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**Title:**

Additive vs non-additive genetic components in lethal cadmium tolerance of *Gammarus* (Crustacea): novel light on the assessment of the potential for adaptation to contamination.

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19 **Abstract**

20 Questioning the likelihood that populations adapt to contamination is critical for ecotoxicological  
21 risk assessment. The appraisal of genetic variance in chemical sensitivities within populations is  
22 currently used to evaluate *a priori* this evolutionary potential. Nevertheless, conclusions from this  
23 approach are questionable since non-additive genetic components in chemical tolerance could  
24 limit the response of such complex phenotypic traits to selection. Coupling quantitative genetics  
25 with ecotoxicology, this study illustrates how the comparison between cadmium sensitivities  
26 among *Gammarus* siblings enabled discrimination between genetic variance components in  
27 chemical tolerance. The results revealed that, whereas genetically determined differences in lethal  
28 tolerance exist within the studied population, such differences were not significantly heritable  
29 since genetic variance mainly relied on non-additive components. Therefore the potential for  
30 genetic adaptation to acute Cd stress appeared to be weak. These outcomes are discussed in  
31 regard to previous findings for asexual daphnids, which suggest a strong potency of genetic  
32 adaptation to environmental contamination, but which contrast with compiled field observations  
33 where adaptation is not the rule. Hereafter, we formulate the reconciling hypothesis of a  
34 widespread weakness of additive components in tolerance to contaminants, which needs to be  
35 further tested to gain insight into the question of the likelihood of adaptation to contamination.

36

37 **Keywords:** adaptation, evolutionary ecotoxicology, *Gammarus*, genetic components,  
38 quantitative genetics.

39

## 39 1. Introduction

40 Questioning whether populations can adapt to contamination is critical for predictive  
41 ecological risk assessment since it is based on the extrapolation of laboratory bioassays to natural  
42 context (Medina *et al.* 2007; Millward and Klerks 2002). Although reported in numerous aquatic  
43 species for different toxicants, some authors conclude from the examination of published  
44 impacted field population surveys that genetic adaptation globally appears to be infrequent,  
45 notably in aquatic animal populations (Klerks 2002; Millward and Klerks 2002; Woods and  
46 Hoffmann 2000). Theoretical explanations are proposed: first, the local scale of water ecosystem  
47 contamination compared to the large home ranges of aquatic animal populations could lead to the  
48 impossibility of genetic isolation due to important gene flows between pristine and contaminated  
49 locations (see Groenendijk *et al.* 2002 for a case study); second, possible fitness costs of  
50 adaptation could counterbalance the selective advantage of increased resistance to contamination;  
51 third, genetically determined differences in resistance to toxic compounds could be insufficient to  
52 permit Darwinian selection. Concomitantly, these last two open questions – the existence of  
53 fitness costs and the lack of genetic variability – are central hypotheses tested by predictive  
54 approaches tackling the question of the evolution of genetic resistance to contaminants.

55 This predictive assessment of adaptive abilities could be performed via multi-generation  
56 artificial selection experiments (*e.g.* Vogt *et al.* 2007; Ward and Robinson 2005; Xie and Klerks  
57 2003). Nevertheless, these protocols imply substantial experimental efforts and they are feasible  
58 only with short life-cycle species. Moreover, the interference with evolutionary processes  
59 induced by laboratory rearing conditions could complicate the interpretation of outcomes (Athrey  
60 *et al.* 2007; Barata *et al.* 2000; Medina *et al.* 2007; Nowak *et al.* 2008; Reznick and Ghalambor  
61 2005; Ward and Robinson 2005). A second approach adopts a more predictive viewpoint; it  
62 focuses on the identification of genetically determined differences in tolerance to a specific

63 compound. Indeed the existence of such differences is a prerequisite for Darwinian selection.  
64 This genetic variation can first be assessed by comparing the sensitivities of genetically  
65 homogenous strains (highly inbred lineages or clones); daphnids as one of the foundation taxa  
66 for aquatic ecotoxicology are extensively employed in this context (Baird *et al.* 1991; Barata *et*  
67 *al.* 1998, 2000, 2002a, 2002b; Lopes *et al.* 2004, 2005; Soares *et al.* 1992). It is noteworthy that  
68 this was done originally for regulatory standardisation purposes rather than to address  
69 evolutionary issues. An alternative methodology consists in comparing the sensitivities of  
70 relatives (*e.g.* parents *vs* offspring or between siblings). Surprisingly this quantitative genetics  
71 approach is very little exploited in aquatic ecotoxicology (see Klerks and Moreau 2001 for one  
72 example). Yet it is achievable with long life-cycle species (sib analysis) and it does not require  
73 lab-specific lineages. In this framework, the potential for adaptation is quantified through the  
74 concept of heritability (Falconer and Mackay 1996), which embraces two components: first the  
75 amount of genetic variability and second the potential to transmit the differences sustaining this  
76 variability. Testing these two prerequisites of Darwinian selection (variation and heredity) is in  
77 fact imperative since what is genetically determined is not necessarily heritable due to the  
78 possibility of non-additive genetic interactions, *i.e.* dominance and combined epistatic effects  
79 (Falconer and Mackay 1996). These mechanisms have been reported in the inheritance of  
80 susceptibility to toxicants through polygenic epistatic systems (Woods and Hoffmann 2000) or  
81 dominance effects (Labbé *et al.* 2007). Yet these two components (variation and heredity) are not  
82 assessed in the former clone approach: in such specific cases the existence of genetically  
83 determined variations is sufficient to guarantee the possibility of selection since transmissibility  
84 from parents to offspring is obvious. Nevertheless the question of transmissibility has never been  
85 elucidated in field populations for aquatic species with sexual reproduction.

86           We wished to test the feasibility of such quantitative genetics protocols for  
87 ecotoxicological concerns with a case study. We chose the freshwater amphipod *Gammarus*  
88 *fossarum* because we control the reproductive cycle of this long life-cycle species in the  
89 laboratory: this offered the opportunity to produce individuals with known pedigrees for  
90 quantitative genetics protocols. Moreover, *Gammarus* being a crustacean, we could refer to  
91 studies conducted with daphnids (Baird *et al.* 1991; Barata, *et al.* 1998, 2000, 2002a, 2002b;  
92 Lopes *et al.* 2004, 2005; Soares *et al.* 1992; Ward and Robinson 2005). Then we could compare  
93 outcomes from procedures based on clonal and sexual modes of reproduction. Given that  
94 important genetically determined differences in tolerance to Cd have been demonstrated in  
95 *Daphnia* (Baird *et al.* 1991; Barata *et al.* 1998, 2000, 2002a, 2002b) and adaptation to lethal Cd  
96 exposure has been studied for this crustacean genus (Ward and Robinson 2005), this metal was  
97 our model contaminant. Therefore, adopting a quantitative genetics approach, our study  
98 illustrates how the comparison of sensitivities among siblings can yield insight into the potential  
99 of a *Gammarus* population to adapt genetically to Cd exposure. For this, neonates were produced  
100 from successive breeding events of acclimatised mating pairs from a non-compromised field  
101 population. Pairing was controlled to supply half siblings. Then we tested the two prerequisites of  
102 Darwinian selection (and thus a potential to evolve resistance) by answering two questions  
103 employing either full-sib or half-sib designs (Falconer and Mackay 1996): (i) Are there  
104 genetically determined differences in lethal Cd-tolerance within a native population of *G.*  
105 *fossarum*? If yes, (ii) are these genotypic differences heritable?

106

## 106 **2. Materials and Methods**

107

### 108 **2.1. Culture conditions and breeding design**

109        Approximately 400 *Gammarus fossarum* adults were collected within an upstream  
110 location of the River Bourbre, Isère, France. Recent demographic and ecotoxicological follow-  
111 ups led us to consider the sampled population as not impacted by environmental contamination in  
112 recent years. After 1 month of acclimatisation to laboratory conditions (natural water;  
113 temperature: 13°C; conductivity: 300  $\mu\text{S cm}^{-1}$ ; photoperiod: 15/9 h light/dark), 24 pairs of  
114 breeders with females in the last stage of their reproductive cycle were isolated in individual 500-  
115 mL beakers with constant water flow. Individuals were fed with alder leaves and weekly supplies  
116 of *Tubifex* larvae. Attention was paid to female size homogeneity. The identification of mating  
117 pairs was easy for this amphipod because of the formation of a characteristic amplexus  
118 (precopulatory guarding behaviour) and the identification of the reproductive status of females  
119 was achieved by direct observation (hatched juveniles in brood pouch, visible gonads, guarding  
120 male). After a few days, 19 females released the juveniles present in their brood pouches. These  
121 140 neonates – each with an identified mother and an unknown father (fertilisation during the  
122 laboratory acclimatisation) – constituted a first set of 19 broods produced in March (Supp. figure  
123 1). Juveniles were isolated in individual 50-mL tubes (BD FalconT) beginning on the day of their  
124 emergence. It should be noted that the accuracy of reproductive stage determination successfully  
125 translated into good synchronisation of offspring release (79% of neonates emerged within 3  
126 days; Supp. figure 1). Soon after neonate release females moulted and shed eggs which were  
127 fertilised by the guarding males. These eggs developed into juveniles released 26 days later  
128 (duration between median dates of emergence). They formed a second set of 14 broods from two  
129 identified parents (105 neonates) still with good synchronisation (70% of releases within 3 days).

130 Taking advantage of the separation of the amplexus after copulation, males were redistributed  
131 between beakers during the reproductive cycle in order to produce a third brood from each female  
132 with a different father (Supp. figure 1). This third set of 11 broods (111 neonates in May)  
133 emerged 25 days after the second one and synchronisation was less but still manageable to  
134 consider neonate exposure as a single experimental group (67% of emergences within 4 days).  
135 Over the 3 months, 356 neonates were thus collected the day of their emergence; the 45 broods  
136 presented a median brood size of eight neonates per female (min=2,  $Q_{25\%}=6$ ,  $Q_{75\%}=9$ , max=20).  
137 The controlled mating scheme involving successive reproductive cycles yielded both paternal and  
138 maternal half siblings (Supp. figure 1).

139

## 140 **2.2. Offspring Cd-sensitivities**

141 The 356 neonates were exposed to a lethal concentration of  $20 \mu\text{g Cd L}^{-1}$  the day after  
142 their release from the maternal brood pouch. The lethal response was chosen as the endpoint in  
143 order to maximise the expression of possible genotypic differences in the toxicological responses  
144 since genetic variability for sublethal responses to Cd tend to be lower than acute responses for  
145 crustacean daphnids (Barata *et al.* 2000). The concentration of  $20 \mu\text{g Cd L}^{-1}$  was determined in a  
146 preliminary test (five Cd concentration levels with three replicates of ten neonates) with the aim  
147 to ensure both sensitivity and specificity (not a too high concentration in order to scatter  
148 mortalities in time, and not a too long exposure in order to attribute mortalities to Cd). During the  
149 preliminary test,  $20 \mu\text{g Cd L}^{-1}$  gave rise to mortalities scattered from 12 h to seven days of  
150 exposure, while only one death occur in the controls. Here, survival was monitored daily during  
151 semi-static exposure in 50-mL tubes randomly ordered within a thermoregulated tank with one  
152 renewal of the water solution every 48 h; mortality was identified via the pleopod ventilatory  
153 activity. Complementary to the individualisation of exposure and to the random spatial

154 distribution of individuals within the experimental system, independence between exposure  
155 conditions and pedigrees was ensured by following a blind protocol and using a common Cd  
156 solution for each of the three monthly sets of broods. To accomplish this, the total volume of  
157 exposure solutions was prepared each month by a unique dilution in culture water of a common  
158 stock solution of 100 mg L<sup>-1</sup> of cadmium (CdCl<sub>2</sub>.H<sub>2</sub>O, Sigma Aldrich, Saint Quentin Fallavier,  
159 France) in ultrapure water acidified (0.2% HNO<sub>3</sub>) for storage. Exposure Cd solutions were  
160 conserved during neonate exposure in high-density polyethylene bottles with bubbling to  
161 guarantee oxygen saturation. In total, considering the 356 exposed neonates, a median (minimum,  
162 maximum, respectively) lethal time of 3 days (1, 9 days, respectively) was recorded.

163

### 164 **2.3. Sib analyses**

165 All statistical procedures were carried out with the R software (R Development Core  
166 Team 2007). In order to employ linear models, log transformations of survival times were  
167 required to verify the homoscedasticity of brood sensitivities (checked by Levene tests:  $P > 0.05$ ),  
168 agreeing with previous findings on the log-normal distribution of Cd-sensitivities in daphnids  
169 (Barata *et al.* 1998, 2002b).

170 First, in order to test the existence of genetically determined differences in Cd-tolerance,  
171 broods were compared in terms of survival time by means of non-parametric tests (Kruskal-  
172 Wallis rank sum tests). The brood effect, *i.e.* similarity among full sibs, was also quantified by  
173 variance decomposition using linear mixed-effect models (Pinheiro and Bates 2000) as  
174 implemented in the nlme package within the R environment. The significance of a brood effect  
175 on Cd-tolerance was assessed by inspecting confidence intervals of the restricted maximum  
176 likelihood (REML) estimators of variance components from a model including a random brood  
177 effect (Falconer and Mackay 1996; Pinheiro and Bates 2000). The possibility of a maternal effect

178 (Falconer and Mackay 1996) was assessed through the examination of correlations (Spearman  
179 correlation tests) between tolerance and maternally mediated parameters tracing a possible  
180 heterogeneity in maternal investment in progenies (brood size, mean length of offspring, mother  
181 length).

182         Second, the similarity in survival times among half-sibs was investigated in order to  
183 assess whether possible genotypic differences in Cd-tolerance are partly heritable. This required  
184 shaping a paternal half-sib design (Falconer and Mackay 1996) taking into account the survival  
185 of 157 neonates from 20 broods corresponding to the progenies of ten males which reproduced in  
186 April and May (Supp. figure 1): ten sires mated with two different dams in quantitative genetics  
187 terms. The heterogeneity in brood size involved an unbalanced nested design for the analysis of  
188 variance. Linear mixed-effect models are suitable in such cases (Pinheiro and Bates 2000). We  
189 therefore built a model considering (i) a random sire effect, (ii) a random dam effect nested  
190 within the sire effect and (iii) a residual environmental variance. The significance of the sire  
191 effect (*i.e.* the similarity among half sibs) was tested by comparing the likelihood of this nested  
192 model with the likelihood of a model considering only one random brood effect (likelihood ratio  
193 test implemented in the nlme package in R). REML estimators were computed only for  
194 significant variance components. Furthermore, we controlled the possibility of a confounding  
195 factor induced by the heterogeneity of exposure conditions between April and May by testing the  
196 introduction of a fixed month effect in these models (ANOVA test) (Pinheiro and Bates 2000).  
197 Complementarily to this paternal half-sib analysis, the survival times of 269 neonates  
198 corresponding to the progenies of 11 females during March, April and May were analysed in the  
199 same manner but as a maternal half-sib design (11 dams mated with three different sires). The  
200 interpretation of resemblance among maternal half-sibs (dam effect) is quite different from the

201 paternal case because maternal effects could be involved in addition to additive genetic  
202 components (Falconer and Mackay 1996).

203

## 203 3. Results

204

### 205 3.1. Genetic determination of Cd-sensitivity: full sib analyses

206 The lethal Cd-sensitivities between broods were compared separately for the three  
207 monthly sets of offspring. This excludes any confounding effect due to a possible heterogeneity  
208 in environmental or exposure conditions between months. Differences in tolerance were observed  
209 between broods (Figure 1): for instance, the most sensitive brood in March presented a median  
210 lethal time of 1 day, while it reached 5 days for the most tolerant one (Figure 1A). Kruskal-Wallis  
211 rank sum tests concluded that these differences could not be explained only by a sampling effect  
212 (induced by small and unequal brood sizes) in March and May ( $P < 10^{-3}$ ). In April, however, a  
213 weaker between-brood heterogeneity ( $P = 0.08$ ) was observed (Figure 1B). The number of broods  
214 considered here was too small to guarantee the detection of between-brood differences by means  
215 of poorly powerful non-parametric tests. This is supported by the significant brood effect for the  
216 three monthly data sets in the analysis of variance using linear mixed-effect models (see REML  
217 estimators of variance components on Figure 1). We note that the 95% confidence intervals of the  
218 REML estimators of the standard deviation associated with this brood effect are large; the limited  
219 number of examined broods also explains this lack of precision. Nevertheless, these combined  
220 results attest that there are significant differences in Cd-tolerance between broods.

221 Before concluding on the genetic determination of these toxicological differences, we first  
222 ruled out a possible confounding effect related to neonate size. Even if a strong between-brood  
223 heterogeneity in neonate size was detected (Kruskal-Wallis rank sum test:  $P < 10^{-8}$ ), individual  
224 survival time did not correlate with individual size (Spearman correlation test:  $P > 0.05$ ). This  
225 agrees with findings for *Daphnia* neonates exposed to Cd (Barata *et al.* 1998). Therefore,  
226 considering that any common environment effect was excluded as a result of the experimental

227 protocol (individual exposure, randomised design, a single Cd solution, 1-day-old neonates), the  
 228 similarity among the sibs of a given brood could only be explained by shared genotypic features  
 229 or by a maternal effect (during egg production and brooding). Nevertheless, even if the latter  
 230 effect cannot be excluded, we have no evidence in favour of it. Firstly, no correlation was  
 231 detected between the size of a female and the Cd-tolerance of her offspring (Spearman  
 232 correlation test:  $P > 0.05$ ). This is notably because breeders were selected aiming to limit female  
 233 size variability (min=9.1 mm,  $Q_{25\%}$ =10.0 mm,  $Q_{75\%}$ =10.1 mm, max=11 mm). Secondly, median  
 234 brood survival times did not correlate with any of the examined maternally mediated parameters  
 235 employed to evaluate the maternal investment in brood (mean length of offspring, brood size)  
 236 (Spearman correlation tests:  $P > 0.05$ ). Thirdly, previous reports for *Daphnia* have suggested a  
 237 clear genetic determination of acute Cd-sensitivity (Baird *et al.* 1991; Barata *et al.* 1998, 2000,  
 238 2002a; Ward and Robinson 2005) and suspect an absence of maternal effects (Barata *et al.* 1998).  
 239 Then all these findings lead to the inference that the between-brood variability observed within  
 240 the three sets of *Gammarus* neonates are likely explained by genetically determined differences  
 241 in Cd-tolerance.

### 243 **3.2. Heritability of Cd-sensitivity: half sib analyses**

244 Only the additive part of this genetic variance – heritability in the narrow sense (Falconer  
 245 and Mackay 1996) – can be regarded as a potential for genetic adaptation since dominance and  
 246 combined epistatic interactions are not transmissible between generations in the case of sexual  
 247 reproduction. Because the phenotypic similarity within half-sibs (contrary to full-sibs) translates  
 248 only a significant additive variance component (plus the probability of a maternal effect for  
 249 maternal half-sibs), we assessed the heritability of Cd-sensitivity within the sampled *Gammarus*  
 250 population by means of classical half-sib designs (Falconer and Mackay 1996). Ten males, called

251 sires, were mated with two successive females, called dams, in order to produce 157 neonates  
252 individually exposed to Cd (April and May; Supp. figure 1). The results of this paternal half-sib  
253 design demonstrate that the between-brood variability in neonate survival time is not significantly  
254 explained by a between-male heterogeneity (Figure 2). A likelihood ratio test of the linear mixed-  
255 effect models confirms the non-significant sire effect, in other words a weak similarity between  
256 paternal half-sibs (Table 1). We conclude that this pattern does not result from a confounding  
257 effect induced by heterogeneity in experimental conditions between April and May (fixed month  
258 effect; ANOVA:  $P > 0.05$ ). Our initial conclusion was that Cd-tolerance is not heritable because  
259 the major part of the genetic variance in Cd-sensitivity (brood effect) appears to be determined by  
260 non-additive components. Analysis of the maternal half-sib design involving 269 individual  
261 records of offspring from 11 dams mated with three successive sires (Supp. figure 1) corroborates  
262 this finding: there was no significant resemblance within maternal half-sibs – *i.e.* a dam effect –  
263 (Figure 3; Table 2), and no confounding effect related to monthly heterogeneity in experimental  
264 conditions could conceal this resemblance (fixed month effect; anova:  $P > 0.05$ ). Furthermore,  
265 the absence of a noticeable dam effect not only confirmed the weakness of the additive genetic  
266 variance in offspring Cd-sensitivity but also revealed that no maternal effect (at least mother-  
267 specific deviations) contributes to the between-brood differences in Cd-tolerance, reinforcing the  
268 hypothesis of genetic determination.

269 We also examined the possibility that lab rearing conditions select resistant breeders,  
270 hence reducing inheritable genetic variation. From Figure 1, it appears that tolerance to Cd  
271 increased in the assayed broods in May, as detected by linear mixed-effect modelling (fixed  
272 month effect, ANOVA:  $P=0.018$ ). Nevertheless, this increase has to be interpreted as  
273 heterogeneity in experimental conditions between months. The possibility that Cd-resistant  
274 genotypes among breeders were favoured in the lab is indeed not consistent with the unchanged

275 between-brood variability from March to May (random month effect on between-brood  
276 variability, likelihood ratio test:  $P > 0.05$ ). Note that this month effect was no more detected within  
277 subsets of broods used in the half-sib designs.  
278

#### 278 4. Discussion

279 Genetic determination of the variability in acute Cd-sensitivity was demonstrated here  
280 within a *Gammarus fossarum* population. This finding agrees with reported between-clone  
281 differences in the lethal response to Cd for crustacean daphnids (Baird *et al.* 1991; Barata *et al.*  
282 1998, 2000, 2002a; Ward and Robinson 2005) and other metals (Baird *et al.* 1991; Barata *et al.*  
283 1998, 2000; Lopes *et al.* 2004). Strikingly, the within-population genetic variability observed in  
284 lethal time of Cd-exposed neonates for *Gammarus* was comparable in magnitude to observations  
285 on natural populations of *Daphnia* (Barata *et al.* 2002a). However, the interpretation of this  
286 genetically determined variability in terms of potential to evolve resistance to prolonged acute  
287 stress diverges: the quantitative genetics methodology applied here to assess whether these  
288 genetic differences in lethal tolerance are heritable between generations (half-sib analyses)  
289 reveals that the observed genetic variance in Cd-sensitivity is mostly explained by large non-  
290 additive variance components. As a consequence, the heritability in the narrow sense, which  
291 quantifies responsiveness to selection, is negligible.

292 This failure to detect any significant additive genetic variance could result from the  
293 restricted number of breeding pairs employed in the study, which could give rise to weak  
294 statistical power. Nevertheless, this conclusion, even if based on a negative result, has to be  
295 analysed considering the strong between-brood variability translating clearly detectable genetic  
296 variability in our data set. We can therefore state that large non-additive genetic components are  
297 present in the determination of lethal Cd-sensitivities within the sampled population, whereas  
298 additive components in the observed genetic variance are weak. That leads to the preliminary  
299 conclusion that such a population would not adapt genetically to prolonged acute Cd exposure  
300 despite the existence of a significant genetic variability in Cd-sensitivity. Nevertheless, keeping  
301 in mind that only a lethal response was considered in this pilot study, and that this parameter

302 provides a non-exclusive measure for chemical tolerance, similar questions on the genetic  
303 determination of sublethal responses should be scrutinised before concluding.

304         This finding with crustacean gammarids seems to contrast with the outcomes from studies  
305 with parthenogenetic crustacean daphnids, which describe genetically determined differences in  
306 resistance to metals (Baird *et al.* 1991; Barata *et al.* 1998, 2000, 2002a, 2002b; Lopes *et al.* 2004,  
307 2005; Soares *et al.* 1992) and suggest a possible increase in mean tolerance of populations during  
308 multi-generation artificial selection experiments (Ward and Robinson 2005) or within  
309 historically-contaminated field contexts (Lopes *et al.* 2004, 2005). Nevertheless, as pointed out  
310 by Lopes *et al.* (2004, 2005), the apparent increase in mean metal tolerance resulted from the  
311 disappearance of sensitive clones and not from the appearance of resistant ones within  
312 populations. This pattern, where no higher resistant genotypes are revealed during selection  
313 induced by exposure, is not falsified regarding the outcomes from the artificial selection test with  
314 Cd (Ward and Robinson 2005), notably considering that only asexual reproduction was allowed  
315 in this experiment. The fact that after decades of metal exposure no clones with higher resistance  
316 emerge within field daphnid populations (Lopes *et al.* 2004, 2005) could be surprising  
317 considering that genetically determined differences in tolerance exist. Nevertheless, in view of  
318 our findings, these observations could be explained by a weakness of additive components in the  
319 genetic variance of lethal metal tolerance. In that case, the apparent adaptation (increased  
320 tolerance in contaminated populations) would be a transitory state reached each year in field  
321 populations due to the loss of sensitive clonal lineages. Therefore, because of a lack of  
322 heritability, this state of apparent adaptation would disappear at each sexual reproduction event  
323 occurring when ephippial eggs are produced before each winter in field populations.

324         From a broader perspective, the hypothesis of a weakness of additive components in the  
325 variability of sensitivity to contaminants is also consistent with the reported similarity between

326 the range of sensitivities observed among different laboratory populations and the variability  
327 among or within field populations (Barata 2002b). This potentially explains why selective  
328 processes related to laboratory rearing or to natural habitat conditions do not result through  
329 pleiotropic effects in population divergence in terms of resistance to contaminants, whereas  
330 genetic variability is attested. Strikingly, the importance of non-additive genetic components in  
331 stress tolerance is also reported in other arthropods for the resistance against parasite infection  
332 (Wegner *et al.* 2008). These results are also pertinent to the current debate on the relative part of  
333 additive components in the determination of the variation of complex phenotypic traits (Hill *et al.*  
334 2008; O'Hara 2008). Because the genetic architecture of complex traits implies polygeny and  
335 epistasis, this question of additive versus non-additive components is of primary importance to  
336 understanding the specific evolutionary behaviour of complex traits, as exemplified by disease  
337 susceptibilities (Blekhman *et al.* 2008). Then, analogous questioning should investigate tolerance  
338 to contaminants.

339  
340 Overall, the identification of genetic variability in tolerance to contaminants within field  
341 or laboratory populations does not necessarily indicate a strong potential to evolve genetic  
342 resistance; indeed non-additive components seem to be able to contribute substantially to the  
343 genetic variance of tolerance. The involvement of large non-additive effects may not be restricted  
344 to the model of polygenic inheritance of contaminant susceptibility through multiple minor genes  
345 implying epistasis. Even in case of inheritance through single major genes (Barata *et al.* 1998;  
346 Woods and Hoffmann 2000), non-additive effects through dominance interactions could also  
347 operate (*e.g.* Labbé *et al.* 2007). The hypothetical widespread weakness of additive components  
348 in chemical tolerance could reconcile on one hand the observation that genetic adaptation in  
349 contaminated contexts is infrequent (Klerks 2002; Millward and Klerks 2002; Woods and

350 Hoffmann 2000) and on the other hand the frequent reports of genetically determined differences  
351 in tolerance to toxic compounds. Then, following this hypothesis and as previously claimed from  
352 the apparent mismatch between laboratory and field outcomes on the inheritance of insecticide  
353 resistance (Reznick and Ghalambor 2005), the exceptional cases of adaptation of field  
354 populations to contamination would be permitted only by the fixation of rare alleles of major  
355 genes (Woods and Hoffmann 2000). Thus, quantitative genetics protocols could provide insight  
356 into the question of genetic adaptation for ecological risk assessment. Indeed it is crucial to test  
357 the hypothesis that additive genetic variation for tolerance to contaminants is generally weak, and  
358 to consider the possibility that evolution of tolerance relies more often on exceptional events in  
359 which rare alleles become fixed in exposed populations.

360

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364

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434 **Tables**

435

<i>observational variance component</i>		<i>REML estimator</i>	
sires	$\sigma_S$	$2.4 \cdot 10^{-5}$	<i>not significant</i>
dams within sires	$\sigma_D$	<b>0.24</b>	[0.14;0.39]
residual	$\sigma_{RESIDUAL}$	<b>0.47</b>	[0.42;0.53]

157 neonates; ten males mated with two females

436

437

438 **Table 1.** Paternal half-sib analysis. REML estimators of the variance components from a linear  
 439 mixed-effect model considering log-transformed lethal time of 157 neonates from the paternal  
 440 half-sib design (figure 2) as a response explained by (i) a random sire effect, (ii) a random dam  
 441 effect nested within the sire effect and (iii) a residual environmental variance. The significance of  
 442 the sire effect (*i.e.* the similarity among paternal half-sibs) was tested through a likelihood ratio  
 443 test comparing this nested model and a model with only one random brood effect. 95%  
 444 confidence intervals are provided for significant effects.

445

445

<i>observational variance component</i>		<i>REML estimator</i>	
dams	$\sigma_s$	$3.9 \cdot 10^{-5}$	<i>not significant</i>
sires within dams	$\sigma_D$	<b>0.26</b>	[0.18;0.38]
residual	$\sigma_{RESIDUAL}$	<b>0.49</b>	[0.44;0.53]
269 neonates; 11 females mated with three males			

446

447

448 **Table 2.** Maternal half-sib analysis. REML estimators of the variance components from a linear  
449 mixed-effect model considering log-transformed lethal time of 269 neonates from the maternal  
450 half-sib design (figure 3) as a response explained by (i) a random dam effect, (ii) a random sire  
451 effect nested within the dam effect and (iii) a residual environmental variance. The significance  
452 of the dam effect (*i.e.* the similarity among maternal half sibs) was tested through a likelihood  
453 ratio test comparing this nested model and a model with only one random brood effect. 95%  
454 confidence intervals are provided for significant effects.

455

455 **Figure captions**

456

457 **Figure 1. Between-brood heterogeneity in Cd-tolerance.** Box-and-whisker plots of log-  
458 transformed lethal times of neonates during 20- $\mu\text{g Cd L}^{-1}$  exposure. The three panels (A, B, C)  
459 correspond to the records of the three monthly sets of broods (March, April, May). Labels of  
460 horizontal axes report the brood sizes (*i.e.* the number of neonates tested per brood). The REML  
461 estimators of variance components of a linear mixed-effect model considering a random effect of  
462 brood on log-transformed lethal time are presented below each panel (standard deviation and  
463 95% confidence interval). Boxes extend from the first to the third quartile of lethal times within  
464 each brood with a bold segment for the median lethal time; the whiskers extend to the most  
465 extreme data points which are no more than 1.5 times the interquartile range, and open circles  
466 represent outliers.

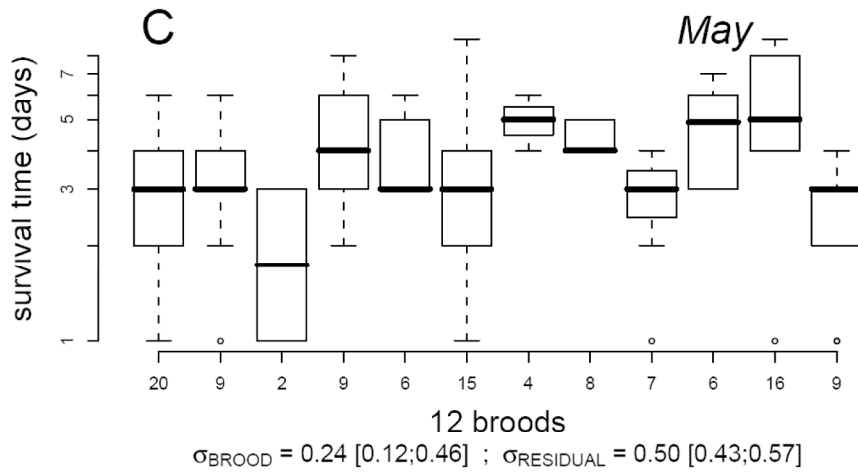
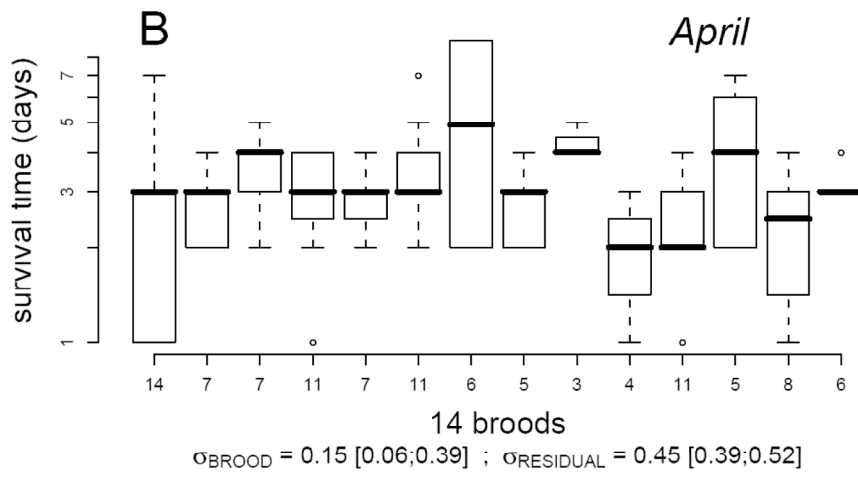
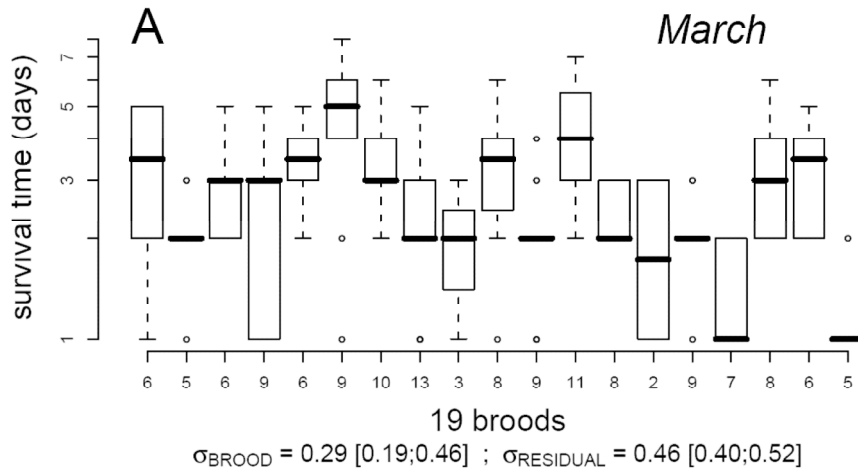
467

468 **Figure 2. Paternal half-sib design.** Box-and-whisker plots (same conventions as figure 1) of  
469 log-transformed lethal times (20- $\mu\text{g Cd L}^{-1}$  exposure) of 157 neonates from the breeding of ten  
470 males called sires (one grey level per sire) with two successive different females called dams  
471 (two successive boxes). Labels of the horizontal axis report the number of neonates tested per  
472 brood.

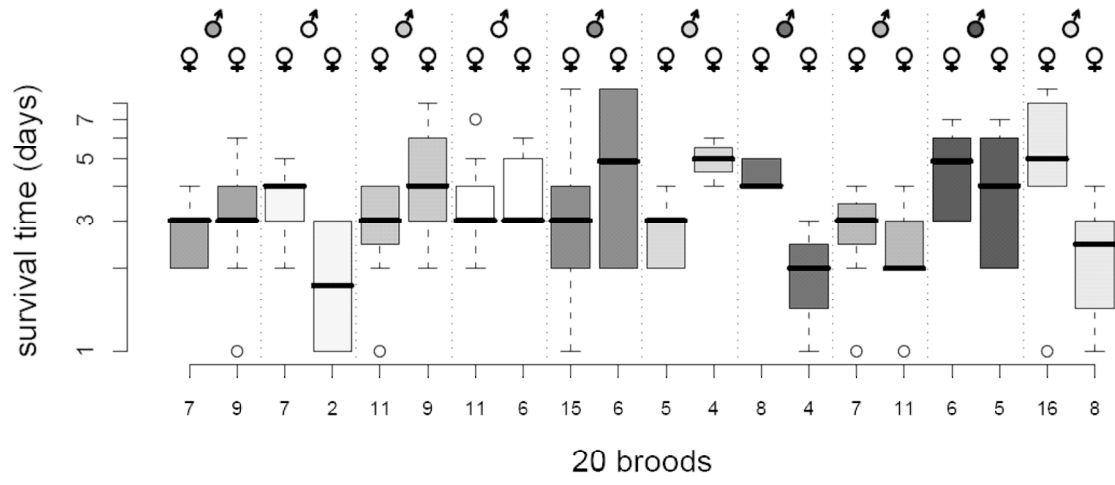
473

474 **Figure 3. Maternal half-sib design.** Box-and-whisker plots (same conventions as figure 1) of  
475 log-transformed lethal times (20- $\mu\text{g Cd L}^{-1}$  exposure) of 269 neonates from the breeding of 11  
476 females called dams (one grey level per dam) with three successive different males called sires  
477 (three successive boxes). Labels of the horizontal axis report the number of neonates tested per  
478 brood.

479 **Supp. figure 1. Breeding design.** Following one month of acclimatisation, mating pairs of  
480 *Gammarus fossarum* breeders were selected with females in the last stage of their reproductive  
481 cycle (hatched juveniles in brood pouch). After a few days, neonates were released away (pink  
482 arrows) and were collected to form a first set of broods (in March) for Cd exposure. Afterward,  
483 females moulted and shed eggs, which were fertilised by the guarding male (green arrows) and  
484 yielded neonates (violet arrows) 3 weeks later (second set of broods in April). After copulation,  
485 males were redistributed (orange arrows) in order to supply a third brood from each female  
486 (emergence in May). In total, 356 neonates were tested individually. Coloured boxes  
487 (respectively blue and pink) underline sib relatedness between broods from the different months  
488 (respectively paternal and maternal half sibs).  
489



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491

