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Exoskeletal deformities in Palaemonidae: Are they a threat to survival?

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ABSTRACT

Shrimps of the *Palaemon* genus have been affected for over 15 years by a problem of exoskeletal deformities, particularly in the Gironde estuary (France). Given the large numbers of individuals affected, this study focus on a better description of the phenomenon to estimate its impact on shrimps. This study, on *Palaemon longirostris* and *Palaemon macrodactylus*, is based on samples collected from 1992 to 2007 in the Gironde estuary with particular focus on the year 2007, and on a 4-month rearing of adult shrimps in experimental system. The different approaches reveal the relative persistence of the phenomenon since 1992 (with on average more than 58% of ovigerous females affected) and its persistence during shrimp life cycle, even after several moults. Important consequences associated with deformities at the individual level were demonstrated: a higher mortality, a lighter egg mass and a lower weight for a given size. The presence of exoskeletal deformities is now a major phenomenon, with profound effects on individual shrimps, and which may in turn have an important impact on the survival of the *P. longirostris* population in particular.

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1. Introduction

Exoskeletal deformity is a major phenomenon that has affected some shrimp of the *Palaemon* genus (*Palaemon longirostris*, *Palaemon macrodactylus* and *Palaemon serratus*) for over 15 years (Béguer et al., 2008). This phenomenon has recently been observed and briefly described for *Palaemon* genus shrimp populations in the Gironde estuary, but it also affects, although to a lesser degree, shrimp in other French and European estuaries, especially the Scheldt (Belgium) and the Rhine (Rotterdam, Netherlands) (Béguer et al., 2008). In 2006, on average, over 40% of shrimp observed in the Gironde population were suffering from deformities (Béguer et al., 2008). Such a phenomenon, affecting a very large proportion of individuals from wild populations, has never been described before. Indeed, although morphological anomalies remain a very well-known and a common occurrence among crustaceans (Aguirre and Hendrickx, 2005; De Grave and Mentlak, 2008; Delphy, 1921; Dutt and Ravindranath, 1974), they are usually observed in isolated individuals or sometimes in farm rearing.

In the Gironde estuary shrimps, and particularly in *P. longirostris* and *P. macrodactylus*, these deformities are mainly in the cephalothorax, which appears either wrinkled or bent instead of being smooth and straight. The rostrum is also often partly affected: either bent to one side, or pointing downwards. Some often spectacular dissymmetries, caused by scaphocerite or uropod hypertrophy, have also been described for both species (Béguer et al., 2008).

P. longirostris is the most common and abundant shrimp in the Gironde estuary (Aurousseau, 1984; Girardin et al., 2008; Sorbe, 1983). It is a typical estuary species, completing its entire life cycle in this environment (Campbell and Jones, 1989; Gonzalez-Ortegon and Cuesta, 2006; Gurney, 1923). It is fished commercially in the Gironde estuary in large quantities using traditional methods and every year, for over a century, between 27 and 82 tonnes have been fished and consumed (Girardin et al., 2008; Holthuis, 1980). Shrimps are also an important link in the food web in this estuary, since they are prey for many species of exploited fish, like the bass or the meagre (Pasquaud, 2006; Pasquaud et al., 2008). *P. longirostris* has been monitored via monthly faunal surveys in the Gironde since 1978 (Girardin et al., 2008) and today its numbers seem to be in decline compared with the abundant quantities observed at the beginning of the monitoring period.

Deformities first appeared in *P. longirostris* before 1992 (oldest preserved samples), but after the beginning of the 1980s, since neither Sorbe (1983) nor Aurousseau (1984) observed this phenomenon during their studies. Deformities have also been observed in *P. macrodactylus*, an exotic species that has colonised the Gironde estuary for about a decade (Béguer et al., 2007) and which is now found here very frequently. This invasive species is very similar to the native species, *P. longirostris*, both in its morphology and its ecology, especially its feeding habits (Sitts and Knight, 1979; Sorbe, 1983).

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The causes of the deformities so far remain unknown, although recent studies seem to dismiss microorganisms, viruses or bacteria as a possible origin, and also parasites (Béguer, unpublished data). Some serious hypotheses regarding the action of organic or other types of micropollutant have since been suggested. Given the scale of this phenomenon, its impact both at the level of the individual and of the population could be considerable, and it needs to be assessed.

The aim of the present study is twofold: (i) to provide a better description of the phenomenon of exoskeletal deformity on the basis of recent samples from the Gironde estuary (2007) and others collected since 1992, and (ii) to assess its consequences by observing individuals kept in captivity for several months, in a controlled environment.

2. Materials and methods

The part of the study based on preserved samples deals with *P. longirostris* and *P. macrodactylus*, the two species most frequently found. However, as *P. longirostris* is present in greater numbers and has similar deformities as *P. macrodactylus*, detailed results are presented for this species only. The experimental part (rearing) deals exclusively with *P. longirostris*, a species that is easier to harvest in sufficient numbers and for which there was already a methodological basis for rearing them (Aurousseau, 1984).

2.1. Strategy to demonstrate the progress and scale of the phenomenon

Since 1979, a large part of the estuary had been sampled monthly for fauna survey (mainly fishes and crustaceans, Girardin et al., 2008) (Fig. 1). The protocol for these campaigns is described in Béguer et al. (2007). Temperature and salinity were recorded at each station. Shrimp samples from each of these campaigns have been preserved since 1992. To assess the scale of the deformity phenomenon and its evolution over the years, sub-samples were observed. For every year until 2007, about thirty ovigerous *P. longirostris* shrimps collected from the upstream part of the estuary during May or June, depending on abundance, were measured and examined. Carapace length (CL, to the nearest 0.1 mm) was used as standard size for all specimens. It was decided to observe and measure shrimp of the same sex, females carrying eggs, as they are easily recognisable, and give a good representation of the general state of health of the population. The period and the place where sampling took place were similar every year. In all, 539 female shrimps were examined under a binocular microscope to detect the presence of deformities.

2.2. Strategy to characterise deformities and relate them to environmental factors

From November 2006 to November 2007, monthly campaigns to collect small mobile fauna (from the bottom and the surface) were carried out over a 60 km length of the Gironde estuary, included the 20 km area around the nuclear power plant (Fig. 1). The protocol was the same as that used since the beginning of the 1980s. During these fishing campaigns, standard physico-chemical parameters were taken: temperature, salinity, conductivity and turbidity. Collected shrimp were preserved in 70% alcohol and sub-samples were prepared in the laboratory: about 30 individuals per station, per month and, where possible, per species were chosen at random. Each shrimp was then measured (CL, to the nearest 0.1 mm), sexed, and weighed (Fresh weight, *W*, to the nearest 0.0001 g). In all, 1937 individual females, 3145 males and 111 juveniles of *P. longirostris* were measured. A total of 2235 *P. macrodactylus* were examined in a similar way (776 females, 694 males and 765 juveniles). The eggs from 247 ovigerous *P. longirostris* females were detached by immersing whole individuals in a 10% bleach solution; the shrimp were then weighed (Fresh weight, *W*, to the nearest 0.0001 g). The eggs in each clutch were counted and characterised according to stage of development: visible eye (stage II) or no visible eye (stage I). For *P. macrodactylus*, 89 clutches were weighed and the eggs counted.

2.3. Observation of shrimp in experimental environment

2.3.1. Capturing the shrimps

During February 2008, a batch of *P. longirostris* was caught using bow nets with mesh size 5 mm, close to the port of Pauillac, about 80 km from the mouth of the Gironde estuary (Fig. 1). Captured specimens were observed for damage to their body or appendages, and only undamaged shrimps (360, CL 6.0–16.6 mm) were selected for an experiment of 4-month duration. Half the individuals, which had no external visible morphological deformity, were the control. The other half had at least one deformity.

2.3.2. Rearing conditions

The shrimps were gradually acclimatised to the rearing conditions, which was different from their natural environment in terms of salinity (5 PSU), for about 3 h. Fresh well water was used, maintained at a dissolved oxygen concentration of 10.3 mg l^{-1}, pH 8.2, and salted artificially at 15 PSU. Temperature was 15 °C at the beginning of the rearing period and 20 °C at the end, in accordance with the increase observed in the natural environment. Temperature fluctuations were avoided by the use of a thermoregulated chamber. These parameters were determined from a previous successful rearing of shrimps (Aurousseau, 1984). Before the beginning of the experiment, the shrimps were quarantined for 2 weeks, in order to select the least fragile individuals. Individual units were designed and built specially for this experiment (Fig. 2). It was essential that all the shrimp were kept separate from each other, (i) to avoid problems of cannibalism that can frequently occur, (ii) to ensure that all individuals were fed identically, and (iii) to be able to monitor and recover the exuvia from each shrimp. The submerged units, consisting of a PVC pipe, 8 cm in diameter and 9 cm high, provided each shrimp with a vital volume of 453 cm^3, sufficient to allow it to move around and to carry out the moulting processes. The submerged part had a 3 mm mesh which allowed good circulation of the water (oxygenation and waste removal). The units were grouped in batches of six and placed in two square tanks, 1.98 m × 1.95 m. These two tanks formed a closed system, with the water circulating at a rate of 45 l/min though two sand filters and a 25 W UV filter.

Every 2 days the shrimps were allowed to feed ad libitum on an artificial food (Biomar Ecostart No. 1.9, composed of 56% protein, 18% fats, 12.2% brute ash and 0.3% cellulose). The shrimps were kept in the dark throughout the entire experiment (except when being measured), as these conditions are similar to those found in the natural environment (the Gironde estuary is very turbid) and thus limited any stress related to captivity.

2.3.3. Observations and measurements

Every 2 days, when the shrimp were feeding, the following physico-chemical parameters were measured: temperature, oxygen, pH, nitrates and nitrites. Each individual shrimp was also checked at this time. When a moult was observed, the shrimps underwent a series of measurements 2 days later, this delay was in order to give the new carapace time to harden (Nouvel-Van Rysselberge, 1937). The shrimps could then be manipulated without serious risk of causing trauma. For each shrimp, CL (to the nearest 0.1 mm), *W* (to the nearest 0.0001 g) and any deformities (location,

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