

Identity, reproductive potential, distribution, ecology and management of invasive *Pomacea maculata* in the southern United States

Romi L. Burks¹, Jennifer Bernatis², James E. Byers³, Jacoby Carter⁴, Charles W. Martin⁵, William G. McDowell^{3,6} and Jess Van Dyke⁷

¹Southwestern University, Department of Biology, 1001 East University, Georgetown, Texas 78626, USA. Email: burksr@southwestern.edu

²Florida Fish and Wildlife Conservation Commission, 7386 NW 71st Street, Gainesville, Florida 32653, USA. Email: Jennifer.Bernatis@myfwc.com

³University of Georgia, Odum School of Ecology, 140 E. Green Street, Athens, Georgia 30602, USA. Email: jebyers@uga.edu

⁴United States Geological Survey, Wetland and Aquatic Research Center, 700 Cajundome Boulevard, Lafayette, Louisiana 70506, USA. Email: carterj@usgs.gov

⁵University of Florida, Institute of Food and Agricultural Sciences, Nature Coast Biological Station, PO Box 878, Cedar Key, Florida 32625, USA. Email: charles.martin@ufl.edu

⁶Colby College, Environmental Studies Program, Mayflower Hill Drive, Waterville, Maine 04901, USA. Email: wgmcdowell@gmail.com

⁷Van Dyke Environmental Services, 16624 Sunray Road, Tallahassee, Florida 32309, USA. Email: jessvd@aol.com

Abstract

Established populations of introduced *Pomacea maculata*, a highly fecund, large species of apple snail native to South America, now occur throughout southeast Asia, in Spain and extensively across the southern United States. Substantial research on non-native apple snails takes place in Southeast Asia and has frequently identified apple snails as *P. canaliculata*. That these Asian populations represent at least two *Pomacea* species, *P. canaliculata* and *P. maculata*, has been confirmed through anatomical and genetic evidence. However, the two species are often still confused because of their similar shell morphologies and life history traits. This contribution reviews the distribution, life history, ecology and management of *P. maculata* introduced to the southern USA. So

far the agricultural impacts of *P. maculata* in the USA fail to match those of non-native applesnails elsewhere, but the invasion of wetlands by this species suggests the need for increased vigilance to prevent further spread and avoid the ecological impacts that have been associated elsewhere with *P. canaliculata*.

Additional keywords: Ampullariidae, apple snail, Gastropoda, introduced, spread

Introduction

Apple snails in the genus *Pomacea* (family Ampullariidae) span the aquatic-terrestrial interface (Fig. 1; Hayes *et al.*, 2009a, b), reach a considerable size (Youens & Burks, 2008), and tend to act as true herbivores rather than only as algal grazers (Hayes *et al.*, 2015). Due to the damage caused to rice (Joshi & Sebastian, 2006), *Pomacea canaliculata* has been listed among 100 of the world's worst invasive species (Lowe *et al.*, 2004) and is the only freshwater snail on the list. However, some of the studies that prompted the inclusion of *P. canaliculata* among the world's worst invasives probably involved *P. maculata* or a mixture of the two species, as the similar morphologies and reproductive behaviours of *P. canaliculata* and *P. maculata* have resulted in substantial taxonomic confusion (Cowie *et al.*, 2006; Hayes *et al.*, 2012). This confusion has indirectly slowed the pace of rigorous research and awareness of the threats posed by *P. maculata* (senior synonym of *P. insularum*; Hayes *et al.*, 2012) as a separate species from *P. canaliculata* (Burks *et al.*, 2016).

Howells *et al.* (2006) were the first to review the state of knowledge of non-native apple snails in the USA at a time when there was substantial concern about the potential impact of non-native *Pomacea* species on rice crops. Fortunately, this anticipated impact has not yet happened and *P. maculata* populations are largely limited to ponds and lakes with aquatic vegetation on which they lay their clutches (Fig. 1). In Texas, the timing and methods of rice farming probably minimised the impacts of apple snails compared to the impacts in other invaded areas such as Southeast Asia (Burlakova *et al.*, 2010). To our knowledge, no other studies have examined agricultural impacts of *P. maculata* in the continental USA. However, we caution against extrapolating the absence of a quantifiable economic impact on rice in Texas to susceptible agricultural areas that have longer periods of standing water, such as rice fields used for waterfowl hunting or crayfish farming. In this review, we focus on the identity, reproductive potential, distribution, ecology and management of *P. maculata* in the southern USA and reference studies

reviewed in more detail elsewhere (Burks *et al.*, 2016). There are populations of *P. canaliculata* in California, Arizona, Florida (Rawlings *et al.*, 2007) and Hawaii (Tran *et al.*, 2008), but none of these populations has expanded to the extent that *P. maculata* has across the southern USA (Byers *et al.*, 2013).

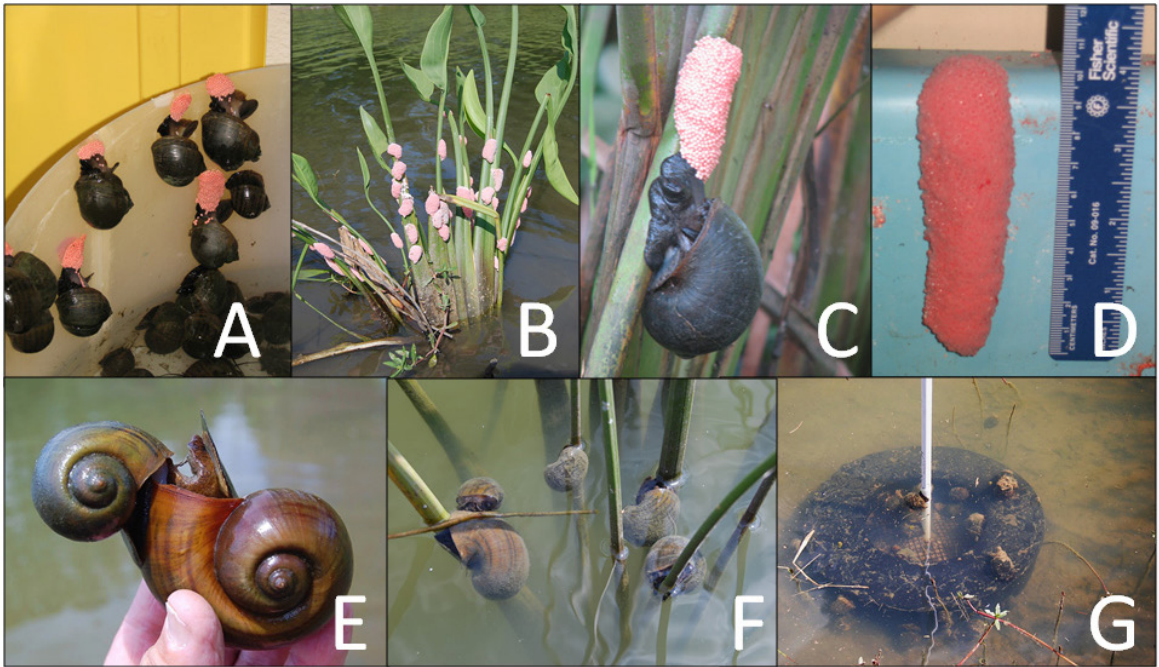


Fig. 1. *Pomacea maculata* and its environment. A: moderately sized (30-50 g) *P. maculata* females depositing clutches following collection. B: abundant egg clutches deposited on emergent vegetation in Florida. C: female laying a large clutch in Armand Bayou, Texas (it took about 45 minutes). D: nearly 10-cm long clutch deposited by *P. maculata* in the laboratory (Southwestern University, Georgetown, Texas). E: mating pair collected from Florida that illustrates the size difference between the sexes (smaller snail is the male). F: adult *P. maculata* stripping vegetation in Wellman Pond, Florida. G: snail trap (design by J. van Dyke) deployed in shallow water of Wellman Pond, Florida.

Identity

Management responses to invasion by *P. canaliculata* and *P. maculata* may be similar, but their potential for spread, ecological impacts, susceptibility to predation and rate of population growth may differ. For example, it remains unclear why *P. maculata* has expanded across the southern USA while *P. canaliculata* has not, despite both species occurring in Florida (Rawlings *et al.*, 2007; Bernatis *et al.*, 2016). We recommend that the research community and the public use the scientific names *P. canaliculata*

and *P. maculata* for the two most widespread invasive apple snails. Consistent, clear communication will help stop the conflation of these two species that is often found in the literature, websites and social media. Readers should recognise that accounts of apple snail impacts, especially in the popular press, may mix information on multiple congeneric species by relying on common names rather than the scientific names (Hayes *et al.*, 2009a; 2012).

Taxonomy

Hayes *et al.* (2012) recognised the species name *Pomacea maculata* as the senior synonym of a number of other *Pomacea* species, including *P. insularum*, the name formerly used for it in North America (e.g. Rawlings *et al.*, 2007) as well as in Asia (Hayes *et al.*, 2008). Rawlings *et al.* (2007) and Hayes *et al.* (2012) included several taxonomic and nomenclatural changes relevant to the non-native ampullariid fauna of the USA since the review of Howells *et al.* (2006) was published. These taxonomic developments resulted from years of investigation, a comprehensive and ongoing review of the genus and family (Cowie & Thiengo, 2003; Hayes *et al.*, 2008, 2009a, b, 2015; Cowie & Héros, 2012; Cowie, 2015; Cowie *et al.*, 2015, 2017) and a targeted comparison of morphological, reproductive and genetic traits of *P. canaliculata* and *P. maculata* (Hayes *et al.*, 2012).

By designating the same specimen as the neotype of *P. maculata* and the lectotype of *P. insularum*, as well as the neotype of *P. gigas* (a name that has also been used in the invasive apple snail literature in Asia), Hayes *et al.* (2012) rendered them objective synonyms. The names *P. insularum* and *P. gigas* are no longer valid names for the species and should not be used. Although the common name ‘island apple snail’ still appears in some recent publications (e.g. Meza-Lopez & Siemann, 2015; Smith *et al.*, 2015), it also lacks meaning as it reflects the no longer valid name ‘*insularum*’, which means ‘of the islands’. The correct name, *P. maculata*, should instead be used in all studies from this point forward (Burks *et al.*, 2016). Furthermore, because of past difficulty of distinguishing *P. maculata* from *P. canaliculata* and of distinguishing both of these species from other *Pomacea* species, it is important that any study deposit voucher specimens in a publicly accessible museum collection so that future researchers can confirm the identity of the material.

Perhaps predictably, the question of an accurate common name often arises in work associated with apple snails. The name ‘*maculata*’ (in English ‘maculate’) means spotted or stained, but unfortunately that translation does not apply to a consistent morphological feature of *P. maculata*. Therefore, use of the terms spotted or stained as common names does not inform identification of the species and risks the likelihood that someone will substitute other terms in the future, unleashing more ambiguity and amplifying the taxonomic confusion. For example, use of “Amazonian apple snail” does not make scientific sense because many species of apple snails occur within the Amazon basin (Hayes *et al.*, 2009a). Due to the extensive diversity in the genus *Pomacea* (Hayes *et al.*, 2009a), *P. maculata* may be confused with other large apple snails with channelled sutures. Some agencies employ the common names ‘giant apple snail’ or ‘channeled [USA spelling] apple snail’ but these common names have just created more problems, especially as ‘channeled’ refers to the channelled sutures from which *P. canaliculata* gets its scientific name.

In addition, the abbreviation of giant apple snail to ‘GAS’ creates the potential for confusion with the name ‘golden apple snail’, also referred to as ‘GAS’, and which has been widely used for the invasive species in Asia. The term ‘golden apple snail’ was thought originally to be only *P. canaliculata*, but we now know it has also been used for *P. maculata* (Hayes *et al.*, 2012). The acronym GAS may also cause confusion, as it is also used for the giant African snail, *Lissachatina fulica* (previously known as *Achatina fulica*), itself a problematic species (e.g. Fontanilla *et al.*, 2014; Iwanowicz *et al.*, 2015). Therefore, in this contribution, we do not use a common name for *P. maculata* nor do we recommend one.

Taxonomic confusion among apple snails also extends into the aquarium trade in the USA. *Pomacea maculata* differs substantially from *Pomacea diffusa*, the scientific name of the so-called ‘spike-topped’ apple snail found in the aquarium trade (Rawlings *et al.*, 2007). Howells *et al.* (2006) treated it as *P. bridgesii*, of which *P. diffusa* was then considered a subspecies (Cowie & Thiengo, 2003), but Rawlings *et al.* (2007) recognised *P. diffusa* as a valid species, with ‘spike-topped’ apple snail as its common name. *Pomacea diffusa* is still often misidentified as *P. bridgesii* (often mis-spelled ‘*bridgesi*’) in the aquarium trade, or it is frequently incorrectly labelled as a generic ‘mystery’ snail (Rawlings *et al.*, 2007; Hayes *et al.*, 2008). We provide this update as some proportion of *P. maculata* introductions probably occur from aquarium releases by consumers mistakenly sold the wrong species (Karatayev *et al.*, 2009).

Genetics and hybridisation

Less genetic research has focused specifically on *P. maculata* than on *P. canaliculata*. Using the COI gene for barcoding, Rawlings *et al.* (2007) confirmed *P. maculata* as the identity of several populations of non-native apple snails in Florida, Georgia and Texas, and identified four mitochondrial haplotypes. Martin *et al.* (2012) later documented *P. maculata* with a haplotype shared by snails in Florida and Georgia. To our knowledge, all the non-native ampullariid populations across the southern USA that have been genetically characterised consist of *P. maculata* (Teem *et al.*, 2013; Deaton *et al.*, 2016), with a few notable exceptions in Florida of *Marisa cornuarietis*, plus a species identified incorrectly as *P. haustum*, and a few populations of *P. canaliculata*, which also occurs in Arizona and California (Rawlings *et al.*, 2007). Only a limited number of haplotypes occur within the populations of *P. maculata* (Burks & C. Savrick, unpublished). Recently, Matsukura *et al.* (2016a) published a set of microsatellites for both *P. maculata* and *P. canaliculata* that will be useful in future genetic studies of introduced populations.

While Hayes *et al.* (2012) showed that *P. canaliculata* and *P. maculata* are distinct species, they also speculated about the possibility of hybridisation or incomplete lineage sorting. Research in both the non-native (Asia) and native (South America) ranges implies that hybridisation does occur (Matsukura *et al.*, 2013, 2016b; Yoshida *et al.*, 2014), but these results cannot yet distinguish the mechanisms underlying genetic exchange or answer whether hybridisation happened before or after invasion in non-native ranges. Matsukura *et al.* (2016b) suggested that hybridisation has the potential to alter environmental tolerances and facilitate invasion in non-native ranges. In the USA, documented overlapping populations of *P. canaliculata* and *P. maculata* only occur in Florida (Rawlings *et al.*, 2007), but no studies of hybridisation in these populations have been published.

Life history and reproductive potential

Clutch and egg size

Clutches of bright pink *P. maculata* eggs are laid on emergent substrates above the water line, generally during dawn and dusk, and deposition of a large clutch can take 30 minutes or more (Kyle *et al.*, 2011). The number of eggs in a clutch averages around

1500-2100 but varies considerably up to a recorded maximum of 4751 (Barnes *et al.*, 2008; Burks *et al.*, 2010; Kyle *et al.*, 2011; Hayes *et al.*, 2012). These numbers contrast with those for *P. canaliculata* clutches, which contain on average 200-300 eggs (Hayes *et al.*, 2012). The diameter of eggs of *P. maculata* is smaller (~2 mm) than that of *P. canaliculata* (~3 mm) (Barnes *et al.*, 2008; Hayes *et al.*, 2012). For the untrained eye, it may still be difficult to assess the differences in egg size in the field. Consequently, genetic confirmation should still accompany any identification of a newly established population. The relative threat potential of *P. maculata* may be assessed, in part, by comparing its overall fecundity to that of other species. Based on the average number of eggs in a clutch, *P. maculata* may be even more likely to become a nuisance species than the already destructive *P. canaliculata* (Barnes *et al.*, 2008).

Hatching rates and clutch submersion

Pomacea maculata lays its eggs in clutches deposited on hard surfaces above the waterline. They typically take 10-14 days to hatch completely and can yield hundreds of hatchlings (Barnes *et al.*, 2008). Three mechanisms may explain when low hatching efficiency or total clutch failure occurs, i.e. non-fertilisation, female age or water damage, all of which warrant additional investigation. In one Florida culture, older *P. maculata* (> 3 years) appeared to have less successful hatch rates than younger snails (Bernatis, unpublished). As estimates vary widely, more studies need to examine the trade-offs between time to reproduction, female size or age, and hatching efficiency, as has been done for *P. canaliculata* (Tamburi & Martín, 2009, 2011). To test the effects of freshwater inundation, Martin & Valentine (2014) used subsections of clutches (less than 1 week old), but did not find any statistical differences among treatments. However, their reported hatching rates were considerably lower (0-17 %) compared to studies with *P. canaliculata* (Pizani *et al.*, 2005). Horn *et al.* (2008) sectioned large clutches into three parts and found more hatchlings emerged from clutch segments above water than from submerged segments. To date, all studies that have examined hatching efficiency of *P. maculata* have focused on fresh water, but submergence in salt water should also be considered.

Eggs need to remain dry and out of the water for a minimal amount of time (i.e. 6-9 days) to maximize hatching success. In a laboratory study (Burks & M. K. Trawick, unpublished), clutches of different ages attached to vertical plant stems were submerged

for 8 or 24 h per day, while control clutches were completely above the water. Hatching efficiency in control clutches was 83 %, whereas submergence generally reduced hatching efficiency compared to controls (Fig. 2). Clutch age at first submergence offset the impact of submergence as 9 day old clutches still exhibited 38-87 % overall hatching efficiency, whereas clutches that were 3 and 6 days old at the start of the experiment essentially failed to hatch when submerged completely for 24 h per day and exhibited reduced hatching success when submerged for 8 h per day (Fig. 2). Horn *et al.* (2008) suggested that management efforts might use the negative effects of immersion on clutches by flooding areas with high abundances of clutches. For clutches immersed for a brief period of time, egg development may still occur. More mature clutches that become only partially submerged, or submerged for shorter periods of time, may still yield substantial numbers of hatchlings (Burks & M. K. Trawick, unpublished). No research to date has examined how flooding would affect dispersal of eggs and subsequent long-term survivorship of hatchlings, the costs of which must be considered when balanced against the benefits of reducing hatching success with flooding.

Maturation time, longevity and overall fecundity

Life history traits of *P. maculata*, including life span, age-specific growth rates, age and size at maturity, size-dependent fecundity and total longevity, remain largely undocumented. In outdoor mesocosms (Bernatis, 2014), *P. maculata* first copulated when 3-4 months old (shell length 30-33 mm), a similar age to that implied by Yoshida *et al.* (2014) and Matsukura *et al.* (2016b). Although not yet confirmed by life history studies, stable isotope analysis of a limited number of *P. maculata* shells indicated a lifespan of 1-3 years (Arnold *et al.*, 2014), which seems noteworthy given the size this species can reach as adults. It is worth considering the potential outcomes of a three-year lifespan. If females start to produce average sized clutches (~1500 eggs; Barnes *et al.*, 2008) during their first year, live for one or two more years, and produce clutches once a week for three summer months (probably an underestimate of their reproductive season in the southern USA) during two or three summers, then one female could contribute 36,000 (two summers) to 54,000 (three summers) eggs in her lifetime. Even if just 0.01 % of those eggs survived to reproductive age (1 in 10,000), a single female would have replaced herself with three to five offspring.

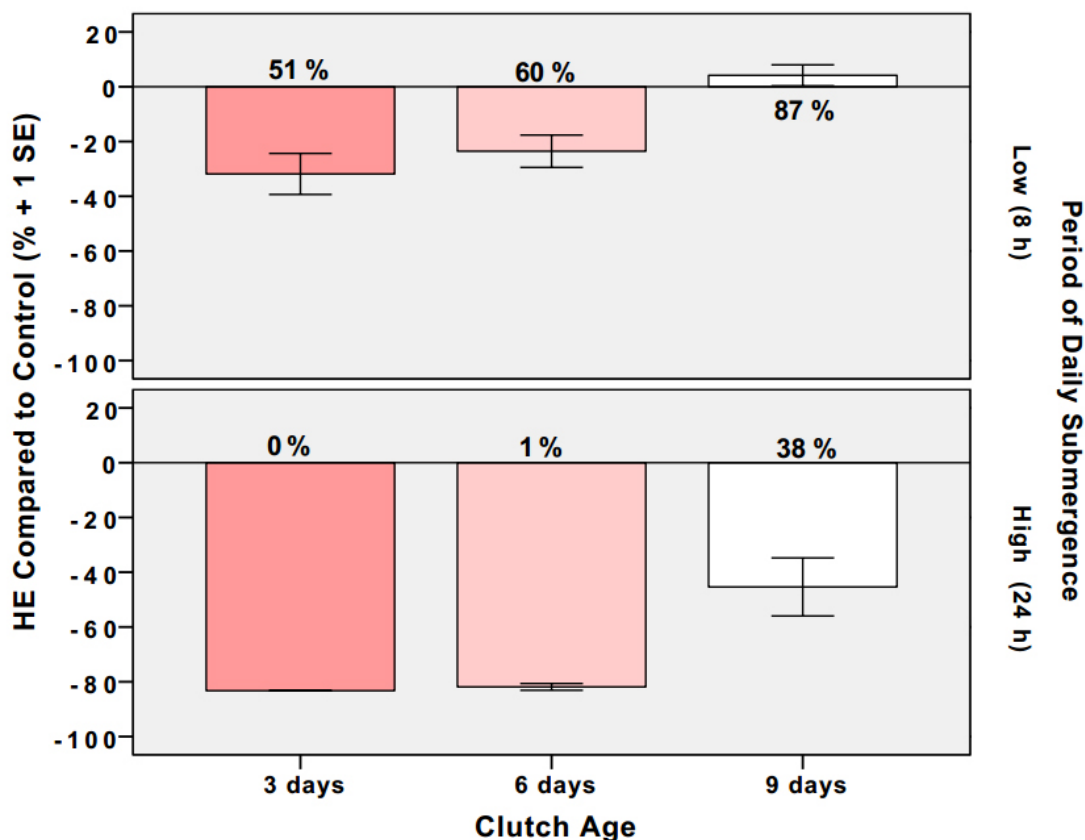


Fig. 2. Hatching efficiency (HE) of clutches of different ages (3, 6 and 9 days old) in response to different periods of full submergence each day (8 h or 24 h), compared to control clutches that were never submerged. Control clutches achieved 83 % HE and submergence reduced HE of most treatments as represented by bars below the 0 % comparison line. Percentages associated with each bar indicate HEs. At the one extreme, immature clutches (3 and 6 days old) failed to hatch when fully submerged for 24 h per day. At the other extreme, 9 day old clutches submerged for 8 h per day hatched at a rate not significantly different from control clutches.

Distribution and abundance

The native range of *Pomacea maculata* includes much of Brasil and extends to Uruguay and parts of Argentina (Hayes *et al.*, 2012). Genetic evidence suggests that *P. maculata* in the southern USA came from either Argentina or Brasil (Rawlings *et al.*, 2007). Given the propensity for the aquarium trade to include *Pomacea* species (Karatayev *et al.*, 2009), we speculate that the current distribution of *P. maculata* in the southern USA resulted from repeated introductions and subsequent expansion of the

populations. *Pomacea maculata* has spread rapidly from its initial introduced populations in Texas (1989) and Florida (1991) and now occurs throughout the coastal plain of seven southern states (Texas, Louisiana, Mississippi, Alabama, Georgia, Florida and South Carolina; Rawlings *et al.*, 2007; Byers *et al.*, 2013). Fortunately, not every introduction of apple snails persists. In 2009, a few *P. maculata* egg masses in a park in Louisiana were discovered early enough in the invasion process to destroy them and no further egg masses have been seen at that location (Carter, unpublished). Early hand removal efforts (Bernatis & Warren, 2014) may help prevent establishment.

While some introductions fail to establish, others do take hold. In southern Louisiana several isolated introductions have led to range expansions into bayous that lead to intersections with the Gulf Intracoastal Waterway, a canal that connects wetlands from Texas to Florida. Isolated colonies have also been reported in separate sections of the Atchafalaya Basin, the largest bottomland hardwood swamp in the USA (Carter & G. LaFluer, unpublished). This westerly range expansion across Louisiana has led to concern that the snails may begin to affect rice and crayfish cultivation in southwestern regions of the state. The separate, isolated colonies have led to speculation that some of them originated from local residents releasing snails purposely to control overgrowth of plants in their own private ponds or to attract wetland birds, or as accidental bait release (Carter, unpublished).

Few publications provide density or abundance data for *P. maculata*. In southeastern Texas, in permanent habitats such as ponds and lakes, Burlakova *et al.* (2010) documented low densities (< 2 snails/m²) and stable populations, with higher densities in the macrophyte-dominated zone than in open water. Juveniles were scarce despite an abundance of egg clutches. In ephemeral ponds in Florida, Smith *et al.* (2015) found a negative correlation between *P. maculata* abundance and frequency of preferred plant species, but no relationship with any other variable. In flooded ephemeral agricultural habitats, Burlakova *et al.* (2010) found extremely high densities (> 130 snails/m²) and speculated that differential survivorship of hatchlings and young snails drove the differences in population dynamics in these habitats compared to the permanent habitats noted above. Kyle *et al.* (2009) also reported dominance of large-sized snails in another population, but with limited evidence of recruitment over a three-year period. Even 1 % successful recruitment might produce devastating effects given the reproductive potential of *P. maculata* (Kyle *et al.*, 2011).

Influence of abiotic conditions on distribution

The characteristics of the habitats into which organisms invade play a key role in determining colonisation success (Lockwood *et al.*, 2013). Abiotic conditions of a system may cause the immediate death of an organism, allow it to thrive, reproduce and become a problem, or any level of success between these extremes. For aquatic snails, the key abiotic variables are likely to be availability of calcium carbonate, levels of pH, dissolved oxygen, salinity and temperature, and the frequency at which habitats dry out.

Calcium carbonate

As calcium is important for shell formation, regeneration and growth (White *et al.*, 2007), calcium carbonate availability is one factor that generally limits the distributions of freshwater molluscs (Lodge *et al.*, 1987). A substantial proportion of aquatic systems in the southern USA have calcium carbonate levels well above the limiting threshold of 29.8 mg/L reported for *P. canaliculata* by Martín *et al.* (2001); in fact, in some it is > 61 mg/L (Scott *et al.*, 2002; Perlman, 2016). Therefore, in the southern USA, calcium carbonate levels probably play a minor role in limiting establishment of *P. maculata*, except in combination with pH.

pH

Many water bodies on the coastal plain of the southern USA have low pH because of the large amounts of decaying organic matter and slow moving water. Low pH makes it extremely difficult for snails to construct shells made of calcium carbonate (Batzer *et al.*, 2005). As a result, water bodies like the Okefenokee Swamp in southern Georgia have no shelled mollusc species (Kratzer & Batzer, 2007). Water bodies with a pH of 4 or lower may be a barrier to apple snails (Ramakrishnan, 2007) and pH of 5.5 or lower is lethal to *P. maculata* hatchlings (Bernatis *et al.*, 2016). The Okefenokee Swamp has a low enough pH to preclude *P. maculata* from invading and establishing a permanent population. Although available pH data capture general regional patterns (Byers *et al.*, 2013), there can be small areas with more neutral pH among these systems, particularly in flowing water, that may be able to support apple snails in otherwise low pH areas. This may be the explanation for two populations that appear to exist in low pH areas (i.e. pH 5.5) in Georgia (Byers *et al.*, 2013). Overall, the majority of the inner coastal plain of

North Carolina appears inhospitable because of large areas with low pH within areas with favourable climate (Byers *et al.*, 2013).

Although apple snails may be able to tolerate relatively low pH environments, reduced growth rates may negatively impact reproduction and changes in shell strength may increase susceptibility to predation and accidental damage. For both *P. maculata* and *P. canaliculata* (Bernatis, 2014), erosion and brittleness of shells was higher in experimental treatments at pH 5.5 than at higher pH values. Given its influence on survival and reproduction, the full extent of the direct and indirect effects of pH on *P. maculata* warrants additional investigation.

Oxygen

Dissolved oxygen levels represent another obvious and potentially limiting factor for aquatic snails. However, given the amphibious nature of *Pomacea* species (Hayes *et al.* 2009b, 2015), their possession of both a lung and a gill lessens the likelihood that dissolved oxygen will influence their distributions (Seuffert & Martín, 2009a). Several studies have looked at the influence of oxygen during different life stages of *Pomacea* spp., and in particular, during aestivation or aerial exposure. In general, *Pomacea* spp. obtain oxygen either aerially or from the water and may rely on aerial respiration to compensate for less than adequate dissolved oxygen in the water (Seuffert & Martín, 2009a, b).

Salinity

Exposure to periodic salinity may play an important role in determining the distribution of freshwater organisms that are exposed to estuarine conditions (Jordan & Deaton, 1999). In many coastal environments, normal periodic changes in salinity occur but occasional extreme conditions may also occur after an environmental disturbance (e.g. flooding). Changes in salinity can cause osmotic stress, which may result in a variety of responses, both behavioural and physiological (McMahon, 1983). Given their ability to take refuge in a shell, several species of non-native, invasive freshwater molluscs show variable degrees of tolerance to salinity (Verbrugge *et al.*, 2012).

Martin & Valentine (2014) measured salinity tolerance and growth of 10 day old *P. maculata* hatchlings and found greater than 40 % survival rates up to 15 ppt. Both Ramakrishnan (2007) and Bernatis *et al.* (2016) investigated salinity tolerances of

juvenile and adult *P. maculata* and reached similar conclusions. For example, *P. maculata* tolerated chronic salinity exposure of 6.8-8.0 ppt for 28 days (Bernatis *et al.*, 2016) and multiple *P. maculata* populations occur within 10 km of the mouth of the St. John's River (Florida) where salinity reaches 4 ppt. Consequently, weakly tidally influenced systems, i.e. with salinity of < 8 ppt, may serve as acceptable habitat for *P. maculata* to thrive (Martin & Valentine, 2014).

Temperature

Temperature regimes limit distributions, alter metabolic rates and influence behaviour of many organisms, including apple snails (Byers *et al.*, 2013). Both low and high temperatures critically influence life history traits of apple snails and hence their distributions. Deaton *et al.* (2016) found that *P. maculata* collected in Louisiana, and other snails from a laboratory population, experienced no mortality and remained active at 15 °C after 10 days. After 10 days, temperatures of 10 °C and 5 °C reduced activity and resulted in 50 and 60 % mortality, respectively. For snails at 0 °C, 100 % mortality occurred after only 5 days.

Buenos Aires, with a mean minimum temperature of the coldest month of 7.6 °C, is possibly the coldest area in the native range of *P. maculata*. Bernatis (2014) reported that adult *P. maculata* survived in water temperatures as low as 2 °C in outdoor mesocosms during winter in Florida. Despite the presence of a thin layer of ice on the water surface, some snails occasionally moved around and fed. In addition, occasional freezing temperatures have not eliminated apple snails from the Ebro River delta in Spain, which is the most northerly non-native location where *P. maculata* occurs (Andree & López, 2013). Invasive success in other non-native ranges indicates the need for more research to determine the extent to which *P. maculata* can spread further north in the USA.

In contrast to cold tolerance, in aquatic systems that periodically dry up, animals unable to retreat to pools of water have evolved several coping techniques, including aestivation during hot months (Darby *et al.*, 2008; Hayes *et al.*, 2015). Regarding upper thermal limits, *P. maculata* tolerated experimental temperatures of 15.2-36.6 °C (Ramakrishnan, 2007), and growth occurred without mortality at both 15 °C and 35 °C (Gettys *et al.*, 2008). To examine the combined influence of temperature and relative humidity (RH), Ramakrishnan (2007) tested how long juvenile and adult *P. maculata* could survive without immersion in water by using small chambers with a platform situated above different salt solutions. Adult *P. maculata* survived for 70 days at 30 °C

and > 95 % RH and at least 308 days at 20-25 °C in 75-95 % RH, with smaller snails suffering proportionately greater water loss (Ramakrishnan, 2007). Bernatis *et al.* (2016) reported similar results for both *P. maculata* and *P. canaliculata*. As RH increased above 80 %, adults of both species survived 365 days on moist sand, while surviving only 22 weeks in lower RH (< 60 %) and on a dry sand substrate.

Modelling future distributions

Newly introduced species take time to expand throughout invaded ranges and reach an equilibrium population density. Consequently, estimating even their current distributions can often be difficult. Even when small, new populations can serve as a source of propagules for range expansion (Ackleh *et al.*, 2007; Lockwood *et al.*, 2013). Niche modelling is a tool to estimate the areas that are environmentally suitable for a given non-native species. Non-native species might come to occupy all suitable areas assuming sufficient time and no barriers to dispersal (Guisan *et al.*, 2013). Accordingly, one can predict areas where the species might occur given enough time to spread and reach spatial equilibrium. Byers *et al.* (2013) modelled the projected distribution of *P. maculata* in the southeastern USA. Their MaxEnt model identified minimum temperature in the coldest month and precipitation in the warmest quarter as the most important climatic determinants of the distribution of *P. maculata* and indicated that it could spread throughout the coastal plain of the southeastern USA, both on the Atlantic and Gulf coasts.

Ecological interactions

When *P. maculata* invades wetlands, it may modify the structure and function of food webs indirectly by competing with native species (Connor *et al.*, 2008; Posch *et al.*, 2013) or directly by consuming plants (Morrison & Hay, 2011a; Smith *et al.*, 2015) and providing novel prey for native predators (Cattau *et al.*, 2016) (Fig. 3). Such invasions might also lead to shifts in feeding patterns among other organisms or provide a new host for parasites, including the rat lungworm, *Angiostrongylus cantonensis* (see section below on *P. maculata* as a parasite vector). Through consumption of macrophytes, certain invasive *Pomacea* species can alter whole ecosystems by causing shifts between alternative stable states (Carlsson *et al.*, 2004a). Such shifts, from clear to turbid water, have yet to be reported in areas of the USA invaded by *P. maculata*. However,

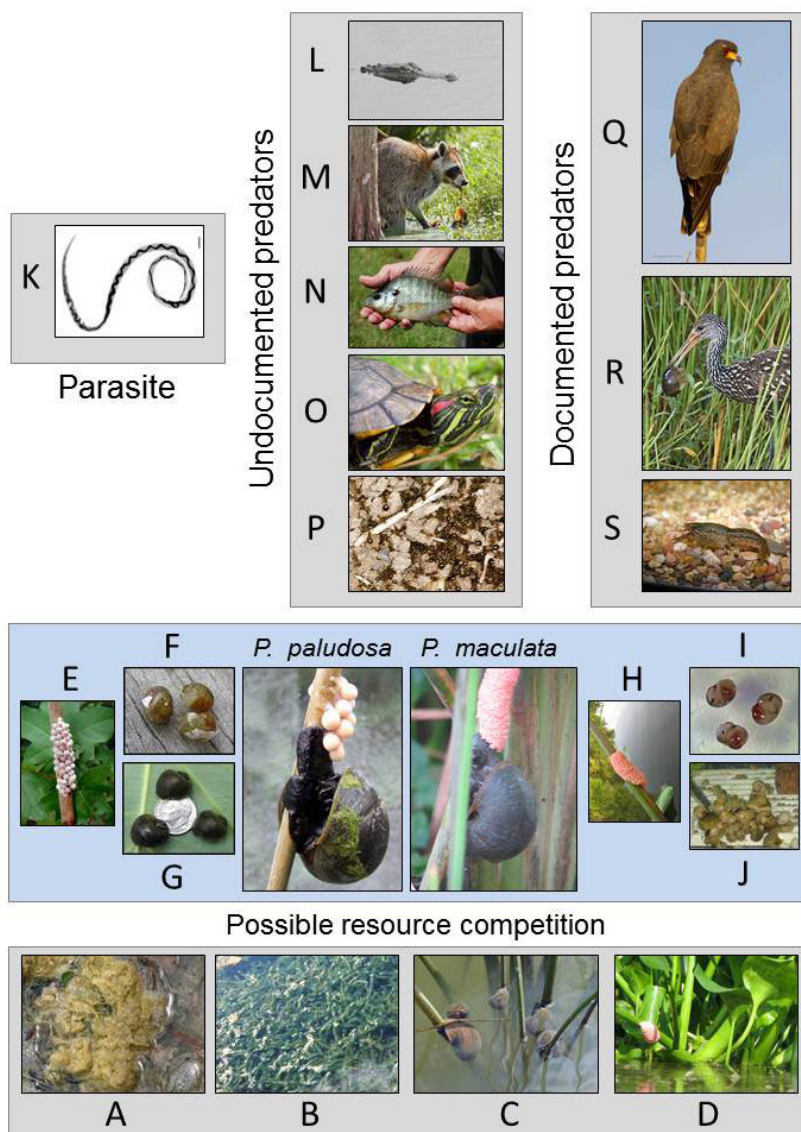


Fig. 3. Simplified three-tiered figure emphasising the intermediate positions of *Pomacea maculata* and *Pomacea paludosa* in aquatic communities in the southern USA. The ranges of these two species overlap in Florida and Georgia. The bottom tier includes primary producers consumed by *Pomacea* species to different extents: A, periphyton; B, submerged macrophytes, C, emergent macrophytes; D, freely-floating macrophytes. Competition for these resources could occur between overlapping *Pomacea* species (Conner *et al.*, 2008; Posch *et al.*, 2013). The middle tier includes life history stages: for *P. paludosa*: E, eggs; F, hatchlings; G, juveniles.

Pomacea maculata deposit much smaller eggs (I) that yield smaller hatchlings (I) but which rapidly grow to the juvenile stage (J). Total annual fecundity of *P. maculata* may exceed that of *P. paludosa* by an order of magnitude. The pink colour of *P. maculata* eggs (H) provides a warning to potential consumers that the eggs possess a neurotoxin. Apple snails in the southern USA may provide a reservoir for a medically important parasite, *Angiostrongylus cantonensis* (K) that has been found in Florida. As well as a representative parasite, the top tier also depicts undocumented and documented predators. Referring only to *P. maculata*, the centre pictures show possible predators not yet documented in the literature, including: L, alligators; M, raccoons; N, redear sunfish; O, turtles; and P, fire ants; with the last three as potential egg predators. The right side of the top tier emphasizes avian apex predators: Q, limpkins; and R, snail kites; that forage on similar-sized snails regardless of species (Cattau *et al.*, 2016). The last photo shows a freshwater crayfish (S), which may consume apple snail hatchlings of different species at different rates (Dorn & Hafsadi, 2016).

a comparison of feeding rates among various species of *Pomacea* indicates that feeding of *P. maculata* equals or surpasses that of other invasive *Pomacea* species (including *P. canaliculata*), which implies the potential for similar negative effects (Morrison & Hay, 2011a).

Pomacea maculata as a competitor and consumer

In laboratory experiments, *P. maculata* suffers from intraspecific competition at high densities (Connor *et al.*, 2008; Posch *et al.*, 2013), but the implications of such competition for population dynamics in natural habitats remain largely unknown. Also, when kept in culture, especially with limited access to additional calcium, adult snails will eat the shells of conspecifics (Burks, personal observation), but no study to date has quantified shell condition of snails collected from populations across a density or pH gradient. More attention has been directed at investigating interspecific than intraspecific competition and examining feeding preferences.

Populations of *P. maculata* started to establish in Florida in the late 1990s and now substantially overlap with populations of the native Florida apple snail, *P. paludosa* (Rawlings *et al.*, 2007; Dorn & Hafsadi, 2016; Cattau *et al.*, 2016). Many reports indicate that periphyton is a main part of the diet of *P. paludosa* (*Pomacea* Project, 2016), but only a few studies have measured it (Shuford *et al.*, 2005). Instead of depending primarily on periphyton, *P. paludosa* may thrive on a combination of periphyton and vascular plants as demonstrated by an experiment in which adults grew significantly more when consuming a complex of *Utricularia* sp. and periphyton than loosely attached benthic algae (Sharfstein & Steinman, 2001). *Pomacea maculata*, perceived as a voracious herbivore, feeds on different types of aquatic macrophytes (Burlakova *et al.*, 2009; Burks *et al.*, 2011; Morrison & Hay, 2011a; Fig. 3) but also on periphyton. In aquaria, *P. maculata*, particularly small individuals, grazed periphyton growing on the shells of conspecifics (Howells *et al.*, 2006), and periphyton added to reconstituted *Myriophyllum spicatum* increased consumption (Boland *et al.*, 2008). Consumption by both apple snail species might include periphyton, but also vascular tissue depending on the host plant's structural complexity. Consequently, if food is limiting, competition may occur between native and non-native apple snails (Fig. 3) when *P. paludosa* feeds on vascular plants (Morrison & Hay, 2011a).

Depending on the plant species, *P. paludosa* may feed at a slower rate than *P. maculata*. For example, the feeding rate of *P. maculata* on *Vallisneria americana*

exceeded that of *P. paludosa* nearly 10-fold (Monette *et al.*, 2016). Monette *et al.* (2016) also found that *P. paludosa* ate around the edges of the leaves of *V. americana*, whereas *P. maculata* typically cut the strands into fragments, causing more damage. In experiments, when the two species are placed together, the presence of the larger and more fecund *P. maculata* results directly in negative impacts on growth and survival of *P. paludosa* (Fig. 3; Connor *et al.*, 2008; Posch *et al.*, 2013).

Numerous experimental laboratory studies have assessed feeding preferences of *P. maculata* among diverse macrophyte species (Burlakova *et al.*, 2009; Baker *et al.*, 2010; Morrison & Hay, 2011a, b; Burks *et al.*, 2011; Bernatis, 2014). Most studies found clear preferences and host plant consumption patterns generally agreed for similar plant species, although there were some discrepancies among studies. Such discrepancies are probably explained by the different methodological approaches, different experimental durations and use of different suites of plant species. For example, among all the plant species tested, the greatest differences in consumption tendencies involved wild taro (*Colocasia esculenta*) and water hyacinth (*Eichhornia crassipes*). In none of the studies did *P. maculata* exhibit a strong preference for these two cosmopolitan macrophytes, but the extent to which they did consume them varied substantially. Nonetheless, it is clear that *P. maculata*, despite its preferences, will consume a wide variety of aquatic macrophytes, and given the differences in feeding patterns among the studies, it should never be used for biocontrol of aquatic weeds (see Control section).

Interested in differential herbivory on plant structures, Bernatis (2014) compared consumption patterns between *P. maculata* and *P. canaliculata* and specifically looked at preferences among different parts of the plants. Neither species restricted feeding to just stems, blades or leaves, but also consumed the roots and root structures (e.g. bulbs, rhizomes) of many species. In the case of *C. esculenta*, and to some extent, the water lily *Nuphar advena*, apple snails consumed the root structures even in the presence of other preferred foods. Snails readily fed on new growth, but once stems reached above the water line, there was minimal foraging. In contrast to these two plant species, apple snails seldom fed on new growth of *V. americana*, even when no other food was available. This result using *V. americana* conflicts with the herbivory rate found by Monette *et al.* (2016). Plants may alter their composition to limit palatability in the presence of herbivores (Qiu & Kwong, 2009), so it is possible that some preference patterns or differences between studies may be related to plant chemical defences, which may be influenced by local conditions. However, no studies have measured and isolated specific plant

compounds that may deter apple snail grazing through inducible defences, a possible explanation for the discrepancies in feeding rates among previous studies (Burlakova *et al.*, 2009; Baker *et al.*, 2010).

Most studies on feeding of *P. maculata* have involved laboratory experiments under controlled conditions and a number of questions are open for investigation. For example, the possibility exists that *P. maculata* may not be strictly herbivorous. *Pomacea canaliculata* consumes amphibian eggs (Karraker & Dudgeon 2014) and preliminary food trials indicated that *P. maculata* ate raw meat when provided (Carter, unpublished). Following the example of studies with *P. canaliculata*, ecologists and managers must gather more ecologically relevant measures of the consumptive impact of *P. maculata* by undertaking more field measurements and experiments (Horgan *et al.*, 2014). At the same time, such future studies must exercise extreme caution to prevent introduction of snails to novel habitats and must heed regulations that seek to limit their spread.

Pomacea maculata as a prey resource

Pomacea maculata occupies an intermediate position in the food web (Fig. 3) and may serve as a food source for a number of predators similar to those known for *P. canaliculata* (Yusa 2006; Yusa *et al.*, 2006; Hayes *et al.*, 2015). However, with the exception of studies on snail kites, there are fewer published studies of predation on *P. maculata* than *P. canaliculata*. Swamp crayfish (*Procambarus clarkii*) and red-eared slider turtles (*Trachemys elegans scripta*) both consumed egg clutches in laboratory settings (Burks & A.L. Plantz, unpublished), but the extent to which this occurs in the field remains unknown. Adult *P. maculata* also readily eat conspecific egg masses and smaller individuals (Horn *et al.*, 2008). However, given the number of eggs produced, egg or juvenile cannibalism probably does not significantly hamper reproductive potential of *P. maculata*, although it would be interesting to develop a model of survivorship. The near absence of predation on *P. canaliculata* eggs (Yusa, 2001) seems also to be the case for *P. maculata* eggs in both their native and non-native ranges (Burks, unpublished). A carotenoprotein, similar to ovorubin in the eggs of *P. canaliculata* (Dreon *et al.*, 2010, 2013) occurs in *P. maculata* eggs (Pasquevich *et al.*, 2014) and these proteins play a role in the defence of embryos (Giglio *et al.*, 2016), probably limiting the overall impacts of predation.

Only a few studies have investigated predators of recently hatched, juvenile or adult *P. maculata* (Horn *et al.*, 2008; Dorn & Hafsadi, 2016; Cattau *et al.*, 2016). Given the

chemical defences of the eggs (Pasquevich *et al.*, 2014, Giglio *et al.*, 2016) and the large size of the adults, hatchlings and juveniles probably experience the highest predation rates. Possible predators of *P. maculata* (Fig. 3) include a similar suite of species as prey on the better studied *P. canaliculata*: crustaceans (shrimp, crayfish, crabs), insects (dragonfly larvae and beetles, among others), fish (including catfish, carp, tilapia, perch, among other benthic predatory fishes), reptiles (turtles), leeches, birds (ducks, crows, storks and various wading birds) and mammals (rats and other mammals feeding in littoral areas) (see Carlsson *et al.*, 2004b; Yusa, 2006; Yusa *et al.*, 2006).

Among invertebrate predators, the red swamp crayfish (*Procambarus clarkii*) consumed individuals of *P. maculata* under 4 mm in size in feeding trials (Carter, unpublished). Furthermore, in experimental wetland mesocosms (Dorn & Hafsadi, 2016), predation by native crayfish (*Procambarus fallax*) on hatchlings was eight times stronger on non-native *P. maculata* than on native *P. paludosa*. Crayfish differentially selected the smaller *P. maculata* hatchlings, to the extent that none of them survived the 44 days of exposure to predators. Regarding vertebrate predators, *P. maculata* has been found in the gut contents of blue catfish (*Ictalurus furcatus*) in Texas and alligators in Florida (Bernatis, unpublished). Glossy ibis (*Plegadis falcinellus*) have been seen feeding on *P. maculata* stranded in a recently drained pond (Howells *et al.*, 2006). Limpkins (*Aramus guarauna*) specialise on snails (though not exclusively) and in the southeastern USA primarily occur in Florida (Kale & Maehr, 1990). Limpkins eat *P. maculata* in South America and limpkins have been photographed eating *P. maculata* in Florida, although there have been no published studies.

However, in Florida, the best-known apple snail predator (Fig. 3) is the Florida snail kite, *Rostrhamus sociabilis plumbeus*, the natural food of which was the native Florida apple snail, *P. paludosa*. These birds initially appeared less efficient at consuming the larger *P. maculata* than the smaller *P. paludosa* (Cattau *et al.*, 2010) and studies recently documented a preference for medium-sized snails regardless of species (Wilcox & Fletcher, 2016; Fig. 3). Thus, feeding patterns appear to depend more on size of the snails. Management efforts have sought to help increase the kites' efficiency of extracting larger *P. maculata* from their shells by providing perches that offer a closer, more stable platform than nests, which may be further away (Pias *et al.*, 2012). Although not clearly predicted early on in the invasion, the abundance of *P. maculata* has benefited kite populations (Cattau *et al.*, 2016; Wilcox & Fletcher, 2016). Water fluctuations may also affect which apple snail the kites consume. For example, in 2011, a drought

and agricultural water withdrawal caused water levels to drop in certain areas in Lake Okeechobee. This water level drop in turn caused localised populations of *P. padulosa* to collapse. However, *P. maculata* did not appear to suffer the same high level of mortality. Consequently, in 2012, *P. maculata* represented nearly 100 % of the snail kite's diet in certain locations (Gray, 2013). Even if management actions could help to increase the number of Florida snail kites, their populations are unlikely to increase enough to control invasions of *P. maculata* adequately. Furthermore, if the birds did increase significantly, they could have a negative effect on the declining populations of *P. paludosa*.

Pomacea maculata as a parasite vector and toxin transmitter

Along with many other snail species, *P. maculata* acts as an intermediate host of the parasitic nematode, *Angiostrongylus cantonensis* (rat lungworm) (Kim *et al.*, 2014). Humans are infected by *A. cantonensis* when they ingest raw snails containing the parasite larvae. In the southern USA, infected *P. maculata* have been reported in Louisiana (Qvarnstrom *et al.*, 2013; Teem *et al.*, 2013). However, Teem *et al.* (2013) did not find any infected *P. maculata* in the sites they sampled in Texas, Mississippi and Florida, although the parasite is present in southern Florida in giant African snails (Iwanowicz *et al.*, 2015; Stockdale-Walden *et al.*, 2015). However, very few locations were sampled and the true extent to which *P. maculata* is infected across the southern USA remains unknown.

Angiostrongylus cantonensis is not the only health threat posed by *P. maculata*. In its native range (i.e. Brasil), *P. maculata* can act as a host for a parasitic trematode (*Stomylotrema gratusis*) (Pinto *et al.*, 2015). No doubt *P. maculata* can act as a host for a large number of parasites, just as can *P. canaliculata* and other ampullariid species (Damborenea *et al.*, 2017). As parasites may modify the anti-predator responses and behaviour of their intermediate hosts to facilitate infection of their final hosts (Poulin, 2011), the distribution of *P. maculata* parasites, infection intensity and possible resulting changes in behaviour merit further study.

In addition, in a laboratory feeding study, Dodd *et al.* (2016) found that *P. maculata* transferred an undescribed cyanotoxin associated with avian vacuolar myelinopathy to domestic chickens. Other *Pomacea* species are also capable of toxin bioaccumulation (Berry & Lind, 2010) and this capacity also warrants further investigation as a threat to human and ecosystem health.

Management

To some degree, there exists a prevailing sense that *P. maculata* may not be as harmful as *P. canaliculata*, despite its rapid range expansion in the southern USA. However, long-term impacts may just be emerging and the history of invasion in the genus warrants application of the precautionary principle. Options that could have realistically resulted in early eradication did not receive enough attention. Almost in a self-defeating manner, United States Federal Aquatic Nuisance Species Task Force admits “there is no way to eradicate all invasive apple snails once established” (Pasko *et al.*, 2011). The United States Department of Agriculture through its Plant Protection and Quarantine Program prohibits interstate transport of *P. maculata* without a permit but such regulations are difficult to enforce. The rapid proliferation of snails in Florida and Texas allowed populations to grow swiftly and ultimately spread throughout the southeastern USA. Annual surveys of Florida’s 460 public water bodies conducted by the Florida Fish and Wildlife Conservation Commission noted an increase in the number of lakes infested (Table 1; R. Kipker, pers. comm.). Management of apple snails also faces budgetary issues, especially in Florida where resources are spent battling a number of invasive species, which costs millions of dollars per year (Dodds *et al.*, 2014). The efficacy of control measures depends on frequency, intensity and accuracy of application.

Table 1. Number of Florida lakes and total area infested with invasive apple snails, primarily *Pomacea maculata*. Data from annual lake surveys by the Florida Fish and Wildlife Conservation Commission.

Year	Number	Hectares
2006	4	1934
2007	46	275,423
2008	59	299,704
2009	81	308,822
2010	98	317,982
2011	110	337,188
2012	126	342,449
2013	147	365,546
2014	164	366,871

Control strategies

Prohibiting or restricting apple snail species

Prevention measures represent the best front-line strategy for combatting any invasive species. This premise holds especially true for species that exhibit extremely high fecundity (Keller *et al.*, 2007), such as *P. maculata* (Barnes *et al.*, 2008; Kyle *et al.*, 2013). Along with *P. canaliculata*, *P. maculata* is listed as a prohibited species in some states (Texas, Florida, Louisiana, South Carolina) but not all. Other states where snails have invaded (i.e. Alabama, Mississippi, Georgia) should consider similar measures. Often, even if *P. maculata* appears on a prohibited list, little enforcement of the prohibition takes place. However, some stakeholders take the problem more seriously. For example, to prevent the further spread of *Pomacea* species, the State of Louisiana made buying, selling or possession of apple snails illegal in August 2012. When the State of Louisiana becomes aware of commercial activity, receives commercial permit requests, or learns of individuals threatening to spread the snails, they have taken enforcement action immediately (Carter, unpublished). Unfortunately, the trade still continues informally and established populations get dispersed through events such as flooding. Overall, detection, prevention and management of apple snails all require constant diligence.

Manually removing snails and eggs

Hand removal involves a substantial time commitment and is tedious work (Bernatis & Warren, 2014). Dense shoreline vegetation greatly complicates the task, as does steep bathymetry and deep organic sediments. However, this simple action often constitutes the first response by managers who simply want to do something quickly. The appearance of pink egg clutches signals the start of invasion and examination of the clutches can help species identification (Hayes *et al.*, 2012). In addition, understanding the mechanisms and preferences behind oviposition behaviour may help identify particular areas where snails congregate (Burks *et al.*, 2010; Kyle *et al.*, 2011). Unless snails invade a very small area, handpicking may be unaffordable without the help of volunteers (Martin *et al.*, 2012).

Reducing egg-laying habitat

Pomacea maculata prefers sturdy emergent vegetation for egg deposition (Burks *et al.*, 2010; Kyle *et al.*, 2011), although snails will also seek out any other hard substrate when emergent plants are not available. From observations in South Carolina, apple snails may also climb shoreline plants to escape water treated with copper sulphate or chelated copper (M. Hook, pers. comm.), so that removal of emergent vegetation not only reduces their ability to escape from the copper treatment but also reduces oviposition sites. Consequently, managed removal of emergent plants, especially ones that are also non-native, invasive species, may reduce the reproductive success of *P. maculata*. Consequently, management efforts should target multiple life history stages using an integrated pest management approach such as hand removal of egg clutches, application of deterrent substances to artificial substrates and removal of nearshore vegetation to effectively cut down oviposition sites.

Baiting and trapping

Pomacea maculata appears highly susceptible to baiting and trapping, but as with manual removal, trapping also requires intensive labour and some costs for materials. By tending to traps weekly in Wellman Pond, Florida (see following case study), this effort temporarily reduced the snail population to the point that the emergent littoral vegetation (which was being heavily consumed) stabilised (Van Dyke, unpublished). In Langan Lake, Alabama (see case study), trapping proved an effective way to monitor trends in the snail population (D. Armstrong, pers. comm.).

Stocking predators

The redear sunfish (*Lepomis microlophus*; Fig. 3) is commonly called the “shellcracker” for good reason. They use thick, pharyngeal teeth to crack open shells of small molluscs. Larger *P. maculata* are too big to be vulnerable, but these fish could readily consume hatchling and juvenile snails. The largest management effort to date, which included stocking redear sunfish in an Alabama lake and downstream portions of a tidal creek, achieved some success in combatting snail invasion (Martin *et al.*, 2012). Biological control with crayfish may also be possible, as red swamp crayfish (*P. clarkii*), native to states bordering the Gulf of Mexico, do eat *P. maculata* (Carter, unpublished)

and native *P. fallas* readily consume *P. maculata* (Dorn & Hafsadi, 2016). However, given the propensity for crayfish to be invasive themselves, this species may not be the best choice in non-native habitats as their preference for *P. maculata* over other prey items remains unknown.

Chemical control

Copper and other metals can be toxic to snails and other aquatic invertebrates because metals can disrupt metabolic processes by damaging cell membranes, denaturing enzymes and binding to proteins and lipids (Dummee *et al.*, 2015). In 2007, Florida lake managers attempted to control an isolated population of *P. maculata* in Newnan's Lake with granular copper sulphate. In spite of three treatments, the snails spread throughout the system, and there was unacceptable mortality of non-target organisms, e.g. odonates, fish and shrimp (Bernatis & Warren, 2014).

At identical rates, chelated copper is just as acutely toxic to sensitive organisms as inorganic copper. In either case, copper does not remain in the water column, but accumulates in the sediments. Precipitation takes longer for chelated forms than for inorganic forms, thus requiring less copper to kill target organisms. Therefore, compared to copper sulphate, the relatively expensive, chelated copper products are effective at lower rates, thus reducing potential for chronic non-target impacts (Leslie, 1992). In 2009, four chelated copper products were tested on *P. maculata* in the laboratory. A double-chelated product, "Captain" by SePRO (28.2 % copper ethanolamine complex), proved to be the most effective. Effects were directly related to dose and water temperature. At 23 °C, the snails produced copious mucus at 0.2 ppm and, at 0.4 ppm, they lay on their sides with opercula open and died within 96 h. At 27 °C, 0.2 ppm was lethal after 48 h (Van Dyke, unpublished).

In the future, use of copper treatments may wane as alternative chemicals become available for snail control that pose fewer threats of non-target mortality than copper. For example, "Bayluscide" by Bayer (17.1 % niclosamide) is used extensively to control the snail hosts of schistosomiasis in other countries (Takougang *et al.*, 2006). In laboratory studies, label rates of niclosamide effectively killed *P. maculata*, but did not appear to harm red swamp crayfish (*P. clarkii*) or redear sunfish (*L. microlophus*) (Carter, unpublished). Niclosamide is approved for use in commercial fishponds in the USA but has not been used to control *P. maculata* because of a lack of registration for that specific use by the United States Environmental Protection Agency (USEPA). Perhaps, the most

environmentally safe option for aquatic snail control is iron phosphate. “Ferroxx AQ” by Neudorff (3 % iron phosphate) is a pelletised formulation that includes a snail feeding attractant. This product was tested on snails with 0.5, 1.0, 2.0 and 4.0 g of pellets per 5.7 L of water, plus a “control” of flour-based pellets (eight snails per treatment). Feeding was suppressed in all treatments and ceased completely at rates of 2.0 g and 4.0 g. At those higher rates, 75 % of the adult snails were dead 6 days after treatment (Van Dyke, unpublished). The registration of “Ferroxx AQ” for aquatic use is currently under review by the USEPA.

Adjusting water level

As suggested by experimental data, a rapid change in water level can have lethal consequences for the egg clutches of *P. maculata*. A rapid rise floods the eggs and drowns some proportion of the embryos. However, such manipulations require careful consideration because some proportion of the clutch may still produce viable offspring, depending on its age (see above). Also, flooding may unintentionally result in increased snail dispersal. On the other hand, a rapid decline in water level may reduce water availability for hatchlings or expose adults to extreme desiccation and predation, as occurs in some South Florida water treatment areas (Bernatis, unpublished). In Wellman Pond (see below), for instance, the invasive, non-native red imported fire ant (*Solenopsis invicta*) quickly invaded the exposed littoral vegetation and attacked adult snails and eggs (Van Dyke, unpublished).

Case studies

While the initial reactions to most *P. maculata* invasions in the southern USA have been minimal, we report on three noteworthy case studies from Florida, Alabama and South Carolina that provide insight into the control of new populations of *P. maculata*.

Florida

Prior to plant restoration, hand collection of eggs and adult snails began in Wellman Pond (6.1 ha, Leon County, Tallahassee, Florida) in the summer of 2007. In spring 2008, efforts were begun to control the snail population using various baiting and trapping methods. The snails were easily attracted to combinations of certain grains and sugars

(Van Dyke, unpublished). They also tended to hide in black plastic containers made of high-density polyethylene (HDPE). Based on these observations, a large (7 L) trap, bait and method of use were designed and patented (Van Dyke *et al.*, 2013). After being attracted to the non-toxic bait, the snails entered the trap from above where a floating grate blocked their exit. In 2008 and 2009, 30 traps were deployed, and baited and emptied weekly (Van Dyke, unpublished). The traps performed well in shallow wade-able habitat with a hard substrate. As workers went from trap to trap, they also collected any eggs and adults observed in the pond. By the end of 2008, the management project removed 170,000 snails (3800 kg) and 2135 egg masses. In 2009, 35,000 snails (800 kg) and 1106 egg masses were removed, reflecting a smaller population.

Extensive landscaping occurred along the entire shoreline of Wellman Pond and involved planting 250,000 native plants (*Juncus effusus*, *Sagittaria* spp., *Pontederia cordata*). However, after one growing season, *P. maculata* had consumed 75 % of these plants. Besides deploying traps, native, snail-eating redear sunfish (*L. microlophus*) were stocked into the pond to control juvenile snails. Managers also painted a large interlocking metal sheet-pile wall, a retaining structure constructed to retain water within the pond, with chelated copper to discourage clutch deposition by snails left in the pond (Van Dyke, unpublished).

This program temporarily stabilised the emergent plant community, but littoral macrophyte abundance declined in subsequent years. All 27,500 plantings of pickerelweed (*Pontederia cordata*) disappeared, although soft-stemmed rush (*Juncus effusus*), smartweed (*Polygonum* spp.), and maidencane (*Panicum hemitomon*) appeared somewhat resistant to the voracious herbivory of *P. maculata*. Thus, partial removal of snails slowed impacts on plant community structure.

Alabama

The Wildlife and Freshwater Fisheries Division of the Alabama Department of Conservation and Natural Resources and the United States Fish and Wildlife Service initiated an on-going ambitious effort involving cooperation among numerous experts, volunteer organisations and public agencies, to eradicate *P. maculata* in autumn 2009 in the Three Mile Creek watershed, Mobile County (Martin *et al.*, 2012). Langan Lake (16 ha) flows into Three Mile Creek and while both these habitats have their own ecological and social importance, the urgency of this effort comes from their connection to the Mobile-Tensaw delta, the second largest river delta in the USA. In summer 2008,

P. maculata had established a population only 12 km upstream from the delta. As one of the most biologically diverse deltas in the USA, more than 780 km² of marshes, swamps and bottomlands within this National Natural Landmark are at high risk from invasion by *P. maculata*.

The control project involved collecting eggs, trapping adults, treating with copper sulphate and reducing emergent vegetation. In Langan Lake, managers initially relied on only copper sulphate because of its relatively low cost but copper concentrations declined rapidly and reached undetectable limits only 24 h after the applications (B. Ricks & A. Ford, personal communication). Volunteers have played a large role in egg collection (Martin *et al.*, 2012). Traps served not only to capture snails but also to assess the efficacy of copper sulphate treatments. For instance, 60-70 % of the snails inside the traps were dead after the initial treatment. Granular copper sulphate was first applied to Langan Lake using a blower to achieve the target concentration of 2.54 ppm elemental copper. No fish died. The treatment resulted in the added bonus of killing non-native, invasive Asiatic clams (*Corbicula fluminea*). Routine applications of copper sulphate now occur in Langan Lake and tidal portions of Three Mile Creek. Any impacts on the fauna downstream of the application sites have not been reported.

As additional management efforts, volunteers continued to remove snails manually. Volunteers also applied herbicides to reduce the abundance of native, but rapidly spreading, giant cutgrass (*Zizaniopsis miliacea*), the plant the snails appeared to prefer for egg deposition. In addition, a copper-based paint was applied to various human-made structures to discourage egg deposition. Finally, native redear sunfish have been stocked annually at a rate of 2500 ha⁻¹ to provide biological control of juvenile snails. *Pomacea maculata* also invaded two other ponds (< 8 ha) in Alabama, in Baldwin and Coffee counties. Quick agreements with landowners that facilitated copper sulphate treatments, trapping and hand picking of egg masses eliminated both populations. The goal of complete eradication in Alabama remains elusive (Martin *et al.*, 2012), but the effort continues.

South Carolina

In May 2008, *P. maculata* was discovered in 35 water bodies (0.25-7.12 ha) and a portion of the Waccamaw River near Socastee (M. Hook & C. Page, pers. comm.). Immediately, surveys of 200 water bodies in the area were undertaken and 39 contained apple snails. Most of these water bodies consisted of interconnected drainage ditches

and retention ponds within housing communities and golf courses. Less than a month after the discovery of *P. maculata*, all infested ponds were treated with granular copper sulphate at a rate of 11.2 kg ha⁻¹. The extent of control varied, so the ponds were retreated in September.

The ponds were surveyed again in May 2009 when egg production had begun. In June, ponds with snails were treated again with copper sulphate and eggs were removed by hand. Unfortunately, this control effort was not effective and snails persisted into July. Subsequently, the infested ponds were treated with double chelated copper at 0.3 ppm (an order of magnitude lower than applications in Alabama), with a follow-up treatment in August to ensure a long exposure time. Initial treatment resulted in high mortality. While doing informal surveys the week after the second treatment, managers stopped counting at 500 shells of dead snails when they examined a single 5 m section of shoreline in several of the ponds. By September 2009, there was little to no egg production in all ponds except two, which were retreated with the same low dose of chelated copper. This level of application did not eliminate other wildlife in the ponds including fish, ducks and turtles, although other invertebrates were not monitored (M. Hook, pers. comm.). By November, no reproduction was observed in any of the ponds, although seasonality probably contributed to reduced snail activity. Through the summer of 2014, routine use of multiple treatments of double chelated copper maintained low abundances when snails were active. Since 2014, chemical control of algae by homeowners and management associations in some of the ponds has helped to keep snail abundances low (M. Hook, pers. comm.). However, the interconnectedness of the systems allows apple snails to persist and the remnant populations that survive make continued monitoring essential.

Future research

Based on these three case studies, no one single approach works for all systems. Management efforts must weigh the ecological costs of ignoring *P. maculata* invasions against the time and economic costs of fighting an on-going battle with snails that exhibit high fecundity (Barnes *et al.*, 2008). Continued spread of *P. maculata* in the southern USA warrants increased concern about the possibility that it may follow the devastating path of *P. canaliculata* in other parts of the world. *Pomacea maculata* has a broader geographic distribution in the continental USA, as *P. canaliculata* only occurs in a few localities in Arizona, Florida and California (Rawlings *et al.*, 2007). There is less published research on *P. maculata* than *P. canaliculata*. We reiterate the need for more

taxonomic rigour and advocate for use of scientific names rather than common names to emphasise the diversity of apple snails present. Given recent taxonomic and phylogenetic clarity (Hayes *et al.*, 2012), more studies are appearing regarding the threat of *P. maculata*, correctly identified, as an expanding, invasive species. In this contribution, we sought to synthesise what is known about *P. maculata* in the USA and conclude by identifying research questions that deserve further attention (Table 2). We hope that this chapter serves as a wake-up call to pay attention to *P. maculata* as a species that warrants concern.

Table 2. Future research areas to advance our understanding of *Pomacea maculata*.

Area	Open research topics	Related references
Identity and genetics	<ul style="list-style-type: none"> • Investigating invasion via genetic analysis • Possibility/likelihood of hybridisation • Microsatellite approach to determine paternity • Sequence of entire genome 	Hayes <i>et al.</i> , 2008 Hayes <i>et al.</i> , 2012 Yoshida <i>et al.</i> , 2014 Matsukura <i>et al.</i> , 2016a
Fecundity, growth and reproduction	<ul style="list-style-type: none"> • Biochemistry of eggs and predation effects • Age and size at first reproduction • Hatchling growth and survival • Maximum reproductive potential per female • Longevity of different sexes 	Dreon <i>et al.</i> , 2010; 2013 Giglio <i>et al.</i> , 2016 Dorn & Hafsadi, 2016 Kyle <i>et al.</i> , 2011
Distribution	<ul style="list-style-type: none"> • Determining vectors of introduction • Refinement of ecological niche modelling • Dispersal ability of life history stages • Proportion of snails infected by <i>A. cantonensis</i> 	Byers <i>et al.</i> , 2013 Teem <i>et al.</i> , 2013
Abiotic tolerances	<ul style="list-style-type: none"> • Hatching efficiency and hatchling survival in a range of environmental conditions • Ability to survive low water levels 	Barnes <i>et al.</i> , 2008 Martin & Valentine, 2014 Ramakrishnan, 2007

Trophic interactions	<ul style="list-style-type: none"> • Quantitative measures of consumption of macrophytes in natural field settings • Determination of trophic position • Role as parasite vector and infection rates 	Connor <i>et al.</i> , 2008 Cattau <i>et al.</i> , 2010, 2016 Posch <i>et al.</i> , 2013 Kim <i>et al.</i> , 2014
Impact	<ul style="list-style-type: none"> • Potential for agricultural impact on rice • Threats to endangered species • Screening of snails for <i>A. cantonensis</i> 	Burlakova <i>et al.</i> , 2010 Teem <i>et al.</i> , 2013
Control	<ul style="list-style-type: none"> • Effective, non-toxic ways to remove snails • Effects of water level fluctuations on dispersal 	Martin <i>et al.</i> , 2012
Education	<ul style="list-style-type: none"> • Aquarium and aquaculture industry outreach • Establish clearinghouse for new information 	<i>Pomacea</i> Project, 2013
Big picture	<ul style="list-style-type: none"> • Ability to shift alternative ecological stable states • Contributions to invasional meltdown theory • Model organisms for amphibiousness 	Carlsson <i>et al.</i> , 2004a Meza-Lopez & Siemann, 2015 Hayes <i>et al.</i> 2009b

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