

# Prime waterfront real estate: Apple snails choose wild taro for oviposition sites

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**Abstract** While difficult to prevent introductions, scientific research can help guide control efforts of exotic, invasive species. South American island apple snails *Pomacea insularum* have quickly spread across the United States Gulf Coast and few control measures exist to delay their spread. Usually occupying cryptic benthic habitats, female apple snails crawl out of the water to deposit large, bright pink egg clutches on emergent objects. To help identify the most likely place to find and remove clutches, we conducted four lab experiments to investigate what specific object qualities (i.e. material; shape and height; plant species; natural and artificial) attracted *P. insularum* females to lay clutches. In our fourth experiment, we specifically examined the relationship between female size and reproductive output. To further understand reproductive output, we quantified experimental clutch characteristics (height above water, dimensions, mass, approximate volume, number of eggs, hatching efficiency). *Pomacea insularum* females laid more clutches on plant material, chose round over flat surfaces and failed to differentiate between tall and short structures. In comparison to a common native plant in the eastern US, *Pontederia cordata*, snails clearly preferred to lay clutches on a widely distributed exotic, invasive plant (wild taro, *Colocasia esculenta*). Unexpectedly, smaller snails showed higher overall total fecundity as well as more eggs per clutch than larger snails. Therefore, hand removal efforts of large females may not be enough to slow down clutch production. Collectively, our results indicate that conservationists and managers should search emergent plants for *P. insularum* clutches carefully to guard against established populations [*Current Zoology* 57 (5): 630–641, 2011].

**Keywords** *Colocasia*, Wild taro, Fecundity, Substrate, Reproduction, Facilitation

Stopping the invasion process of exotic invasive species (EIS) begins with preventing the introduction of exotic organisms (Lodge et al., 2006). Although most introductions fail (Kolar and Lodge, 2001), the few that succeed require control measures to lessen their ecological and economic damage (Pimentel et al., 2005). To successfully battle already established EIS populations and detect new introductions, managers and conservationists need to understand the ecology of these biological threats and have access to well-researched, efficient pest population control strategies (Lodge et al., 2006). Recently, new methods for EIS detection have emerged, such as using environmental DNA to test for the presence of invasive Asian carps (*Hypophthalmichthys molitrix* or *H. nobilis*) in the Great Lakes (Jerde et al., 2011). However, for most EIS, we do not have the genetic sequences or resources to employ these new high-tech molecular means of detection. Instead, most control efforts must still occur at the ground level.

South American apple snails (Ampullariidae) represent a notorious group containing invaders with the ability to disrupt ecosystems (Carlsson et al., 2004), consume substantial amounts of aquatic plants (Qiu and Kwong, 2009), spread disease (Rawlings et al., 2007), and out-compete native species (Morrison and Hay, 2011). Using mitochondrial DNA haplotype comparisons, scientists recently confirmed the identity of channeled apple snail populations along the coast of the Southeastern U.S. (Fig. 1A; Rawlings et al., 2007) and Southeastern Asia (Hayes et al., 2008) as *P. insularum* (d'Orbigny 1835), the island apple snail—a close relative of another channeled species, the better known golden apple snail *P. canaliculata* (Lamarck 1828).

Although only recently recognized as an environmental threat, scientists have documented early impacts of *P. insularum* which include consumption of native Florida plants (Baker et al., 2010), competition with the native Florida apple snail *P. paludosa* (Connor et al., 2008; Morrison and Hay, 2011), and the ability to ex-

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exploit ephemeral habitats (Burlakova et al., 2010). *Pomacea insularum* now occurs in eight states across the United States Gulf Coast (Fig. 1A; Karatayev et al., 2009) with discovery of new populations occurring frequently. Despite these early indicators that *P. insularum* represents an exotic species warranting serious and immediate concern, we know of no publications that focus on how to improve our ability to control the spread of *P. insularum*.

In addition to their large size (adults can reach fist size and 175g; Kyle et al., 2009) and ravenous consumption of aquatic macrophytes (Morrison and Hay, 2011), *P. insularum* stands out among invasive species due to its staggering reproductive abilities. Female *P. insularum* lay eggs in large clutches averaging  $1626.3 \pm 121.7$  eggs but ranging up to nearly 5,000 (Barnes et al., 2008). These clutches can hatch within three weeks of oviposition (Howells et al., 2006) with variable but alarmingly high hatching efficiency (70.8% mean field hatching efficiency across treatments with a range of 0.9 to 94.3%; Barnes et al., 2008). While researchers have not yet quantified annual fecundity of *P. insularum* or know if fecundity depends on age or size, these snails can produce multiple clutches throughout the warm seasons and may need only nine months to reach reproductive maturity (Burks, personal observation). This rapid reproductive rate allows these invaders to reach high densities quickly, overwhelm aquatic flora, and then spread to new environments.

Currently, only three methods exist to control infestations of *P. insularum*: physical removal of snails, physical removal of eggs, or the use of broad-spectrum poisons such as copper sulfate and Rotenone (Cowie, 2002; Van Dyke, 2010). Obviously, conservationists should only use broad-spectrum poisons under the most dire of circumstances. While the cryptically-colored snails can often be difficult to find, the detection and removal of noticeably pink egg clutches represents a strategy of great potential. Egg removal requires no special equipment, can potentially be accomplished with volunteers and directly reduces the reproductive output of a population. However, to transform this recommendation into a feasible on-the-ground control strategy, we need to understand more about the behavior and reproductive biology of *P. insularum*.

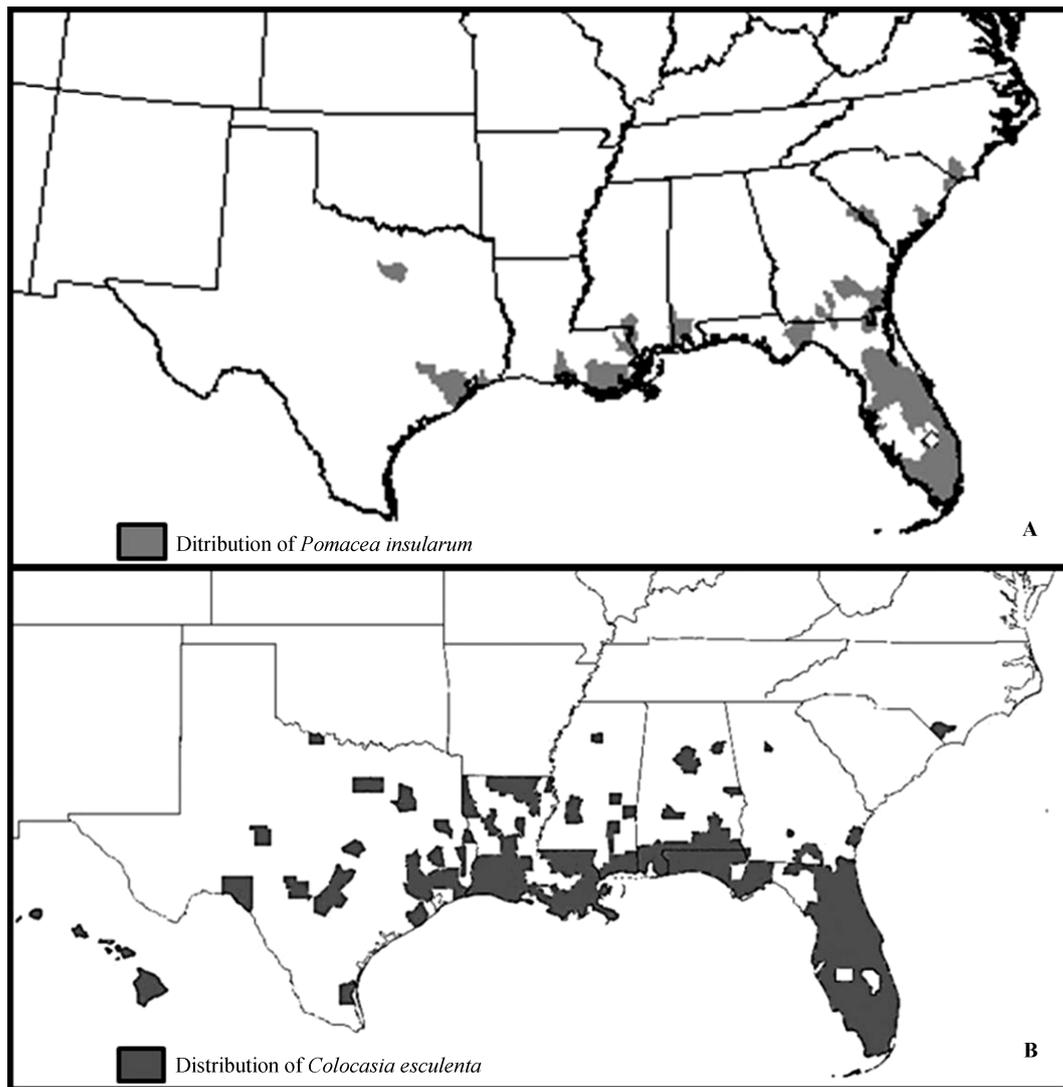
When ready to lay a clutch, a female *P. insularum* climbs out of the water onto an emergent object and lays her eggs, which are vibrant pink. Oviposition can take hours because individual females form clutches by sliding individual eggs along their foot onto the substrate.

At the end of this reproductive event, the female has formed an ellipsoidal clutch held together and attached to the substrate by a gelatinous matrix. Apple snails place their eggs out of water ostensibly to avoid aquatic predation but also to avoid water stress. Fresh eggs of other species do not hatch successfully when submerged (Turner, 1998; Pizani et al., 2005). Yet, for the female, a trade-off likely also exists between the time spent laying eggs and exposure to aerial predation (Cattau et al., 2010). Consequently, we expect female apple snails to choose a substrate that allows her to best negotiate this trade-off (Turner, 1996).

Females use a wide range of objects as oviposition substrates. Burks et al. (2010) conducted the first census of common substrates utilized by *P. insularum* as oviposition sites along a 2-km stretch of bayou in Houston, Texas. This study found that *P. insularum* females utilized a wide range of structures (plants, trees, concrete pillars, sewer cisterns, etc.) but appeared to demonstrate preferences for certain structures, especially the widespread exotic invasive plant wild taro *Colocasia esculenta*. Wild taro, an emergent aquatic macrophyte, grows in dense stands of thick, sturdy stalks topped by broad leaves. Historically brought from Asia to North America as a food and landscaping resource (Akridge and Fonteyn, 1981), wild taro now exists across the Gulf Coast landscape from Texas to North Carolina, which encompasses all established *P. insularum* populations in the U.S. (Fig. 1B; Center for Invasive Species and Ecosystem Health, 2010).

Besides serving as a place to deposit clutches, wild taro may also appeal to apple snails as a food source. Unfortunately, conflicting accounts of taro consumption by apple snails exist in the literature. For example, in 96-hour non-choice feeding trials, Burlakova et al. (2008) found that *P. insularum* consumed 75.9% ( $\pm 14.4\%$ ) of the *C. esculenta* offered whereas Baker et al. (2010) did not detect consumption of wild taro in similar non-choice experiments. As the question remains whether or not wild taro provides a good food resource for apple snails, we focused on its role as a substrate for oviposition. Because of their documented relationship and overlapping distributions (Fig 1A, B), we think wild taro may be another important component in the success story of exotic invasive *P. insularum* in North America.

To improve our ability to detect new introductions and slow spreading populations, we seek to determine where conservationists will most likely find *P. insularum* egg clutches and how female size influences



**Fig. 1 Documented distributions of *Pomacea insularum* and *Colocasia esculenta* in North America**

A. Documented distribution of island apple snails *P. insularum* in North America (Benson, 2011). B. Documented distribution of wild taro *C. esculenta* in North America by the Center for Invasive Species and Ecosystem Health (Center for Invasive Species and Ecosystem Health, 2010).

reproductive output. First, we tested oviposition preference of female snails with four laboratory experiments where we presented different substrates with equal area or availability. Second, we determined overall fecundity of females across a size gradient and examined how egg clutch size related to female snail size. Third, we examined characteristics of *P. insularum* egg clutches laid on different substrates to assess if substrate influenced oviposition or hatching efficiency (HE). Finally, we tested if *P. insularum* reproduction in our lab experiments mimicked reproduction in the wild. Based on previous field observations, we hypothesized that female snails would choose natural substrates when available and that they would demonstrate a positive attraction to wild taro (Burks et al., 2010).

## 1 Materials and Methods

### 1.1 Oviposition substrate preference experiments

To discover mechanisms underlying trends in oviposition, we designed four experiments that investigated what substrate characteristics appealed to female *P. insularum* (Table 1). Each experiment presented one, two or three *P. insularum* adult females with different substrate types selected from field observations. We used multiple snails to boost the likelihood of getting enough clutches for statistical analysis. To ensure substrate size did not affect snail preferences, presented substrates possessed approximately the same surface area within the same experiment. Substrate height differed by design in our second experiment (Table 1).

**Table 1 Characteristics of each oviposition preference experiment ( $\pm 1 SE$  when appropriate)**

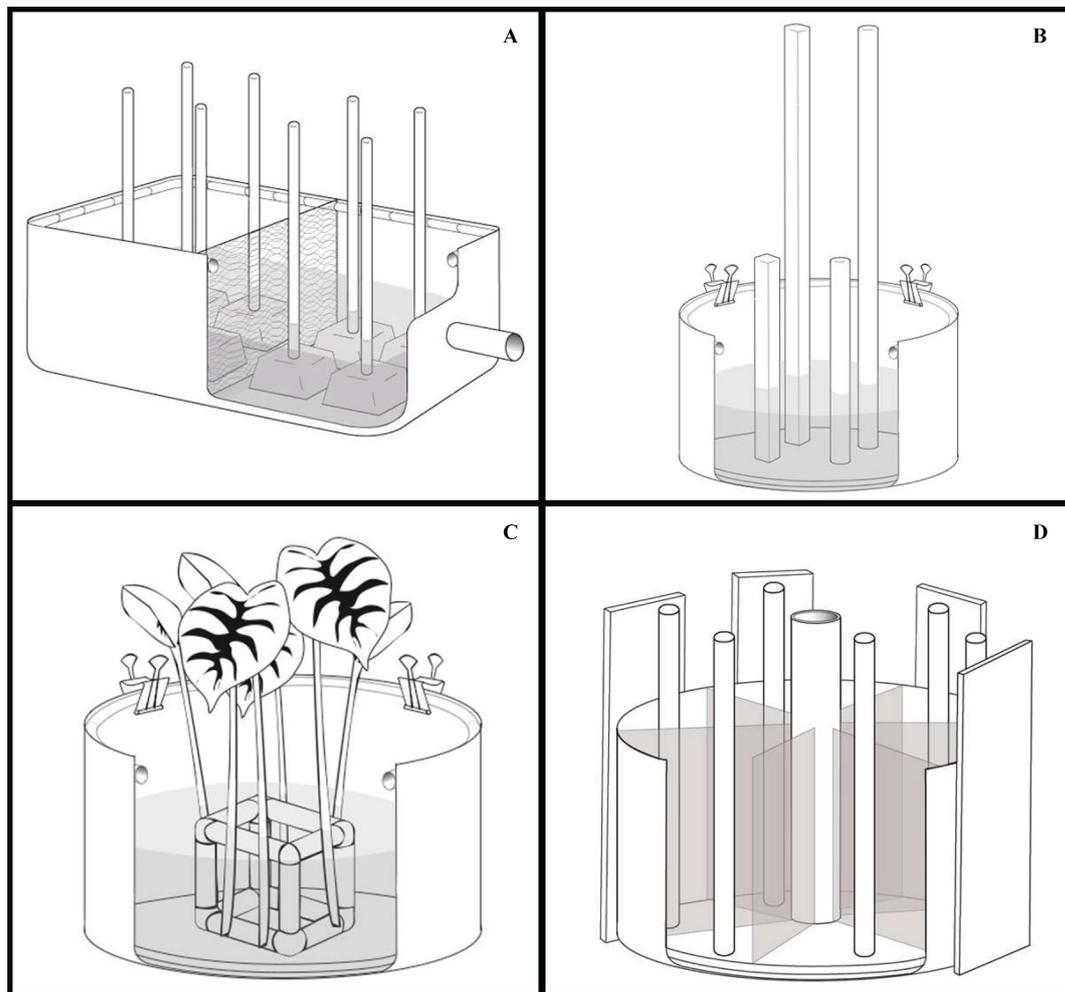
Preference tested	Number of separate tanks (n)	Tank size	Days	Substrates available [diameter (d), length (l)]	Water depth (cm)	Approximate water temp. (Nearest °C)	Food	Females per replicate	Snail operculum width (mm)	Snail mass (g)
Substrate material	12	Dimensions (54.3 × 54.3 × 45.7 cm)	19	Plant, wood, metal, plastic (l = 76.2 cm, d = 1.9 cm)	15.2	26°C	<i>Ad libitum</i>	2	47.6 ±0.710	105 ±5.18
Substrate shape and height	5	Diameter (50.8 cm) Height (33.0 cm)	21	Round (d = 3.2 cm) Square (2.54 x 2.54 cm) Short (l = 50.8 cm) Tall (l = 81.3 cm)	20.3	28°C	140 g/day	3	58.6 ±0.541	112.4 ±4.09
Plant species	5	Diameter (50.8 cm) Height (33.0 cm)	14	<i>C. esculenta</i> <i>P. cordata</i> (l = 53.3 cm)	20.3	28°C	140 g/day	3	60.4 ±0.733	124.4 ±5.23
Clutch size Versus female size	6	Diameter (50.8 cm) Height (33.0 cm)	35	Wooden Pole (d = 3.2 cm; l = 81.3) PlexiGlas Rectangular Sheet (12.5 x 20 cm)	28.0	28°C	<i>Ad libitum</i>	6	N.A.	74.01 ±14.30

Each tank also contained one adult male snail. Fresh romaine lettuce served as food for snails.

Although each setup contained specialized features, our four experiments possessed similar designs (Table 1; Fig. 2). We used different groups of adult *P. insularum* snails in each experimental setup and randomized substrate position within each tank. In all experiments, 1000 L/h, Fluval 305 multi-stage filters pumped, filtered and aerated the water in the tanks. Aquarium heaters (300 W) maintained the water temperature (Table 1). For the first three oviposition preference experiments, we engineered the system to make the walls undesirable egg-laying surfaces by pumping the water into regularly perforated rubber tubing that lined each tank's rim (Fig. 2A-C). In general, this design discouraged snails from depositing eggs on places other than the presented substrate poles. For the fourth experiment, we filled the tanks to the brim with water to prevent oviposition on the tank walls (Fig. 2D). Despite the deterrent design of the tanks, a few snails managed to lay small clutches on

gaps in the water flow or on the tubing. Because these clutches possessed a notably smaller size and more irregular shapes than the substrate clutches, we excluded them from our data analysis ( $n=8, 5, 10,$  and  $6$  for our four experiments, respectively).

In our Experiment 1 (Substrate Material), we simultaneously offered female snails four substrates composed of different materials frequently encountered in exotic environments (Burks et al. 2010): plant (*C. esculenta* stalk), wood (round wooden dowel), metal (galvanized metal pole), and imitation plant material (green Astroturf wrapped around PVC pipe). We obtained all *C. esculenta* stalks from Town Lake, Austin, Texas. To house this experiment, we constructed a flow-through tank system comprised of six rectangular 189 L containers divided in half by metal mesh, which created 12 snail containment areas (Fig. 2A). PVC pipe connected the ends of each large tank to a reservoir tank from



**Fig. 2** Experimental tank designs for preference experiments

A. Substrate material. B. Substrate shape and height. C. Plant species. D. Clutch size versus female size. See Table 1 for design specifics for each experiment.

which a pump drew and filtered water. In total, the tank system used four reservoir tanks and pumps. We secured the base of each substrate to keep it upright.

In Experiment 2 (Substrate Shape and Height), we tested what physical features of a substrate most appealed to reproducing *P. insularum*. Although other variables may affect oviposition, this experiment tested two varying factors in surface characteristics: shape (round or square) and height (tall or short). The two variables combined to create a  $2 \times 2$  design with four different wooden egg-laying structures (tall/round, tall/flat, short/round, and short/flat; Table 1). We conducted the experiment in individual round tanks, each with a filter and a heater maintaining the water quality (Fig. 2B). We chose to test round versus flat substrates as a surrogate for more naturally round structures found in nature in contrast to more manmade structures that have flat surfaces. A test of short versus tall substrates questioned the tradeoff between potential water exposure of eggs versus snails' innate tendencies to limit time out of the water. We cut circular sections of plywood that sat on the bottom of each tank (diameter = 48 cm) and attached four bolts to each base to stabilize substrate poles. The bolts fit into the base of the wooden poles and held the poles vertically stable.

In Experiment 3 (Plant Species), we tested if female snails differentiated between exotic wild taro *C. esculenta* and North American native pickerelweed *Pontederia cordata* when depositing clutches. We chose *P. cordata* because of its recommendation as a native alternative to wild taro in landscaping and its use as a substrate by native Florida apple snails, *P. paludosa* (Turner, 1996). To maintain available live plants, we transplanted *P. cordata* individuals from a pond in Pasadena, Texas, into a local greenhouse. We presented freshly cut stocks of each plant with intact leaves as the oviposition substrates. The experiment used three, similarly-sized stalks of each plant species zip-tied to bamboo sticks for support (six total stalks per replicate). We built cubic PVC pipe frames (pipe diameter = 2 cm; frame dimensions =  $20 \times 15 \times 15$  cm<sup>3</sup>) to support the plant stalks. The experiment occurred in the same individual round tanks with bases as the previous shape and height experiment (Fig. 2C). Each frame sat squarely on the plywood base, held in place by bolts.

In Experiment 4 (Natural versus Artificial), we kept each female in isolation and provided two emergent surfaces for oviposition: one round (wooden dowel) and one flat plastic surface (PlexiGlas). In all four oviposition experiments, we removed clutches carefully using a

razor blade, recorded the number of newly laid clutches per substrate, monitored water temperatures using digital thermometers daily and fed lettuce to snails daily (Table 1). Our data included number of clutches per substrate per day and the clutch height above the water level as measured from the base of the clutch. To test for significant patterns in substrate choice, we used a randomized blocked ANOVA with tanks as our block and substrate type as our main factor. In addition, we employed a paired *t*-test to contrast the average number of clutches laid on plant versus wood substrates.

### 1.2 Clutch size versus female size

To investigate how female size impacted reproductive output, we tracked clutch deposition of individual snails over 5 weeks in Experiment 4. To examine the relationship between total fecundity, clutch size and female snail size, we used a gradient of female sizes ranging from 17.92 g to 168.68 g (average  $\pm 1$  SD =  $73.34 \pm 35.91$  g; median = 71.45 g), grouped snails into sets of six and recorded snail size every week to match with each individual clutch laid. To isolate the females, we divided the round tanks described above into six equally-sized compartments using plastic-coated chicken wire that we secured with clips and zip-ties (Fig. 2D). We randomly assigned one snail from each set of six to each of six tanks. Independent filters circulated water from the center of each tank to ensure consistent water quality for each compartment. Although *P. insularum* females can store sperm for weeks (Howells et al. 2006), we rotated an adult male snail through each of the tanks (24 hrs per compartment) to ensure the potential for copulation. We ran linear regressions to look for predictive relationships between clutch characteristics and female snail size. For regression with clutch dimensions (length, width and height), our analyses included 61 clutches.

### 1.3 Clutch characteristics and hatching efficiency

During oviposition preference experiments, we collected and measured the characteristics of clutches laid to observe the impact of different substrates on clutches. We also weighed and hatched clutches from the Shape and Height and Plant Species experiments to examine egg number and HE. When analyzing clutch characteristics, we first tested consistency in snail laying activity between our five tanks using non-parametric Kruskal-Wallis tests. We pooled all clutches and tested for treatment differences using Independent Sample *t*-tests when equal variance or no significant variation occurred. Due to the general lack of clutches on square substrates, we only statistically compared clutches laid on tall and

short substrates. In the Plant Species Experiment, only the height of clutches differed significantly across replicate tanks. Consequently, for this variable only, we used the mean height of clutches from each tank. However, the number of data points ( $n$ ) available differed noticeably for each treatment ( $n = 26$  clutches on *C. esculenta*;  $n = 6$  on *P. cordata*). Accordingly, we utilized Mann-Whitney  $U$  tests to compare Plant Species characteristics. We also conducted regressions between HE and substrate characteristics to determine to what degree these characteristics predicted HE.

#### 1.4 Lab and field comparison

To ensure female behavior in our laboratory closely depicted behavior in the field, we used wild *P. insularum* adults recently collected from areas in and around Armand Bayou, Houston, TX (lat 29°56'38"N, long 95°07'18"W). We determined snail gender based on aperture shape according to Cazzaniga (2006) or by separating mating pairs. To verify the appropriateness of extrapolating our results to the field, we investigated whether *P. insularum* reproductive behavior in our laboratory via Experiments 1 and 3 mimicked field reproduction. Specifically, we compared physical characteristics of clutches laid in laboratory experiments with clutches laid in the field.

We measured clutch height (distance from water surface to base of the clutch), dimensions (length, width, depth) and approximate volume (length  $\times$  width  $\times$  depth) of *P. insularum* clutches laid on *C. esculenta* in both the field and laboratory. We sampled field clutches from Armand Bayou in May 2007 ( $n=20$ ), May 2008 ( $n=20$ ), and August 2008 ( $n=35$ ). In the field, we first measured clutch heights then clipped plant stems to collect clutches and transported intact clutches wrapped in wax paper to the laboratory for additional measurements. After completing all measurements, we dissolved a subset of laboratory and field clutches in 0.1 M NaOH to break down the gelatinous binding matrix and allow counting of individual eggs. We allowed other clutches to hatch to calculate hatching efficiency (HE).

To hatch the eggs, we placed each clutch on a modified Tupperware container containing spring water (Barnes et al., 2008) into which emerging hatchlings fell. To standardize hatching conditions, we incubated the clutches at 28.5°C for 14 days. After this period, we dissolved the remaining eggs in 0.1 M NaOH solution and counted the number of eggs that hatched (i.e. hatchlings) versus intact eggs remaining from each clutch. The sum of the number of hatchlings and intact eggs equaled the total egg number for that clutch. We

then calculated HE by dividing the number of hatchlings by the total egg number in a clutch. Because we compared two sets of clutches that held normal distributions, we utilized Independent-Sample  $t$ -tests to determine differences in clutch characteristics between the laboratory and the field. Our data regarding clutch width required log-transformation to meet the assumption of normality.

## 2 Results

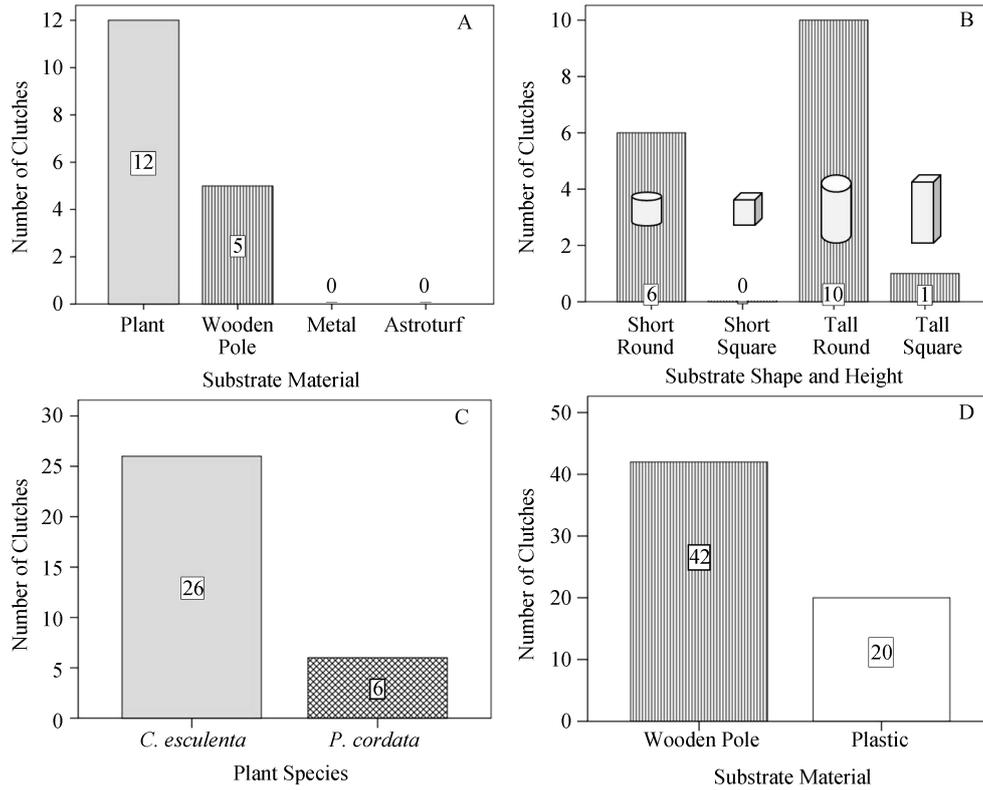
### 2.1 Oviposition substrate preference experiments

During Experiment 1 (Material), pairs of snails produced a total of 17 total clutches on the presented substrate poles and laid the most clutches on the plant substrate ( $n=12$ ). Significantly different number of clutches occurred per substrate (Fig. 3A; randomized block One-Way ANOVA,  $F_{1,45} = 17.69$ ,  $P < 0.0009$ ). We did not find a single clutch on either metal or AstroTurf. Per tank, average numbers of clutches did not differ between plant and wood substrates (paired  $t$ -test,  $t_{11} = 1.4$ ,  $P = 0.1891$ ).

In Experiment 2 (Substrate Shape and Height), trios of *P. insularum* females demonstrated a preference for laying clutches on rounded materials versus squared materials (Fig. 3B;  $F_{1,17} = 5.263$ ,  $P = 0.03429$ ). Only one clutch out of 17 occurred on a square substrate. For Experiment 3 (Plant), we found that trios of female snails laid substantially more clutches on exotic *C. esculenta* rather than *P. cordata* (Fig. 3C;  $F_{1,7} = 10.31$ ,  $P = 0.01483$ ). Single female snails in Experiment 4 (Natural versus Artificial) laid 62 viable clutches. More clutches occurred on natural substrate (i.e. wood) than artificial substrate (i.e. plastic) (Fig. 3D;  $F_{1,9} = 16.646$ ,  $P = 0.002758$ ).

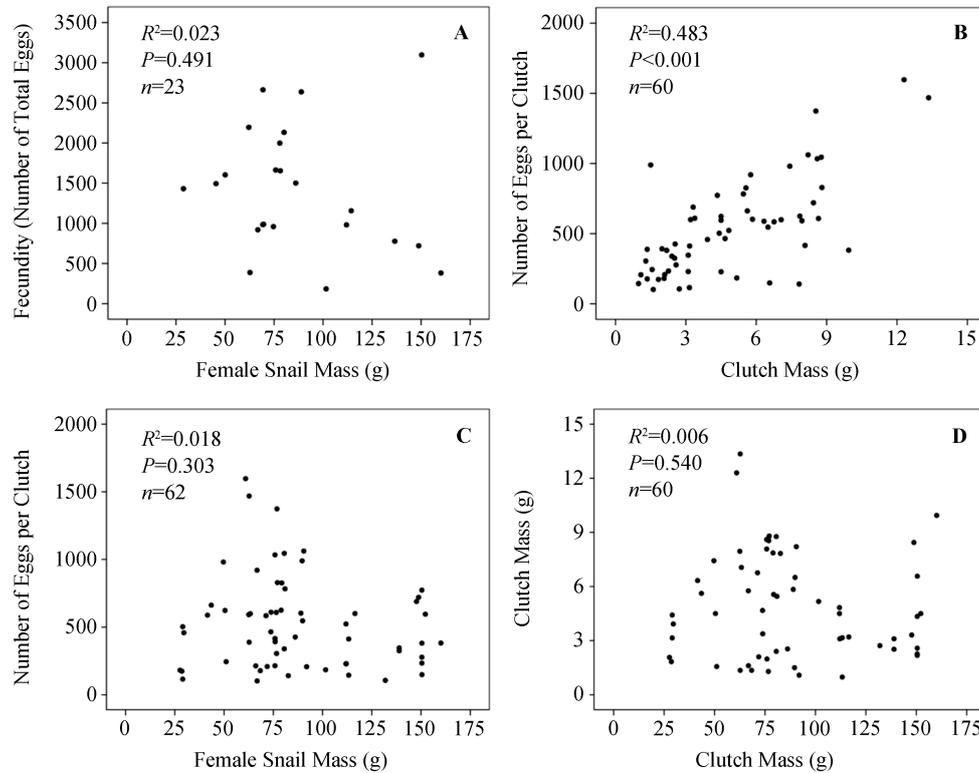
### 2.2 Clutch size vs. female size

In Experiment 4, total fecundity of female snails ( $n=23$ ) did not relate to female mass (Fig. 4A;  $P = 0.491$ ,  $R^2 = 0.023$ ). When broken down into individual clutches ( $n=62$ ), we also failed to see a trend in number of eggs per clutch and female size (Fig. 4B). Most surprisingly, small snails tended to have the highest fecundity in our experiment (Fig. 4A) and laid the largest clutches (Fig. 4B). After excluding four incomplete clutches (i.e. possibly resulting from disrupted oviposition), clutch size still ranged extensively from 102 to 1597 eggs (Table 2). As expected, a significant predictive relationship occurred between clutch mass and number of eggs (Fig. 4C). However, we did not find any clear relationship between clutch mass and female snail size (Fig. 4D). In terms of other characteristics, clutch width showed a



**Fig. 3** Number of clutches laid by *P. insularum* on different substrates in four preference experiments

A. Substrate material. B. Substrate shape and height. C. Plant species. D. Clutch size versus female size. Small numbers within bars indicate number of clutches per substrate type. Note that scales on the Y-axes vary between experiments. See Table 2 for clutch characteristics for each experiment.



**Fig. 4** Scatter plots illustrating relationships between (A) total female fecundity in number of eggs versus average female snail mass ( $n=23$ ); (B) number of eggs in a clutch ( $n=62$ ) and female snail mass; (C) number of eggs per clutch laid by female island apple snails and the mass of the entire clutch ( $n=60$ ); and (D) clutch mass versus female snail mass

Probability and  $R^2$  values derived from linear regressions. Data points represent clutches laid during the Clutch Size versus Female Size experiment.

**Table 2 Mean characteristics of clutches collected from the field as well as laid in each oviposition preference experiment ( $\pm 1 SE$ )**

Experiment or Comparison	Substrate ( <i>n</i> )	Height above water (cm)	Length (mm)	Width (mm)	Depth (mm)	Mass (g)	Total egg number per clutch	Hatching efficiency per clutch (%)
Field and Lab	<i>C. esculenta</i> Field (75)	<b>58.65 <math>\pm</math> 2.91</b>	69.6 $\pm$ 4.65	<b>23.2 <math>\pm</math> 0.76</b>	17.3 $\pm$ 0.49	*	<b>1249 <math>\pm</math> 69</b>	<b>37.4 <math>\pm</math> 3.48</b>
	<i>C. esculenta</i> Lab (37)	<b>26.1 <math>\pm</math> 2.27</b>	62.7 $\pm$ 3.28	<b>20.9 <math>\pm</math> 0.50</b>	16.4 $\pm$ 0.54	*	<b>1523 <math>\pm</math> 128</b>	<b>76.7 <math>\pm</math> 3.57</b>
Substrate Material	Plant (11)	40.52 $\pm$ 3.16	63.7 $\pm$ 4.65	23.0 $\pm$ 0.806	17.5 $\pm$ 0.684	*	*	*
	Wood (5)	32.3 $\pm$ 5.46	75.5 $\pm$ 13.8	26.0 $\pm$ 2.00	16.6 $\pm$ 1.59	*	*	*
Substrate Shape and Height	Short (6)	28.0 $\pm$ 6.94	79.2 $\pm$ 10.8	21.8 $\pm$ 1.29	13.3 $\pm$ 0.914	<b>11.9 <math>\pm</math> 2.19</b>	<b>2046 <math>\pm</math> 184</b>	76.8 $\pm$ 6.06
	Tall (10)	35.4 $\pm$ 3.46	71.3 $\pm$ 10.2	20.4 $\pm$ 0.914	13.6 $\pm$ 0.702	<b>7.29 <math>\pm</math> 0.950</b>	<b>1307 <math>\pm</math> 145</b>	76.4 $\pm$ 6.23
Plant Species	<i>C. esculenta</i> (26)	19.1 $\pm$ 1.96	62.3 $\pm$ 4.28	20.0 $\pm$ 0.534	16.0 $\pm$ 0.70	8.19 $\pm$ 0.792	1524 $\pm$ 129	76.7 $\pm$ 3.57
	<i>P. cordata</i> (6)	18.7 $\pm$ 4.45	49.1 $\pm$ 7.7	21.3 $\pm$ 1.72	17.0 $\pm$ 1.80	7.73 $\pm$ 2.76	1591 $\pm$ 499	50.8 $\pm$ 4.24
Clutch Size to Female Size	Wood (42)	*	57.9 $\pm$ 4.04	17.1 $\pm$ 0.787	10.1 $\pm$ 0.445	5.07 $\pm$ 0.440	517 $\pm$ 82.1	*
	Plastic (20)	*	52.6 $\pm$ 4.73	18.2 $\pm$ 1.31	9.98 $\pm$ 1.01	4.60 $\pm$ 0.739	528 $\pm$ 51.7	*

Because we only hatched a subset of clutches in the Field data set, we used 55 clutches out of the 75 total field clutches laid on wild taro for information about hatchling number, unhatched egg number and HE. For these same limited characteristics, we analyzed 24 lab clutches taken from wild taro in the Plant Species Experiment. Bold text and light gray shading indicates that significant differences occurred between treatments for that characteristic in the experiment. Boxes marked with a star (\*) indicate that we did not measure that characteristic.

significant, although very weak, predictive relationship with female size ( $P = 0.007$ ,  $R^2 = 0.118$ ; Table 2). We saw no evidence of clear relationships between female mass and clutch height, length or mass ( $p$ 's  $> 0.154$ ).

### 2.3 Clutch characteristics and hatching efficiency

We found no difference in clutch characteristics between clutches laid on plant versus wood substrates (Table 2;  $P > 0.05$ ). Because snails laid only one clutch on a square-shaped substrate, we only statistically compared clutches laid on tall and short round substrates. From these comparisons, no significant differences in clutch characteristics occurred between clutches placed on tall versus short round substrates (Table 2;  $P > 0.05$ ). We found, however, that clutches laid on short round substrates possessed significantly higher masses ( $t_{16} = 2.203$ ,  $P = 0.043$ ), hatchling numbers ( $t_{14} = 2.38$ ,  $P = 0.032$ ) and total egg numbers ( $t_{14} = 3.133$ ,  $p = 0.007$ ) than clutches on tall round substrates. However, HE did not differ between short and tall substrates ( $t_{14} = 0.035$ ,  $P = 0.973$ ) despite a range in clutch sizes (Table 2). Together, all clutches in our shape and height experiment hatched with  $76.7 \pm 19.7\%$  efficiency. After comparing clutches laid on different plant species, we found no significant difference between clutches laid on *C. esculenta* and *P. cordata* for any metric (Mann Whitney U-Tests,  $P$ 's  $> 0.05$ ). Clutches laid on wild taro and then incubated exhibited a high HE of 77%, while the six clutches laid on pickerelweed averaged only a 50% HE. Regressions between HE and clutch characteristics revealed no noticeable associations in any experiment ( $R^2 < 0.50$ ).

### 2.4 Lab and field comparison

From our comparison of our lab ( $n=37$ ) and field clutches ( $n=75$ ) laid on wild taro, we found that female *P. insularum* laid clutches of equivalent length and depth ( $t_{110} = -1.871$ ,  $P = 0.064$  and  $t_{110} = -1.095$ ,  $P = 0.276$ , respectively; Table 2). However, in the field, snails laid significantly wider clutches ( $t_{110} = -2.590$ ,  $P = 0.011$ ) at a significantly greater height on the wild taro ( $t_{90} = -8.125$ ,  $P < 0.001$ ; Table 2). Field clutches contained an average of 1249 eggs ( $\pm 515 = 1$  SD). Laboratory snails laid slightly larger clutches ( $1524 \pm 360$  eggs) that significantly differed from those collected from the field ( $t_{77} = 2.031$ ;  $P = 0.046$ ). Most notably, we found that incubated laboratory clutches exhibited a striking 77% HE. In contrast, clutches brought from the field showed much more variability in HE and hatched at only 37%, significantly less successfully than lab clutches ( $t_{63} = 7.875$ ;  $P < 0.001$ ).

## 3 Discussion

Our laboratory experiments clearly demonstrated that *P. insularum* females chose to place clutches on substrates composed of plant material, even in the presence of alternative substrates of equivalent size and availability. Specifically, these exotic female snails preferred exotic invasive wild taro *C. esculenta* as an oviposition site over a native North American plant *P. cordata*. While we do not know the particular mechanism underlying this choice, we might speculate the sturdiness of the taro stem combined with a potential food resource (Burlakova et al., 2008) explains our results. Combined with the field observations of Burks et al. (2010), we feel comfortable concluding that stands of wild taro, if present, will most likely house more *P. insularum* clutches than any other structure in the area.

The perceived relationship between *P. insularum* and wild taro in the field (Burks et al., 2010) might be simply explained by the high availability of wild taro, as the distributions of these species overlap substantially. However, availability cannot explain the relationship fully as *P. insularum* laid more clutches on taro plants than predicted by availability (Burks et al., 2010). In terms of anticipating behavior of these invasive pests, our laboratory experiments presented substrates with equal availability and still agreed with the fieldwork of Burks et al. (2010). Although we did not test more than one alternative plant, the emergent structure, strong stem and broad leaves make pickerelweed a seemingly comparable oviposition site to wild taro. In addition, pickerelweed represents a reasonable surrogate for other leafy emergent plants, including duck potato or broad-leaved arrowhead *Sagittaria latifolia*, narrow-leaved arrowhead or arum *S. cuneata*, and shoots of water lilies *Nuphar spp.*

Furthermore, we compared the behavior of females in our experiments to females in the field to evaluate our ability to successfully extrapolate beyond the laboratory. We expected that if laboratory conditions or our observations altered reproductive behavior, clutches laid in our experiments would be significantly smaller and differently shaped than those laid in the field. However, we collected slightly larger clutches on wild taro in the laboratory than the field and all clutches showed reasonably similar morphological characteristics. Therefore, we believe the female behavior in our experiments accurately reflects that in the field and our conclusions can be extrapolated to wild *P. insularum* populations.

Apart from plant species preferences, we can draw

conclusions about the general characteristics of the structures *P. insularum* females will most likely use in exotic environments. Because adult snails can easily weigh over 150 g (Kyle et al., 2009), some field substrates may not have the strength to support large snails. Therefore, a structure's rigidity or flexibility may play an important role in the probability of its use as an oviposition site (Turner, 1996; Howells, personal communication). Although we ensured all structures possessed sufficient sturdiness for any sized female to lay eggs in our experiments, smaller females may be able to successfully utilize a wider range of substrates for oviposition in the field than larger females. Female snails of all sizes in our study laid clutches of comparable size, implying that less rigid structures may foster high levels of reproduction by smaller individuals. Therefore, we suggest conservationists consider any structure that can support at least a 25 g snail for an extended period, including developing stands of wild taro, as a potential site for clutches of any size. In addition, island apple snails may disproportionately exploit artificial structures that provide stability and safety for clutches, especially within sparse areas of plants (Burks et al., 2010).

The height a structure extends above the water level may also influence its use as an oviposition site as snails face a trade-off between keeping eggs dry and limiting predation (Cattau et al., 2010) and desiccation. In our lab experiments, we found that a substrate's maximum height had little effect on how far females crawled up a stem to oviposit or how well the clutch hatched. Therefore, female snails do not appear to choose substrates based on the structures' emergent height or impact on hatching efficiency. In the laboratory, our substrates never exceeded 81.3 cm in height and snails never placed clutches higher than 54.6 cm above water. In the field, we observed *P. insularum* females climbing as high as 1.2 m and laying on thick stems with a 4.7 cm diameter (Burks et al., 2010). Consequently, our tank design makes it difficult to predict precisely how far above the water a female apple snail may seek to place her clutch. Furthermore, other factors, such as temperature and water stress may alter hatching efficiency and deserve further attention.

Although we predicted that larger females laid larger clutches (Howells et al., 2006), our results failed to confirm this trend for the exotic island apple snail. The lack of correlation between female size and fecundity implies the potential for rapid population growth once a snail reaches a threshold size for reproduction. Overall, the reality that smaller *P. insularum* females demon-

strated some of the greatest fecundity and laid the largest clutches in our experiments does not bode well for environments recently invaded by this species.

A single egg clutch represents great potential for *P. insularum* population growth and prevention of even one clutch from hatching helps control the spread of this invader. After a two-week laboratory incubation, our calculated lab HEs exceeded previous field estimates by only 2%–3% (field mean = 70.8%; Barnes et al., 2008). For reasons that remain somewhat a mystery to us, our clutches collected from the field in 2008 hatched with an unusually low efficiency (i.e. closer to 40% than 75%). These clutches may have experienced water exposure or lacked fertilization, as indicated by a complete lack of hatching in some clutches. Nevertheless, even a 40% HE still produces hundreds of viable hatchlings. A range of HEs up to 75% highlights the potential of *P. insularum* for explosive population growth. Furthermore, our results imply that adult female island apple snails possess this staggering reproductive ability even at small sizes.

Fortunately, the highly recognizable bright pink coloration and greater accessibility makes egg clutches a far easier target for control than the elusive aquatic snails (Barnes et al., 2008; Burks et al., 2010). Therefore, if we can use our newly gained understanding of oviposition preferences, we can theoretically restrict the reproductive potential of *P. insularum* by efficiently finding and removing egg clutches from appealing substrates. In the case of areas containing both the snail and wild taro, removing wild taro stands of all sizes may prove especially helpful. Wild taro certainly seems to serve as a highly advantageous and attractive oviposition site for female apple snails. Unfortunately, we do not yet know the population implications of removing clutches or of island apple snails and wild taro cohabitating a watershed. We believe that a better understanding of the quantitative impacts of different clutch removal strategies will improve our ability to control the environmental damage caused by *P. insularum*. Furthermore, the dispersal relationship between island apple snails and wild taro also represents a research area worthy of investigation.

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## References

- Akridge RE, Fonteyn PJ, 1981. Naturalization of *Colocasia esculenta* (Araceae) in the San Marcos River, Texas. *Southwest Nat.* 26: 210–211.
- Baker P, Zimmanck F, Baker SM, 2010. Feeding rates of an introduced freshwater gastropod *Pomacea insularum* on native and nonindigenous aquatic plants in Florida. *J. Mollus. Stud.* 76: 138–143.
- Barnes MA, Fordham RK, Burks RL, Hand JJ, 2008. Fecundity of the exotic applesnail *Pomacea insularum*. *N. Am. Benthol. Soc.* 27: 738–745.
- Benson AJ, 2011. *Pomacea insularum*. USGS Nonindigenous Aquatic Species Database, Gainesville, FL. <http://nas.er.usgs.gov/queries/FactSheet.aspx?speciesID=2599>. Revision Date: 8 October 2009.
- Burks RL, Kyle CH, Trawick MK, 2010. Pink eggs and snails: Field oviposition patterns indicate shallow aquatic systems susceptible to invasion by *Pomacea insularum*. *Hydrobiol.* 646: 243–251.
- Burlakova LE, Karatayev AY, Padilla DK, Cartwright LD, Hollas DN, 2008. Wetland restoration and invasive species: Applesnail *Pomacea insularum* feeding on native and invasive aquatic plants. *Restor. Ecol.* 17: 433–440.
- Burlakova LE, Padilla DK, Karatayev AY, Hollas DN, Cartwright LD et al., 2010. Differences in population dynamics and potential impacts of a freshwater invader driven by temporal habitat stability. *Biol. Invasions* 12: 927–941.
- Carlsson NOL, Brönmark C, Hansson LA, 2004. Invading herbivory: The golden apple snail alters ecosystem functioning in Asian wetlands. *Ecology* 85: 1575–1580.
- Cattau CE, Martin J, Kitchens WM, 2010. Effects of an exotic prey species on a native specialist: Example of the snail kite. *Biol. Conserv.* 143: 513–520.
- Cazzaniga NJ, 2006. *Pomacea canaliculata*: Harmless and useless in its natural realm (Argentina). In: Joshi R, Sebastian L ed. *Global Advancements in Ecology and Management of Golden Apple Snails*. Nueva Ecija, Philippines: Philippine Rice Research Institute, 37–60.
- Conner SL, Pomroy CM, Darby PC, 2008. Density effects of native and exotic snails on growth in juvenile apple snails *Pomacea paludosa* (Gastropoda: Ampullariidae): A laboratory experiment. *J. Mollus. Stud.* 74: 355–362.
- Cowie RL, 2002. Apple snails (Ampullariidae) as agricultural pests: Their biology, impacts and management. In: Barker JM ed. *Molluscs as Crop Pests*. London, United Kingdom: CABI Publishing, 145–191.
- Center for Invasive Species and Ecosystem Health, 2010. Coco yam, wild taro. In: *Early Detection and Distribution Mapping System*. Available via DIALOG. <http://www.eddmaps.org/florida/species/subject.cfm?sub=5369>. Accessed 28 January 2011.
- Hayes KA, Joshi RC, Thiengo SC, Cowie RH, 2008. Out of South America: Multiple origins of non-native apple snails in Asia. *Divers. Distrib.* 14: 701–712.
- Howells RG, Burlakova LE, Karatayev AY, Marfurt RK, Burks RL, 2006. Native and introduced Ampullariidae in North America: History, status, and ecology. In: Joshi R, Sebastian L ed. *Global Advancements in Ecology and Management of Golden Apple Snails*. Nueva Ecija, Philippines: Philippine Rice Research Institute, 73–112.
- Jerde CL, Mahon AR, Chadderton LW, Lodge DM, 2011. “Sight-unseen” detection of rare aquatic species using environmental DNA. *Conserv. Lett.* 4: 150–157.
- Karatayev AY, Burlakova LE, Karatayev VA, Padilla DK, 2009. Introduction, distribution, spread, and impacts of exotic freshwater gastropods in Texas. *Hydrobiologia* 619: 181–194.
- Kolar CS, Lodge DM, 2001. Progress in invasion biology: Predicting invaders. *Trends Ecol. Evol.* 16: 199–204.
- Kyle CK, Trawick MK, McDonough JP, Burks RL, 2009. Population dynamics of the invasive apple snail *Pomacea insularum* in suburban southeast Houston, Texas. *Tex. J. Sci.* 61: 323–327.
- Lodge DM, Williams S, MacIsaac HJ, Hayes KR, Leung B et al., 2006. Biological Invasions: Recommendations for U.S. Policy and Management. *Ecol. Appl.* 16: 2035–2054.
- Morrison WE, Hay ME, 2011. Feeding and growth of native, invasive and non-invasive alien apple snails (Ampullariidae) in the United States: Invasives eat more and grow more. *Biol. Invasions* 13: 945–955.
- Pimentel D, Zuniga R, Morrison D, 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. *Ecol. Econ.* 52: 273–288.
- Pizani NV, Estebenet AL, Martin PR, 2005. Effects of submersion and aerial exposure on clutches and hatchlings of *Pomacea canaliculata* (Gastropoda: Ampullariidae). *Am. Malacol. Bull.* 20: 55–63.
- Qiu JW, Kwong KL, 2009. Effects of macrophytes on feeding and life-history traits of the invasive apple snail *Pomacea canaliculata*. *Freshwater Biol.* 54: 1720–1730.
- Rawlings TA, Hayes KA, Cowie RH, Collins TM, 2007. The identity, distribution, and impacts of non-native apple snails in the continental United States. *BMC Evol. Biol.* 7: 97–134.
- Turner RL, 1996. Use of stems of emergent plants for oviposition by the Florida applesnail *Pomacea paludosa* and implications for marsh management. *Fla. Sci.* 59: 34–49.
- Turner RL, 1998. Effects of submergence on embryonic survival and developmental rate of the Florida applesnail *Pomacea paludosa*: Implications for egg predation and marsh management. *Fla. Sci.* 61: 118–129.
- Van Dyke J, 2010. Control. In: *Snailbusters*. <http://snailbusters.wordpress.com/control/>. Accessed 1 February 2011.