

## The shy and the bold: Behavioural differences in native and invasive apple snails

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### Abstract

Differences in behaviour of species or populations may be important in survival and adaptation to the wild. The difference in shyness or boldness is one major component of personality trait. Here, we examine the behavioural differences associated with shyness and boldness of the native apple snail (*Pila pesmei*) compared to the invasive apple snail (*Pomacea canaliculata*) in Thailand. We used the proportion of space use and distance moved from the release area to explore the novel open space to assess shy/bold behaviour in the laboratory. We found that the invasive apple snail (*P. canaliculata*) occupied out of the release area and moved further than the native apple snail (*Pila pesmei*), suggesting that the invasive apple snail is significantly bolder than the native apple snail. These behavioural differences may have implication for invasion success of the non-native invasive species. In addition, we examined whether shy/bold behaviour (taken as the distance moved) and shell size covaried in *Pila pesmei* and *P. canaliculata*. However, we found that distance moved from the release area was not correlated with size in either species. These findings suggest that differences in behavioural traits may be an important factor influencing the interaction between native and non-native invasive species.

**Keywords:** shyness, boldness, behavioural trait, native species, invasive species

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### 1. Introduction

Over the last decade, there has been considerable interest in the study of consistency in the behavioural differences of animals [1]. Behavioural differences may influence species or populations survival and adaptation to the wild [2] and thereby may play an important role in the ecology and evolution of animals [3, 4]. Furthermore, behavioural traits may influence the success or failure of non-native species in novel environments [5 - 7]. Shy/bold behaviour is one of the commonly considered measures of “personality traits”, and is described as the propensity of an individual to approach unfamiliar objects or situations and take risks in the novel environmental conditions they encounter [8, 9]. By definition, shy individuals (often referred to

as risk averse) react to objects or unfamiliar situations by retreating, reducing activity levels, and becoming quiet or vigilant, whereas bold individuals (risk-prone) become more active (i.e., increase activity levels, quick to approach novel objects) and display exploratory behaviours in novel situations [7 - 10].

Several studies have shown a link between shy/ bold behaviour and animal movement, as bold individuals tend to move further than shy ones [2, 11 - 13]. This could have implications for ecologically important processes, such as dispersal, which enables spreading of a population [11] and may be a key trait for successful invasion of new areas [14, 15]. As bold individuals are more prone to move through and explore novel environments to discover novel food

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sources or habitats, they may outcompete native species and succeed in expanding populations [6, 7].

Here, we investigated the differences in a behavioural trait associated with shyness and boldness in two species of apple snails from Thailand, a native and an invasive species. The freshwater apple snail (*Pomacea canaliculata*) is native to South America but has been introduced in many parts of the world as a decorative snail in aquaria and a food source for people [16, 17]. However, market demand for this snail declined because consumers did not find it sufficiently appetising, and it was intentionally released from many snail farms, or, in many instances, the snails escaped aquaculture facilities into the wild, becoming one of the most important pests of rice and other crops and causing yield loss and increasing economic costs [16 - 18]. It also has negative effects on human health, natural environment and aquatic biodiversity as well as impacts on the native aquatic fauna and native snail populations [16, 17]. In Thailand, native apple snails (*Pila* spp.) are economically important in providing food, especially for farmers [19]. However, *Pila* spp. have decreased in abundance in their natural habitats [20].

As mentioned above, the existence of behavioural differences in a population may play an important role in invasion success of individuals. We choose to study shy/bold behaviour as this is one behavioural trait associated with invasion success. A measurement of movement is one way of estimating shyness and boldness of an individual [2]. Therefore, in this study we examine shyness and boldness by comparing the space use and the distance moved from the release area between the native species (*Pila pesmei*) and the non-native invasive species (*P. canaliculata*). We specifically hypothesized that the invasive apple snail would be bolder than the native apple snail. Furthermore, we examined whether different shyness and boldness (distance moved) of native and invasive apple snails were correlated with their size. Based on current knowledge of animal personality in general and in studies of other aquatic animals we predict that the size of these apple snails would have no effect on the shy/bold behaviour.

## 2. Materials and methods

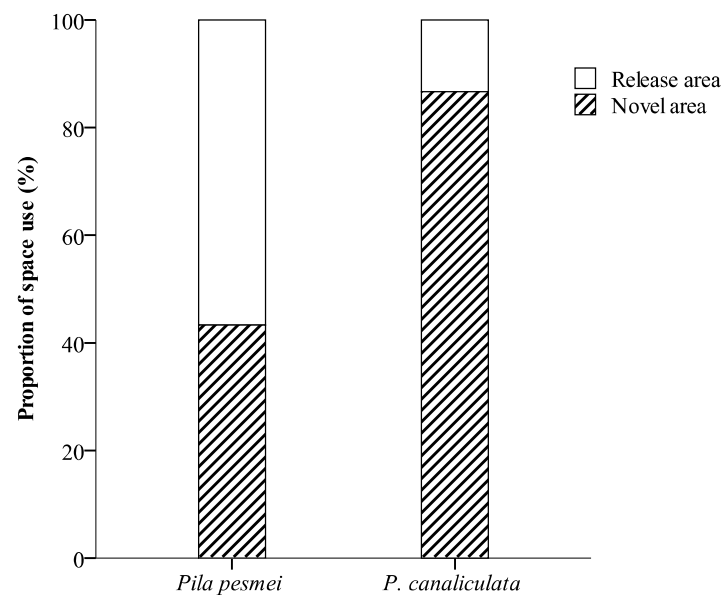
A total of 87 native apple snail (*Pila pesmei*) and 121 invasive apple snail (*P. canaliculata*) were collected from two different study sites in Hua Chang Subdistrict, Chaturaphak Phiman District, Roi Et, Thailand (WGS84: 15°51'N 103°34'E): *Pila pesmei* were dug up from an upland rice field, while *P. canaliculata* were hand-collected in a lowland rice field. All apple snails were brought to the laboratory at Burapha University in Chon Buri, Thailand and identified by morphological characteristics using the identification key of Keawjam [19], Brandt [21] and Hayes *et al.* [22]. Individuals of each species were kept in separate cement ponds (42 x 42 x 33 cm), filled with tap water at approximately

29°C, exposed to natural photoperiod, and allowed for at least 1 week of acclimatization time to adjust to laboratory conditions. Each pond was covered with nylon net (mesh size 2 mm) and overlaid with bricks to prevent apple snails escaping. All apple snails were fed *ad libitum* once daily with soft plants such as Chinese cabbage (*Brassica pekinensis*), lettuce (*Lactuca sativa*) and water spinach (*Ipomoea aquatic*). All procedures performed in studies involving animals were in accordance with the ethical standards of Burapha University and the National Research Council of Thailand at which the studies were conducted.

We randomly selected 30 *Pila pesmei* and 30 *P. canaliculata* (26-30 mm in shell height) from cement ponds to examine shyness and boldness traits. Experiments were conducted during nighttime, between 18.00 and 23.00, under dim lighting, simulating the natural conditions under which the apple snails are most active [23]. In each experiment, one individual of each species was randomly introduced into separate test tanks (30 x 30 x 30 cm filled with 3 L of dechlorinated tap water) on one side of the tank and covered with a plastic basket (17.5 x 12.5 x 7 cm). Following a 10 min acclimatisation period, the basket was carefully removed and the apple snail was free to emerge and crawl throughout the test tank for a 60-min period. The snails were observed from outside the test tank every 15 min during this period. The position of each apple snail was photographed before and after the experiment. This observational assessment is to collect data that are unbiased (i.e., measured in a way that does not underestimate or overestimate the true value). The 60-min experiment was repeated thirty times using different individuals.

In this study we defined the differences in shy/bold behaviour as differences in space use by the end of the 60-min trial (as the proportion of apple snails occupying the release area and the novel open space) and distance moved from the release area to enter the novel open space (as measured by a displacement that is the shortest distance from the initial to the final position of the locomotion), measures that have been used by Bremner-Harrison *et al.* [2], Fraser *et al.* [11], Magnhagen *et al.* [13] and Rehage and Sih [14]. These measures provide a means of examination of shyness and boldness in an individual that reflects a personality trait, thereby providing a method for predicting the behavioural response of individuals to novel subjects or situations after release. Hence, a bolder apple snail was expected to be more likely to preferentially occupy the novel open space and move further away from the release area than a shy apple snail.

Nonparametric tests were used throughout these experiments as none of the data conformed to a normal distribution and could not be satisfactorily transformed. Fisher's exact test modified for data arranged in a 2 x 2 table was used to analyze the relative difference in the space use between *Pila pesmei* and *P. canaliculata*.



**Figure 1** Proportion of space use of *Pila pesmei* and *P. canaliculata* by the end of the 60-min trial

In order to Fisher's exact test can determine the significance of a difference between the proportions in the two groups that is categorical variables with small sample size [24], we compared the proportion of individuals that were found at the release area and the novel open space between *Pila pesmei* and *P. canaliculata*. Furthermore, Mann-Whitney *U* test was used to determine significant differences in the distance moved from the release area to the novel open space between *Pila pesmei* and *P. canaliculata*. Finally, Spearman rank correlation coefficient was used to assess the relationship between shy/bold behaviour (the distance moved after release) and shell height (a measure of body size) for each apple snail species. Exact tests were used throughout. For all statistical tests,  $p < 0.05$  were considered to be significant. All analyses were conducted in R software version 3.2.3 [25].

### 3. Results and discussion

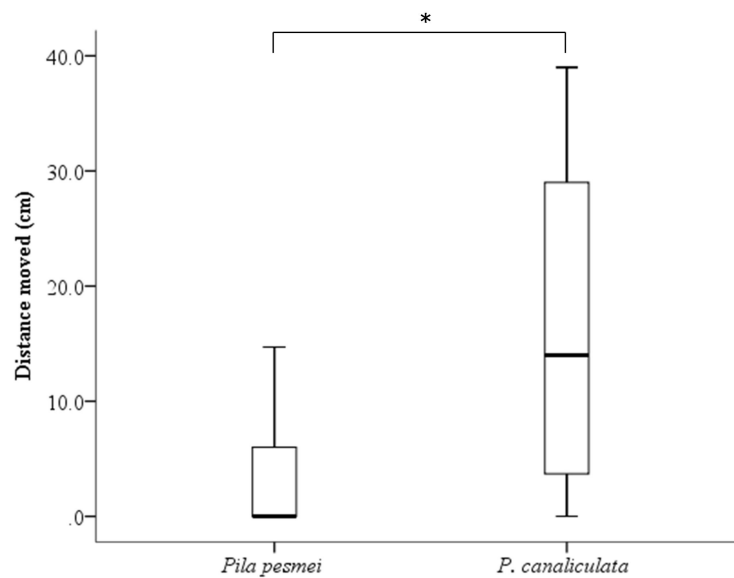
There was significant difference in the proportion of space use of *Pila pesmei* and *P. canaliculata* (Fisher's exact test,  $n_1 = 30$ ,  $n_2 = 30$ ,  $p < 0.001$ ; Figure 1). At the end of the 60-min trial, 43.33% of *Pila pesmei* occupied out of the release area, whereas the remaining 56.67% returned to the release area from the open space. On the other hand, *P. canaliculata* preferentially occupied the novel open space (86.67%) to a larger extent than in the release area (13.33%). The comparison of the distance moved from the release area into the novel open space revealed a significant difference between *Pila pesmei* and *P. canaliculata* (Mann-Whitney *U* test,  $n_1 = 30$ ,  $n_2 = 30$ ,  $U = 210.0$ ,  $p < 0.001$ ), whereby *P. canaliculata* moved away from the release area further than *Pila pesmei* (Figure 2).

These suggest that *P. canaliculata* was significantly bolder than *Pila pesmei*.

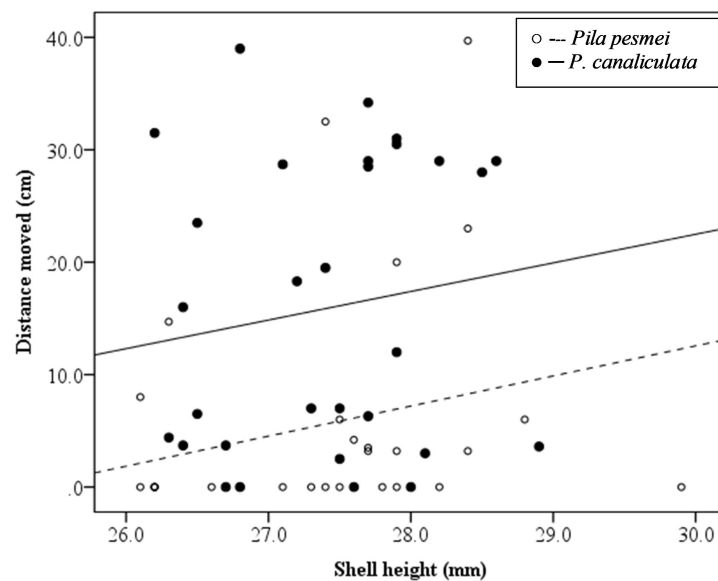
Our study is consistent with previous studies on mosquitofish [14] and crayfish [26] which revealed that invasive species are bolder than native species. It has been suggested that differences in shy/bold behaviour might play a role in the competitive displacement and invasion success of a species [7, 14, 26]. Bolder individuals (those that are more active, more prone to disperse into new habitats to explore novel objects or environments, and more likely to approach predators) may be more likely to expand their range further than shy ones and become a successful invader [7, 27, 28]. In addition, a species that is more exploratory is likely to be confronted with novel food resources which increase its survival and chances of successful reproduction and hence this behavioural ability may lead to the success of the species in a novel environment [29]. The results of the present study, along with other data on snail characteristics (i.e., environmental tolerance, high adaptability, high reproductive rate and rapid dispersal), is important for predicting invasion success of *P. canaliculata* in invaded habitats [17, 30 - 32].

However, we found no relationship between distance moved (the distance apple snails moved from the release area into the novel open space) and shell height for either *Pila pesmei* (Spearman rank correlation,  $n = 30$ ,  $r_s = 0.351$ ,  $p = 0.058$ ) or *P. canaliculata* ( $n = 30$ ,  $r_s = 0.109$ ,  $p = 0.565$ ; Figure 3).

This may be due to the small tested size range (26-30 mm) and therefore future studies should address the shy/bold behaviour in smaller and larger individuals than those studied here. Previous studies of behavioural traits in freshwater fishes [10 - 12, 33] and crabs [34, 35]



**Figure 2** Boxplot of the distance moved by *Pila pesmei* and *P. canaliculata* from the release area into the novel open space. \*Indicates a significant comparison where  $p < 0.05$



**Figure 3** Relationship between the distance moved from the release area to the novel open space and shell height of *Pila pesmei* and *P. canaliculata*. Regressions: *Pila pesmei*:  $y = 2.68x - 67.72$ ; *P. canaliculata*:  $y = 2.54x - 53.83$

have also did not find any effect of body size on boldness traits. However, other studies of freshwater fishes [9, 36 - 38] and squid [39, 40] have shown that size may play an important role in determining the expression of personality traits in which small individuals are usually bolder than large ones. One possible explanation for the influence of body size on the expression of shyness and boldness found in previous studies might be related to the energetic costs associated with metabolic requirements. Smaller individuals have proportionally greater energy demands due to higher metabolic rates and fewer body fat reserves than larger ones [36, 41, 42] and therefore

small individuals should be more prone to take the risk to find food by leaving a safe environment sooner than larger individuals. Another possible explanation for the size-effects could be that body size often correlates with body condition, and consequently affect behavioural traits such as boldness. The body condition is influenced indirectly by environmental variables such as food availability that is an individual with poor body condition might be more motivated to take risks or encounters to find food than an individual with good body condition [35, 43]. It could be that individuals that are hungrier are more likely to take greater risks to investigate novel objects and obtain

food in new environments than well fed individuals [10, 42]. All apple snails used in this study were of small tested size range and well fed prior to being tested (standardized condition), suggesting that they had similar energetic conditions. Hence, it appears that differences in shyness and boldness of the two apple snail species are not related to the metabolic requirement hypothesis [12].

#### 4. Conclusions

Our results provide evidence that a non-native invasive snail species was significantly bolder than the native species, which illustrates the importance of understanding behavioural traits as a key factor determining invasion success. Although the space use and the distance moved within a novel compartment, like the one used in this study, have proved to be useful measures of shyness and boldness [2, 11, 13, 14], they have been rarely used to test predictions in invasion ecology. Even though there is some evidence that varying nutritional condition and size of snails as well as other measures would induce differences in shyness and boldness [9, 35 - 40, 43], this still requires further investigation in our system. Nevertheless, this study suggests that it will be useful to look for similar individual differences in behavioural traits of other native and non-native invasive species.

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