

# PREDATION IMPACTS OF THE INVASIVE FLATWORM *PLATYDEMUS MANOKWARI* ON EGGS AND HATCHLINGS OF LAND SNAILS

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(Received 25 November 2009; accepted 13 February 2010)

## ABSTRACT

Some species of terrestrial planarians (flatworms) are among the predators of land snails, but their predatory impacts have not been sufficiently studied. Flatworms are known to follow snail trails to find prey and enter shells to consume snails; however, eggs (which do not have trails to follow) and snails whose shells are too small for flatworms to enter may not be eaten. To determine whether an invasive flatworm, *Platydemus manokwari*, preys on snail eggs or small land snails, we conducted a laboratory experiment in which we fed five eggs or five hatchlings (2 mm in diameter) of a common land snail species found in Japan to *P. manokwari* individuals of various sizes. Egg predation did not occur within 10 days, but hatchling predation commenced on the first night; only 9% of the hatchlings remained on Day 10. *Platydemus manokwari* did not recognize early-stage eggs as food, but started preying on eggs just before they hatched. Flatworms can therefore be a significant predator on land snails, preying on even tiny land snails, leaving only early-stage eggs free from predation.

## INTRODUCTION

Some species of terrestrial planarians (flatworms) are among the predators of land snails. Relatively few studies have examined the impacts of their predation so far, and even the typical prey types of many planarian species remain unknown (Winsor, Johns & Barker, 2004). Even before their predatory characters have been determined, flatworm species have been unintentionally introduced into many tropical islands via human activities involving soil and other materials (Winsor, 1983; Boag, Yeates & Johns, 1998), or intentionally introduced as biological control agents for invasive land snails, such as *Achatina fulica* (Waterhouse & Norris, 1987; Muniappan, 1990). As alien flatworms invade more locations, their potential effects as predators on native land snails raise concerns; therefore, research examining these impacts is required (Ogren & Kawakatsu, 1998; Ducey, McCormick & Davidson, 2007).

Flatworms locate prey by chance through physical contact, or by using an 'ambush' strategy, or by tracking prey using sensory cues (Ogren, 1995). Once a flatworm finds a prey item (i.e. a land snail), it 'either embraces it in its folds so that the proboscis can enter the aperture of the shell, or it crawls into the shell and out again, forming a U-shaped fold that carries the proboscis deeply into the body whorl' (Mead, 1963). These reported predation behaviours imply that eggs, which do not have trails to track, may not be recognized as food items, and that a flatworm may be unable to prey on snails that are so small that it cannot insert its folded body into the shell. Although observations that flatworms can prey on snails as small as 5–5.5 mm (hatchlings of *A. fulica*, Mead, 1963; size cited from Bequaert, 1950) or 20–70 mg (Ducey *et al.*, 2007) have been reported, no empirical studies have confirmed their ability to prey on tiny land snails. Predation on eggs has also not been examined.

*Platydemus manokwari* de Beauchamp (Tricladida: Rhynchodemidae) has been introduced into many tropical islands (Muniappan *et al.*, 1986; Hopper & Smith, 1992;

Cowie, 2002), and strong impacts on native land snail species, such as *Mandarina*, have recently been found in the Ogasawara Islands, Japan (Chiba, 2003; Sugiura, Okochi & Tamada, 2006; Ohbayashi *et al.*, 2007). In the Ogasawara Islands, *P. manokwari* preys on a broad range of food items including slugs, earthworms and isopods (*Armadillidium vulgare*) as well as land snails (Kaneda, Kitagawa & Ichinohe, 1990; Ohbayashi *et al.*, 2005; Sugiura, 2010); however, *P. manokwari* prefers land snails to earthworms or isopods (Sugiura, 2010). Among land snail prey, predation rates are higher on smaller snails (0.11–0.19 g) than on large snails (0.63–1.72 g) (Sugiura, 2010). Thus, land snails, and especially small-bodied species and individuals, might be exposed to high predation pressure from *P. manokwari*. Nonetheless, small-bodied individuals or land snail species in the Ogasawara Islands and in other locations in the Pacific are often smaller than 5 mm (Vagvolgyi, 1976; Azuma, 1982), whereas the body size of adult *P. manokwari* can reach 100 × 10 mm (Kaneda *et al.*, 1990). Large *P. manokwari* individuals may not feed on extremely small snails even if they prefer smaller individuals, and extremely small snails may only be preyed on by much smaller *P. manokwari* individuals. To determine whether *P. manokwari* preys on eggs or small land snails, we conducted a laboratory experiment in which *P. manokwari* individuals of various sizes were offered eggs or hatchlings of a land snail species that is common in Japan.

## METHODS

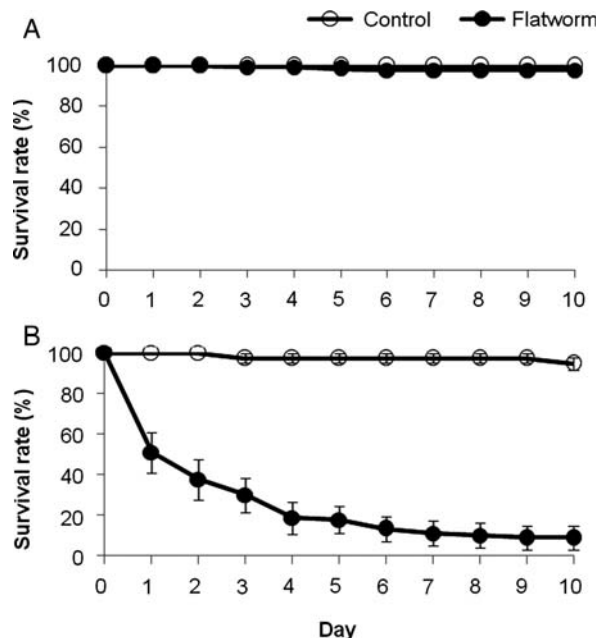
*Platydemus manokwari* were collected from Okinawa (26°09'N, 127°44'E) in July 2009. Each worm was placed singly in a plastic airtight container (490 ml, 10-cm diameter, 6-cm height) by itself (control) or with soaked sphagnum moss and five snail eggs or five newly hatched land snails (treatment). *Platydemus manokwari* were starved for at least 1 week prior to the experiment. Containers were kept at 25°C with a 16:8 light:dark photoperiod. Egg and snail survival was checked every day for 10 days.

Because endemic land snails in the Ogasawara Islands are seriously threatened, eggs and hatchlings of the introduced species *Acusta despecta sieboldiana* (Pfeiffer) and *Bradybaena similis* (Férussac) were collected from Tsukuba (36°0'N, 140°7'E) in July 2009 for use as potential prey species. Adult shell sizes (diameters) are about 23 mm for *A. d. sieboldiana* and about 18 mm for *B. similis* (Azuma, 1982). Three experiments were conducted using different combinations of land snail species and life stages (eggs or hatchlings) according to the availability of sufficient numbers of individuals. Experiment 1 examined predation on *A. d. sieboldiana* eggs, experiment 2 examined predation on *B. similis* eggs and experiment 3 predation on *A. d. sieboldiana* hatchlings. Replicates were made 27 and 12 (treatment and control), 17 and 9, and 18 and 8 times for experiment 1, 2 and 3, respectively. Sizes of *P. manokwari* were 5–223 mg for experiment 1, 6–148 mg for experiment 2 and 2–270 mg for experiment 3. Egg size and hatchling shell size were fairly uniform; they were 1.8–2.1 mm (mean  $\pm$  SD,  $1.9 \pm 0.2$ ;  $n = 35$ ) in diameter for both species. Eggs of *A. d. sieboldiana* were laid 1 day before the start of experiment 1, but the date on which *B. similis* eggs were laid was not determined. Thus, experiment 2 was terminated on the day the first hatchling was observed (Day 5). The hatchlings used in experiment 3 were less than 1 week post-eclosion.

A generalized linear model (GLM) with a binomial error distribution and a logit link (i.e. logistic regression) was used to examine the effect of flatworm presence on egg or snail survival in each container on Day 10. Statistical analyses were performed using R v. 2.10.0 (R Development Core Team, 2009).

## RESULTS

On Day 10 of experiment 1, all of the *Acusta despecta sieboldiana* eggs in the control treatment remained intact, and only three eggs from two replicates in the flatworm treatment were observed to be dead (Fig. 1A). Each dead egg had a tiny hole



**Figure 1.** Mean survival rates of eggs (A, experiment 1) and hatchlings (B, experiment 3) of *Acusta despecta sieboldiana* under predation by *Platydemus manokwari* (flatworm) and without predation (control). Five eggs or hatchlings were placed in a container with one individual *P. manokwari* on Day 0. Bars indicate SE.

in it and had become desiccated, which sometimes happens even when eggs are reared without flatworms (N. Iwai, personal observations). The effect of flatworm presence on the survival of eggs to Day 10 was not significant (GLM,  $z = -0.005$ ,  $P > 0.05$ ). In experiment 2 involving *Bradybaena similis* as potential prey, the first hatchlings were observed on Day 5, at which time all of the eggs in both the control and flatworm treatments remained intact. However, flatworms immediately preyed on newly hatched individuals; only the small transparent shell was left after predation.

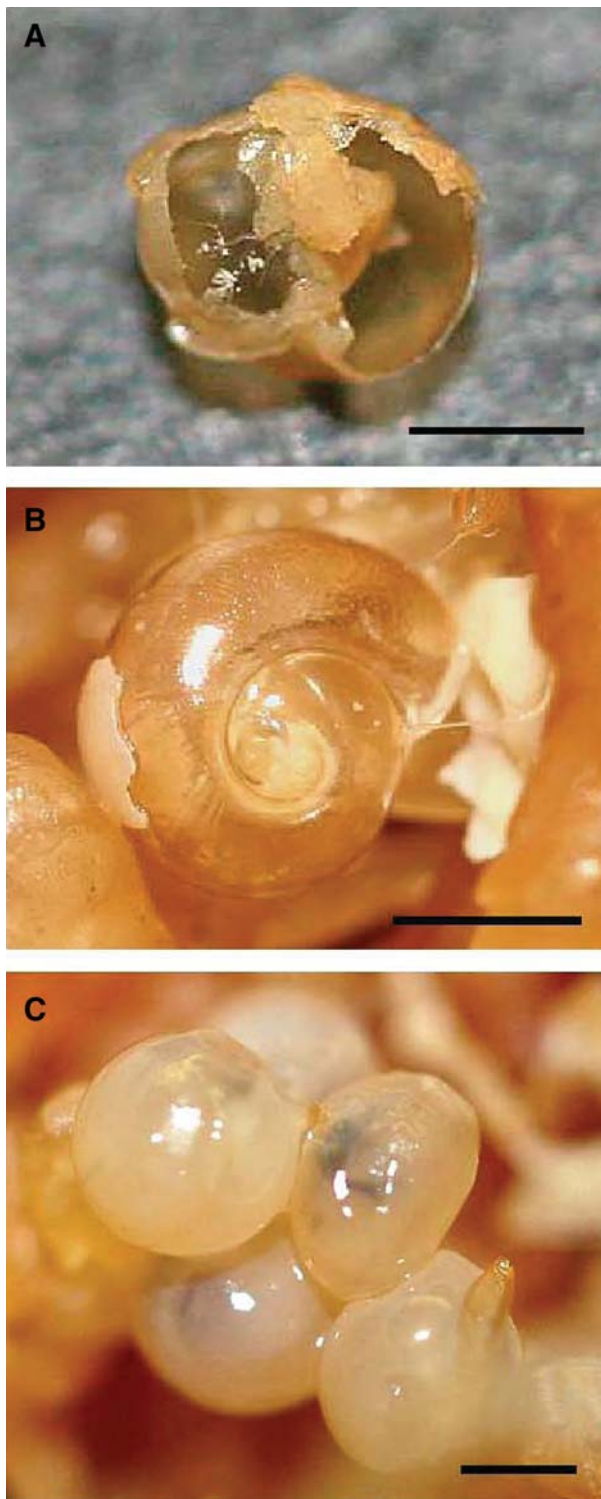
In experiment 3, in six of the 18 replicates, all five *A. d. sieboldiana* hatchlings were eaten on the first night, and predation commenced within the first 4 days in all but one replicate (Fig. 1B). Predation by flatworms was obvious because snail shells remained; the inside of a shell was cleared and mucus was still attached to it. On Day 10, almost all of the snails (91%) had been consumed in the flatworm treatment, and snails remained in only two replicates; five snails remained in a replicate with a 2-mg flatworm, and three snails remained with a 181-mg flatworm. In the replicate containing a 181-mg flatworm, two of the five snails were eaten on Day 4, but further predation did not occur before the end of the experiment. In the control treatment, only two hatchlings were found dead, on Days 3 and 10, from separate replicates. The effect of flatworm presence on the survival of hatchlings to Day 10 was significant (GLM,  $z = -6.47$ ,  $P < 0.001$ ).

## DISCUSSION

Predation by *Platydemus manokwari* on *Acusta despecta sieboldiana* and *Bradybaena similis* eggs did not occur during the experiment. Some *P. manokwari* individuals were observed crawling on the eggs, but they did not seem to recognize them as food. However, once snails hatched, *P. manokwari* individuals of various sizes (5–270 mg) preyed on hatchlings of *A. d. sieboldiana*, although the smallest *P. manokwari* specimen (2 mg) did not. Predation behaviours were not observed, but one *P. manokwari* individual was found holding a cleared snail shell, covering it with its ventral body near the pharynx. *Platydemus manokwari* should be able to move small snails to its pharynx using its muscular body; thus, no ‘gape limit’ for predation on land snail hatchlings should exist. Although insufficient number of *B. similis* hatchlings prevented us from conducting predation experiments on hatchlings of this species, hatchlings of *B. similis* were also predated right after hatching, indicating that our result should be robust for multiple species of snails.

The 2-mg *P. manokwari* specimen did not feed on hatchlings of *A. d. sieboldiana*. This individual may have been too small to attack a snail by itself. As gregarious attacks by *P. manokwari* allow them to prey on larger land snails (Sugiura, 2010), small *P. manokwari* individuals may only be able to feed on land snails when joining larger *P. manokwari* in attacking snails. Kaneda *et al.* (1990) reported that the size of *P. manokwari* hatchlings ranged from 0.8 to 26.4 mg (mean  $\pm$  SD,  $11.77 \pm 6.56$ ), and extremely small hatchlings usually died within a few days. In our experiment, the smallest *P. manokwari* individual that preyed on snail hatchlings was 5 mg, which suggests that almost all active *P. manokwari*, regardless of size, could prey on snails as small as 2 mm.

When the containers from experiments 1 and 2 were left in the same condition (both eggs and flatworms present) after their trials were terminated, newly hatched land snails were quickly eaten, usually within 2 days following hatching. Predation even occurred immediately before hatching, and cleared snail shells with a broken eggshell attached were often observed (Fig. 2A, B). It seems that predation can occur once an embryo has developed to an advanced stage (where, to the naked human eye, the



**Figure 2.** **A, B.** Cleared snail shells after predation by *Platydemus manokwari*. Predation appeared to occur just before hatching; a broken eggshell is still attached to the snail shell. **C.** Snail eggs in their final stage. Miniature snails can be seen from outside the egg. Scale bars = 1 mm.

miniature snail could be seen from outside) (Fig. 2C). However, it appears that *P. manokwari* was not able to recognize eggs in earlier developmental stages as food, nor did it demonstrate ambush behaviour, such as remaining near eggs. Sugiura & Yamaura (2009) suggested that, in the field, *P. manokwari*

followed the trails of land snails to find prey. Although the mechanism by which *P. manokwari* follow trails has not been determined, it is likely that it has some type of chemoreceptor that can detect the chemical cue of prey, as has been predicted in other flatworms (Winsor *et al.*, 2004). Land snail embryos may lack this cue during early stages, but may start secreting it near hatching, thereby becoming vulnerable to predation by *P. manokwari*. Because *P. manokwari* have eyes capable of detecting only light direction and a very crude image (Land & Nilsson, 2002) together with the fact that they consume dead snails that are not moving (Kaneda *et al.*, 1990), it is less likely that they use visual cue such as movement of embryos inside the egg to locate their prey.

To find final-stage eggs, *P. manokwari* might have to cross over an egg by chance, because no trail would exist for the flatworm to follow. *Platydemus manokwari* and land snails are often found beneath stones or logs, sharing microhabitats that are close to each other (Winsor *et al.*, 2004), and encounters between *P. manokwari* and land snail eggs should occur frequently. As eggs cannot escape, predation by *P. manokwari* on final-stage eggs should have a strong influence on snail mortality rates. Moreover, invasive flatworms have been found in many Pacific islands, where minute genera, whose shells are less than 10 mm, constitute 60% of the snail fauna (Vagvolgyi, 1976). For effective conservation of land snails, we need to recognize that flatworms can act as significant predators on them, including small-bodied snails and hatchlings, leaving only early-stage eggs free from predation.

## ACKNOWLEDGEMENTS

We thank Dr S. Abe for his help collecting flatworms in Okinawa, Dr A. Iguchi for collecting land snails and Dr Y. Yamaura for statistical advice. *Platydemus manokwari* were reared under the permission of the Japanese Ministry of the Environment. This study was supported by the Global Environment Research Fund (F-051).

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