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How does the bopyrid isopod *Gyge branchialis* interfere with trace metal bioaccumulation in the mud shrimp *Upogebia* cf. *pusilla*?

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Abstract

Parasites are widespread in natural environments, and their impacts on the fitness of their host and, at a broader scale, on ecosystem functioning are well recognized. Over the last two decades, there has been an increasing interest in the effects of parasites in conjunction with other stressors, especially pollutants, on the health of organisms. For instance, parasites can interfere with the bioaccumulation process of contaminants in their host leading to parasitized organisms exhibiting lower pollutants burdens than unparasitized individuals for example. However, the mechanisms underlying these patterns are not well understood. This study examined how the bopyrid parasite *Gyge branchialis* could lower the cadmium (Cd) uptake of its mud shrimp host *Upogebia* cf. *pusilla*. When exposed to water-borne Cd, parasites were able to bioaccumulate this trace metal. However, the uptake of Cd by the parasite was low and cannot entirely explain the deficit of Cd contamination of the host. The weight of gills of parasitized organisms was significantly reduced compared with unparasitized organisms. We suggest that by reducing the surface for metal uptake, parasites could lower the contaminant burden of their host.

Keywords Host-parasite interactions · Metal contamination · Physiological alteration · Mud shrimp

Introduction

Increasing human population densities and associated activities have led to the release of important contaminants in aquatic environments, leading to high levels of contamination locally (Tueros et al. 2009; Pan and Wang 2012). These pollutants are of major concern because some have strong deleterious impacts on the health of organisms (e.g., Felten et al. 2008; Al Kaddissi et al. 2014).

Living organisms are naturally exposed to a broad variety of stressors, which can have cumulative effects on their fitness. In particular, the process of contaminant bioaccumulation, as well as the effects of contaminants on organisms, depend on several other environmental parameters (see

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Holmstrup et al. 2010 for a review). Amongst these factors, the influence of parasites in contaminant bioaccumulation is now well recognized (Sures and Siddall 1999; Coors and De Meester 2008; Paul-Pont et al. 2010). By definition, parasites benefit from their host and are harmful to them. In a contamination context, parasites can further negatively impact their host through enhancing their sensitivity to pollutants (Baudrimont and de Montaudouin 2007; Coors and De Meester 2008). In contrast, parasites have also been shown to reduce the contaminant burden of their host (Sures and Siddall 1999; Evans et al. 2001; Paul-Pont et al. 2010). The mechanisms underlying this pattern of reduced contaminant burdens in parasitized organisms are not fully understood but parasites could diminish the capacity of their host to absorb and bioaccumulate contaminants by altering their physiological status and/or by reducing their activity levels (e.g., Evans et al. 2001; Paul-Pont et al. 2010). Moreover, parasites can bioaccumulate contaminants to concentrations which are orders of magnitude higher than their host (Nachev and Sures 2016). Therefore, they could also directly interfere with contaminant bioaccumulation processes by acting as pollutant sinks. For example, the concentration of lead in the acanthocephalan parasite *Pomphorhynchus laevis* was 3- to 30-fold higher than the concentrations found in the intestine of its fish

host, the chub *Squalius (Leuciscus) cephalus*. The parasite *P. laevis* interferes with the fish's hepatic-intestinal cycling of lead, thus directly reducing the lead uptake through the intestinal wall of the host (Sures and Siddall 1999).

In a previous study, unparasitized mud shrimp, *Upogebia* cf. *pusilla* Petagna, 1792, bioaccumulated 3.6-fold and 2.1-fold more Cd in their hepatopancreas and abdominal muscle, respectively, than parasitized organisms after an exposure period of 14 days to water-borne cadmium (Cd) (Dairain et al. 2018). In this study, we investigate how the bopyrid parasite *Gyge branchialis* Cornalia & Panceri, 1861, may interfere with the process of trace metal bioaccumulation in its mud shrimp host *U.* cf. *pusilla*, using Cd as a model contaminant. The female bopyrid parasite lives in one of the gill chambers of its host and feeds on internal host body fluids (Tucker 1930). Thus, trophic contamination by trace metal taken up by the host is likely for this parasite. The female bopyrid parasite represents ca. 6.6% of the total host-parasite biomass for this host-parasite combination. Therefore, we firstly evaluated whether the parasite acts as an important Cd sink in this system. In addition, the mud shrimp *U.* cf. *pusilla* filters water regularly for respiration purposes, and consequently the gills are a major site of metal uptake from contaminated water. Hence, secondly, due to the parasite position in the gills, we evaluated whether the bopyrid parasite damages the gills of the host, potentially affecting its uptake of Cd.

Materials and methods

The host-parasite association

The crustacean *Upogebia* cf. *pusilla* is a gebiidean mud shrimp occurring in intertidal and upper sublittoral zones along the Northeast Atlantic and Mediterranean coasts (de Saint Laurent and Le Loeuff 1979; Dworschak 1983). It lives in a deep (up to 49 cm) and complex burrow connected to the sediment-water interface by several distant openings (Pascal 2017). The fossorial lifestyle of the mud shrimp is associated with extensive bioturbation activity (Pascal 2017). The bopyrid isopod *Gyge branchialis* is a parasite of *U.* cf. *pusilla* (Tucker 1930). A sexually mature female lives in one (very rarely both) gill chamber of its host (Pascal et al. 2016), with a dwarf male attached to her. This ectoparasite disrupts the mud shrimp's reproduction (Tucker 1930; Pascal et al. 2016) and also reduces its activity levels (Pascal 2017).

Trace metal bioaccumulation in the bopyrid parasite

The bioaccumulation of Cd in female bopyrids has been investigated by conducting a 14-day contamination experiment on parasitized mud shrimp. Naturally Cd-uncontaminated mud shrimp were used in this experiment. They were sampled in the middle of Arcachon Bay (44° 40' N, 1° 08' W), France, using a bait piston pump. Only adult male mud shrimp of similar size (total length TL = 47.6 ± 0.5 mm, mean ± SE) were selected for the experiment. Experimental units consisted of Plexiglas tubes (diameter 9.5 × 40 cm) filled with sediments up to 20–22 cm depth. The rest was filled with seawater (water column = 1.06 L). Mud shrimp were placed in experimental units (one organism per core) and allowed to burrow for 3 days. This time was sufficient to observe a complete burrow (two open ends). Two series of treatments with four replicates per sampling time were conducted: (1) parasitized mud shrimp unexposed to Cd-contaminated seawater ("unexposed") and (2) parasitized mud shrimp exposed to Cd-contaminated seawater ("exposed"). Metal contamination was initiated by supplying seawater with a Cd stock solution (added as $CdCl_2$, concentration = 1 g L^{-1}) at a rate of 180 mL h^{-1}. Throughout the experiment, the Cd concentration in the water of contaminated experimental units was 7.4 ± 0.2 µg L^{-1} (mean ± SE), whereas Cd concentrations were systematically below the detection limit (DL) in the water column of uncontaminated experimental units (DL = 0.34 µg Cd L^{-1}).

Mud shrimp were sampled after 3, 7, and 14 days of incubation. Their hepatopancreas and abdominal muscle were sampled for Cd quantifications. The female bopyrid parasite was removed from its host under a stereomicroscope (SMZ1500; Nikon) and measured (total length, TL) (NIS-elements 0.4.00.00 software) before Cd analyses were performed.

After being dried (at least 48 h at 45 °C), biological samples were firstly weighed (dry weight, DW), then placed in polypropylene tubes and finally digested with nitric acid (HNO_3 65%) at 100 °C for 3 h (HotBlock; Environmental Express). Ultrapure water (Milli-Q) was added after cooling to dilute samples and obtain a final nitric acid concentration of 11%. Then, Cd concentrations were determined in mud shrimp's abdominal muscle, hepatopancreas, and in bopyrid parasites by atomic absorption spectrophotometry with Zeeman correction (Agilent 240Z AA; Agilent). Method blanks and certified reference materials (DOLT-5, dogfish liver, NRCC-CNRC) were included in each analytical batch, and treated and analyzed in the same way as biological samples (DL = 0.075 µg g^{-1} DW).

Prior to any statistical analysis, a Levene test was performed to assess homogeneity of variances. Bopyrid parasites (females only) TL and DW were tested for significant differences between experimental conditions using a Wilcoxon test. There were no significant TL nor DW differences between the bopyrid parasites examined (TL = 10.0 ± 0.3 mm; Wilcoxon

test, $W = 0.88$, $p = 0.50$, and DW = 33.8 ± 2.7 mg; Wilcoxon test, $W = 73$, $p = 0.08$). Then, the effects of experimental condition ("unexposed" and "exposed") and time of experiment (3, 7, and 14 days) on Cd bioaccumulation in bopyrid parasites and in the mud shrimp's abdominal muscle and hepatopancreas (log-transformed data) were assessed using a two-way ANOVA. Concentrations of Cd were compared between female bopyrids and the abdominal muscle and the hepatopancreas of the host using a paired t test and a Wilcoxon test for paired variables, respectively. Quantities of Cd in each organ were deduced from Cd concentrations and DW. Results are reported as the mean $\pm$ SE of N replicate measurements. Differences were considered significant for $p < 0.05$.

Influence of the bopyrid parasite on the mud shrimp's gills

The effect of the bopyrid parasite *G. branchialis* on the gills of the mud shrimp *U.* cf. *pusilla* has been determined by comparing the weight of gills between parasitized and unparasitized organisms. Mud shrimp were sampled on a mud flat in Arcachon Bay (44° 40' N, 1° 08' W), France, in May–June 2016 using a bait piston pump. Once collected, each specimen was individually isolated in a plastic pot containing seawater, to avoid fighting between the mud shrimp. In the laboratory, all mud shrimp were rinsed of sediment with seawater, and frozen (− 20 °C) before being dissected. A total of 51 unparasitized and 39 parasitized mud shrimp were processed.

Each *U.* cf. *pusilla* was measured from the rostrum tip to the telson (total length, TL) using a digital caliper. Then, the left and right gill chambers were checked for the presence of the female bopyrid parasite *G. branchialis*. If present, the

parasite was delicately removed from the gill chamber of its host under a stereomicroscope (SMZ1500; Nikon), before the left and right gills were carefully sampled. Gills were dried (at least 48 h at 60 °C) before being weighed (dry weight, DW). In a similar way, mud shrimp without their gills were dried and weighed.

Prior to any statistical analysis, a Levene test was performed to assess homogeneity of variances. The effect of the bopyrid parasite on the relationship between the total DW of gills (right and left gills together) and the mud shrimp TL was investigated by applying an analysis of covariance (ANCOVA) on log-transformed data. Regarding parasitized mud shrimp, the DW of the gill infested with the bopyrid parasite and the DW of the gill unparasitized were compared using a Wilcoxon test for paired variables. Differences were considered significant for $p < 0.05$.

Results and discussion

Trace metal bioaccumulation in the female bopyrid parasite

The Cd concentrations in "unexposed" parasites were low throughout the experiment, whereas "exposed" parasites showed significantly higher levels of Cd (Fig. 1; Table 1). At experiment completion, Cd concentrations in "exposed" parasites were 7.0 times higher than in "unexposed" bopyrids. There was no effect of time and no interactive effect between experimental condition and time on the bioaccumulation of Cd by bopyrid parasites (Table 1). The ability of marine parasites to accumulate

Fig. 1 Concentrations of cadmium (Cd, µg g^{-1} DW; median, first, and third quartiles; logarithmic scale) in the abdominal muscle and hepatopancreas of mud shrimp *Upogebia* cf. *pusilla* and in their bopyrid parasites *Gyge branchialis* exposed ("exposed") and unexposed ("unexposed") to Cd contamination over 14 days. $N = 4$, and $N = 3$ for "exposed" organisms at day 7
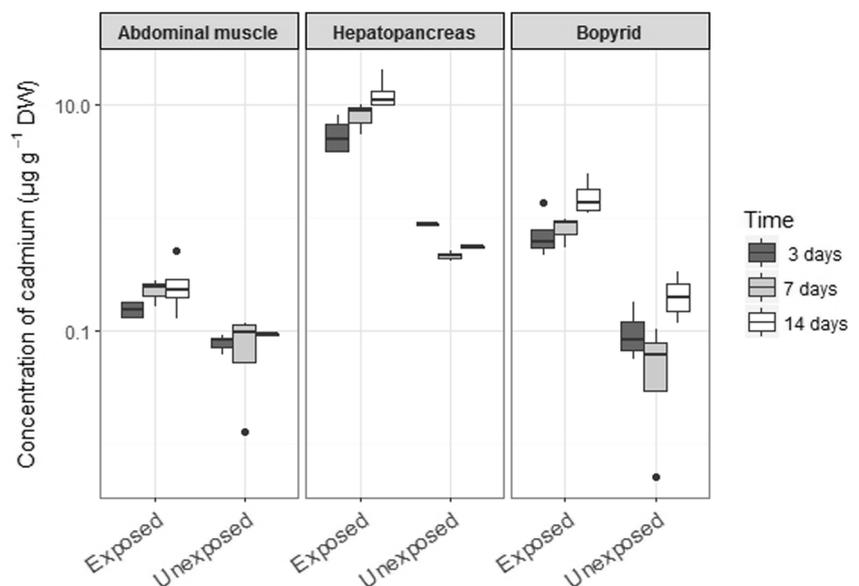
Table 1 Results of two-way ANOVA evaluating the influence of time of exposure (3, 7, and 14 days) and experimental conditions ("exposed" vs. "unexposed") on cadmium concentrations in the abdominal muscle and the hepatopancreas of the mud shrimp *Upogebia* cf. *pusilla* and in their bopyrid parasites *Gyge branchialis*. *p* values in bold indicate significant effect

	Factor	Df	*F*	*p*
Mud shrimp's abdominal muscle	Time (1)	2	1.20	0.35
	Experimental condition (2)	1	17.02	**< 0.05**
	(1) × (2)	2	1.03	0.38
Mud shrimp's hepatopancreas	Time (1)	2	3.30	0.062
	Experimental condition (2)	1	613.96	**< 0.05**
	(1) × (2)	2	13.84	**< 0.05**
Bopyrid parasite	Time (1)	2	3.51	0.058
	Experimental condition (2)	1	45.38	**< 0.05**
	(1) × (2)	2	1.00	0.39

contaminants is well recognized (Nachev and Sures 2016), even though studies targeting ectoparasites, such as bopyrids, are scarce. The literature suggests that ectoparasites have a reduced ability to bioaccumulate contaminants (Bergey et al. 2002) compared to endoparasites, such as acanthocephalans, which often display higher contaminant levels than their host (Sures and Siddall 1999; Nachev and Sures 2016). Furthermore, in this study, the Cd concentrations were significantly higher in "exposed" parasites than in the abdominal muscle of their "exposed" host (paired *t* test, $t = 4.76$, $p < 0.05$) but lower than in the "exposed" host's hepatopancreas (Wilcoxon test for paired variables, $V = 0$, $p < 0.05$) at each of the sampling times (Fig. 1). A mass balance calculation showed that there were on average 0.24 ± 0.06 μg of Cd in the hepatopancreas, and 0.01 ± 0.00 μg of Cd in the abdominal muscle, of "exposed" parasitized mud shrimp, while bopyrids contained 0.05 ± 0.02 μg of Cd on average at experiment completion. On the other hand, there was on average 0.38 ± 0.19 μg of Cd in the hepatopancreas, and 0.03 ± 0.01 μg of Cd in the abdominal muscle, of unparasitized mud shrimp exposed to Cd contamination over 14 days (Dairain et al. 2018). Thus, the difference in Cd bioaccumulation between parasitized and unparasitized mud shrimp cannot only be due to a direct effect of the bopyrid parasite acting as a sink in mud shrimp.

Effect of the bopyrid parasite on the gills of mud shrimp

The bopyrid parasite was negatively associated with the relationship between the total DW of gills and mud shrimp TL (ANCOVA, $F_{1,87} = 19.40$, $p < 0.05$), with the gills of parasitized organisms being lighter than those of unparasitized individuals, when standardized for TL (Fig. 2). These results are in accordance with those of Pascal et al. (2016) who showed that parasitized organisms were almost 10% lighter than uninfested specimens,

when weight was standardized for TL. In addition, we noticed that the gills harboring the bopyrid parasite were significantly lighter than the opposite unparasitized gill (Wilcoxon test for paired variables, $V = 142$, $p < 0.05$). Together, these gill weight results suggest that the bopyrid parasite has a particularly detrimental impact on the gills of its host. The mud shrimp *U.* cf. *pusilla* is primarily a suspension feeder (Dworschak 1987) but also filters water for respiration purposes. Thus, gills are a major site of direct Cd uptake. By reducing the weight of gills of the mud shrimp, it is likely that the bopyrid parasite reduces the surface area available for Cd uptake by the host. To properly confirm this, an experiment comparing Cd bioaccumulation in the gills of parasitized and unparasitized organisms, as well as between the two gills of parasitized mud shrimp, is required.
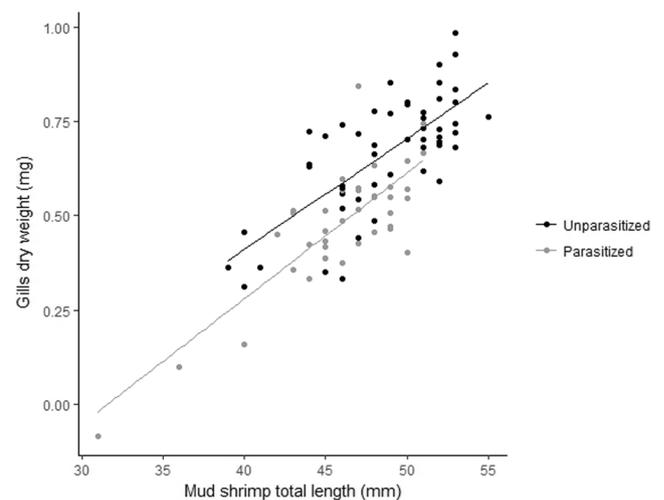
Fig. 2 Influence of the bopyrid parasite *Gyge branchialis* on the biometric relationships between the gills dry weight (log-transformed data; mg) and the mud shrimp *Upogebia* cf. *pusilla* total length (mm). $y = 0.029x - 0.76$ for unparasitized mud shrimp; $y = 0.033x - 1.05$ for parasitized mud shrimp (ANCOVA, $p < 0.05$). $N = 51$ unparasitized mud shrimp, and $N = 39$ parasitized mud shrimp

Conclusion

We found that the bopyrid parasite *Gyge branchialis* bioaccumulates Cd when its host, the mud shrimp *Upogebia* cf. *pusilla*, is exposed to this trace metal. Levels of Cd were higher in the parasite than in the abdominal tissue of the host. However, this was not the case for the hepatopancreas of the host which showed the highest Cd levels recorded. Additionally, the parasite damages the gills of the mud shrimp, which might reduce the surface area of the gills and lessen the Cd uptake by parasitized organisms. Finally, it should be considered that the parasite may also reduce the Cd uptake of its filter feeder host by lessening its filtration and/or ventilation activities (Stier et al. 2015; Pascal 2017). However, the influence of this bopyrid parasite on the filtration activity of the mud shrimp in regard of the bioaccumulation process of Cd and other contaminants has still to be investigated.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval All applicable international, national, and/or institutional guidelines for the care and use of animals were followed.

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