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Review

Can recently-hatched crayfish cling to moving ducks and be transported during flight?

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ABSTRACT

The red swamp crayfish (*Procambarus clarkii*) is a freshwater invasive species which has become a worldwide problem. Recent work on ectozoochory of freshwater macrocrustacean species indicated that there might be a possibility of transport of recently-hatched crayfish by birds. In this context, we applied a new set of methods to quantify the probability of transport of recently-hatched crayfish, namely with moving animal vectors. First, we tested the desiccation resistance of crayfish and the capacity of crayfish to cling to mallard's feet, depending on the standing time of the feet. We also determined the ability of recently-hatched crayfish to cling to an artificially moving freshly dead mallard (*Anas platyrhynchos*) and finally, we determined the time required for the death of 50% and 90% of the individuals of recently-hatched crayfish under conditions similar to those of mallard flight.

Recently-hatched crayfish were able to survive up to 225 min out of water, withstanding longer at a lower temperature and therefore transport does not seem to be limited by desiccation survival. The duration of the standing period of duck's feet positively affected the probability of transport of crayfish. Recently-hatched crayfish were able to cling to a moving duck and the probability of clinging was significantly affected by the water depth, being lower at greater depths. Moreover, when transported on a freshly dead duck under flight simulation conditions the time required for the death of 50% and 90% of the individuals were 2 min 14 s and 4 min 53 s, respectively. These flight durations correspond to transport distances of 2.8 km and 6.1 km, respectively, which is enough for transport to another aquatic system. The results demonstrate that passive transport of recently hatched *P. clarkii* by actively moving waterbirds is possible, and therefore it will likely enhance the local process of invasion.

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Introduction

The introduction and spread of invasive species is currently considered one of the greatest threats to global biodiversity (McCarthy et al., 2006). It may cause major changes in the functioning of ecosystems, as invaders affect the distribution and abundance of native species, which can lead to its decline (Lodge et al., 1998; Correia and Anastácio, 2008; Strayer, 2012). Crustaceans are, among all aquatic invaders, the most successful group and crayfish invasions have been increasing in recent years (McCarthy et al., 2006; Dana et al., 2011; Hanfling et al., 2011; Van Leeuwen et al., 2012).

Originally from the southern U.S. and northern Mexico, the Louisiana red crayfish *Procambarus clarkii* (Girard, 1852) (Decapoda, Cambaridae), was introduced in Europe in the 1970s (Holdich et al., 2009) and is now present in five continents – Africa, Asia, Europe, North America and South America (Capinha et al., 2010). In Portugal the first occurrence of the species was in 1979 in the Caia River (a tributary of the Guadiana River) (Ramos and Pereira, 1981) and in 1986 it was well established in southern and central Portugal. Soon after, damage in rice production was observed (Anastácio et al., 2005). This species disperses mainly by human transport and active dispersal (Ferreira, 2011). It is classified as one of the most invasive species in Europe (DAISIE, 2008).

Invasive aquatic invertebrates and plants often have a widespread distribution, despite the isolation between freshwater habitats (Waterkeyn et al., 2010a). Passive dispersal is a key factor for these distribution patterns and constitutes a crucial process for the maintenance of species and genetic diversity), and also plays an important role with regard to climate responses (Jenkins and Buikema, 1998; Bilton et al., 2001; Figuerola and Green, 2002a; Bohonak and Jenkins, 2003; Waterkeyn et al., 2010b; Van Leeuwen et al., 2012). Many organisms achieve a broad distribution by active means such as flying, but others are unable to disperse by themselves over long distances. These depend on other factors such as animals, wind or water for a passive scattering, which in case of ectozoochory often involves a stage resistant to desiccation, specific to the life cycle (Bilton et al., 2001). Although there is much information about the animal-mediated dispersal in terrestrial ecosystems, little information exists about the processes affecting long-distance dispersal (LDD – distances above 10 km) (Green and Figuerola, 2005) between aquatic habitats (Figuerola and Green, 2002a).

Recognised as an important factor in long distance dispersal, waterbirds are a dispersal vector for some organisms, due to their abundance, widespread distribution, and high frequency of movements within and between habitats (Figuerola and Green, 2002a,b; Ferreira, 2011; Raulings et al., 2011; Van Leeuwen et al., 2012). Moreover, the fact that aquatic seeds and invertebrates are included in the diet of most species of the family *Anatidae* (which include ducks, geese and swans), increases the chances of internal or external transport (Green et al., 2002; Van Leeuwen et al., 2012). The dispersal of propagules by birds may occur in three ways: through the feathers, feet and beak; by regurgitation of the oesophagus contents; and through the intestine by faecal deposition (Green et al., 2002; Raulings et al., 2011). Morphology and size of propagules affects transport frequency (De Bie et al., 2012; Figuerola et al., 2005). As an example, experimental studies demonstrated that *Cercopagis pengoi* fouled to the feathers of *Aythya affinis* (Makarewicz et al., 2001). This allows the invasion among wetlands, since it is possible that a waterbird transports this animal with eggs between lakes at a distance of about 50 km However, this vector is less important than human-mediated transport (Makarewicz et al., 2001).

The processes affecting dispersal among freshwater habitats are not yet well known, particularly with regard to decapods' passive dispersal. Recent findings showed the ability of birds to transport two types of decapods, i.e., juvenile Louisiana red crayfish (*P. clarkii*) (Ferreira, 2011; Anastácio et al., 2013) and river shrimps (*Athyaephyra desmaresti*) (Banha and Anastácio, 2012). In this context, several experiments were performed to test the hypotheses that recently-hatched *P. clarkii* can survive air exposure and that these can cling and can be transported by moving waterbirds. Therefore we tested the desiccation resistance of crayfish, the capacity of crayfish to cling to mallard's feet, the ability of recently-hatched crayfish to cling to an artificially moving freshly dead mallard (*Anas platyrhynchos*) and the LT_{50} and LT_{90} of recently-hatched crayfish under conditions similar to those of mallard flight.

Materials and methods

Recently-hatched *P. clarkii* were caught in Paúl de Magos, in Salvaterra de Magos, and Divor dam, Portugal with 1 mm mesh dip nets, or were obtained through laboratory reproduction from females captured at the same locations. The crayfish were kept in the laboratory in tanks with continuous aeration for at least 24 h before each experiment at a temperature of 17 °C and a 12/12 photoperiod and were fed with Tetra® pond koi sticks. The values of the environmental variables (mean air temperature, relative humidity, wind speed, wind direction, precipitation) from the experiment "Survival rate during flight" were obtained from the nearest weather station (Évora's Geophysical Centre – Mitra Station, which is approximately 8 km away from where the experiment was performed). The air temperature and relative humidity from all other experiments were obtained on site using portable devices such as a thermo-hygrometer, and a water temperature, pH, conductivity and dissolved oxygen probe (WTW Multiline F-set). Crayfish total size was measured with a digital calliper. In order to obtain recently-hatched juveniles in the laboratory, 6 adult males and 6 adult females were kept in individual containers (55 cm × 45 cm × 40 cm), for about 20 days using the method described by Huner and Barr (1991). Two mallard ducks were bought on a local food market. The birds were transported in humane conditions with minimum stress to the veterinary hospital of the University of Évora. Subsequently, the birds were euthanized with pentobarbital by a veterinary. The bodies were immediately refrigerated until the start of the experiments. Furthermore, duck's legs were separated just before performing the experiment in which they were used. PASW Statistics 18 (formerly SPSS Statistics) was used for all the statistical analysis.

Desiccation survival

To understand for how long recently-hatched crayfish can remain out of water, two different temperatures were used: 24 and 19 °C, which is within the interval of mean Summer and Autumn temperatures (World Meteorological Organization, 2014) of *P. clarkii*'s current European distribution and suitable areas for expansion (Capinha and Anastácio, 2011). Furthermore summer and autumn correspond to the major recruitment periods of the species (Anastácio and Marques, 1995; Fidalgo et al., 2001; Adão and Marques, 1993). Since it was not possible to obtain a sufficient number of animals from the same source for both temperatures, for the temperature of 24 °C, recently-hatched juveniles were obtained by laboratory reproduction of adults from Salvaterra de Magos, and for the 19 °C temperature the recently-hatched juveniles came from the same river basin of the adults. 49 individuals (mean total length, TL: 8.56 mm ± 0.55 S.D.) were placed in individual plastic cups, with a diameter of 3 cm and 5 cm in height, and kept at a 24 °C temperature and 35% relative humidity. Every 30 min, 7 cups were taken randomly and the number of deaths was recorded. The procedure was repeated with another set of 49 individuals (total

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