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ORIGINAL PAPER

The role of waterfowl and fishing gear on zebra mussel larvae dispersal

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Abstract The zebra mussel, *Dreissena polymorpha* (Pallas 1771), is an invasive freshwater species with major negative impacts, promoting changes in ecosystem structure and function and also contributing to economic losses. Navigation has been considered the primary vector of dispersion and little importance has been given to alternative natural (waterbirds) and other human vectors. Using an experimental approach under field conditions, we evaluated and compared zebra mussel dispersal potential by fishing gear (waders and keepnets) versus mallard ducks (*Anas platyrhynchos*), by examining the adherence and survival rate of zebra mussel larvae on each vector. In addition, we evaluated the survival of zebra mussel larvae under desiccating conditions (i.e., a set of controlled temperatures and relative humidities). Larvae adhered to all types of vectors and survived desiccation under both laboratory and field conditions and thus appear able to be dispersed long distances overland by both ducks and fishing gear. Specifically, on a per-event basis, fishing gear has a higher potential to spread zebra mussel larvae than ducks. Survival was three times higher on human vectors and the number of larvae attached to human vectors was over double of that on the ducks. However, our findings demonstrate that natural vectors, like ducks, can contribute to the transport of zebra mussel larvae at a local scale. Nevertheless, since vectors related to human activity

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presented a higher potential for transport, it is imperative to continue campaigns to raise the awareness of anglers and boaters as well as continue the implementation of legislation to reduce the risk of zebra mussel dispersal.

Keywords Biological invasions · Desiccation · Dispersal · *Dreissena polymorpha* · Fishing gear · Waterbirds

Introduction

The zebra mussel, *Dreissena polymorpha* (Pallas 1771), is one of the world's worse invasive alien species (Lowe et al. 2000). Due to its filtering capacities, high densities and widespread distribution, *D. polymorpha* alters both the structure and function of the invaded environment, causing large shifts in the flow of energy from planktonic to benthic food webs. It also causes changes in water biochemistry, having been described as an ''ecosystem engineer'' (Bailey et al. 1999; Jones et al. 1994, 1997; Karatayev et al. 2002; Mayer et al. 2002; Simberloff and Von Holle 1999; Sousa et al. 2009; Strayer et al. 1998), and due to its fouling nature, this species is also responsible for large declines in the populations of native bivalve species (Sousa et al. 2011; Strayer 2008) and for major economic impacts on water-dependent industries and water-supply systems (Connelly et al. 2007; Durán et al. 2012; Pimentel et al. 2005; Sousa et al. 2014).

One of the most recent zebra mussel invasions in Europe occurred in the Ebro River basin, Spain (Altaba et al. 2001), and estimated costs exceeded 13 million euros in almost one decade (Durán et al. 2012). In the Ebro River basin, sport-fishing activities have grown in importance since the late 1970s as anglers from all over Europe come to this area to fish for wels catfish (*Silurus glanis*), carp (*Cyprinus carpio*) and black-bass (*Micropterus salmoides*). One hypothesis for the introduction of zebra mussel in the area is that larvae were brought in water buckets for the transport of live bait (Binimelis et al. 2007), but other vectors associated with fishing, such as boats or anchors, are also possible (Binimelis et al. 2007). Considering that the principal vectors for the large-scale spread of zebra mussels have been shipping and navigation activities (Bidwell 2010; Carlton 1996; Kraft et al. 2002; Minchin and Gollasch 2002), Spanish authorities adopted important measures and new legislation regarding navigation rules, including a restriction on the number of navigable reservoirs and the introduction of disinfection protocols. Actually, these measures together with an intense awareness campaign appear to have helped to slow down the spread of the zebra mussel (but see Johnson et al. 2006). In addition, disinfection has been recommended for all equipment used for freshwater sport and recreational activities including all associated gear that has been in contact with the water, such as life jackets, boots and other fishing equipment (Durán et al. 2010). Additionally, to prevent new biological invasions, the use of live bait (fishes, crustaceans, mollusks and other aquatic organisms) for fishing is now illegal (Ministerio de Medio Ambiente y Medio Rural y Marino 2007). The measures adopted on boat and shipping activities were implemented in agreement with most users, and there has been effective regulation and monitoring by authorities although the monitoring of the disinfection of smaller equipment, like fishing gear, remains difficult (Durán et al. 2010).

A major gap in the knowledge is the spread of zebra mussel by natural overland vectors (Johnson and Carlton 1996). In a scientific, but mainly in a management context, very low importance has been given to waterbirds as a mechanism of post-establishment spread of zebra mussel (Bidwell 2010; Carlton 1993; Johnson and Carlton 1996). Nevertheless, waterbirds have long been considered a major vector for the dispersal of aquatic organisms because of their abundant and widespread distribution across the world's wetlands, and their capacity to travel long distances (Figuerola and Green 2002). Moreover, the small size of zebra mussel larvae (200–300 μm) and their high densities would likely favor waterbird transport (Bie et al. 2012; Boag 1986; Figuerola and Green 2002). However, during overland transport, larvae would experience desiccating conditions, and they do not appear to have any adaptations for surviving such conditions, unlike the dispersive stages of some other freshwater organisms (e.g., the ephippia of cladocerans). Thus mortality during transport would be expected to be high.

The aim of our study was to investigate the importance of waterbirds, in relation to recreational fishing gear, on the overland transport of zebra mussel larvae. First, we examined the survival of larvae in the

laboratory under desiccating conditions that might cause mortality during transport. Although desiccation tolerance has been examined for juvenile and adult stages (McMahon et al. 1993; Ricciardi et al. 1995; Paukstis et al. 1999), it has never been examined for larvae, despite implications for dispersal. Second, we compared the adhesion of zebra mussel larvae to fishing equipment and bird feathers over different periods of immersion in water and assessed larval survival on these vectors during simulated overland transport. Finally, based on these results, we calculated potential dispersal distances and estimated the relative potential of these vectors as mechanisms of post-establishment zebra mussel spread.

Materials and methods

Survival of zebra mussel larvae

A laboratory experiment was performed to determine how long zebra mussel larvae can survive out of water under specific conditions in the absence of wind. We tested two different temperatures 17.5 and 27.5 °C, which correspond to the lowest and the highest values of the average mean temperatures in summer for the surrounding area of the Ebro River basin. These values were obtained from the governmental agencies "Agencia Estatal de Meteorologia" (AEMET) and "Instituto Meteorologia" (IM) (AEMET and IM 2011). At each temperature we determined survival at three different relative humidities, 30, 50 and 80 % using a refrigerated incubator (IngClimas model EC/E DBO). To obtain a humidity of 80 % an ultrasound humidifier [Honeywell BH-860 E] was placed inside the incubator and for a humidity of 30 %, 1 kg of silica gel was placed inside the incubator. The relative humidity values used were in the range of values occurring for the same area and season referred above (AEMET and IM 2011). These values are also very similar to the values used by McMahon et al. (1993).

The larvae used in this experiment were collected in the "Galachos de Juslibol" lake (41°42′15,022″N; 0°55′36,717″W; Zaragoza, Ebro River basin) by filtering and concentrating water with a 50-μm-mesh plankton net (KC-Denmark®, length of 125 cm, 30 cm diameter). The samples were collected from a 2.5-m vertical plankton tow taken from a boat. The sample was then concentrated into a 1.5 L plastic bottle, stored at the same temperature as the lake and transported to the laboratory (Facultad de Veterinaria, Zaragoza). This process took approximately 1 h before the start of the experiment. A single plankton sample was collected on six different days in 10th; 11th of June and 9th, 17th, 16th, 18th of July 2013. In each day, before sample collection, environmental variables in the lake were registered. Water temperature was 26.6 °C (±0.14 SD), mean pH was 8.1 (±0 SD), mean conductivity was 794 μs cm^{-1} (±9.86 SD) and mean dissolved oxygen was 11.15 g L^{-1} (±1.61 SD). Due to the logistic constraint of having only one incubator, the sample collected on any individual day was used for only a single combination of temperature and relative humidity conditions and thus there was no replication of the six treatments.

The sample was divided in the laboratory into equal parts into a number of cups depending on the abundance of larvae in the sample. The water in each cup was then filtered with a 6-cm-diameter disk of 50-μm Nitex mesh (Sefar Nitex® 03-50/37) so that the larvae were retained on the mesh. The mesh, containing an average of 140 larvae (95 % CI 106–173), was then placed into a plastic Petri dish (Fisherbrand, 90 × 16 mm) and then into the incubator. For each temperature/relative humidity combination, 6–12 such groups of larvae were prepared and then sequentially removed after different periods of air exposure ranging from 90 to 360 min. After each dish was removed from the incubator, 15 mL of larvae-free water from the collection site and 0.7 mL of neutral red solution (Rojo Neutro 10G DC Panreac Ref. 251619.1605) were added to the Petri dish to ascertain the number of surviving larvae (Horvath and Lamberti 1999). The larvae were left for at least 3 h in this solution after which the mesh was brushed to suspend any remaining larvae. The liquid was then centrifuged for 10 min at 1972*g* (Biofuge Primo Sorvall) and the concentrated precipitate was then immediately observed under a dissecting microscope (Nikon Eclipse E200; at 100× with cross-polarized light to find larvae [Johnson 1995] and at 400× to see details) or examined later after adding 1 mL of formalin (3 %). For distinguishing live and dead larvae, the light was changed to normal light, because the red color of the vital stain can only be seen with non-polarized light. The number of live and dead individuals of the different live stages (veliger and pediveliger) were counted with larvae colored with neutral

red considered alive and the larvae not coloured dead (Crippen and Perrier 1974; Horvath and Lamberti 1999). The percentage of live larvae was also quantified in the original water sample at the start of the experiment (i.e., time zero) to determine the initial condition of larvae in the samples of which 62 % were veligers and 38 % pediveligers.

Adhesion of zebra mussel larvae to waterfowl and human vectors

We investigated whether zebra mussel larvae can adhere to waterfowl (e.g., ducks) and to two different human vectors associated with recreational fishing equipment and compared the frequency of attachment to each vector. This experiment was conducted during 3 days on an irrigation pond near the town of Mequinenza (41°19′25,801″N; 0°17′31,981″W). The average water temperature was 23.5 °C (±0.8 SD), average pH was 8.5 (±1.0 SD), average conductivity was 916.2 µs cm^{-1} (±14.3 SD) and dissolved oxygen was 11.4 g L^{-1} (±2.4 SD). We tested two different periods of vector exposure, 1 and 10 min in the water. These two time periods were selected in order to test the effect of time over an order of magnitude, but still be short enough to allow replication. Additionally, these values were in the larger range of immersion times that anglers keep the gear in contact with water or ducks are on the water (pers. obs.). The fishing equipment used was: keepnet (bluefish®; nylon, 3 m long; 0.5 cm mesh; 50 cm diameter), neoprene waders (Storm®; size number 45) and a pair of waders boots (Rapala®, size number 45; felt soles). This equipment was chosen because it is commonly used by anglers on the Ebro River. Additionally, this equipment was studied earlier and was considered the type of fishing gear with the highest potential to disperse zebra mussel larvae (Asensio and Carreras 2009). We only used one wader/boot combination and one keepnet because this equipment is industrially manufactured with little or no variability.

To simulate waterfowl mediated passive dispersal, dead ducks (mallard, *Anas platyrhynchos*; mean weight of 1.15 kg) were used. This species was selected because of its abundance (Cramp and Simmons 1977) and high potential for local and regional migrations (Figuerola and Green 2002; Krementz et al. 2011; Rodrigues et al. 2000). All ducks were euthanized 1 day before the experiment, and kept frozen before use [euthanasia was performed by a registered veterinarian according to the Law of Animal Welfare following the procedure DOMTOR (medetomidine) + Imalgène 1.000 (ketamine) + T-61 (embutraide)]. To balance the need to include natural variation among animals with ethical issues, we used one duck per day (three total) as there was no observable damage to the plumage due to manipulations during the course of the days, and there were no trends in the number of larvae adhered to the plumage over time for any given treatment. To simulate exposure to larvae, the euthanized ducks were pulled with a rope by the same person wearing the waders and boots (a loop on the base of both wings), in the water at a speed of 0.5 m s^{-1}, the highest value of the range typical for duck swimming speed at low metabolic cost (0.35–0.5 m s^{-1}; Prange and Schmidt-Nielsen 1970), and at a depth of 60 cm. During the same period, the keepnet was placed into the water, and kept still as anglers normally use it. After the exposure period, all the vectors were removed and individually rinsed in a plastic box for 1 min using a garden hose with larvae-free water (preliminary trials were used to determine the time necessary to remove larvae). For each exposure period, we ran 30 replicate trials (10 with each duck), alternating between 1 and 10 min trials, over the 3 days (i.e., 60 trials in total). The rinse water from each vector was filtered with a 50-µm-mesh plankton net, and the resulting 100-mL sample was preserved by adding 1 mL of formalin (3 %) and kept on ice (during 3–21-h), being immediately processed on arrival to the laboratory. The total number of larvae in the sample was then determined, but due to the differences in the size, shape and material of the vectors, no attempt was made to standardize larval abundance other than on a "per-event" basis (e.g., the total number of larvae adhered to a pair of waders versus the total number of larvae adhered to a single duck). To determine the natural larval density during the experiment, a sample of pond water was collected each day with an 8-L plastic bucket and filtered using the same plankton net used for the rinse water. Each resulting 100-mL sample was preserved by adding 1 mL of formalin (3 %) and kept on ice during the transport to the lab. In the laboratory, the number of the different larval stages (veliger and pediveliger) was counted for all samples.

Survival of zebra mussel larvae transported on waterfowl versus human vectors

Survival of zebra mussel larvae on the three different vectors (duck, keepnet and waders) was examined in the field under simulated transport conditions. The larvae were obtained as described for the survival experiment under laboratory conditions. Plankton samples were separated into 27 plastic cups of 100 mL each. Nine cups were used per vector, each one corresponding to a certain transport time on the vector. An additional sample from the water was also used to determine the natural larval density on that day. Before use, all cups were kept at 20 °C without exposure to light. For each trial, water from one cup was slowly poured over each vector (one cup per vector). The vectors were then gently shaken for 4 s to remove excess water. Then, the human vectors (waders and keepnet) were placed in separate impermeable storage bags (included with each product when purchased) inside the car. The duck (euthanized one day before the experiment, and kept frozen before use) was suspended by taut cords in a position similar to a live duck during gliding flight (extended neck and wings, legs extended near tail) from a metal structure attached to the top of the car. Since the mean flight speed for *Anas* spp. ranges from 60 to 78 km h^{-1} (Welhun 1994), the car was driven at a constant speed of 75 km h^{-1}, during 20, 40, 60, 80, 100, 120, 180 and 240 min. The human vectors were tested at the same time (i.e., placed in the trunk of the car) for all but the 180 min trial, which was replaced by a longer trial of 930 min (but without vehicle movement). The sequence of time trials was random. After the transport period, each vector was individually and thoroughly washed for 1 min with a garden hose. The water from each vector was collected individually, filtered with the plankton net and placed into a plastic cup with 1 mL of neutral red solution. All samples were kept refrigerated during transport back to the laboratory where they were examined for live and dead larvae as described above. The proportion of dead larvae was estimated in the original water sample at the start of the transportation trials (i.e., time zero) to determine the initial conditions but also at the middle and at the end of the experiment in the control samples. During the experiments, the average air temperature inside the car was 26.7 °C (±2.4 SD) and the average air relative humidity was 39.7 % (±6.9 SD). Outside the car, the average air temperature was 25.4 °C (±1.9 SD), the average air relative humidity was 38.1 % (±4.8 SD) and the wind speed was 2.7 m s^{-1} (±1.1 SD).

Statistical analysis

Statistical analyses were performed using IBM® SPSS® version 20. Probit analysis was used to calculate the probability of zebra mussel larvae survival and the time for 50 % (LT_{50}) and 90 % mortality (LT_{90}) in the laboratory experiment. As we did not replicate the different temperature/relative humidity treatments, differences between trials cannot be strictly interpreted being due to the environmental parameters that we manipulated.

The dependent variable from the adhesion experiment, namely the number of larvae adhered to an individual vector, was transformed [$\log(X + 1)$] to meet the assumptions of a normal distribution and to achieve homoscedasticity. After this transformation, the influence of vector type and immersion time was analyzed using a two-way ANOVA. Time and vector were considered fixed factors. We use the Tukey HSD post hoc test to determine which pairs of vectors differed significantly and to determine differences between exposures for each vector. For the experiment on survival during transport, the mean survival time of larvae on the different vectors was calculated using a Kaplan–Meier test. To assess the differences in survival time between vectors, a pairwise-comparison log-rank (Mantel–Cox) test was used. In this last experiment, we used a different statistical analysis from the first experiment for survival analysis due to some censored data (e.g., for human vectors we did not achieve 100 % mortality) (Banha and Anastácio 2012).

Results

Survival of zebra mussel larvae

During air exposure, the proportion of dead larvae generally increased logistically with time in all trials (Fig. 1). As expected, the larvae survived longer at lower temperatures (17.5 °C) except at the highest relative humidity (RH) value used (80 %), with only 10 % of the larvae alive ("LT_{90}", "lethal time" until 90 % mortality) after approximately 3 h in both trials.

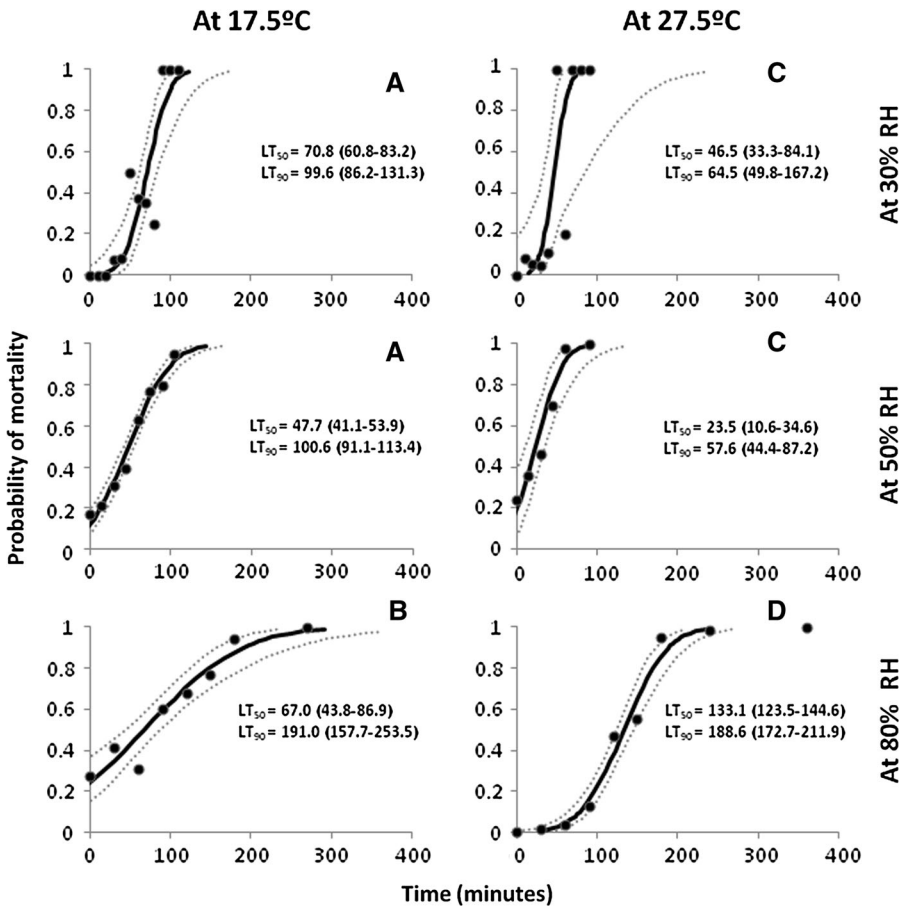

Fig. 1 Zebra mussel larvae mortality as a function of the time spent out of water at six different combinations of temperature (°C) and relative humidity (%). The *black circles* are the observed proportions of dead larvae; the *black line* with the respective 95 % confidence intervals (*dotted line*) was obtained by Probit analysis. *Different letters* represent statistically significant differences in survival between trials [pairwise comparisons Log Rank (Mantel–Cox): $P < 0.005$]

The same pattern occurred for the LT_{50} values, except at 80 % RH where the time to 50 % mortality was twice as long at the higher temperature. This non-intuitive result is likely due to the lack of replication in this experiment (see above). Nevertheless, overall the results support the idea that increasing mortality occurs at higher temperatures and lower relative humidities. More important, regardless of the treatment conditions, survival of larval stages out of water appears to be only a matter of hours, even under the benign laboratory conditions. The mean percentage of live larvae in the original water sample at the start of the experiment (i.e., time zero) was 92.6 % (76–100 % range).

Zebra mussel larvae adhesion to waterfowl versus human vectors

The type of vector significantly influenced the number of larvae adhered (Table 1) as the mean number of larvae adhered to the ducks was lower than the mean number adhered to both human vectors, with a major difference observed for the keepnet (Tukey HSD test, mean difference = −0.336; standard error (SE) 0.076; $P < 0.001$; 95 % CI −0.517 to −0.156) followed by waders (Tukey HSD test, mean difference = −0.188; SE 0.076; $P = 0.039$; 95 % CI −0.368 to −0.008). Indeed, for both submersion periods used, the mean number of larvae adhered to the human vectors was more than double that of the ducks (Fig. 2). However, between the two human vectors no significant difference was observed (Tukey HSD test, mean difference = 0.148; SE 0.076; $P = 0.130$; 95 % CI −0.032 to 0.328).

Overall, the immersion time of the vector also affected the number of adhered larvae (Table 1) with more adhered larvae for the 10-min trials relative to the 1-min trial (Fig. 2). However, the post hoc Tukey HSD test did not show any differences between immersion times for each individual vector (duck: Mean difference = −0.162 larvae adhered/trial; SE

Table 1 Influence of vector type and immersion time on log (number of larvae adhered +1) analyzed using a two-way ANOVA

Source	Type III sum of squares	*df*	Mean square	F	Sig.
Corrected model	4.246	5	0.849	4.868	<0.001
Intercept	154.055	1	154.055	883.089	<0.001
Time	0.763	1	.763	4.373	0.038
Vector	3.408	2	1.704	9.769	<0.001
Time × vector	0.075	2	0.037	0.215	0.807
Error	30.354	174	0.174		
Total	188.655	180			
Corrected total	34.600	179			

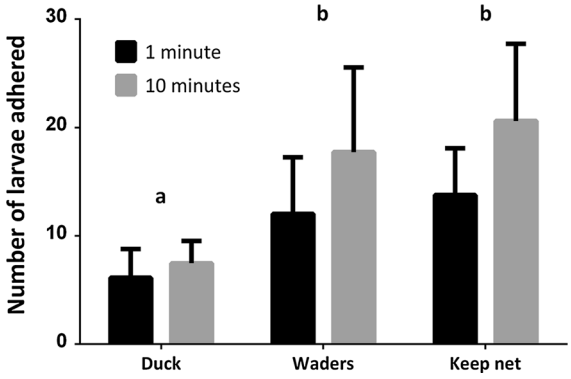

Fig. 2 Mean number of larvae (+SD) adhered to the vectors at two different submersion periods (1 and 10 min). *Different letters* indicate significant differences ($P < 0.05$) between vectors

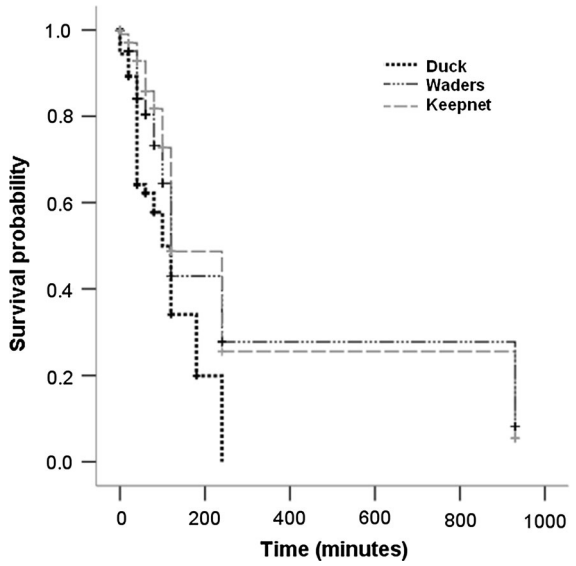

Fig. 3 Survival as a function of time of zebra mussel larvae on natural (ducks) and human vectors (two types of fishing equipment: waders and keepnets) during transport under simulated field conditions (duck flight and storage of fishing equipment in automobiles). Results obtained after Kaplan–Meyer analysis

0.108; $P = 0.661$; 95 % CI −0.473 to 0.149; waders: Mean difference = −0.072 larvae adhered/trial; SE 0.108; $P = 0.985$; 95 % CI −0.383 to 0.238; keepnet: Mean difference = −0.156 larvae adhered/trial; SE 0.108; $P = 0.700$; 95 % CI −0.466 to 0.155). Moreover, the observed increases in the number of adhered larvae were only 15–40 %, in spite of the 10-fold longer exposure. An average density of 12.3 larvae L^{-1} (±9.6 SD; n = 3), with a proportion of 80 % of veliger and 20 % of pediveligers was found in the pond water.

Survival of zebra mussel larvae transported on waterfowl versus human vectors

Survival out of water decreased over time for all vectors (Fig. 3). No larvae were alive after 240 min on the duck vector, but at the longest time period tested, 930 min, 29 % of larvae on the waders were alive and 21 % were alive on the keepnet. The Kaplan–Meier analysis shows a similar mean survival time for the keepnet, with 341 min (SE 29; 95 % CI 285–397 min), and for waders, with 342 min (SE 34; 95 % CI 276–408 min). In fact, there were no differences between the zebra mussel larvae survival time on these two human vectors [Log Rank (Mantel–Cox): $X^2 = 3.432$; $df = 1$; $P = 0.064$]. Yet, for the duck, the Kaplan–Meier calculated a mean survival time of 116.0 min, approximately a third of the survival time for the human vectors (SE 11.0; 95 % CI 94.4–137.5 min). The pairwise comparisons show that there were differences between the survival time on this vector and on the waders [Log Rank (Mantel–

Cox): $X^2 = 16.331$; $df = 1$; $P < 0.001$] but also when compared with the keepnet [Log Rank (Mantel–Cox): $X^2 = 33.421$; $df = 1$; $P < 0.001$]. The mean number of larvae recovered from each vector was 10 ± 7.2 SD (3–24 range) for the duck; 54.1 ± 41.5 SD (17–128 range) for the waders and 56.4 ± 38.6 SD (17–146 range) for the keepnet. The mean number of larvae in each control cup was 172 ± 95.9 SD (70–260 range). The proportion of dead larvae in the controls at the beginning, middle and at the end of the experiment was 16, 40 and 37 %, respectively. 35 % of the larvae were pediveligers and 65 % were veligers.

Discussion

Our work showed that zebra mussel larvae survive out of water for periods that may allow long-distance overland dispersal, i.e., distances over 10 km according to Green and Figuerola (2005). Furthermore, our results are consistent with previous work on this species, namely the negative effect of higher temperatures and low humidity/dry conditions on the survival out of water (Paukstis et al. 1999; Ricciardi et al. 1995). Our estimates of larvae survival time under desiccating conditions in the laboratory (i.e., $LT_{50} = 46.5$ at 27.5 °C and 30 % RH) were much lower than those obtained for the natural vector in the field (mean survival = 116.0 min at 26.7 °C and 39.7 % RH) even when comparable air temperature and relative humidity conditions were used. It is possible that the plumage maintained the moisture, resulting in a higher survival rate on the duck, in spite of the wind which may amplify desiccation.

Waterbirds and fishing gear, like waders and keepnets, constitute potential vectors of transport. According to our findings, and assuming a flight speed of 75 km h^{-1} (Welhun 1994), the zebra mussel larvae could be transported 145 km by ducks, with a 50 % chance of survival. Moreover, anglers driving between sites could transport zebra mussel larvae with 50 % survival rates for 500–700 km on keepnets and waders, given speed limits on roadways (90 km h^{-1}) and highways (120 km h^{-1}) in Portugal and Spain. As shown by the lab experiment, these transport distances could be affected by weather conditions, increasing in cooler and more humid conditions and decreasing in opposite scenarios. Additionally, these values could even be higher under real conditions since the zebra larvae in our experiments may have suffered some stress or damage due to the capture, handling and transport process. On a per-event basis, our study showed that human vectors (fishing gear) have more potential to spread zebra mussel larvae than natural vectors such as ducks, especially as they transport more larvae (approximately double). Nevertheless, this difference is small relative to a previous comparison of human and natural vectors (Johnson and Carlton 1996), where there was a three-orders-of-magnitude difference between larvae transported in boat live wells relative to ducks.

Surprisingly, and contrary to our expectations, the immersion time of the studied vectors did not play an important role in the number of larvae that adhered to it. According to our findings, a 1000 % increase in exposure time to water containing zebra mussel larvae only results in a 15–40 % increase in the number of larvae adhered to the vector. Therefore the number of larvae adhered to a vector seems to be more affected by vector characteristics (e.g., surface) than by the exposure time, and anything that comes into contact with contaminated water, even for very short periods, needs to be disinfected or thoroughly dried.

The importance of any particular vector will depend on the stage of the life cycle that is transported, the number of surviving mussels transported per dispersal event, the frequency of such events, and the spatial patterns of vector movement (Johnson and Padilla 1996). Our assertion of higher risks associated with fishing gear relative to waterfowl is largely due to the threefold higher survival of larvae when transported by human vectors. The lower survival on waterfowl is likely due to the exposure of larvae to the wind, which will dry plumage faster than on fishing gear kept inside a vehicle. Additionally, the number of larvae that adhered to the human vectors was more than double that of the natural vector, which could also contribute to a higher dispersal risk by these vectors (i.e., higher propagule pressure Simberloff 2009). Finally, the maximum speed is higher for human vectors than for birds.

However, our findings show that waterbird-mediated dispersal of zebra mussels may be a more relevant process than previously acknowledged (Bidwell 2010). In fact, our values of larvae adhesion to ducks were 6–7 times higher than the ones found by Johnson and Carlton (1996) in which they obtained less than 1 zebra mussel larvae/bird. Considering our findings of

adherence and survival rate on ducks, we estimate that a single duck can transport 3–4 live larvae for more than 100 km.

Compared with our results, recent studies on waterbird dispersal of other aquatic organisms, namely larger crustaceans, have shown a smaller number of individuals adhered per event, 3–7 times lower, and shorter transport distances (Águas et al. 2014; Anastácio et al. 2013; Banha and Anastácio 2012; Rachalewski et al. 2013). Short-distance mallard flights are more common than large movements. Mean flight distances are between 1 and 2 km for foraging away from roost sites (Legagneux et al. 2009), and are 15 km for female mallard movements between diurnal and nocturnal sites (Link et al. 2011). Taking into account our results and the fact that a duck only needs 2–12 min to fly those distances, we conclude that the zebra mussel larvae transported in such a dispersal event would present a survival probability near 100 %. So, transport within these distances is very likely, because vector movements are frequent and the survival rate of the propagules is very high. Therefore, the transport of zebra mussel larvae by ducks (and possibly other bird species) should be considered an important process at a local scale.

The high survival of larvae on the human vectors shows that they can survive overnight. Therefore the implementation of disinfection protocols by anglers is essential to block the spread of zebra mussels by these vectors. The Ebro River is not only a hotspot for European anglers from countries already invaded by zebra mussel, like France, but also from currently uninvaded regions of the Iberian Peninsula. Therefore, our findings show that zebra mussel larvae are likely to expand to the northwest or the south-west of the Iberian Peninsula due to transport by anglers. A crucial fact that favors zebra mussel dispersal by the vectors examined here is that the peak of larvae abundance occur in the summer months (Durán and Anadón 2008; Mackie 1991), which matches periods of high abundance and activity of both types of vectors, with *Anas platyrhynchos* highest density in August (Holgado and Menárguez 2012) and the summer holidays when many anglers from other counties visit the Ebro River region (Gomez 2005).

Our work provides a first step in investigating the dispersal of zebra mussel by comparing vectors on a "per event" basis. Future studies on the frequency, movement distance and routes of different vectors should be assessed by further fieldwork and would complement our work. This information could be used to model dispersal probabilities and develop spatially-explicit maps of invasion risk. However, our work has some limitations. First, the experimental design of our survival experiments did not include replication of the different treatments (e.g., combinations of temperature/relative humidity in the laboratory; vectors in the field) and thus need to be interpreted cautiously. Also, the use of a dead duck does not replicate exactly the movements or conditions of a live duck. For example, duck feet were not used for swimming and during the simulated flight only gliding was replicated.

As shown in earlier studies on zebra mussels (Johnson and Carlton 1996) and other aquatic invertebrates (Águas et al. 2014; Anastácio et al. 2013; Banha and Anastácio 2012; Frisch et al. 2007; Rachalewski et al. 2013), our findings show that natural vectors, like ducks, can transport zebra mussel larvae between waterbodies. We suggest that dispersal of larvae by natural vectors may lead to secondary spread and that such natural spread may be less of a "mussel myth" than previously asserted (Johnson and Padilla 1996). However, in order to prevent zebra mussel spread, quantitative and comparative knowledge of the risks of different vectors is needed, both among different vectors associated with human activities (Johnson et al. 2001; Kelly et al. 2013; this study) and relative to natural vectors (Johnson and Carlton 1996; this study). We also conclude that it is essential to continue awareness campaigns for anglers and boaters (Simberloff et al. 2013) as well as the implementation of further legislation to manage human vectors in the context of the risk of biological invasions.

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