Wild record of an apple snail in the Waikato River, Hamilton, New Zealand, and their incidence in freshwater aquaria

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Abstract

We report the discovery of a single specimen of a live apple snail *Pomacea diffusa* Blume 1957 (Ampullariidae: Prosobranchia), from the Waikato River, Hamilton city, central North Island, New Zealand. This species, along with the congeneric *P. insularum*, is imported for the aquarium trade, and its occurrence in the river likely stemmed from an aquarium release. A survey of 55 aquaria belonging to 43 hobbyists revealed 27 apple snails, with one owner having 22 snails. Assessment of environmental tolerances and impacts of *P. diffusa*, based largely on studies of the closely related and commonly confused congener *P. bridgesii*, suggests that direct habitat impacts by this species are likely to be minor. However, there could be indirect influences on native biodiversity through predation on eggs or competition for food supplies with other detritivorous species if densities were to become high. Water temperatures in the Waikato River below Hamilton (10-23˚C in 2009) may enable released individuals to persist for an extended period, and over summer may exceed the threshold required to enable breeding. However, population establishment would be most likely in locations where water is heated through geothermal influences or industrial cooling water discharges.

Key words: Ampullariidae – *Pomacea diffusa* – *Pomacea insularum* – aquarium trade – biosecurity – geothermal habitats.

Introduction

Apple snails (Ampullariidae) have been introduced into the wild in many tropical and sub-tropical countries, including south-east Asia, Guam, Hawaii, Papua New Guinea, the Dominican Republic, Panama, parts of the southern United States mainland, Australia, and most recently Spain, where they can spread rapidly among suitable freshwater habitats (Cowie 1998; Rawlings *et al*. 2007; Hayes *et al*. 2008). Two species native to South America, *Pomacea canaliculata* and *P.*

insularum, have caused concern in rice-growing areas where they have become serious pests (Cowie 2002). Indeed, high densities of *P. canaliculata* have been reported to cause a complete shift in ecosystem state and function by virtually eating all aquatic plants and increasing nutrient and phytoplankton biomass in some tropical wetlands (Carlsson *et al.* 2004). *Pomacea* species can also influence ecosystems indirectly through predation (Kwong *et al.* 2009). In some Central and South American countries, apple snails are used as a food source due to their high levels of calcium, phosphorus, protein and amino acids, and they have been used therapeutically as a source of dietary minerals, leading them to be considered by some as a possible aquaculture species (Alves *et al.* 2006). Indeed, consumptive value has prompted the introduction of at least one large apple snail species outside of its natural range (Cowie 1998).

Apple snails have various adaptations that enable them to withstand a wide range of environmental conditions, primarily in tropical and sub-tropical areas where they inhabit swamps, ditches, ponds, lakes and rivers (Bronson 2002). Snails can respire using a gill and a lung, enabling them to withstand alternating periods of wet and dry conditions (Cowie 2002). *Pomacea* also has an operculum, a feature of the gastropod subclass Prosobranchia, which it closes when exposed, and a tubular siphon which can be used to breathe air. These adaptations make *Pomacea* tolerant of fluctuating water levels and low oxygen water, and also less vulnerable to terrestrial predators. Snails may remain active throughout the year or enter periods of aestivation by burying themselves in mud and slowing down metabolism in response to adverse environmental conditions, often reflecting water temperature, rainfall and food availability. Apple snails are generally herbivorous although many feed on eggs of other snails and decaying animals, and they can become cannibalistic when food is very short (Bronson 2002).

We report a wild record of the apple snail *P. diffusa* Blume 1957 from the Waikato River, central North Island, New Zealand, evaluate its potential to become established within this catchment, and assess its potential effects on freshwater ecosystems. *P. diffusa* is often incorrectly identified as *P. bridgesii* (Cowie *et al.* 2006) which was originally described from Beni in Bolivia and is rare in the wild. The type locality for *P. diffusa* is Santa Cruz in Bolivia, although this species is widespread throughout the Amazon basin (Perera & Walls 1996; Rawlings *et al.* 2007). Recent genetic analyses have confirmed that the species introduced overseas is *P. diffusa* (Hayes *et al.* 2008), and earlier work attributed to *P. bridgesii* acquired from the aquarium trade is most likely to have been on *P. diffusa*. Because these species are closely related and their identities have been confused in the past, we use published information from both species in our evaluation.

Methods

Invertebrate sampling was carried out in littoral habitats at selected sites along the Waikato River in Hamilton city on 11 March 2010 for an unrelated project. A D-frame net (0.5 mm mesh) was used to sweep through macrophyte beds (predominantly *Egeria densa*), and large rocks, where present, were turned over and brushed upstream of the net. During sampling of a site at Swarbicks Landing on River Road (NZMG 2709892E, 6380866N), the presence of a large apple snail was noted. This snail was retrieved from amongst riprap comprising angular boulders on a steeply sloping bank of the river, at around 0.3 m water depth. Physicochemical measurements made at the collection site in March 2010 indicated a daytime spot water temperature of 20˚C, dissolved oxygen concentration of 8.6 g m^{-3} (YSI 550A meter) and specific conductivity of 155 µS cm^{-1} (YSI EC 300 meter). pH of the lower river ranges from 7.0-8.5 (annual median 7.4-7.7; Beard 2010).

An assessment of the potential for apple snails to survive and breed in ambient freshwater was made based on continuous monitoring of water temperature in the Waikato River from loggers deployed at Hamilton city and the lower river around 80 km below Hamilton. In addition, we report the results of a survey of home aquaria undertaken between 5 March 2007 and 8 March 2008, in which the presence of apple snails was noted during a survey for small incidental fauna (see Duggan 2010a). Volunteer participants were found by approaching aquarium owners known to one of the authors (ICD), word of mouth, using a bulk email message to University of Waikato staff and through two newspaper articles that described the project and asked for participants. Most participants (37; 86%) were in the Waikato region, while the remainder (6; 14%) was recruited in the Wellington region.

The apple snail retrieved from the Waikato River (preserved in 100% isopropanol), along with two golden apple snails and two 'mystery snails' bought live from a local pet shop, were subjected to molecular analysis using approximately 25 mg of foot tissue excised from directly behind the opercula of each individual. Total genomic DNA was extracted using the Zymo Research Quick-gDNATM MiniPrep kit following the manufacturers recommended procedures. Following extraction, an approximately 680-bp fragment of the mitochondrial cytochrome c oxidase subunit I (COI) gene was amplified using the primers LCO1490 and HCO2198 (Folmer *et al.* 1994). PCR amplification of each individual was carried out in a 20 µL reaction containing ca. 20 ng of extracted DNA, 5 pmol of each primer and 10 µL of i-Taq™ 2X PCR master mix (iNtRON Biotechnology, Gyeonggi, South Korea). Thermocycling conditions were: 94˚C for 5 min followed by 5 cycles of initial denaturation and polymerase amplification (94˚C for 1 min, 48˚C for 1.5 min, 72˚C for 1 min) followed by 36 cycles of 94˚C for 1 min, 52˚C for 1.5 min and 1 min at 72˚C, followed by 5 min at 72˚C. All PCR products were visualised on 1.5% agarose gel with a 100 bp ladder standard. Prior to sequencing 10 µL of each PCR product was purified using 3 µL mastermix containing 0.1U of Shrimp alkaline phosphatase, 1U of Exonuclase I and 2.7 µL of ultra pure water. Sequencing was performed directly on a capillary electrophoresis ABI 3130XL genetic analyser (Perkin-Elmer Applied Biosystems Inc., Foster City, CA, USA) at the University of Waikato DNA sequencing facility using the HCO2198 primer. Individual sequences were confirmed as being derived from applicable taxa using the GenBank BLASTn algorithm. Electropherograms were checked for errors and aligned using Geneious Pro v5.1.4 (Drummond *et al.* 2010). All sequences were deposited in GenBank (GenBank Accession numbers HQ908055 – 57)

Results and discussion

The apple snail found in the Waikato River was morphologically identified as *Pomacea diffusa* Blume 1957 (Figure 1). Additionally, the mtDNA COI sequence

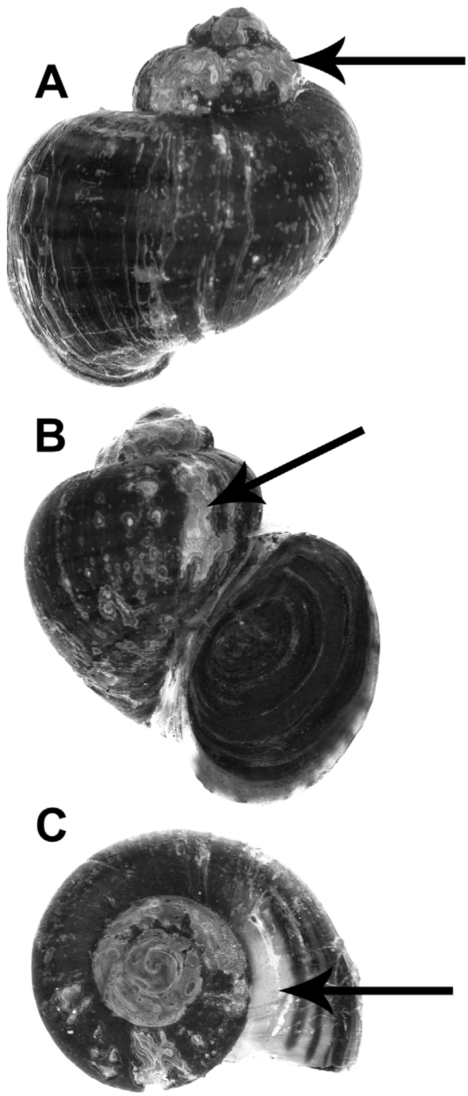

Figure 1. Photos of the apple snail found in the Waikato River showing erosion scars on the spire (A) and shell (B), and banding on the outer whorl (C).

data was 100% identical at over 611 bp to that of several *P. diffusa* deposited on GenBank (Hayes *et al*. 2008; Accession numbers EF515065 – 66; EF515074 – 81). The two 'mystery' snails purchased at a local pet shop were also identical at over 600 bp with that of the wild *P. diffusa* found in the Waikato River (Accession numbers HQ908055; HQ908056).

P. diffusa, also referred to as the 'spike-topped apple snail' (Cowie *et al*. 2006), has 5-6 whorls that are distinctly stepped with square shoulders and sutures angled at almost 90° leading to an apex (Perera & Walls 1996; Bronson 2002). The specimen found in the Waikato River was 29 mm in height and 26 mm wide, of undetermined sex, and dark colour with light markings on the outer whorl and erosion scars evident on the spire (Figure 1). Colour of these snails can be highly variable ranging from yellow, green to brown, sometimes with spiral bands; the yellow colour morph is common in the aquarium trade where it can be referred to as the 'golden mystery snail' (Perera & Walls 1996).

P. diffusa is widely available in New Zealand pet stores, specialist aquarium stores and via the internet. Also available is the golden apple snail which has more deeply incised whorl sutures than *P. diffusa*. Golden apple snails are sometimes advertised as *P. linearis* (more correctly *P. lineata*), although genetic analyses indicate that those sold by at least one pet store are in fact *P. insularum*. mtDNA COI sequence data obtained from the pet shop purchased golden apple snail was 100% identical at over 650 bp to that of several *P. insularum* sequences found on GenBank (Accession numbers GU133205 – 06; GU236489 - 91; EF515026 – 27 , 34, 36 & 37). In fact the observed COI haplotype is the most common *P. insularum* haplotype found in both the wild and in pet shops worldwide (K. Hayes, University of Hawaii, pers. comm.). *P. insularum* is morphologically very similar to *P. canaliculata* (Cowie *et al.* 2006), a species that has been listed as "established in New Zealand" by Biosecurity New Zealand (2005), although our results suggest it is more likely to be *P. insularum*.

During our aquarium survey, 55 home aquaria were visited belonging to 43 people, yielding 27apple snails in total belonging to four owners (i.e., in 9% of households visited). Two owners had single snails, one had three individuals, while the remaining tank had 22 individuals; the latter owner noted that snails were successfully breeding in their tropical tank. An additional owner stated they had kept an apple snail previously that had since died. Apple snails typically live 1-2 years in aquaria where they prefer water temperatures over 20˚C (Perera & Walls 1996). The presence of apple snails in home aquaria, and the discovery of our specimen adjacent to a highly accessible part of the Waikato River, suggests it is most likely to have originated from an aquarium release. Sampling conducted previously and subsequently using the same methods at this site has not revealed the presence of other apple snails, suggesting that either densities were very low, this snail had entered from outside the sampling area, or that the find was an isolated occurrence.

Our wild record of *P. diffusa* increases the number of introduced invertebrates known from the Waikato River to nine, of which seven are freshwater snails (11 gastropod snails are known in total from the river; Collier & Hogg 2010). With this record, at least 18 introduced macroinvertebrates including 10 introduced snails have been reported from various freshwater habitats around New Zealand (ICD unpubl. data). DNA evidence indicates that *P. diffusa* currently in the aquarium trade originated from a single source in Brazil (Hayes *et al.* 2008). It has also been reported from the wild in Florida, Cuba, Australia, Sri Lanka and Hawaii, although it has now apparently disappeared from Hawaii due in part to competition with the larger and more aggressive *P. canaliculata* (Rawlings *et al.* 2007; Hayes *et al.* 2008; Vázquez & Perera 2010).

P. bridgesii has been reported to gain weight rapidly (1.7% per day at 27.6 ˚C under laboratory conditions; Alves *et al.* 2006), but physical damage to habitats where these species have been released into the wild appears to have

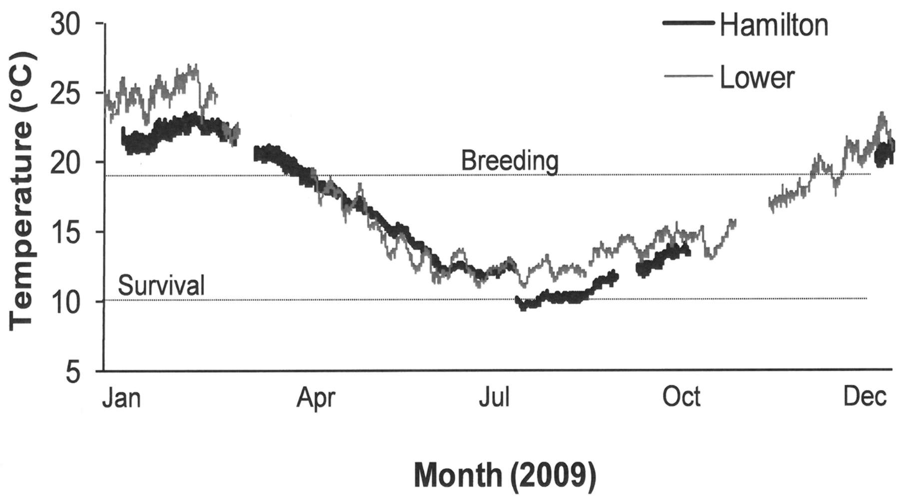

Figure 2. Continuous temperature data from two sites on the Waikato River: Hamilton city near the apple snail collection site and a site in the lower river 80 km downstream (data from Environment Waikato; gaps indicate missing data). Indicative temperature thresholds for survival and breeding are shown based on available information.

been minor and in the United States *P. diffusa* is generally regarded as innocuous (Rawlings *et al.* 2007). Because it feeds mostly on decaying vegetation or algae, *P. diffusa* is not considered an agricultural pest or a problem for macrophytes like *P. canaliculata* or *P. insularum* which can feed voraciously on live plant material high in nitrogen (Morrison 2010; Wong *et al.* 2010). However, potential direct and indirect effects on natural habitats are unclear, and *P. diffus*a may compete for food with native scavengers such as crayfish, shrimps and some fish species (Rawlings *et al.* 2007). In addition, laboratory trials indicate feeding on eggs of other freshwater snails (Aditya & Raut 2002), suggesting potential for an impact on native biodiversity. Moreover, some apple snails may act as intermediate hosts for other introduced species; for example 28% of *P. bridgesii* snails examined by Gorni & Alves (2006) were found to harbour Naididae worms, and some *Pomacea* species can transfer the rat lungworm nematode (*Angiostrongylus cantonesis*), schistomes and intestinal flukes (Cowie 2002).

Pomacea typically lay eggs on hard substrates and plants, usually above the water line to protect eggs from predation by other aquatic species or in response to low oxygen tension (Cowie 2002). Egg masses of *P. diffusa* have an irregular honey-combed appearance and a tan to salmon colour, although they are white when freshly-laid (Rawlings *et al.* 2007). Under ideal conditions some apple snail species produce one clutch of eggs every 4-7 days and grow into adults in less than three months (Bronson 2002). Unlike many other snail species, apple snails are not hermaphroditic and both sexes are required for reproduction, although females can store sperm for several months. Temperature and food availability are the main factors regulating the rate of egg production. Apple snails start to enter dormancy at temperatures less than 18°C and mortality rate increases below this, with snails generally absent where minima fall below 10°C (Bronson 2002), although *P. canaliculata* and *P. insularum* at least can survive short periods at much colder temperatures (Cowie 2002; Ramakrishnan 2007). Breeding appears possible at temperatures over around 18˚C, with egg incubation time linked to water temperature.

An examination of continuous water temperature data from the Waikato River for 2009 indicates that temperatures below Hamilton rarely fell below 10˚C with water temperatures greater than 18˚C occurring for around five months (Figure 2). Water temperature maxima exceeded 23˚C regularly over summer in the lower river but rarely in Hamilton city in 2009 (Environment Waikato, unpubl. data). Some geothermal tributaries in the upper Waikato River (e.g., Waiotapu Stream) maintain year-round temperatures of 14-25˚C, and geothermal inputs maintain minimum temperatures in Lake Ohakuri above 12˚C (Duggan 2002; Rutherford 2010). A number of streams with geothermal influences extending over several kilometers in the central North Island would provide suitable and relatively constant temperatures throughout the year, and have been the sites of establishment for other tropical snails and fish species (e.g., Golden Springs; Duggan 2002, 2010b). However, suitable sites will be restricted to neutral-alkaline springs, as the acidity in sulphide springs will usually be high enough to dissolve snail shells (Duggan 2002; Duggan *et al.* 2007). In addition, discharges of cooling water from thermal power stations also have the potential to elevate river water temperatures to levels capable of sustaining apple snails. For example, summer temperatures of 21-29˚C have been recorded in the river below a power station outfall in the upper Waikato River, and a maximum water temperature of 25˚C is normally permitted one kilometre downstream of a thermal power station discharge in the lower river with thermal effects evident for several kilometres downstream (Rutherford 2010).

In summary, water temperatures within the Waikato River catchment may enable released individuals of *P. diffusa* to persist in some locations. Population establishment could potentially occur in localised areas, especially where water is heated sufficiently through geothermal influences or industrial cooling water discharges. As this species of apple snail feeds mostly on algae or decaying vegetation, direct effects on habitat, for example by removing macrophyte beds, are likely to be minor. However, if populations were to establish in high densities, *P. diffusa* has the potential to impact native biodiversity through predation and competition for food resources. More significant impacts are likely if populations of the voracious herbivore *P. insularum* or related species were to establish in New Zealand freshwaters. Elsewhere, species of apple snails have apparently developed behavioural strategies to enable overwintering in harsh climates (Hayes *et al.* 2009), indicating that vigilance is required to ensure that self-sustaining wild populations of apple snails do not establish in New Zealand freshwaters.

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