SURVIVAL OF MALE AND FEMALE *CERASTODERMA GLAUCUM* (BIVALVIA) DURING AERIAL EXPOSURE

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AERIAL EXPOSURE
CERASTODERMA GLAUCUM
SEX-BIASED DISPERSAL

ABSTRACT. – The lagoon cockle, *Cerastoderma glaucum* (Poiret, 1789) usually inhabits small, often isolated, water basins. Dispersal among these basins can take place overland (human mediated) or by migratory birds, when cockles are attached to their bodies. It requires the capacity of the cockle to survive aerial exposure. The time of survival of cockles in air was tested. The LT_{50} was of 80 h at 15 °C and 43 h at 22 °C. There was no significant difference in survival time neither among size classes, nor between males and females. The similarity between male and female mortality curves and the strong differences between the results coming from two thermal conditions suggest that the experiment was reliable.

INTRODUCTION

The lagoon cockle *Cerastoderma glaucum* (Lamellibranchia: Cardiidae) is a euryhaline and eurythermic (Rygg 1970) bivalve present across Europe from the Caspian to the Baltic Seas (Brock 1979). The distribution area of *C. glaucum* even if wide, is very fragmented. It usually inhabits isolated or semi-isolated, shallow, non-tidal biotopes, *e.g.*, brackish lagoons, estuaries, bays and lakes. It was previously thought that the main limiting abiotic factor for this cockle was its intolerance to air exposure provoked by tides (Russell 1971, 1972). However, apparently, the nature of sediment is more important than the direct influence of tides. This species never occurs on bottoms with loose sediment structure typical of regions exposed to tides, currents and waves (Brock 1979).

The pelagic larval stage of the lagoon cockle is restricted to 1-2 weeks (Lauckner 1972, Kingston 1974, Wołowicz 1987). As the distribution areas of this species are often separated by unsuitable habitats (insularity), alternative dispersal modes necessarily allowed the founding of such populations. *C. glaucum* is probably not dispersed by man for aquaculture purposes, because it only has a marginal commercial value (Arjonilla *et al.* 1994). However, it may be transported as a result of anthropogenic activities, such as constructing canals connecting separated basins, or pumping water from one water basin and releasing it into another (*e.g.* for the needs of power plants) [for a freshwater bivalve, *Dreissena polymorpha* (Pallas, 1771) Quaglia *et al.* 2008]. Furthermore, the spat of *C. glaucum* sticks to the surface of submerged plants, and juveniles climb and attach to substrates with their byssal gland (Pearson 2003, Reise 2003). They may therefore be transported among water basins with these plants attached to the engines of small trailerable boats or to other recreational equipment (Quaglia *et al.* 2008). More-

over, migrating birds seem a plausible vector provoking gene flow (discussed in Tarnowska *et al.* 2010). The role of birds in the dispersal of *C. glaucum* has been previously evoked by many authors (Boyden & Russell 1972, Rose 1972, Gasse *et al.* 1987, Spencer & Patchett 1997, Reise 2003, Nikula 2008). Interestingly, Cadée (1995) suggested a possibility of internal transport of adult cockle, as a specimen of *Cerastoderma edule* (Linnaeus, 1758) was observed alive after regurgitation by a herring gull and found in the act of burrowing again in the tidal flat. However, for mollusks external transport seems to be more common (Wesselingh *et al.* 1999). Adult bivalves may be dispersed attached to birds feet (*e.g.*, Darwin 1878, Kew 1893, Boycott 1936, Rees 1965, Green & Figuerola 2005). Larvae and spat may also be transported externally, for example on birds plumage (Green & Figuerola 2005 and references therein). Additionally, spat and juveniles of *C. glaucum* may be transported by birds together with plants (Reise 2003). Many bird species perform regular migrations between the southern and the northern parts of Europe. They rest and feed in lagoons and estuaries which are often inhabited by *C. glaucum* (Wołowicz 1991). Any overland transport or the transport of cockles attached to birds' feet would require resistance to aerial exposure.

Recent genetic studies of *C. glaucum* populations from almost the whole distribution area revealed very different genetic structure patterns between mitochondrial DNA and microsatellite markers (Tarnowska *et al.* 2010). In mitochondrial DNA, contrary to the nuclear DNA, some geographical discontinuities were found in genetic structure. As mitochondrial DNA is transmitted in the maternal lineage and nuclear DNA is transmitted by both parents (Chenuil 2006), such differences suggest migration rate differences between males and females. It seems unlikely that a male and a female larva behave or even survive differently. However, adult males and females often display

different physiological conditions (thus different survival rates facing stress) due to differences in reproductive efforts in animals (Tarnowska *et al*. 2009). Gender-biased dispersal could hypothetically be driven by gender differences in survival during aerial exposure and starvation involved with passive dispersal mechanisms (*e.g.,* transport by migrating birds or human activity). Gender differences in survival rates, susceptibility to diseases and life span are not rare phenomena (*e.g.,* in human: Olivetti *et al*. 1995, Macintyre *et al*. 1996, Arbuckle 2006) and are even theoretically expected, for instance, in hybrid zones according to sex determinism mechanisms (*e.g.,* Haldane 1922, Chenuil *et al*. 2004). A recent genetic study on the lagoon cockle revealed a striking similarity in the mitochondrial marker of cockles from the Iberian Peninsula and the Baltic Sea (Tarnowska *et al*. 2010). Some waders (Charadriiformes) that undergo particularly long migratory flights use the East Atlantic Flyway, which is a major route for long-distance transport between these two regions (Sánchez *et al*. 2006, Sánchez *et al*. 2007). This suggested that long distance dispersal *via* migrating birds and gender differences in survival during this transport may together explain the particular genetic structure observed in the lagoon cockle at mitochondrial and nuclear markers. Theoretically, even if long distance dispersal occurs for a single individual, it may enable its genotype (or mitochondrial haplotype) to invade an already settled population due to genetic drift or natural selection. This hypothesis led to this experimental study.

MATERIALS AND METHODS

Cockles used for the experiment were sampled on 8th June 2010 in the Berre Lagoon (Plage du Jaï) on the French coast of the Mediterranean Sea (43°24'N, 5°08'E). After sampling, the 84 cockles were transported to the laboratory (30 minutes car trip) in sea water at the same temperature as the sampling site (22 °C). The specimens were sampled from the surface of the sediment. The cockles were divided into two lots, in a way respecting similar size distributions. They were placed on a dry tray, on a laboratory table, in the same orientation, the distance among specimens being of a few centimeters. The first lot was placed at 22 ± 1 °C (exposed to indirect sunlight during the day, in a laboratory room with air conditioning) and the second at 15 ± 1 °C (in the dark). The light conditions differed because of the laboratory conditions constraints. The air temperature was controlled. The number of dead individuals was controlled at more or less regular time intervals. Cockles were considered dead when the shell was slightly open and when applying a contact to the shell did not provoke its closing due to the action of adductor muscles. Each time a cockle was found dead the duration of the survival in air was recorded. The LT_{50}, which is the exposure time required to kill 50 % of the test population, was calculated for each temperature. Then shell length, width and height were measured with a slide caliper accurate to 0.01 mm.

The dead cockles were placed individually in small plastic bags filled with sea water at 4 °C and stored until observation (maximum 2 days). Gonad tissue (equivalent to a volume of a few microliters) was spread on a glass slide (those classically used for histology) and the slide was placed on a heating plate at 50 °C for a few seconds. A few drops of toluidine blue were spread at the slide for coloration and the excess of it was rinsed for a few seconds under distilled water flow. The sex of each individual was then determined under an optical microscope according to a reliable protocol, since eggs and sperm are distinguishable regardless of gonad development stage (Wołowicz 1987, 1991).

The exact test of Fisher was applied to test the significance of differences in the sex ratio. The 95 % confidence intervals were calculated for the proportion of females (Newcombe 1998). Other statistical analyses were performed using XLstat v.2011. The normality of the data distribution was checked using the Shapiro-Wilk test (significance level 5 %). The significance of the correlations of the survival time in air and every dimension of the shell (length, width and height) was checked with the Spearman (non-parametric) test. The effects of sex and size (three size groups chosen *a priori* attributing equal number of individuals to each group) on survival time in air were investigated with the Kruskal-Wallis non-parametric test.

RESULTS

The proportion of females was 0.37 ($\pm$ 0.1), but the females to males ratio did not significantly differ from 1: 1 (p > 0.05). The observed survival time data did not have a normal distribution. The temperature of the room strongly influenced survival time, which was significantly longer at 15 °C than at 22 °C (p < 0.0001) (Fig. 1). The LT_{50} was 80 h at 15 °C and 43 h at 22 °C. The mortality exceeded 80 % after about 100 h at 15 °C and after about 50 h at 22 °C. No significant difference was found between the survival time of males and females in both samples (p = 0.644 at 15 °C, p = 0.540 at 22 °C) and the whole curves were very similar (Fig. 1). The survival time at both temperatures slightly diminished when cockle size increased, but neither the correlations (p = 0.346 and 0.635 respectively at 15 °C and 22 °C) nor the Kruskal-Wallis tests (p = 0.838 and 0.628 respectively at 15 °C and 22 °C) were significant.

DISCUSSION

Survival time and the role of air temperature

In our study the LT_{50} of *C. glaucum* during the aerial exposure was of 80 h (more than 3 days) at 15 °C and
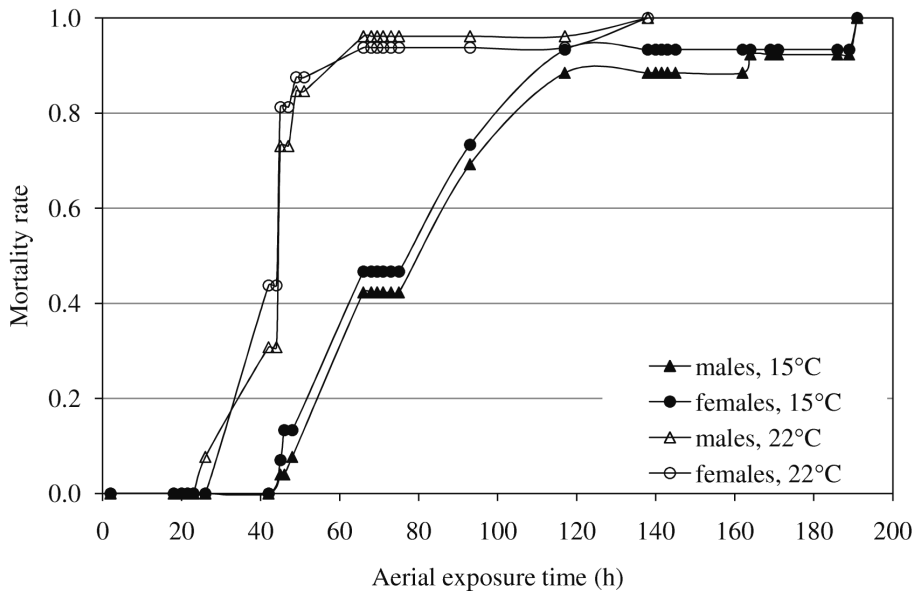
Fig. 1. – Mortality of cockles during the aerial exposure for each sex at 15 °C and 22 °C.

of 43 h (less than 2 days) at 22 °C. These durations were slightly shorter than the ones found by Boyden (1972): LT_{50} of 87 h (almost 4 days) at 15 °C and of 63 h (almost 3 days) at 20 °C. Comparisons among these studies are difficult to perform in a reliable way since numerous conditions were different. In particular, our study did not control and did not record humidity values, and light conditions were not identical between the two temperature conditions. Survival time of the common cockle, *Cerastoderma edule* was longer than that of *C. glaucum*, reaching 129 h at 15 °C and 89 h at 20 °C (Boyden 1972). This is because some mollusks, especially those inhabiting intertidal regions, like *C. edule*, have a capacity to air-breathe (Boyden 1972 and references therein). *C. glaucum* cannot air-breathe (Boyden 1972). Dance (1958) reported that a freshwater mussel survived 12 months out of water.

The bivalves which cannot air-breathe initially utilize the oxygen dissolved in the water present in the shell. Afterwards, the conditions become anoxic. The ability to survive in anoxic conditions or low oxygen contents varies among bivalves reaching 2 weeks for *Nucula sulcata* (Bronn, 1831) (Taylor *et al.* 1995), 5-17 days for *Nuculoma tenuis* (Montagu, 1808) (in water with extremely low oxygen content) (Moore 1931) or 5 days for *Modiolus demissus* (Dillwyn) (in nitrogen) (Lent 1968).

The principal physiological means of resisting to both aerial exposure and hypoxia is metabolic rate decrease thus energy saving (Storey & Storey 1990). Prolonged aerial exposure provokes tissue hypoxia and metabolic processes leave the aerobic pathways for the anaerobic ones (Ahmad & Chaplin 1984, Eertman & De Zwaan 1994). In bivalves, this leads to end products (*e.g.*, short-chained organic acids or alcohols) that can be reused or easily removed from the cells (Oeschger 1990, De Zwaan & Eertman 1996).

Another factor influencing survival in air is also tolerance to desiccation. *C. glaucum* may survive the loss of 33 % of water from the tissues. Air temperature is crucial during aerial exposure. In the lagoons inhabited by *C. glaucum*, like the Berre Lagoon, where the cockles were sampled, the temperature may exceed 30 °C in the summer (Stora *et al.* 1995, Gouze *et al.* 2005). This species is adapted to these extreme conditions. However, during aerial exposure, temperature increase causes an increase in evaporation of water from the body. Cockles are closed when exposed to air, which limits evaporation (Boyden 1972). But high temperatures may also increase metabolic activity by increasing oxygen demand (Clarke 1991, in *C. glaucum*: Tarnowska *et al.* 2009).

A longer survival of bivalves subject to aerial exposure or hypoxia at lower temperature was reported previously (Matthews & McMahon 1999). In a freshwater bivalve, *Corbicula fluminea* (O.F. Müller, 1774) subject to periodic emersion in its natural habitat, the ranges of median tolerance to aerial exposure were: 23.8-24.9 h at 35 °C, 71.4-78.2 h at 25 °C, and 248.5-341.6 h at 15 °C (Byrne *et al.* 1988). Apart from high temperatures, environments rich in hydrogen sulphide, and high or low salinity can all negatively affect air survival (Shumway *et al.* 1983, Veldhuizen-Tsoerkan *et al.* 1991). Anoxic and aerial survival were demonstrated to provide early warning indicator of contaminant induced stress (De Zwaan & Eertman 1996).

The influence of size on aerial exposure survival time

We did not evidence a significant effect of size on survival during aerial exposure. Both our study and the previous one (Boyden 1972) were performed on adult individuals. In *Corbicula fluminea* (Bivalvia) larger individuals were proved to lose water at a slower rate and survived longer in air (at 25 °C and 35 °C) than smaller ones

(Byrne *et al.* 1988). Larger individuals were more tolerant to anoxia than smaller ones in other bivalves [Borsa *et al.* 1992 for *Ruditapes decussatus* (Linnaeus, 1758), Matthews & McMahon 1999 for *Dreissena polymorpha*]. However, the pattern may be inverted with larger specimens being less tolerant of anoxia than smaller ones (Matthews & McMahon 1999 for *C. fluminea*). One of the main sources of anaerobic metabolic substrate in mollusks is glycogen (De Zwaan 1983). Therefore, lower tolerance of larger specimens to anoxia might be due to the reduction of tissue glycogen level correlated with reproductive cycle (Tarnowska *et al.* 2009 and references therein).

In general, smaller, younger bivalves appeared more sensitive to other types of environmental stress, such as intoxication (*e.g.,* Ringwood 1993, Markich 2003). However, in *Mytilus edulis* (Linnaeus, 1758) the resistance to intoxication with copper declined gradually, being significantly correlated with shell length (Hoare & Davenport 1994).

The influence of sex on the aerial exposure survival time

No difference was recorded in the survival time in air between males and females. Although the absence of significant difference does not prove that survival is the same among sexes, curves are rather smooth and similar, which can be compared to the conspicuous differences observed among temperature conditions in the same Figure (Fig. 1), and suggests that the sample sizes provided a reasonable statistical power. In previous studies, the females of bivalves were often suggested to be more fragile to various perturbations than males. Stress caused by invasive species *Dreissena* provoked an increased mortality in females in comparison to males in the native bivalve *Lampsilis radiata* (Gmelin, 1791). It was considered to be due to the fact that in female stresses related to breeding added to stresses incurred from *Dreissena* encrustation produced lethal effects (Haag *et al.* 1991). The resistance of females to aerial exposure could decrease during gonad maturation and spawning, as it demands a lot of energy. The gonad development stages were not taken into account in this study. We did not trust gonadic stages inferred from our data, because the stress provoked by aerial exposure may provoke immediate spawning. Reproduction is less seasonal in the Berre lagoon than in other investigated areas (Tarnowska *et al.* 2009) thus this location may not be the most susceptible to reveal sex differences due to reproductive effort.

The consequences of the results for gene flow

The survival times of *C. glaucum* in air, although shorter than found by Boyden (1972), are long enough to enable a long-distance dispersal of cockles *via* birds. Theoretically, the dispersal distances may be quite impor-

tant, as for example a duck flies at 60-78 km h⁻¹ (Welham 1994). Moreover, most bird migrations are at an altitude ranging from 150 m to 600 m, although they may also exceed several thousand meters (Williams 1950). The air temperature is known to decrease with altitude, which is favorable for bivalves survival during birds mediated transport. On the other hand, in our experiment the cockles were placed in order to avoid airflow. The cockles attached to migrating birds are exposed to a high airflow rate likely to accelerate desiccation and death.

Although we confirmed that the time of survival in air is long enough for such a transport, the present study does not support differences in survival times between males and females, which may have explained the different patterns of genetic structure between mitochondrial and nuclear DNA revealed by previous studies (Tarnowska *et al.* 2010). We argued above that absence of statistical significance appeared unlikely to be caused by insufficient sample sizes. However, the survival time in air was studied only on adult individuals. Eggs and juveniles can be transported by birds as well. Their physiology is different than the one of adults and the existence of differences between the two sexes in the aerial exposure survival at these stages cannot be excluded. We collected cockles from the surface of the sediment (not the buried ones), which are more prone to infection by trematodes (Bowers *et al.* 1996). However, birds' feet or necks do not burry into the sediment, so surface sampling is not biased relative to our assumption (bird dispersal). A differential survival among genders during dispersal is not the only possible cause of dispersal differences. In some bivalves, behavior differs between sexes. In *Macoma balthica* (Linnaeus, 1758) females prevailed among the crawlers, compared to the buried individuals (Mouritsen 1997). It is also possible that mitochondrial DNA does not reflect simply neutral processes, but that this marker is subject to natural selection. If mitochondrial haplotypes are differentially adapted to given environmental conditions, then the geographical (or spatial) distribution of mitochondrial genetic diversity does not reflect gene flow (Ballard & Whitlock 2004).

To conclude, in the present study we revealed the influence of air temperature on the survival time of cockles. However, we could not prove that the differences in genetic structures revealed by nuclear and mitochondrial markers were provoked by survival differences to aerial exposure (*i.e.*, birds-mediated transport). The hypothesis of differences among sexes in aerial survival cannot be ruled out. Similar tests could be carried out in other locations, where reproduction is more seasonal, and using more individuals of different sizes. We indicated the non-significant p-values of our tests to allow future meta-analyses to be carried out by computing Fisher's combined probability test. Alternative hypotheses to explain the genetic observations are that (i) differences between the effective dispersal of male and female are provoked by

other mechanisms than aerial survival time of adults (*e.g.*, different behavior) or (ii) the differences between the results revealed by the two genomes reveal an effect of natural selection on mitochondrial DNA or nuclear DNA.

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