenagrionidae, Hydrophillidae and Dytiscidae individuals spawn the eggs on vegetation growing in the water<sup>7, 11</sup>. The removal of plants may therefore be more effective for the extirpation of these water insects than drying the pond. The larvae of water insects that gradually increased in size to some extent, yet remained very small after refilling the pond, were not considered to reflect predation pressure. However, adults of *Anisops* spp. were sampled only 3 days after the refilling of water in the pond. *Anisops* larvae reach adult size within approximately 2 months<sup>3</sup>. It is clear that the adult *Anisops* spp. collected in the pond had migrated by flying there from other habitats. Our field survey conclusively showed that *Anisops* spp. represented the most abundant water insects at the study site and were assumed to be the most important causative species for the mass mortality of larval fish by predation in nursing ponds in Lao PDR.

In the predation experiments, the mean number of dead fish within 24 h in the control group was very low (0.2 larval fish). The mean number of larval fish that died per day in the experimental groups ranged from 2.5 to 5.5 for silver barb, 0 to 5.5 for Indian carp, and 0 to 5.0 for common carp. From the results, we can conclude that the mortality in the experimental groups was caused by predation by A. bouvieri. This can be supported by our direct observation of predation of larval fish by A. bouvieri, and by observation of dead fish under a microscope. Although the mortality of silver barb fluctuated throughout the experimental period, the mortality rates of the Indian carp and common carp decreased with increasing size (Fig. 3). The growth rates of the latter 2 species exceeded that of the silver barb. The Indian carp and common carp reached total lengths of 8.8 and 7.8 mm 6 days after hatching, and 12.0 and 9.4 mm 9 days after hatching, respectively. The mortality of these 2 species decreased with growth and the number of dead fish was less than 2 larval fish per day 9 days after hatching. There are 2 possible interpretations for this decrease. One is that the predators become satiated by eating a smaller number of larger prey due to the increased body mass of the latter. The other is that predation pressure may decline as the size of the prey increases. It is not possible to evaluate the adequacy of these 2 hypotheses based on the results presented in this study. However, assuming the triplicate ratio of total prey length reflects the level of predation, the relative daily predation can be estimated by multiplying body size by the number of prey and the estimated relative total predation would be constant with the growth of the prey. The relative level of predation decreased with growth in the Indian carp and common carp. This simple and provisional calculation supports the latter hypothesis, although further experimental confirmation is required.

Previous studies have shown that an adult *A. bouvieri* kills 1 larval fish every 2 h<sup>5</sup>, and that the mean predation rate of *A. bouvieri* on mosquito larvae remains stable over a 6-day feeding period<sup>9</sup>. In the present study, a single *A. bouvieri* preyed on a mean of 2.8, 5.5, and 4.8 larval fish (3 days after hatching) of silver barb, Indian carp, and common carp, respectively, within a 24-h period. When we ignore the encounter rate between predator and prey, *Anisops* spp. have the potential to prey upon 51.8 larval fish (3 days after hatching)/m<sup>2</sup> of silver barb, 101.8 larval fish/m<sup>2</sup> of Indian carp, and 88.8 larval fish/m<sup>2</sup> of common carp when the mean density of *Anisops* spp. is 18.5 individuals/m<sup>2</sup> (observed in the present study). These estimates suggest that daily mortalities may reach 10.4, 40.7, and 46.7%, at larval densities of 500, 250, and 190 larval fish/m<sup>2</sup> (the standard recommended densities by FAO), respectively. This result clearly reveals the serious negative impacts of *Anisops* spp. on seed production in Lao PDR.

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#### References

- 1. Adeyemo, A. et al. (1997) Predation by aquatic insects on African catfish fry. *Aquacult. Int.*, **5**, 101–103.
- Bardach, J. E. et al. (1972) Aquaculture. John Wiley, New York, p. 136.
- Brooks, G. T. (1951) A revision of the Genus Anisops (Notonectidae, Hemiptera). *The University of Kansas Science Bulletin*. University of Kansas, 1, 301–519.
- Gonzalez, A. V. & Leal, J. M. (1995) Predation potential of some aquatic insect (*Pantala, Coenagrion, Tropisternus, Notonecta* and *Sigara*) on Common carp fry. *J. Appl. Aquacult.*, 5, 77–82.
- Gorai, A. K. & Chaudhuri, R. D. N. (1962) Food and feeding habits of *Anisops bouvieri* Kirk. (Heteroptera: Notonectidae). J. Asiatic Soc., 4, 135–139.
- Kawai, T. & Tanida, K. (2005) Aquatic Insects of Japan: Manual with Keys and Illustrations, Tokai University press, Kanagawa, Japan, 129–641.
- Marco, P. D., Jr., Latini, A. O. & Reis, A. P. (1999) Environmental determination of dragonfly assemblage in aquaculture ponds. *Aqua. Res.*, **30**, 357–364.
- Meenakarn, S. & Funge-Smith, S. (1998) Small-scale fish hatcheries for Lao PDR. FAO, Bangkok, Thailand, 1–45.
- Saha, N. et al. (2007) A comparative study of predation of three aquatic Heteropteran bugs on *Culex quinquefasciatus* larvae. *Limnology*, doi: 10.1007/s10201-006-0197-6.
- Siriwardena, S. N. (2007) Role of freshwater fish seed supply in rural aquaculture. In Assessment of freshwater fish seed resources for sustainable aquaculture. FAO technical paper 501, eds. Bondad-Reantaso M. G., FAO, Roma, 563–578.
- William, D. D. & Feltmate, B. W. (1992) Aquatic Insects. CAB International, Wallingford, UK, 25–76.