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# Survival of an invasive aquatic snail to overland translocation in non-aquatic media: Implications for spreading

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ABSTRACT

Invasive species are a threat to aquatic ecosystems worldwide. Aquatic snails have a limited ability for an active dispersal. Therefore, their ability to survive to transport in non-aquatic media may help explain their spread across unconnected habitats. We assessed the ability of New Zealand Mud Snail (NZMS) (*Potamopyrgus antipodarum*) to survive attached to different materials. Two studies were conducted: (1) a laboratory study to assess the tolerance of a laboratory population of NZMS to non-aquatic media attaching snails to leaf litter, sediment and clay and (2) a field-laboratory study to assess the survival of an invasive field population after being left imbedded in clay in the laboratory and subsequently transferred to a new river reach. All laboratory animals died after 3 days in leaf litter, while in the case of sediment and clay all snails died after 5 days. After being imbedded in clay and subsequently transferred to the river, the survival of the field population was lower than that of the laboratory populations. We conclude that NZMS can be dispersed by mechanisms which imply spending some time in non-aquatic media, and that this species has a relatively high tolerance to translocation between reaches with contrasting environmental properties.



## 1. Introduction

Invasive species are a threat to the biodiversity and functioning of ecosystems worldwide (Everett, 2000; Kolar and Lodge, 2000). All biogeographical barriers have been overtaken by humans which has facilitated the global spread of exotic species (Everett, 2000; Nentwing, 2007). Some of these species are causing economic impacts (Everett, 2000) and are threatening native species (Kolar and Lodge, 2000). Established invasive species are generally difficult to eradicate. Therefore, to assess the risk of successful establishment we need to know the rate of survival of these species in the new ecosystems. In the case of aquatic environments, the rate of species introduction is continuously increasing worldwide (Ricciardi and Rasmussen, 1998; Rahel, 2000; Ricciardi and MacIsaac, 2000) being one of the most endangered by exotic species (Gherardi, 2007).

An exotic species is considered invasive when it proliferates, spreads and persists in a new ecosystem causing negative impacts in the environment (Mack et al., 2000). Aquatic snails are considered as non-efficient dispersers because of their slow movements (Dillon, 2000; Kappes and Haase, 2012). However, several aquatic snails, such as New Zealand mudsnail (*Potamopyrgus antipodarum*), the apple snail (*Pomacea sp*) or the bladder snail (*Physella acuta*) are invasive worldwide (Strayer, 1999; Karatayev et al., 2009; Alonso and Castro-Díez, 2008, 2012a; Banha et al., 2014). Malone (1965) showed that gastropods can attach themselves to the external surface of birds, or to the mud that gets attached to birds. Mud containing snails may be also transported by cattle, wild mammals, anglers and cars (Facon et al., 2004; Richards et al., 2004; Wilmer et al., 2008; Kappes and Haase, 2012; van Leeuwen and van der Velde, 2012; Banha et al., 2014). This fact indicates that passive dispersal mechanisms are important for some mollusk species as drivers of spread (Kappes and Haase, 2012; Banha et al., 2016). In this case, the ability of aquatic mollusks to survive for some time in a non-aquatic medium is a key factor explaining their capacity to spread across unconnected aquatic ecosystems (i.e. lateral dispersal, Kappes and Haase, 2012). This has been also shown for other animal groups (Frisch et al., 2007; Águas et al., 2014). Lateral dispersal usually implies that invertebrates are imbedded in any type of substratum (e.g. mud, sand, macrophytes, leaves, feathers, etc.) which is moved by terrestrial/aerial animals (Johnson and Carlton, 1996; Alonso and Castro-Díez, 2008; Waterkeyn et al., 2010; Wood et al., 2011; Kappes and Haase, 2012; Banha et al., 2016; Reynolds et al., 2015). Unfortunately, most studies assessing the tolerance of

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mollusks to non-aquatic media are conducted with animals directly exposed to air, which may be an unrealistic situation. Substrata may preserve moisture for some time, therefore increasing the chances of survival during lateral dispersal. Moreover, most studies have been conducted in laboratory, where the animals, after air-exposure, are usually returned to the initial controlled aquatic media (i.e. stable water temperature, same physicochemical water properties previous to air exposure) (Paukstis et al., 1999; Suemoto et al., 2004; Alonso and Castro-Díez, 2012b; van Leeuwen and van der Velde, 2012). By contrast animals arriving to new ecosystems normally have to face novel environmental conditions (e.g. temperature, physicochemical water properties, etc.) which can reduce their chances of survival. All the above suggests that classical laboratory experiments may sub- or overestimate tolerance of natural populations to field translocations in non-aquatic media.

One of the species that has colonized new habitats worldwide through lateral dispersal is the New Zealand mud snail (NZMS) (*Potamopyrgus antipodarum*, Hydrobiidae, Mollusca) (J.E. Gray, 1843). This parthenogenetic mollusk is native to New Zealand and adjacent islands, but it has successfully established in a wide range of aquatic ecosystems (Alonso and Castro-Díez, 2008, 2012a). Several studies have shown or suggested that this snail can move across catchments by means of human and/or animal-mediated vectors, such as anglers, boats and waterfowl (Richards et al., 2004; Kappes and Haase, 2012; van Leeuwen and van der Velde, 2012). NZMS can be directly transported attached to the surface of these vectors or indirectly, attached to mud, sand, sediment, macrophytes or leaf litter that are transported with these vectors. However, the knowledge on how the type of substratum affects the chances of survival of NZMS is scarce and no information on post-transport survival under field conditions is available so far.

The aims of this study are: (1) assessing the NZMS survival in non-aquatic media simulating transportation attached to different substrata (leaf litter, sediment and clay) under laboratory conditions; (2) assessing the post-transport survival under field conditions; (3) comparing post-transport survival between a laboratory and a field experiment; (4) assessing whether the origin of populations (laboratory vs field) affects the tolerance of NZMS to non-aquatic media. We tested the hypotheses that substrata preserving more moisture will increase the post-transport survival of NZMS (hypothesis 1), that the survival of NZMS in field scenarios will be lower than under laboratory conditions (hypothesis 2), and that the laboratory population will present a similar tolerance to non-aquatic media than the field population (hypothesis 3). This study represents a step forward in the understanding of the NZMS post-transport survival in realistic scenarios, which is relevant for an efficient management of this invasive snail.

## 2. Materials and methods

Two studies were conducted: the first one was carried out in the laboratory to assess the survival of a laboratory population of NZMS after being in different non-aquatic media simulating animal transportation on different substrata (*laboratory bioassay*). The second was a field-laboratory study (*field-laboratory bioassay*) to assess the survival of an invasive field NZMS population after a laboratory simulation of transport in non-aquatic media and a subsequently transfer of the animals both to a new river reach and to initial laboratory conditions.

### 2.1. Laboratory bioassay

We used a laboratory population of NZMS (Department of Life Sciences, University of Alcalá). This population was started in January 2009 with snails collected from an upper reach of the Henares River (41° 6′ N; 2° 36′ W) (Guadalajara, Spain). Animals were kept in 60L glass aquaria with USEPA moderately hard water (96 mg $NaHCO_3$, 60 mg $CaSO_4{}^*2H_2O$, 4 mg KCl, 122.2 mg $MgSO_4{}^*7H_2O$ per l of deionised water) (US Environmental Protection Agency, 2002), enriched with calcium carbonate (10 mg $CaCO_3$ per l of deionised water). The culture was kept at 20–22 °C and animals were fed with fish food (Tetra© GmbH, Germany) and dry algae (0.10 mg of Spirulina per animal and day) (Sera Spirulina Tabs GmbH, Germany).

We simulated the conditions during a natural transportation event by embedding the animals into three types of substrata, frequently found in aquatic ecosystems: leaf litter, sediment and clay. (1) **Leaf litter** was obtained by collecting senescent leaves of *Fraxinus angustifolia* in autumn and letting them dry at laboratory temperature. This plant is frequently found in riparian ecosystems of Central Spain where NZMS dwells. Dried leaves were placed into aquaria with deionized water and an air pump. Leaves were kept in these conditions during one week prior to the bioassay with daily water renew. Then, leaves were cut in pieces of a mean size of 12.2 × 13.5 mm and kept in water before the bioassay. (2) An artificial **sediment**, mimicking natural sediments, was made with a mixture of ground (<1 mm) dry leaves of *F. angustifolia* (10%), fine sand (0.25–0.5 mm) (30%), coarse sand (0.5–1.0 mm) (30%) and commercial non-toxic red clay (30%) (100% natural clay, Sio-2®, Barcelona). All these components were mixed up in a glass vessel with a magnetic stirrer and deionized water. After 30 min the mixture was filtered and 1.1 g (wet weight) cubic portions were made. (3) Commercial red **clay** (Sio-2®, Barcelona) was cut in cubic portions (mean wet weight = 5.2 g) and submerged in deionized water until saturation during 10 min previous to the bioassay.

Laboratory snails (mean shell size 3.4 ± 0.3 mm) were selected and kept in glass aquaria with USEPA moderately hard water (1.5 l per aquarium). Aquaria were then kept in a climatic chamber (ANSONIC) at 18 °C (12 h:12 h photoperiod) for a week previous to the bioassay (mean ($n$ = 10) water properties: pH = 8.0 ± 0.04, water temperature (°C) = 18.2 ± 0.72 and dissolved oxygen (mg $O_2$/l) = 9.9 ± 0.08). Animals were fed and water was renewed every two days. The bioassay consisted on two crossed treatments: (1) substratum type (leaf litter, sediment and clay) plus one control for each substratum. In each control, animals were handled in the same way as in each treatment but they were immediately returned to water. (2) Exposure time (3, 4 and 5 days in each substratum). Each treatment combination was in triplicate. Each replicate consisted on a plastic ice-tray (25 cm long × 13 cm wide, with 50 wells of 3.5 ml each) with ten animals that were individually placed in each of the ten wells. Therefore, a total of 270 animals (27 trays) were used for non-aquatic exposure plus 90 animals (9 trays) for substratum controls. In the leaf litter treatment, each animal was placed in the bottom of the well with the aid of forceps, and covered with one piece of leaf. In the sediment and clay treatments, each animal was embedded in one side of the sediment/clay cube with a gentle pressure and then the cube was introduced into a well with the animal downwards. Groups of three randomly chosen ice-trays were put into polyester bags and kept in the climatic chamber at 18 °C. The ice-trays simulated the sole of a wader and the polyester bags simulated the bag used by anglers to transport waders. This approach aimed at increasing the realism of the bioassay to assess the capacity of NZMS to survive under suboptimal conditions. However, our layout does not allow us assessing the effects of transport (e.g. maintenance of animals in boots, dropping during transport, etc.).

The substratum moisture at the beginning of the bioassay was calculated by weighing cubes or leaf litter ($n$ = 3) with no animals (made with remaining material of the bioassay) before and after ≥48 h in the oven at 60 °C (leaf litter) and at 105 °C (sediment and clay). During the bioassay substratum moisture was calculated