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Establishment of the Invasive Island Apple Snail *Pomacea insularum* (Gastropoda: Ampullaridae) and Eradication Efforts in Mobile, Alabama, USA

CHARLES W. MARTIN, KEITH M. BAYHA, AND JOHN F. VALENTINE

Species invasions are thought to be among the most detrimental of all anthropogenic disturbances. Invasive consumers severely impact native ecosystems through the consumption of and competition with native species. Worldwide, snails of the Family Ampullaridae have successfully colonized a wide range of habitats outside their native range. This is of great ecological concern because once established, these snails frequently exhibit rampant herbivory in the recipient ecosystem. Here, we chronicle the recent invasion of island apple snails (*Pomacea insularum*; d'Orbigny 1837) in a tributary of the Mobile–Tensaw Delta, Alabama, in order to establish a baseline against which future assessments of apple snail expansion can be measured. In addition, we discuss the current, albeit largely unsuccessful, efforts to eradicate these invaders post-invasion as well as the possible implications of the continued growth of this population should geographic expansion occur. We also provide the first genetic evidence, from the mitochondrial cytochrome c oxidase I (COI) sequence, positively identifying these snails as *P. insularum*. All individuals were identical for COI and genetically identical to invasive animals in Georgia and portions of Florida, as well as native animals near Buenos Aires, Argentina, indicating a possible secondary invasion from nearby invasive U.S. populations but, ultimately, an Argentinian origin. Based on our evidence, we suggest that the best control measures for apple snail invasions include increased proactive enforcement to prevent future invasions elsewhere in the southeastern United States (and similar areas), the adoption of research into new management strategies designed to prevent future invasions and slow the spread of established invasive populations, and rapid and overwhelming control and eradication efforts at the first sign of invasion.

Biological invasions are among the gravest of all anthropogenic threats to native ecosystem structure and function (e.g., Mooney and Drake, 1986; Vitousek et al., 1996; Mack et al., 2000). Invasive species proliferation and their subsequent domination of native organisms can have marked negative impacts on the distribution and abundance of native fauna and flora in a wide variety of ecosystems (Cohen and Carlton, 1998; Mack et al., 2000). Even more alarming is the fact that the expanding global economy has led to greater rates of biological invasion than ever before (e.g., Cohen and Carlton, 1998). For this reason, many have expressed concern that the proliferation of invasive species in historically unoccupied areas will alter the successional trajectory of recipient ecosystems to the extent that their structure and function may differ markedly from that based on current theory and long-term historical records (e.g., Mills and Leach, 1993; Ruiz et al., 1999).

While it is known that, contrary to many paradigms (such as loss of overall biodiversity due to invasions) set forth by Elton (1958), some invasions have little effect or even positive impacts

(Levine and D'Antonio, 1999; Lonsdale, 1999; Cleland et al., 2004; Smith et al., 2004; Capers et al., 2007; Davis, 2009; Martin and Valentine, 2011), invasive consumers often exhibit strong, detrimental impacts in recipient ecosystems (Martin, 2011). Invasive consumers often impact nonnative regions via runaway consumption of native prey and plants (e.g., Grosholz et al., 2000; Grosholz, 2002; Sax and Gaines, 2008; Martin et al., 2010). Although comparatively fewer studies have been performed on higher trophic level invaders (Pysek et al., 2008), the extinction of birds on islands colonized by invasive snakes (Conry, 1988; Wiles et al., 2003) and mammals (Blackburn et al., 2004) supports this notion. Likewise, recent invasions of voracious predators and stalwart competitors, such as constricting snakes (Reed, 2005), lionfish (Albins and Hixon, 2008), and cichlid fish (Martin et al., 2010), all threaten the structure of ecosystems in the southeastern United States. The costliness and general unpredictability in the effectiveness of eradication efforts have led many environmental managers to suggest that the best approach is to avoid initial invasions (Mack et al., 2000).

Snails of the Family Ampullaridae have an impressive record of successfully invading and thriving in novel environments throughout the world (Britton, 1991; Cowie, 1995, 2002; Howells, 2001a,b; Howells et al., 2006; Rawlings et al., 2007). These snails have been transported worldwide for a variety of commercial reasons, including for food (Cowie, 1995, 2002; Su Sin, 2003), as biological control agents (Okuma et al., 1994; Pointier and Jourdan, 2000), and for the aquarium industry (Cowie, 1995, 2002). The genus *Pomacea* has been particularly successful in proliferating in areas outside its native geographic range in South America, including the Philippines (Acosta and Pullin, 1991), throughout mainland Asia (Mochida, 1991), Hawaii (Lach and Cowie, 1999), and a number of warmer regions in the United States (reviewed in Howells et al., 2006). In the United States, these snails were originally identified as a single species, *Pomacea canaliculata*, based on their channeled shell morphology. However, recent genetic evidence shows that there have been three distinct species that have invaded in the United States: *Pomacea canaliculata* in Arizona and California, *Pomacea haustorium* in Florida, and *Pomacea insularum* in Florida, Georgia, and Texas (Howells et al., 2006).

Snails of the genus *Pomacea* are known to have significant negative impacts on plants in many of these invaded areas. Their grazing can extensively damage agricultural crops (Cowie, 2002; Joshi and Sebastian, 2006), and the snails are also potential intermediate hosts for parasites that may infect humans (Cowie, 2002). Perhaps most alarming, however, is the damage they can inflict on wetlands. A recent field experiment (Carlsson et al., 2004) showed that grazing by these snails triggered a phase shift from clear waters dominated by submerged aquatic vegetation to turbid waters in which planktonic algae became the dominant primary producer.

Here, we review the temporal trajectory of the invasion of *P. insularum* in Mobile, AL, using a number of nonscientific (i.e., periodical articles) and scientific sources to document the chronology of their establishment, proliferation, and the (as-yet) unsuccessful measures taken to eradicate them. Moreover, we detail for the first time conclusive evidence of the genetic identity of these invaders and their likely source region(s). Our goal is to use the Mobile, AL, invasion as a case study that will lead cognizant environmental management agencies to proactively take precautionary measures to avoid invasion, as well as strong action in the face of a new invasion.

INTRODUCTION TO LANGAN PARK, MOBILE, AL

The first known occurrence of invasive apple snails in Mobile, AL (Fig. 1), was reported in Howells et al. (2006) as *P. canaliculata*. An update to this reference indicates that many of the specimens originally referred to as *P. canaliculata* were in fact *P. insularum* (though individuals from Mobile, AL, were not analyzed; Howells et al., 2006). According to Howells et al. (2006), a population has persisted in Langan Park (Mobile, AL) since 2003, and its establishment coincided with the sale of snails at a local pet store.

The 720-acre Langan Park (also called Municipal Park) was opened in 1957 by the City of Mobile and includes two large lakes (13.3 and 5.3 ha), home to many resident waterfowl species as well as fish, including largemouth bass (*Micropterus salmoides*), several species of sunfish (*Lepomis* spp.), mosquitofish (*Gambusia affinis*), and invasive Nile tilapia (*Oreochromis niloticus*) (C. W. Martin, pers. obs.). These lakes drain into Three-Mile Creek (Fig. 1), which feeds directly into the Mobile–Tensaw Delta (MTD; Fig. 1). The MTD is an area of extremely high biodiversity (Lydeard and Mayden, 1995) containing numerous wetland plants and 24 species of submerged aquatic vegetation (Chaplin and Valentine, 2009). In addition to these potentially vulnerable plants, the MTD also supports a diverse mixture of terrestrial, freshwater, and estuarine consumers, including some 115 species of fish (Boschung et al., 2004) that rely on these wetland plants for food and habitat. Many of these consumers are recreationally important fish [e.g., largemouth bass (*M. salmoides*), speckled trout (*Cynoscion nebulosus*), flounder (*Paralichthys* spp.), and redfish (*Sciaenops ocellatus*), to name a few] and commercially important decapod crustaceans [e.g., blue crab (*Callinectes sapidus*), Penaeid shrimps (*Farfantepenaeus* spp.), etc.]. The presence of 67 species of endangered, threatened, rare, and imperiled species in the MTD (TNC, 2012) indicates that this area provides a critical sanctuary for a number of plants and animals and that it is critical for management authorities to monitor and develop proactive management strategies to protect the natural integrity of this oligohaline ecosystem. Moreover, the nearby Port of Mobile could serve as a jumping-off point from which stowaway snails in ballast water or eggs laid on hulls could be exported all over the world, if snails/eggs can survive increased salinity.

GENETIC ANALYSIS

As previously noted, traditional approaches have been an obstacle to accurately identifying

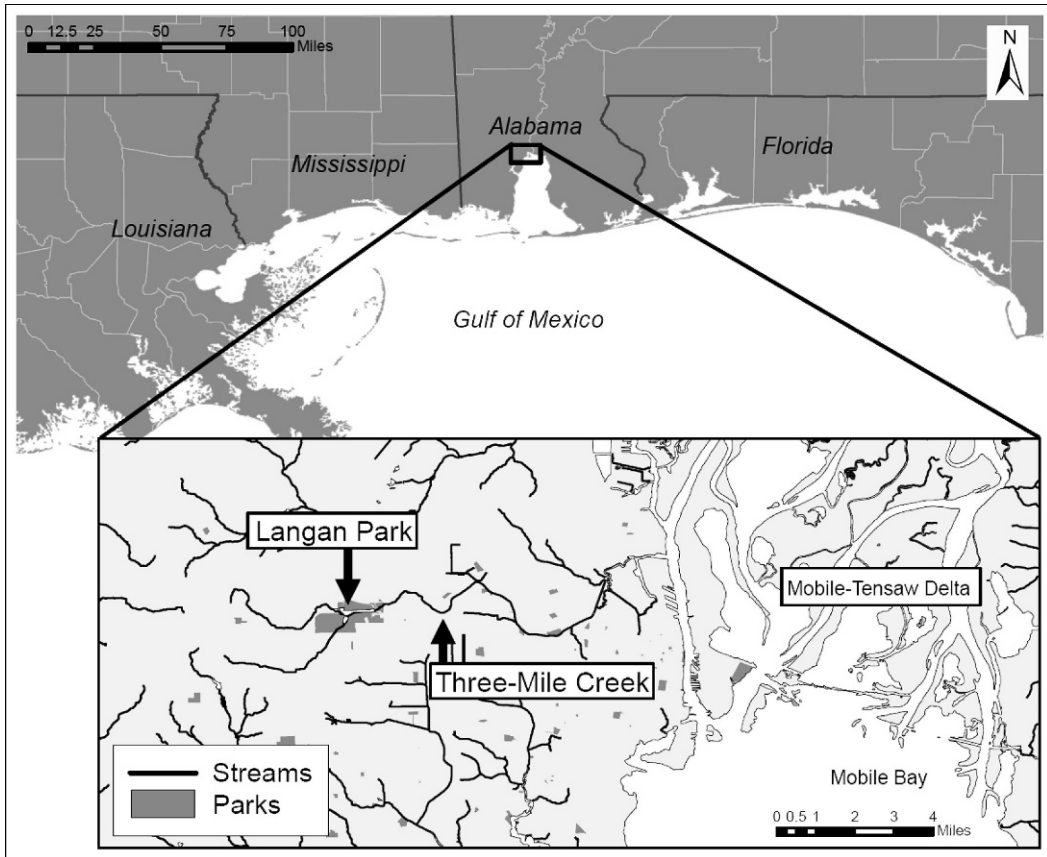


Fig. 1. The invaded area of the island apple snail described in this account. Initial release was in Langan Park, presumably from aquarium specimens. Snails later colonized the downstream watershed (Three-Mile Creek) almost to the Mobile–Tensaw Delta.

snails of the genus *Pomacea* to the species level (Howells et al., 2006; Rawlings et al., 2007). Such misidentifications may lead managers to incorporate ineffective strategies to control the spread of these snails. Since the identity and origin of snails in Langan Park has yet to be conclusively identified, we collected eggs from five individual egg masses taken from Langan Park and DNA sequenced one egg per egg mass for mitochondrial cytochrome c oxidase I (COI). DNA was extracted with a standard CTAB (cetyltrimethylammonium bromide) protocol (Winnepenninckx et al., 1993), and a 709–base pair region of mitochondrial COI was amplified by polymerase chain reaction (PCR) using the primers LCO1490 and HCO2198 (Folmer et al., 1994), with conditions consisting of 94°C for 120 sec; 38 cycles of 94°C for 30 sec, 48°C for 45 sec, and 72°C for 75 sec; followed by 72°C for 600 sec and refrigeration at 4°C. PCR success was assessed by running products out on a 2% agarose gel. PCR purification and cycle sequencing was performed by Beckman Genomics

(Danvers, MA). Electropherograms were assembled in Seqman v. 8.1.5 (DNASTar, Inc.) and checked for errors by eye. Species identity of the genetic data was assessed by a BLASTn search (Altschul et al., 1997). DNA sequences have been deposited in the NCBI GenBank (accession No. JX845573). Sequences collected for this study were aligned with published *P. insularum* sequences from native and invasive ranges [Argentina (NCBI accession Nos. EF514945, EU528481–EU528495, EU28526–EU528531); Brazil (EF14986–EF515012, EU528538–EU528575); Florida, United States (EF14942–EF514944, EF514985, EF515026–EF515053, EF515057–EF515058, FS15045); Georgia, United States (EF15054–EF515056); Texas, United States (EF15013–EF515025); Spain (GU133205–GU236491); China (FJ946828); Japan (AB433776–AB433781); Thailand (EU528496); and Vietnam (EU528502)] using CLUSTALX (Larkin et al., 2007), and a statistical parsimony network was constructed using TCS 1.2.1 (Clement et al., 2000).

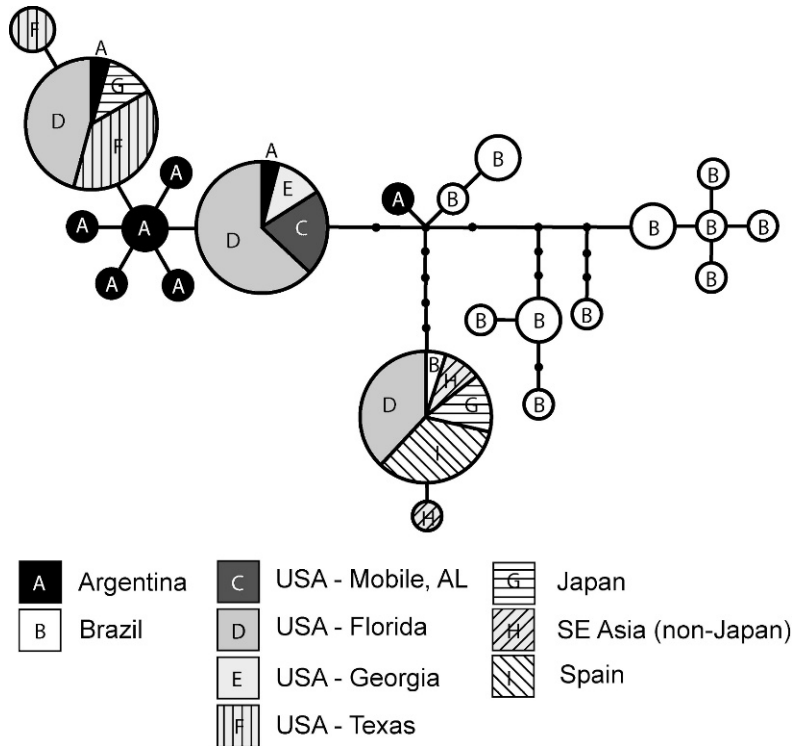


Fig. 2. Statistical parsimony network of COI haplotypes for native and invasive *Pomacea insularum* constructed using TCS 1.2.1 (Clement et al., 2000). Each circle represents a COI haplotype, and circle size denotes the number of individuals with that haplotype. Each solid dot represents a single mutational step. Color/letter combinations indicate geographic region of collection. Note that the Langan Park animals (red) share a COI haplotype with animals from Argentina (Buenos Aires and Santa Fe Provinces), Florida (central and southern Florida), and Georgia (southeastern).

All COI sequences collected from Langan Park snail eggs were identical to each other, and BLASTn searches were a 100% match to *P. insularum* from Argentina (NCBI accession No. EU528481), indicating positive identification as *P. insularum*, based on published taxonomic designations and identifications by Hayes et al. (2008). A statistical parsimony network (Fig. 2) indicated that the Langan Park snails contained the same COI haplotype found in snails from 1) Buenos Aires and Santa Fe Provinces, Argentina; 2) southeast Georgia; and 3) various regions of Florida. However, our COI sequences do not match those taken from native populations in Brazil nor from invasive populations in Texas, Asia, or Spain (Fig. 2). These data indicate that the Langan Park *P. insularum* have a source region in the vicinity of Buenos Aires, Argentina, like most of the U.S. invasive snails, or represent a secondary invasion from populations in Florida (central Florida, in the vicinity of Tampa and Orlando, and southern Florida, in Everglades National Park) or Georgia (southeastern Georgia), but definitely not Texas (near Houston). It should be noted that

these conclusions are based on currently published, yet possibly incompletely geographically sampled, data of worldwide *P. insularum* populations. In addition, our analyses are based on five sequences from five different egg clutches, and while only one haplotype was recovered, it is possible that they do not include all haplotypes to be found in the Langan Park population. Therefore, future, more exhaustive sampling and analyses will likely deliver firmer conclusions regarding the complete invasion history of *P. insularum* into Langan Park.

POPULATION EXPLOSION AND CONTROL EFFORTS

A full timeline of known events related to the invasion of apple snails in Mobile, AL, is given in Figure 3. From 2003 through 2008, the snail population in Langan Park was reportedly small (Howells et al., 2006). In 2008, officials from the State of Alabama Division of Wildlife and Freshwater Fisheries recognized the population had grown to the point that it represented a potential threat to vegetated habitats in the Three-Mile Creek Tributary and began to devel-

op a plan for their eradication (Brantley, 2009). However, several technical and legal problems arose (Brantley, 2009), resulting in an additional year's delay before action could be taken. During that time, snails colonized large areas of Three-Mile Creek, expanding geographically to within 1 mile of the MTD (Brantley, 2009; Raines, 2009a).

In October 2009, two "killing doses" of copper sulfate, an algacide also toxic to snails, were applied to the waters of Langan Park and Three-Mile Creek with the expectation that several subsequent treatments would be necessary to eradicate these snails (Raines, 2009a). To date, approximately 4 tons of copper sulfate have been applied to the area to eradicate the apple snails (Werner, 2010). Copper sulfate (containing 25% elemental copper) was applied to Langan Park on 6 Oct. 2009 and again on 15 and 16 Oct. 2009, while the waters of Three-Mile Creek were treated on 7 and 9 Oct. 2009 (Ricks and Ford, 2009). Copper concentrations declined rapidly and were not detectable 24 hr after application (Ricks and Ford, 2009).

Initial reaction to the toxic treatment was positive, as dead snails were seen floating at the water's surface, and others were seen lying motionless in the shallow waters (Raines, 2009b). Catches in snail traps, used by state and federal officials to monitor snail abundance, dropped from around 74/d before treatment in October 2009 to 3–4/d in December 2009 (Raines, 2010). This, however, coincided with the onset of colder weather, when snails are known to burrow to seek refuge from the colder waters (Dute, 2010). State officials estimated snail mortality from the copper treatments at 50–75% (Ricks and Ford, 2009).

Even though the initial prospects of the snail eradication program were positive (Raines, 2009b), live snails were found a week after the treatment (Raines, 2009c,d). Snails survived well in Three-Mile Creek, where it was more difficult to treat the entirety of the habitat with the correct dosage, and seemingly the snails were lethargic after the treatment (Raines, 2009d, 2010). State officials noticed altered snail behavior during the treatment, as snails produced excess amounts of mucus, demonstrated difficulty in retracting their operculum, and some even filled their shell with air and floated downstream, a reaction that could potentially have resulted in their spread to new areas (Ricks and Ford, 2009; Raines, 2010).

In addition to the copper sulfate treatment, volunteers removed visible eggs laid on emergent vegetation and other objects (Ferrara, 2009; Raines, 2009a). Apple snails crawl out of the

water to lay their eggs on any structure over the water, allowing the hatchlings to fall into the water as they emerge from the egg. While some volunteers searched the shorelines of park waters, others paddled the length of Three-Mile Creek in kayaks to remove the bright pink eggs (Ferrara, 2009). Eggs were immediately frozen on dry ice and were later buried (Ferrara, 2009). Furthermore, much of the emergent vegetation (primarily *Typha*) around park waters was removed to improve the ability of volunteers to locate eggs (Raines, 2010). In all, several hundred volunteer man-hours were spent removing eggs (Werner, 2010).

On 22 Jan. 2010, 14,000 native redear sunfish (*Lepomis microlophus*) were released by the State of Alabama into park waters to help combat apple snails (Dute, 2010). These centrarchid sunfish specialize in feeding on small gastropods (Froese and Pauly, 2012). While adult apple snails are too large for sunfish to consume, sunfish can consume smaller snails. These sunfish, native in many areas of the United States, including Alabama, have been purposely stocked to help control the abundance of other invasive mollusks, including quagga mussels (Tavares, 2009) and zebra mussels (French and Morgan, 1995), with varying levels of success. To date (May 2012), there is no evidence that these sunfish have produced any significant declines in the apple snail population in Three-Mile Creek.

LESSONS FROM MOBILE, AL

Despite the financial and time commitments made by state officials and volunteers, apple snails remain in Langan Park, and their bright pink eggs could still be seen on concrete walls lining the lakes as late as 2011 (C. W. Martin and K. M. Bayha, pers. obs.). While the effort was not entirely futile, with many snails and eggs destroyed and snail abundance noticeably reduced, this effort was ultimately unable to eradicate the invasive snails.

There are several lessons to be learned from this snail invasion and the unsuccessful efforts to eradicate them. The findings reported here deliver a strong warning to other areas that invasion by these snails should not be taken lightly, based on the difficulty in eradication efforts and the pervasive impacts on native vegetation. This includes areas with similar climates found throughout the Southeastern United States and around the Northern Gulf of Mexico. The best strategy is likely prevention of the initial snail invasion (Stachowicz et al., 1999) or rapid and overwhelming eradication efforts at the first sign of invasion. Community education

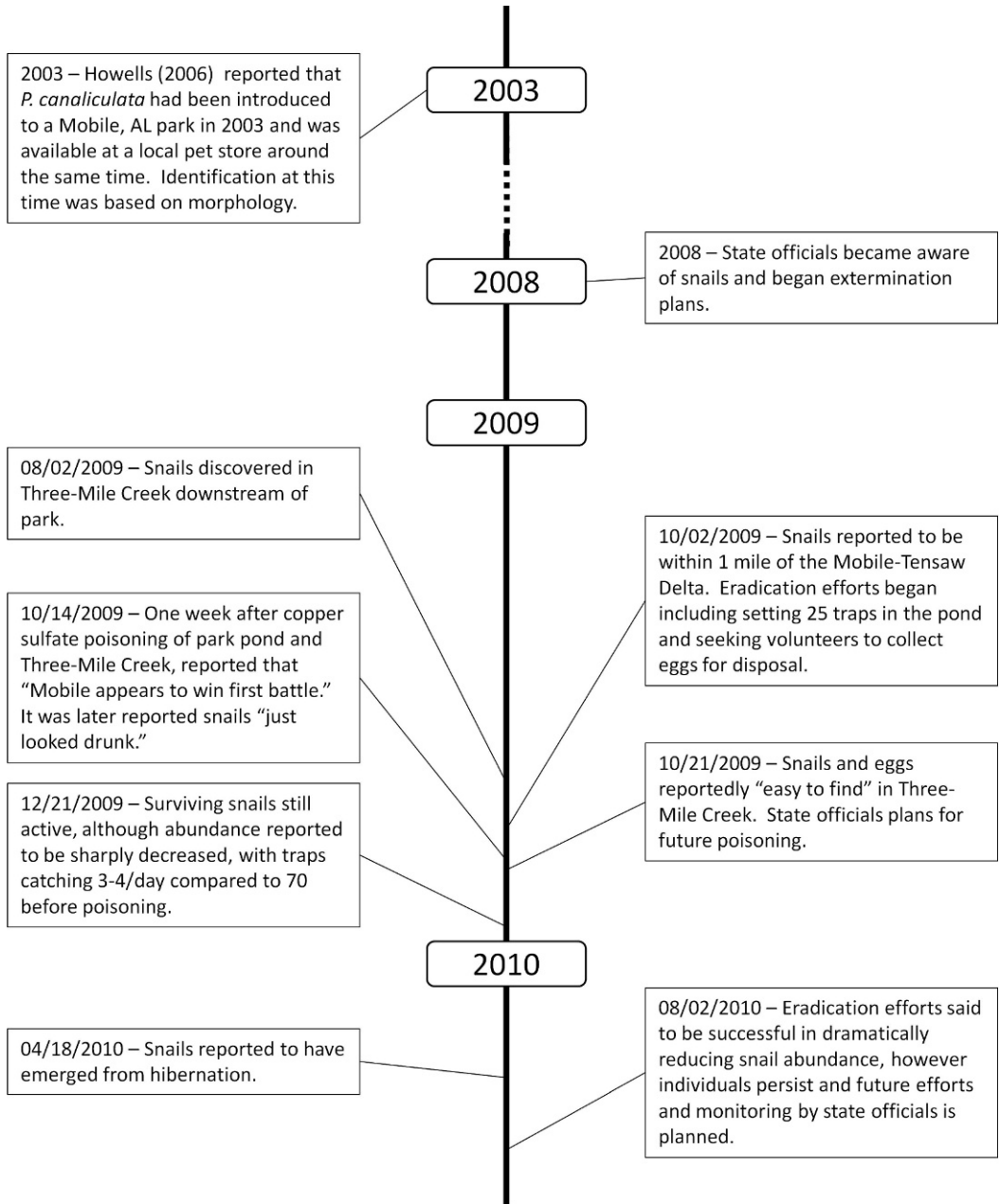


Fig. 3. Timeline documenting the *Pomacea insularum* invasion of Langan Park in Mobile, AL.

programs on invasive species through formal seminars, public notices, or signs along park waters and in pet stores warning people not to release aquarium specimens may help to successfully prevent releases of such nonnative species. Furthermore, in the case of Langan Park, eradication efforts were likely undertaken after the initial presence of apple snails in 2003 (Howells et al., 2006); this effort was too late to

be effective. Control efforts, such as removing egg masses hidden in emergent vegetation or even copper sulfate treatment, may have been more successful during the early stages of the invasions, when fewer individuals are present. Therefore, we propose an early reporting system through public education at public parks and locations where release often occurs to aid in identifying the individuals to remove before they

become a nuisance. To date, the story of snail invasion of Mobile, AL, has not been a positive one, and it is our hope that managers and officials in other areas heed this account, lest it be repeated.

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