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Is there a link between shell morphology and parasites of zebra mussels?

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ABSTRACT

The shell morphology of zebra mussels, *Dreissena polymorpha*, was analyzed to determine if alterations in shell shape and asymmetry between valves were related to its infection status, i.e. infected or not by microparasites like ciliates *Ophryoglena* spp. or intracellular bacteria Rickettsiales-like organisms (RLOs), and by macroparasites like trematodes *Phyllodistomum folium* and *Bucephalus polymorphus*. For microparasites, two groups of mussels were observed depending on shell measurements. Mussels with the more concave shells were the most parasitized by ciliates. This could be more a consequence than a cause and we hypothesized that a modification of the water flow through the mantle cavity could promote the infection with a ciliate. There were more RLOs present in the most symmetrical individuals. A potential explanation involved a canalization of the left–right asymmetry as a by-product of the parasite infection. Trematode infections were associated with different responses in valve width. Females infected by *P. folium* displayed significantly higher symmetry in valve width compared with non-infected congeners, whereas the infection involved an opposite pattern in males. *B. polymorphus* was also linked to a decrease in valve width asymmetry. This study suggested that a relationship exists between parasitism and shell morphology through the physiological condition of host zebra mussels.

1. Introduction

The shell of bivalve molluscs provides not only a substantial protection from predators and mechanical damage, but also a better control of the internal environment, and an efficient circulation of water currents through the mantle cavity (Seed, 1980). The formation of this external skeleton may be subject to considerable ontogenic variations, providing the opportunity to study the effects of biotic and abiotic factors experienced by organisms. A lot of natural factors can arguably control shell morphology, such as the nature of substratum, food supply, oxygen but anthropogenic changes can also affect shell morphology (Sokolowski et al., 2008). Among these environmental factors, pollution has been reported to cause shell abnormalities and growth depressions in oyster and clam populations (Alzieu et al., 1990; Li, 2002; Sokolowski et al., 2002). However, Voets et al. (2006) did not show any relationship between shell morphology in zebra mussel (*Dreissena polymorpha*) and multicontamination of freshwater system.

Among biotic factors parasitism could represent a stressor involving morphological aberrations. Several studies showed that parasites are associated with elevated developmental instability in their host, which is generally a direct and significant cause of a decrease in fitness (Møller, 1992; Møller and Manning, 2003; Polak, 1997; Thomas et al., 1998). Dingemanse et al. (2009) have observed different head morphologies in three-spined stickelbacks, depending on the infection intensity of *Schistocephalus solidus* (Cestoda). The heads of infected fish were reduced in size and differently shaped compared to those of non-infected congeners, which involved different foraging capacities. In the freshwater snail *Potamopyrgus antipodarum*, the infection by trematode parasites prevented spine production and the shell became wider (Levri et al., 2005). Møller (1996) suggested that the correlation between infection and the instability of development resulted from three main phenomena: (1) hosts showing abnormalities may be immunologically more susceptible to parasitism compared with organisms perfectly symmetrical, (2) they may be more often exposed to parasites and, (3) parasites, themselves, may be a direct cause of instability when they disrupt the development of their host. Studies reporting an effect of infection on the host morphology concerned mainly trematode parasites (Alda et al., 2010; Hay et al., 2005; Krist, 2000; Levri et al., 2005).

The zebra mussel, *D. polymorpha*, is an invasive species which has successfully colonized a wide range of ecosystems throughout Europe and North America where it became common over wide areas (McMahon, 1996). *D. polymorpha* have been documented to be the host for a variety of parasites (e.g. bacteria, ciliates or trematodes) using it as a unique or as an intermediate host in their life cycle (Molloy et al., 1997, 2001, 2005). The species name '*polymorpha*' comes from the large variety of shell shapes and colors it can

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display (Biochino, 1994; Marsden et al., 1996; Van Beneden, 1835). Comparing several zebra mussel populations, some authors have observed different shell morphologies related to environmental factors, such as the water depth or physico-chemical conditions (Latjner et al., 2004; Trichkova et al., 2008).

In the present study, we analyzed the data to see if shell morphology and valve asymmetry of zebra mussels could be related to their infection status. We hypothesized that organisms with abnormal shells would be more infected than their symmetrical congeners. The shell morphometric parameters were assessed in three populations of freshwater mussels, *D. polymorpha*, depending on the investigated parasites. The organisms were collected in the Moselle River, downstream in the vicinity of Metz (Northeast of France), in the Meuse River at Troussey and in the Vilaine River at Langon. The shells were measured and scanned by X-ray computed tomography and studied by image analysis. Each individual was dissected and treated for histological procedures to reveal its parasites and its gender. Also, the morphological traits were linked to infection parameters.

2. Material and methods

2.1. Field sampling

Zebra mussels were randomly handpicked from under rocks on the shores of three Rivers of the Northern half of France, depending on the investigated parasites. (1) For ciliates *Ophryoglena* spp. (Oph) and intracellular bacteria Rickettsiales-like organisms (RLOs), mussels ($N = 63$) were sampled in the Moselle River, 900 m downstream the wastewater treatment plant of Metz, in France (49°09′08.67″N, 06°12′07.02″E). (2) To obtain enough organisms infected by the trematode *Phyllodistomum folium* or *Bucephalus polymorphus* a sampling effort was realized. Zebra mussels ($N = 564$) were collected in the Meuse River at Troussey for *P. folium* (48°42′13.89″N, 5°42′02.99″E) and (3) in the Vilaine River at Langon (47°42′56.95″N, 1°50′13.41″W) for *B. polymorphus* ($N = 905$). Herein, the different geographical distributions of the studied parasites could be related to the environmental quality (Minguez et al., 2011). All these species are found in the native range of zebra mussels and are host-specific except for bacteria which are generalist (Molloy et al., 1997, 2001, 2005). The shell length range of zebra mussels from the Moselle River and Langon was 15–21 mm, and 23–37 mm for organisms from Troussey. All the sampled organisms were adult.

2.2. Parasite inventory

The procedure for parasite inventory is described by Minguez et al. (2009). Briefly, classic histological methods were used after the fixation of each organism in Bouin's fixative, i.e. rinsing in water, embedding in paraffin after dehydration in an ascending ethanol–roti-histol series, section 5 µm thick, staining in Gill II hematoxylin/eosin. A semi-quantitative parasite inventory was made by observing 30–40 tissue sections per individual, representing one twentieth of the whole digestive gland. However, all the organs are visible on sections. Parasites were identified following Molloy et al. (1997, 2001, 2005). The two trematode species *P. folium* and *B. polymorphus* have been previously described by molecular techniques in French samples (Petkevičiūtė et al., 2003; Stunžėnas et al., 2004). Ciliates belong to the genus *Ophryoglena*. Two species, infecting the digestive gland lumina, have been observed: the larger identified as *Ophryoglena hemophaga* and a smaller species still undescribed (Molloy et al., 2005). Moreover, bacteria have been recognized as Rickettsiales-like organisms (Molloy et al., 2001). Standard epidemiological parameters were used to assess the level of infection (Bush et al., 1997): prevalence (percentage of infected mussels) and mean intensity (mean number of parasites read on the 30–40 sections, i.e. 1/20 of the whole digestive gland). This last was determined only for parasites that could be enumerated, e.g. individual cells of *Ophryoglena* spp., and cytoplasmic inclusion bodies of Rickettsiales-like organisms.

The size of each bacterial inclusion was also calculated by image analysis (cell^P software, Olympus) linked with a graphic tablet (Wacom® Cintiq® 21X pen display).

2.3. Biometry

The shell parameters were only assessed on organisms selected following the parasite inventory, according to their infection status and/or host gender. For the macroparasites, the study of shell morphology was performed on 50 individuals for the *P. folium* group ($N = 25$ infected, identified after dissection and 25 non-infected mussels randomly chosen after histological observations) and on 48 individuals for the *B. polymorphus* group ($N = 24$ infected, identified after dissection and 24 non-infected mussels randomly chosen after histological observations). After thoroughly cleaning all shells for any surface deposits or epibionts, the maximum length (L), height (h) and width (w) were measured with a calliper rule to the nearest 0.01 mm (Fig. 1). The weights (W) of each valve (r: right and l: left) and the entire shell (W_t) were measured on a Pioneer PA214CM balance to the nearest 0.1 mg. To obtain the inner volume (V) of the shell (r: right, l: left and t: entire shell), scans were performed using a medical X-ray computed tomography (BrightSpeed Excel by GE Healthcare), with a slice thickness of 0.625 mm. The images were then analyzed using cell^P software (Olympus) and a graphic tablet (Wacom® Cintiq® 21X pen display). For the analysis, the different morphological traits of shells were studied as ratio, i.e. total volume and total weight were reported to shell length, and an index related to the shell concavity was calculated as the ratio of length to width.

Absolute asymmetry, defined as the unsigned difference of right and left characters, was measured for valve width, inner volume and weight. The tested linear regressions showed their independence with the shell length ($Rs < 0.10$; $p > 0.05$). The percentage of asymmetry for these three parameters was thus calculated as

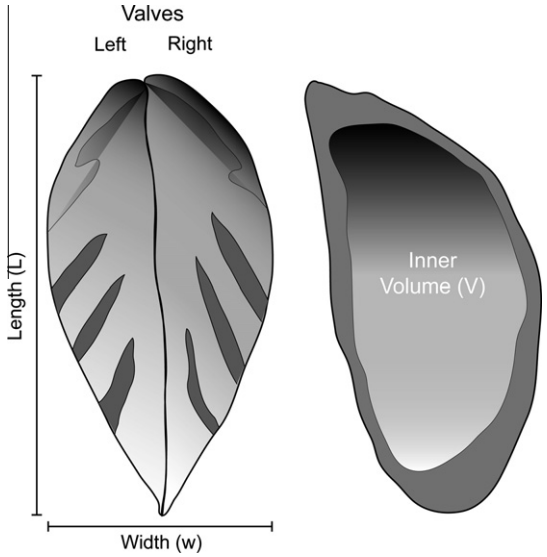

Fig. 1. Morphological parameters measured for each valve. V: inner volume, L: length, w: width.