

CORRECTED PROOF

Management in Practice

Detection and possible elimination of the first recorded population of the zebra mussel (*Dreissena polymorpha*) in Portugal from a reservoirDavid Catita¹, Mafalda Gama², Rita Azedo¹, Filipe Banha², João Pinto², Ana Ilhéu¹ and Pedro Manuel Anastácio^{2,*}¹EDIA – Empresa de Desenvolvimento e Infraestruturas do Alqueva, Beja, Portugal²MARE – Marine and Environmental Sciences Centre. Departamento de Paisagem, Ambiente e Ordenamento. Universidade de Évora. Rua Romão Ramalho, 59. 7000-671 Évora, PortugalAuthor e-mails: dcatita@edia.pt (DC), mafaldagama@uevora.com (MG), r_azedo@yahoo.com (RA), filipebanha@uevora.com (FB), jfgpinto95@gmail.com (JP), ailheu@edia.pt (AI), anast@uevora.pt (PMA)

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Abstract

The zebra mussel, *Dreissena polymorpha*, has strong economic and environmental effects within a wide range of habitats, including reservoirs, and there is a need to monitor in advance of an arrival so that an elimination might be possible. We report the establishment of the zebra mussel in the Alfândão Reservoir, a small reservoir in central Portugal, 190 km away from the closest known population in Spain. The area has been monitored using suspended ropes since 2016, and during October 2019, mussels of 5–43.8 mm shell length were recorded for the first time in the Alfândão reservoir. The shallow lined reservoir was then chlorinated and drained. To date, this treatment appears to have been successful in eliminating this population. It is unknown how this mussel arrived, since the reservoir area has a surrounding exclusion zone.

Key words: first record, biological invasions, bivalve, irrigation, impact**Introduction**

The zebra mussel (*Dreissena polymorpha* Pallas, 1771) is a small dreissenid freshwater and brackish water bivalve native to the Black and Caspian region, occurring in lakes or slow-moving low salinity waters (Durán and Anadón 2008; Karatayev et al. 1998). Zebra mussels have a pelagic veliger microscopic larval stage for up to ~ 20 days and a sessile and gregarious juvenile and adult form normally attached to hard substrates or to other invertebrates (Minchin et al. 2002). The adults often reach a size between 25 and 35 mm and attain populations of up to 750,000 individuals m⁻². Females may reproduce within 6–7 weeks of settlement (Mackie 1991) having attained 8 mm. Fertilization occurs externally (Ram et al. 1996) following broadcast spawning once water temperatures reach 12–15 °C, but spawning is optimal at 14–20 °C, which may take place over a period of 3–5 months (GISD 2015).

D. polymorpha causes severe economic impacts (bij de Vaate et al. 2010) and may also have negative ecological impacts on these habitats. The zebra

mussel can damage hydraulic infrastructures, clog boat engine water intakes, and foul hulls. This species clogs water pipes distributing water in urban, agricultural, and industrial zones. It forms large populations in drains, floodgates, and irrigation networks, obstructing or decreasing water flow (Minchin et al. 2002). It also causes damage within pressurized irrigation pipes used for sprinklers and in drip irrigation, accelerating pipe corrosion and thereby increasing maintenance costs. In North America, there has been a cost to power plants and municipal drinking-water plants during 1989–2004 estimated at \$267 million (Connelly et al. 2007). Other studies suggested a potential loss of \$244 million over 20 years in Florida (Lee et al. 2007). The Ebro hydrographic confederation (CHE) conducted an economic study in 2005, which estimated a cost of approximately 40 million Euros over 20 years (Durán et al. 2010).

The zebra mussel expanded its distribution from Black and Caspian Sea basins in the first part of the 19th century to ports in northern Europe (van der Velde et al. 2010) then extending through different river basins by shipping along linking canal systems. It appeared in Britain in the 1820s, it was recorded in Italy in the 1970s and Greece in the early 1980s. Mountain ridges (such as the Alps and the Pyrenees) were most likely crossed by *D. polymorpha* attached to recreational/sports fishing boats on trailers, which led to the invasion of the Iberian Peninsula, reaching the Ebro river by 2001 (Durán and Anadón 2008). The species now exists in several reservoirs of the Ebro basin (Durán and Anadón 2008), in the Júcar basin since 2005, in the Segura basin since 2006 (CNPN 2007), and in the Guadalquivir basin since 2009 (Junta de Andalucía 2010). Spain had a population of zebra mussels detected in the 1980s in the Llobregat river, but this population failed to become established (CNPN 2007). At the end of the 19th century, shells of *D. polymorpha* were detected in the margin of the Douro River, near Oporto, Portugal (Castro 1873), but the species did not become established. This paper reports the first finding of an established population of the zebra mussel in Portugal and its removal from the Alfundão reservoir.

Description of Alfundão and upstream Pisão reservoirs

The Alfundão Reservoir (Figure 1), stores 16.9 dam³ of water upstream of a pumping and filtering station supplying water to a 609 ha irrigation area (Azevedo and Paulo 2011). The reservoir is 110 m long, 62 m wide, and 3.5 m deep, at an elevation of 136.5 to 140 m, and has an impermeable white geomembrane of high-density polyethylene (HDPE). It is directly connected to the nearby, and much larger, upstream Pisão Reservoir by 4.4 km of link of steel pipes with diameters ranging from 0.9 to 2 m under gravity, with no tanks and only a valve chamber near Alfundão (Figure 2). Water in the Alfundão Reservoir comes directly from the Pisão Reservoir. Whenever agricultural irrigation takes water from Alfundão, Pisão water is passed

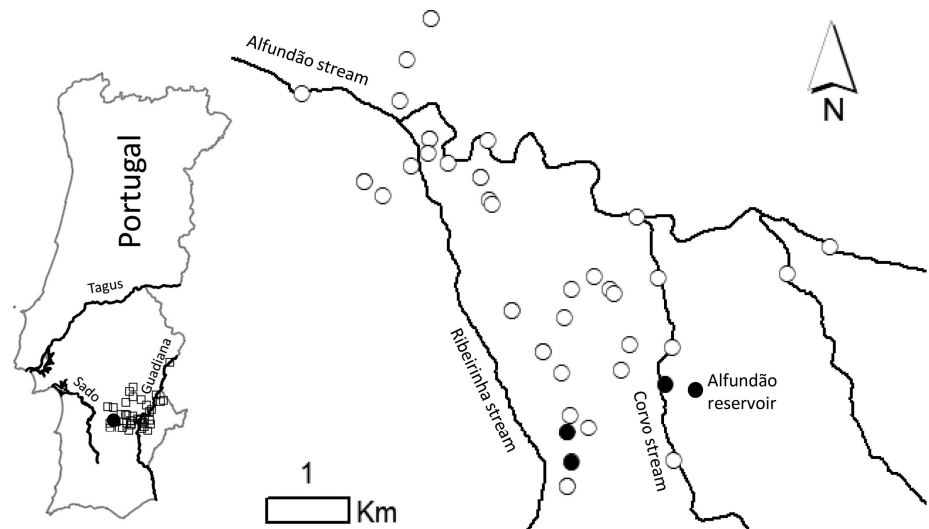


Figure 1. On the left, a map of Portugal shows the detection area (black circle), the 60 monitoring points (empty squares) and the 3 closest rivers. The main image (on the right) shows the location of the Alfundão reservoir and the hydrants and stream points, monitored after detection. Black circles indicate infested locations.

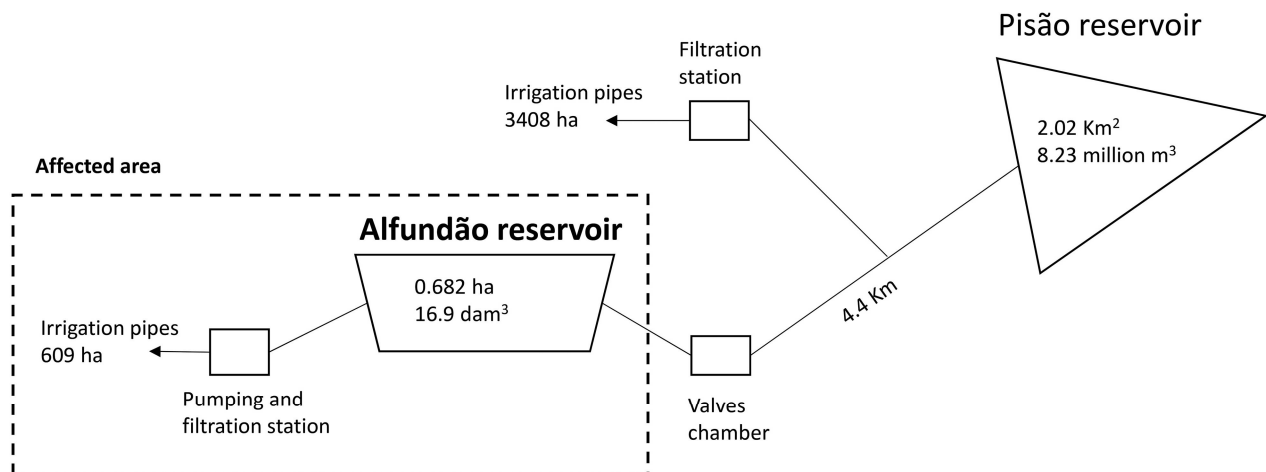


Figure 2. Hydraulic connections around the Alfundão reservoir. The area affected by zebra mussels is indicated by a dashed line.

downstream. The Alfundão Reservoir perimeter is totally fenced, with limited staff access and with no public access for any activity. As there is no vegetation due to the lining of the reservoir, crayfish are rare, but some fish species are present, and occasionally birds visit for shelter opportunities.

The larger Pisão Reservoir has an area of 2.02 km², storing 8.23 million m³ of water (Figure 2). This is a naturalized reservoir with aquatic vegetation and excellent conditions for winter birds, and it is also important during their reproductive season.

Materials and methods

Since July 2015, the EDIA (Empresa de Desenvolvimento e Infraestruturas do Alqueva) developed a plan for the early detection and prevention of the zebra mussel in the Alqueva Reservoir and tributaries (Guadiana and Sado river basins) in Alentejo region. Monitoring involves 60 sites within natural



Figure 3. Photos taken during the local detection and management process. A – Monitorization rope used for the initial detection of the species. B – The empty Alfundão reservoir during the cleaning operations while the sediment was being removed. Photos by David Catita.

and artificial reservoirs over an area of approximately 8,500 km². Preventive measures were also used, involving boat cleaning stations and local advertising campaigns. Larvae detection by PCR and by cross polarized light microscopy has been used intermittently in reservoirs and adjacent areas including in the Pisão but not the Alfundão (EDIA 2018). From December 2016, single vertically hung ropes of 18 mm diameter braided nylon were knotted at 2 m intervals for sixty separate water bodies and in the Alfundão Reservoir knotted at 0.5 m intervals to 3.5 m to detect settling zebra mussels (CHE 2009) (Figure 1). There is an inflow to the Alfundão Reservoir from the Pisão Reservoir (Figure 2), and water quality is only monitored in Pisão approximately six times per year. Water temperature, conductivity, pH, and dissolved O₂ are measured in situ at a 2 m depth, and calcium is quantified in the lab according to ISO 11885.

Following detection of zebra mussels on ropes, the reservoir and associated pipe system water was chlorinated and drained, and sediments were removed (CHE 2014) (Figure 3). This took place over two days, and the reservoir subsequently dried out over seven days. The downstream pipe system only remained dry for three days. The inflowing and outflowing pipe system from Alfundão was checked and cleaned. The area surrounding Alfundão was visually checked for the presence of zebra mussels in pipes, hydrants, streams, and reservoirs (Figure 1). This included diving in the Pisão Reservoir. The length of the largest individuals found by the cleaning crew in the pipe system was registered, and 100 random individuals on the detection rope (0 to 3.5 m depth at bottom) were also measured.

Results

Species detection

Zebra mussels ranging from 5 to 23 mm shell length were detected on a rope from a depth of one meter to 3.5 m on the 1st of October 2019 in the

Table 1. Water quality in the Pisão Reservoir, during 2016 to 2019 at a 2 m depth. The Pisão Reservoir supplies the water for the Alfundão Reservoir.

	Mean	S.D.	Range
Water temperature (°C)	19.2	5.9	9.2–26.6
pH	8.4	0.4	7.3–8.9
Dissolved Oxygen (mg/L)	8.9	1.8	1.38–11.52
Conductivity (µS/cm)	590.7	82.5	425–739
Calcium (mg/L)	44.9	7	29.5–58.7

Alfundão Reservoir (Figure 3). The reservoir and its associated pipes, gates, and pumps, and surrounding streams were then examined to show a dense localized population solely within the Alfundão Reservoir. To this date, no zebra mussel larvae were detected by PCR or by cross polarized light microscopy at any of the locations monitored by these techniques, including in the nearby Pisão Reservoir.

Population structure and invaded structures

Live zebra mussels were detected in the Alfundão Reservoir in its inflow gate and outflow pipes and gates, in the pumping and filtering systems, and in a nearby stream (a few individuals) where excess water from this system is usually drained away (Figure 1). No zebra mussels were found during rope monitoring and shores and underwater examination in the Pisão Reservoir, which supplies water to the Alfundão (Table 1). Some shells were found in two irrigation water outlets downstream the Alfundão Reservoir, at the end of the intervention in the system for species control (Figure 1).

The largest densities were found within the outflow 90 cm diameter, 100 m long steel pipe. From this, a crust of zebra mussels of up to 8 cm in thickness provided a wet biomass of ~ 300 kg once removed. Maximum observed zebra mussel length in the rope was 22.26 mm and the maximum length in the pipes was 43.78 mm. After the intervention for cleaning and disinfection, a thorough visual examination of all of the system showed no live specimens.

Discussion

The population in the Alfundão Reservoir is in a straight-line distance of ~ 190 km of the nearest known locality in the Guadalquivir river in Spain, corresponding to a driving distance of 237 km and driving time of three hours. Local scale secondary dispersion of larvae by other vectors, such as birds or crayfish, can occur after long distance dispersal (Banha et al. 2015; Gonçalves et al. 2013; Johnson and Carlton 1996), but birds seldom visit this reservoir even if abundant in the nearby Pisão reservoir. In the region, there are crayfish fishing which are prone to accidental transport of invasive species (Banha and Anastácio 2015). This activity often involves fishermen transporting crayfish and fishing gear among Portugal and Spanish Andalusia areas already invaded by the zebra mussel. However, crayfish are very rare in the Alfundão Reservoir. Unintentional introduction by professional or sports fisheries activities or recreational kayaks are

unlikely since this is a fenced area with restricted access. No firewater has been collected from this region by plane. There is also a strong agricultural activity in the region by Spanish companies.

Whatever the introduction vector, individuals in the Alfundão outflow pipes may have established before the ones in the reservoir since both the biomass and sizes were much larger (max. length in the rope was 22.26 mm and max. length in the pipes was 43.78 mm). Although inflow pipes to Alfundão were only colonized in the final meters, the possibility of a colonization of zebra mussels from the Pisão Reservoir cannot be excluded. The Pisão was monitored from 2016 to 2018 by PCR and cross-polarized microscopy and from 2016 onwards using ropes, and no zebra mussels were detected. Additionally, visual checking and diving in Pisão after detection in Alfundão, showed no specimens and therefore it remains undetected there. However, temperatures in the Pisão allow zebra mussel development and reproduction during most of the year, excluding winter months (GISD 2015). Likewise, calcium concentrations above 20 mg/L occurred in all samples and allow for *D. polymorpha* growth and survival (Cohen 2005). pH levels in Pisão were also adequate for the survival or growth of the species throughout all year (Cohen 2005; Mackie and Claudi 2009).

If the eradication is not successful, *D. polymorpha* may have strong ecological impacts on habitats and biodiversity (see review by Karatayev et al. 2007). They have high filtration rates (Kryger and Riisgård 1988), resulting in increased water transparency and consequently changes in the phyto and zooplankton communities (Fishman et al. 2010). The shifts in the phytoplankton/zooplankton budgets may also lead to increased fish competition due to shifting diets, altering ecological balances in the ecosystems (McNickle et al. 2006; Strayer 2009) and may also decrease fish larvae growth (Raikow 2004). Additionally, they may lead to substrate modification altering energy budgets within the system, also affecting food chains (Birnbaum 2014). Large densities may affect fish spawning and feeding (Birnbaum 2014). Massive colonization by this mussel may lead to decreased oxygen levels due to respiration and increased dissolved organic matter related to excretion and overall nutrient cycling (Bruesewitz et al. 2006). Impacts on native bivalve species were also reported, leading to lower abundance (Dzierżyńska-Białończyk et al. 2018). Zebra mussels grow over unionid shells, aggregating near the posterior siphonal part, usually exposed above sediments, negatively affecting water exchange in the mantle cavity (Bódis et al. 2014). *Unio tumidiformis*, one of the most imperiled Iberian naiad species, occurs in this area, both in the Sado and Guadiana rivers. It was recently described as a separate species from *Unio crassus* (Reis and Araujo 2009) and has a status of vulnerable by IUCN (Araujo 2011). This is an endemic species in the south of Portugal and southwestern Spain and, like other unionids (Dzierżyńska-Białończyk et al. 2018; Sousa et al. 2011; Strayer and Malcom 2007), it may be negatively affected by zebra mussel invasion.

The introduction location is within the Sado river basin, close to the interface where water is transferred from the Guadiana to the Sado river when this is necessary. It is within the area of a large water management project (Empreendimento de Fins Múltiplos de Alqueva – EFMA) by a public company, EDIA – Empresa de Desenvolvimento e Infraestruturas do Alqueva. It includes 69 reservoirs of various sizes, interconnected by over 2,000 km of canals and pipes, with an area of influence over 10,000 km². Most of these structures are susceptible to the invasion by zebra mussel, with potentially large direct economic effects. Due to the potential economic and ecological impacts of the establishment of the species, monitoring will be maintained, focused but not restricted to the detection area.

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