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► To cite this version:

Marie-Agnès Coutellec, Arnaud Chaumot, Elliott Sucré. Neglected impacts of plant protection products on invertebrate aquatic biodiversity: a focus on eco-evolutionary processes. *Environmental Science and Pollution Research*, In press, 10.1007/s11356-024-32767-3 . hal-04501458

HAL Id: hal-04501458

<https://hal.science/hal-04501458>

Submitted on 12 Mar 2024

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Environmental Science and Pollution Research

Neglected impacts of plant protection products on invertebrate aquatic biodiversity: a focus on eco-evolutionary processes

--Manuscript Draft--

Manuscript Number:	ESPR-D-23-12493R1	
Full Title:	Neglected impacts of plant protection products on invertebrate aquatic biodiversity: a focus on eco-evolutionary processes	
Article Type:	Review Article	
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Corresponding Author Secondary Information:		
Corresponding Author's Institution:	INRAE	
Corresponding Author's Secondary Institution:		
First Author:	Marie-Agnes Coutellec	
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Order of Authors Secondary Information:		
Funding Information:	OFB (ECOPHYTO)	Not applicable
Abstract:	<p>The application of plant protection products (PPPs) may have delayed and long-term non-intentional impacts on aquatic invertebrates inhabiting agricultural landscapes. Such effects may induce population responses based on developmental and transgenerational plasticity, selection of genetic resistance, as well as increased extirpation risks associated with random genetic drift. While the current knowledge on such effects of PPPs is still scarce in non-target aquatic invertebrate species, evidences are accumulating that support the need for consideration of evolutionary components of the population response to PPPs in standard procedures of risk assessment. This mini-review, as part of a contribution to the collective scientific assessment on PPP impacts on biodiversity and ecosystem services performed in the period 2020-2022, presents a brief survey of the current results published on the subject, mainly in freshwater crustaceans, and proposes some research avenues and strategies that we feel relevant to fill this gap.</p>	

1 **Neglected impacts of plant protection products on invertebrate** 2
aquatic biodiversity: a focus on eco-evolutionary processes

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19 **Keywords : phytopharmaceuticals, ecotoxicology, evolutionary toxicology, aquatic invertebrates**

20

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23 impacts on aquatic invertebrates inhabiting agricultural landscapes. Such effects may induce

24 population responses based on developmental and transgenerational plasticity, selection of genetic

25 resistance, as well as increased extirpation risks associated with random genetic drift. While the

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28 population response to PPPs in standard procedures of risk assessment. This mini-review, as part of a

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36 **Introduction**

37 One of the ultimate goals of ecotoxicology is to estimate non-intentional effects of anthropogenic
38 pollution on biodiversity and ecosystem functioning, in order to provide decision-makers with tools to
39 alleviate such effects and to maintain ecosystem sustainability and services. In the European
40 framework of chemicals regulation (REACH), ecological risk assessment (ERA) targets this
41 overarching objective by evaluating new substances upstream from placing them on the market.
42 However, this assessment is most often based on short-term standard toxicity tests centered on the
43 organism level (e.g., the great majority of OECD test guidelines) which lack ecological realism,
44 especially regarding the protection goals targeted by the EU (e.g. population level assessment, see
45 WDF for aquatic ecosystems ; see Hommen et al. 2010). While the statement of this regulatory hiatus
46 is no news (e.g., Chapman 2002), it has been more recently extended to the *evolutionary impact* (Van
47 Straalen and Feder 2012 ; Straub et al. 2020), as a consequence of an ever-growing corpus of
48 evidences reported for the three last decades and which covers the tree of life [see e.g. special issues
49 on genetic and evolutionary toxicology in journals such as Environmental Health Perspectives
50 (Anderson et al. 1994), Ecotoxicology (Coutellec and Barata 2011, 2013) or Evolutionary
51 Applications (Brady et al. 2017a)]. Nowadays, the issue of human-induced evolutionary impact finds a
52 particular echo in the current context of Anthropocene (Earth's most recent geologic period during
53 which humanity became the most influential driver of environmental change) and its trail of long term
54 impacts on biodiversity. In the light of earlier findings on the development of resistance to pests
55 targeted by agricultural treatments, the question of the eco-evolutionary effects of chemical
56 contaminants is becoming increasingly important for biodiversity species, particularly in view of the
57 ever-increasing toxic pressure on various components of ecosystem communities caused by the use of
58 agricultural chemical inputs (e.g. Schulz et al 2021).

59

60 This mini-review focuses on evolutionary impacts of plant protection products (PPPs) on aquatic
61 invertebrates and was performed as a contribution to the collective scientific assessment to which this
62 special issue is devoted. More precisely, this work was part of a broader analysis of PPP
63 ecotoxicological impacts on aquatic invertebrates, including studies addressing effects at organism,
64 population, and community levels (see chapter 10 of the full report, Mamy et al. 2022). The literature
65 encompassed in the present survey covers the period 2000-2020 (with a punctual addition of more
66 recent, complementary studies), and includes both experimental and field approaches, which address
67 microevolutionary and transgenerational changes triggered by PPPs (see Table 1). In terms of
68 biodiversity, these studies typically lie at the level of intraspecies diversity- (genetic diversity within
69 and between conspecific populations). Intraspecific diversity is one of the three pillars of biodiversity,
70 upon which population adaptive potential towards environmental change is based (Lande and Shannon

71 1996). Genetic improvement programs have resorted for decades on the exploitation of genetic
72 diversity (Falconer and Mackay 1996), and the positive relationship between genetic diversity and
73 fitness, which is nicely illustrated by the experimental concept of heterosis (hybrid vigor), is today
74 largely documented in numerous non resource species (DeWoody et al. 2021). Likewise, genetic
75 diversity and extinction risks associated with its reduction are at the heart of conservation programs
76 (Frankham, 2010). The central role played by genetic diversity in the maintain of populations exposed
77 to PPPs has been recently demonstrated in a study on daphnids, showing that genetically more diverse
78 populations are able to persist longer in environments contaminated with copper (Loria et al. 2022).
79 This result takes on particular importance regarding previous works, which have revealed loss of
80 genetic diversity within daphnid populations in agricultural landscapes (Coors et al 2009).

81

82 **Table 1.** Summary information on evolutionary impacts of PPPs to aquatic invertebrates

Organisms	Delayed effects, Phenotypic plasticity	Genetic adaptation (resistance)	Maladaptation, Cost of resistance
gammarids (arthropods)	☐ Tolerance induced by pre-exposure (clothianidin ; Siddique et al. 2021)		<ul style="list-style-type: none"> • Increased susceptibility to various PPPs (Schneeweiss et al. 2023) • Fitness cost and increased susceptibility to thermal stress (Siddique et al. 2020 ; 2021)
daphnids (arthropods)	☐ Morphological defenses (carbaryl and endosulfan ; Barry 2000)	<ul style="list-style-type: none"> • Evolutionary rescue (copper ; Loria et al. 2022) • Resistance (carbaryl ; Jenssen et al. 2015) 	<ul style="list-style-type: none"> • Loss of genetic diversity (various PPPs ; Coors et al. 2009) • Increased susceptibility to parasite (Jenssen et al. 2011)
<i>H. azteca</i> (arthropod)	☐ Plasticity of enzymatic systems of detoxification (pyrethroids ; Fung et al. 2021)	<ul style="list-style-type: none"> ☐ Selection of point mutations in target proteins (pyrethrinoids, organophosphates and carbamates ; Weston et al. 2013 ; Major et al. 2018) ☐ Gene duplication (chlorpyrifos ; Major et al. 2020) 	<ul style="list-style-type: none"> • Fitness cost of resistance, lower thermal tolerance, increased sensitivity to additional contaminants (Heim et al. 2018) • Reduced thermal tolerance in interaction with increasing salinity conditions (Fulton et al. 2021)
ephemeropteran (insect)		☐ Selection of genes involved in cuticular resistance (Gouin et al. 2019)	
mosquitoes (insects)		☐ Resistance to various insecticides used in vector control, involving target site mutation, increased metabolic capacities, gene overexpression / amplification (see recent review by Liu 2015)	☐ Fitness cost of resistance (chlorpyrifos ; Delnat et al. 2019)
<i>L. stagnalis</i> (mollusc)			<ul style="list-style-type: none"> • Increased random genetic drift induced by PPP cocktails (Coutellec et al. 2013) • Increase of susceptibility in inbred lines (diquat ; Duval et al. 2016)

83

84

85 **Aquatic invertebrates as non-intentional targets of PPPs**

86 As representative of the main part of animal diversity and biomass, invertebrates do not form a
87 monophyletic group (Lecointre and Le Guyader 2017). Indeed, this grouping covers all metazoan
88 phyla (ctenophora, porifera, placozoa, cnidaria, and all bilaterian sub-phyla). Their biological traits
89 (morphology, body size, development, reproduction, etc.) encompass and underly a great diversity of
90 life histories. In spite of the occurrence of highly conserved pathways, as studied under evolutionary
91 developmental biology and comparative genomics (Carroll et al. 2005 ; Hall 2012), the heterogeneity
92 and divergence of invertebrates is reflected notably in their molecular equipment and in the way their
93 biological functions are regulated (development, metabolism, homeostasis, reproduction...). In the
94 context of ecotoxicology, such discrepancies translate into heterogeneous sensitivity to chemical
95 components such as PPPs, whose toxic modes of action can be highly specific (e.g., inhibition of
96 enzymes or receptors through the binding to specific sites in a particular taxonomic group). This
97 heterogeneity in physiological functioning across large metazoan lineages (non vertebrates) is puzzling
98 and contributes to the difficulty to assess biological disturbance by xenobiotics such as PPPs when
99 they are released into the environment.

100 Aquatic life is represented by all these phyla, sometimes exclusively (e.g., non-bilaterian metazoans).
101 Therefore, understanding the impact of PPPs on aquatic invertebrates implies that diversity and
102 heterogeneity is to be considered as pervasive and ubiquitous, notably in terms of taxonomic
103 biodiversity (the majority of animal species), complexity (e.g., body plan of a placozoan vs a
104 cephalopod mollusc), life style (sessile, mobile, parasitic forms...), life cycle (fully aquatic or not,
105 including dormancy stages), lifespan, voltinism, semel- vs iteroparity, reproductive mode (scissiparity,
106 parthenogenesis, which can be partial, cyclic, or obligatory, hermaphroditism, separate sexes),
107 dispersal (internal vs external fertilization, phoresis, parasitic cycle..), as well as functional ecology
108 and position in foodwebs. With respect to aquatic environments inhabited by invertebrates, diversity is
109 also pervasive, in terms of salinity (freshwater, brackish, and marine waters), typology (lentic vs lotic
110 systems), connectivity (dendritic hydrographic networks, interconnected marshes, estuaries...),
111 anthropization (from ponds and other artificial water bodies to deep ocean waters), and vulnerability
112 against chemical contamination including PPPs (proximity to treated agricultural zones, multiple
113 modes of transfer).

114 Invertebrates exhibit some propensity in terms of sensitivity to toxicants, which results from (1)
115 phylogenetic proximity between some groups and the organisms targeted by PPPs (e.g., aquatic
116 arthropods and crop insect pests), (2) a central position within biotic interactions, especially foodwebs,
117 which increases their ecological vulnerability to the disturbance of lower trophic levels sensitive to
118 pesticides such as herbicides and fungicides, as well as their role in propagating indirect effects to
119 higher levels such as predators, (3) sensitivity of species which phenology is in line with PPP
120 applications (e.g., seasonal reproduction of univoltine organisms and reprotoxic substances), (4)

121 relatively short lifespan and increased risk of micro-evolutionary effects induced by PPPs (multi- and
122 transgenerational effects, evolution of resistance, etc.), and (5) high fluctuation in population size and
123 isolation degree, which exacerbates demographic stochasticity and its consequences (Allee effect,
124 random genetic drift), especially in species with low mobility and dispersal, as well as those with a
125 complete aquatic lifecycle in discontinuous and semi-permanent habitats (marshes and ditches in
126 agricultural landscapes).

127

128 **Eco-evolutionary processes altered or induced by PPPs**

129 With respect to evolutionary impacts of environmental change and degradation, a brief recall of basics
130 may be useful here before focusing on the particular case of PPPs. The ability of natural species to
131 respond adaptively to environmental change relies on a combination of characteristics inherited
132 through macroevolution (since their ancestral lineage) and of on-going microevolutionary
133 (populationlevel) processes that continuously shape their genetic diversity and adaptive potential
134 (Brady et al. 2017b). Such responses may thus either reflect ancestral exposure to stressful conditions,
135 be they abiotic (e.g. oxidative stress and aerobic mode of life) or biotic (e.g. host-parasite and host-
136 pathogen coevolution), or more recent and novel stressors, to which species ancestors were naive
137 (various xenobiotic substances). Adaptation is by itself an ambiguous term, which can be understood
138 either as the phenotypic expression of an already adapted state (the result of past evolution induced by
139 ancient selective pressures, for example, lungs as a breathing system adapted to terrestrial life) or as
140 the evolutionary process « in action » leading to this state, i.e., the on-going selection of advantageous
141 genetic variants (Bateson and Gluckman, 2011). The latter implies that variation at the adaptive trait
142 occurs in the population and has a heritable genetic component upon which natural selection can
143 operate. By contrast, the former (the achieved adapted phenotype) is expected to present limited
144 genetic variation and thereby low ability to respond to new selective factors, due to longstanding
145 evolutionary constraints (see Arnold 1992).

146

147 Compared to purely ecological studies, approaches designed to tackle evolutionary effects of PPPs are
148 recent, and therefore much less numerous. This is in line with the recent awareness that ecological and
149 evolutionary processes may operate at a similar pace, due to intensified human-induced environmental
150 disturbances and invertebrate biodiversity crisis. Appropriately, the ever increasing number of cases of
151 genetic resistances observed in pests, as adaptation to PPPs (see Sparks et al. 2021), illustrates quite
152 well how rapid such evolutionary effects can be in agrosystems. This and the current knowledge on
153 resistance modes of propagation and molecular basis (Paris and Després 2012 ; Ffrench-Constant
154 2013) provide strong arguments to consider evolutionary effects when it comes to address biodiversity
155 impacts of PPPs. However, the demonstration of an evolutionary impact is not an easy task, due to

156 many confusing factors and unknown parameters (population history and level of genetic variation)
157 and studies appropriately designed to this end are not plethora.

158
159 Traditional ecotoxicological approaches mainly focus on direct effects measured or estimated during
160 exposure, which prevents from detecting any post-exposure consequences (see Beketov and Liess,
161 2008), including evolutionary changes. Delayed effects may be lethal or sublethal, affecting organisms
162 later in their life or in their progeny (parental effects, inter- and transgenerational effects, of epigenetic
163 or genetic nature). In turn, such effects have consequences at the population level, in terms of growth
164 rate and fitness, as well as phenotypic and genetic diversity, and trait heritability (see Medina et al.
165 2007 ; Hoffmann and Willi 2008 ; Bickham 2011 ; Coutellec and Barata 2011 ; Oziolor et al. 2016,
166 Brady et al. 2017a). Such delayed effects have been shown in the laboratory after several days
167 following pulse exposure of various invertebrates exposed to thiacloprid at concentrations with
168 moderate toxicity. For example, in the case of the crustacean *Gammarus pulex* exposed to thiacloprid,
169 the LC50 (concentration that kills 50% of test individuals) was found 50 times lower 17 days after a
170 24h long exposure than that estimated one day post-exposure (Beketov and Liess 2008). Likewise, in
171 the damselfly *Coenagrion scitulum* exposed as larvae to the pyrethrinoid insecticide esfenvalerate at
172 environmental concentrations, a reduction of locomotory capacity in adults was observed, leading to a
173 lowered ability to expand to the north as a response to climate change (Dinh et al. 2016). In the
174 trichopteran insect *Brachycentrus americanus*, females- decreased their investment in reproduction
175 when exposed at pupal stage to the same insecticide (Palmquist et al. 2008).

176

177 **Phenotypic plasticity as a response to PPPs**

178 Facing new stressors, natural populations can respond plastically or genetically. The former process is
179 known as *phenotypic plasticity*, the ability of a genotype to produce distinct phenotypes when exposed
180 to different environments throughout its ontogeny (Pigliucci 2005). Phenotypic plasticity can be
181 supported by an epigenetic basis (see Hallgrimsson and Hall, 2011; -Ashe et al. 2021). Despite the
182 confusion around the concept of plasticity and how it differs from immediate and transient homeostatic
183 response to stress, an inclusive definition seems to have become consensual, which considers both an
184 irreversible component, determined during parental or early exposure (*developmental plasticity*)– see
185 Plautz and Salice (2013) for an example of contaminant exposure during embryonic development in a
186 gastropod - and a later, reversible component induced by the environment experimented during the
187 post-embryonic life course of the organism (*phenotypic flexibility*, which can affect behavior,
188 physiology, and morphology) (Piersma and Drent, 2003 ; Beaman et al. 2016 ; Lande 2019). One
189 classical example in ecotoxicology for phenotypic flexibility is the acclimation during pre-exposure to
190 chronic concentrations of a toxicant that increases the acute tolerance in case of subsequent exposure
191 to the same chemical. Although studies addressing the contribution of phenotypic plasticity to PPP

192 tolerance remain extremely rare in comparison for instance with a growing number of studies
193 documenting this mechanism for metallic contaminants, a recent illustration was reported for the
194 tolerance of the freshwater crustacean *Gammarus pulex* preexposed in the laboratory to the
195 neonicotinoid insecticide clothianidin (Siddique et al 2021). The response is moreover considered «
196 adaptive » if the phenotype produced in a given environment effectively increases the genotypic
197 fitness in that environment. This adaptive value implies some level of environmental predictability
198 (e.g., seasonal variation of temperature, light, presence of a predator, etc.). At the population level,
199 plasticity can thus itself be subject to natural selection, although empirical evidences are not
200 overwhelming (Van Buskirk and Steiner 2009 ; Arnold et al. 2019). Phenotypic plasticity can offer a
201 way to adapt in stressful environments when population genetic diversity is already depreciated
202 (Baldanzi et al. 2017). It is often viewed as a way to respond to rapid environmental change (Fox et al.
203 2019), as already suggested as plausible mechanism for population maintain in field contamination
204 context (see an example in the annelid by Kille et al 2013). A spectacular example of such a response
205 is found in daphnids, who develop defense structures (helmets, spines) in the presence of predator cues
206 (Weiss et al. 2004). It is interesting to note that such morphological modifications can also be induced
207 by chemicals such as insecticides carbaryl and endosulfan (Barry 2000), and that more generally,
208 epigenetic alterations can be induced by various xenobiotics (see Vandegehuchte and Janssen 2014)
209 including PPPs (e.g., vinclozolin, Skinner and Anway, 2007). Because some epigenetic changes may
210 be transmitted to next generations along with genetic information (Schmid et al. 2018 ; Nilsson et al.
211 2022), it is now admitted that epigenetics plays a role in adaptation, through interactions with genetic
212 variation and evolutionary forces (Bonduriansky et al. 2012).

213

214 **Selective effects of PPPs**

215 Besides rapid plasticity, population adaptive responses may also rely on the selection of natural genetic
216 variants. This type of response implies that genetic variation pre-exists in the population at genes
217 involved in the responsive traits, and that a positive relationship occurs between the trait value and
218 fitness. In an ecotoxicological context, stress response may be generated by various molecular and
219 cellular mechanisms. The evolution of multiple resistances to pesticides in target species provides a
220 corpus of knowledge about some of these mechanisms. Genetic resistance, as a case of evolutionary
221 adaptation due to selective effects of PPPs, can result from different evolutionary processes : selection
222 of advantageous variants from extant genetic variation in the population, point *de novo* mutation,
223 horizontal transfer, and adaptive introgression by hybridization (Hawkins et al. 2019). Metabolic
224 resistance, which results from a higher capacity to metabolize the pesticide, is supposed to be
225 multigenic and to spread through selection on extant variation. On the contrary, target site resistance is
226 monogenic and may imply spontaneous *de novo* mutations. In target species, selection is strong and
227 may favor a new advantageous mutation more rapidly than in non-target species.

228 The evolution of tolerance to various insecticides in non-target aquatic invertebrates reveals a process
229 of genetic selection induced by chronic or repeated exposure. Such an evolution was observed in the
230 crustacean *Hyalella azteca* species complex as a response to persistent pyrethroids in sediments of
231 californian hydrosystems (Weston et al. 2013; Major et al. 2018). Authors of this extensive study
232 combined laboratory toxicity assessment and genotyping of natural populations sampled in sites with
233 different loads of contamination by pyrethroids. Resistances prove to be multiple, implying parallel
234 evolution of distinct alternative mutations already described in pest insects at the pyrethrinoid target
235 gene, which encodes for the sodium channel protein (*vgsc*). Similarly, another point mutation was
236 discovered that confers resistance to organophosphates and carbamates, especially in agricultural
237 landscapes, by leading to a substitution in the amino acid sequence (glycine to serin at position 119) of
238 acetylcholine esterase (AChE) (Major et al. 2020). The study moreover suggests a duplication of the
239 gene *ace-1* (encoding AChE), which confers resistance to chlorpyrifos. Following the description of
240 the major role played by genetic determinism in the observed tolerance to pyrethroids, a study has
241 complemented this vision of genetic mutants resistant to PPPs, demonstrating that plasticity processes
242 linked to the activation of detoxification enzymatic systems also underpin tolerance in the same
243 *Hyalella* populations chronically exposed to PPPs (Fung et al 2021). Beyond intraspecific
244 (populationlevel) evolutionary impacts associated with the loss of genetic diversity induced by
245 directional selection as well as potential cost of resistance (Coustau et al. 2000 ; see below), the
246 accumulation of pyrethroids in resistant populations of *H. azteca* raises the question of functional
247 consequences at the level of ecosystems, as their (secondary) consumers are expected to concentrate
248 bioaccumulable pesticides in higher amounts than when predated on non tolerant preys (Hartz et al.
249 2021). In the same line, recent works suggest the development of resistance to insecticides in another
250 freshwater crustacean, *Gammarus pulex*, through the observation of differential sensitivity to the
251 neonicotinoid clothianidin between individuals sampled in populations exposed to different
252 concentrations of PPPs (Becker and Liess 2017 ; Shahid et al. 2018 ; Becker et al. 2020). However, the
253 resistance factors in this case are much weaker (of the order of 2 or 3) compared with studies on
254 *Hyalella* (e.g. tolerance factors of up to 550, Weston et al 2013). Authors of these studies invoke
255 factors susceptible to modulate the evolution of genetic resistance in gammarids, such as the proximity
256 of non-tolerant populations (decreasing the efficiency of selection by gene flow from non-adapted
257 populations), as well as low taxonomic biodiversity of the community, which would relax interspecific
258 competition and thereby reinforce intraspecific competition (and selection) in gammarids. Temporal
259 fluctuations in tolerance levels were recorded in the field, with an unexpected pattern of negative
260 correlation with exposure levels along the seasons, explained as a reduction in potential variability of
261 sensitivities within populations at the maximum of the cultural treatments, which may induce high
262 lethal stress in streams (Becker et al. 2020). The genetic or plastic nature of tolerance seems here again
263 not totally exclusive depending on the exposure history of populations (Siddique et al 2021).

264 Furthermore, evolution of resistance to these insecticides is not systematic, especially in environments
265 with more diversified contamination profiles or affected by multiple stressors. This is the conclusion
266 reached by studies of amphipod tolerance, which reported increased susceptibilities to PPPs in
267 agricultural environments (Schneeweiss et al 2023), for example, or in aquatic environments under
268 urban influence, which are also impregnated with insecticides (Švara et al 2021, Grethlein et al 2022).
269 In another vein, a population genomics approach, without *a priori*, designed to identify genomic
270 regions involved in the response to selection (local adaptation) suggests that PPPs affect the
271 distribution of genetic diversity in populations of the ephemeropteran insect *Andesiops torrens* in
272 Chile, through the detection of two genes under selection which are involved in cuticular resistance to
273 insecticides (*LDL receptor-related protein 2* and *Dump*) (Gouin et al. 2019).
274 Under conditions of severe stress, population ability to react through genetic adaptation following a
275 phase of demographic decline is at the base of the evolutionary rescue concept (Bell 2017).
276 Interestingly, the above-mentioned study on daphnid exposure to copper also shows that if the
277 majority of exposed populations finally decline or go to extinction, some of them exhibit a U-shaped
278 dynamics consistent with this phenomenon (Loria et al. 2022).

279

280 **Cost of genetic adaptation**

281 Resistance to PPPs may imply a cost, revealed by a decrease in fitness (defined as the ability of an
282 organism to leave offspring in the next generation of the population) when the selective pressure is
283 alleviated, or when an additional factor is surimposed. The concept of fitness cost reflects this decrease
284 and relies on the observation that in absence of PPP, resistant genes are generally rare in populations
285 (an indication that they would be selected against), and that resistance is often unstable in experimental
286 lines or populations (Coustau et al. 2000). A recent review encompassing 170 studies published on the
287 cost of resistance to insecticides in target insects showed that in 60% of the cases, resistance
288 effectively implies a fitness cost (Freeman et al. 2021). This cost is particularly expressed as a return
289 of the strain to the sensitive state in absence of treatment (reversion), and by a decreased reproduction.
290 However, the generality of this result masks differences among insecticide classes, with a cost less
291 frequently reported for organochlorids, pyrethrinoids, neonicotinoids and Bt. In aquatic invertebrates
292 (not directly targeted by PPPs), although this type of effect is still largely understudied, one can
293 mention the seminal works of Jansen & coll. on the effects of the carbamate carbaryl to *Daphnia*
294 *magna*. These studies showed that the level of tolerance expressed in exposed natural populations
295 lowers under laboratory conditions in absence of the insecticide (Jansen et al. 2015), and that clonal
296 strains resistant to carbaryl (following experimental exposure) prove to be more sensitive to parasites
297 than their non-resistant counterparts (Jansen et al. 2011). Another example is provided in aquatic
298 arthropods by a study showing a cost of resistance to chlorpyrifos in the mosquito *Culex pipiens*, that
299 is expressed in presence of Bti, and translates both by a reduced larval survival and a higher

300 vulnerability to its predator *Plea minutissima* (Delnat et al. 2019). Tolerance to neurotoxic insecticides
301 observed in field populations of freshwater amphipods also documented the potential fitness costs
302 induced by multi-generational exposure to PPPs. Pyrethroid resistance in *Hyaella* has hence been
303 associated to fitness costs for tolerant organisms with increased susceptibility to thermal stress and
304 lower reproductive capacities (Heim et al 2018). Neonicotinoid tolerance in *Gammarus pulex*
305 population from agricultural areas goes with reduced demographic growth when populations are
306 cultured under PPP-free conditions in the laboratory (Siddique et al 2020), and increased susceptibility
307 to elevated temperature (Siddique et al 2021). These observations highlight a major challenge in the
308 context of ongoing global change, characterized by multiple stress situations in the future aquatic
309 environment.

310

311

312 **Non adaptive evolutionary impacts of PPPs**

313 Besides selective effects that may be exerted by the chronic presence of a given family of PPPs, the
314 chemical pressure related to the recurrent use of complex cocktails of molecules in agricultural
315 systems may also affect the genetic diversity of exposed populations, through demographic
316 impairment, as already evocated above for field daphnids populations (Coors et al 2009). This type of
317 effects was also illustrated in the gastropod *Lymnaea stagnalis*, in outdoor mesocosm experiments
318 where mixtures of PPPs were applied, as a result of treatment programs and following various ways of
319 transfer from the treated parcels to the surrounding aquatic systems (aerial drift, run-off, and drainage ;
320 Auber et al. 2011). The experiment showed that in a few generations, treatments decreased population
321 neutral genetic diversity (microsatellite markers) and increased population genetic differentiation, as
322 theoretically expected due to random genetic drift (Coutellec et al. 2013). Similarly, by experimentally
323 manipulating effective population size in order to calibrate demographic reductions potentially
324 induced by toxicants, we also detected a rapid effect of random drift load in this species, by observing
325 significant heterosis among small populations (Coutellec and Caquet 2011). This effect is indicative of
326 impaired adaptive potential associated with reduced population genetic diversity, and of increased
327 extirpation risk due to the random accumulation of spontaneous slightly deleterious mutations
328 (Whitlock et al. 2000). Although not directly triggered by PPPs in the cited study, this finding points to
329 the genetic risk incurred by populations when their demography is recurrently impaired by PPPs. Last,
330 among other evolutionary forces able to interact with the selective potential of PPPs, inbreeding needs
331 to be mentioned. Using experimental evolution in *L. stagnalis*, a synergistic interaction could be
332 highlighted between the pro-oxidant herbicide diquat and inbreeding induced by self-fertilization as
333 compared to cross-fertilization (Duval et al. 2016).

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Research needs and perspectives

While evolutionary processes become better incorporated into ecology, their study remains limited in ecotoxicology, despite early awareness of the scientific community (Van Straalen and Timmermans 2002). However, the level of knowledge of such effects is continuously improved. Regarding PPPs, aquatic invertebrates, as a group of unintentional targets particularly vulnerable, deserves specific attention in this respect. However, face to the challenge that represents the demonstration of evolutionary impacts on natural populations (the response of which depends upon population genetic diversity, isolation, and exposure history) and of their consequences at the ecosystem level (which may be masked by a number of others factors or missed because unanticipated), it seems unrealistic to directly incorporate evolutionary principles into ecological risk assessment procedures. Nonetheless, from the information compiled in the present study, it appears that ecological risk assessment of PPPs would gain a significant step in scientific soundness if it could more explicitly consider evolutionary components of population responses. We hereby propose a few avenues that might help progress in this way. First, the consideration of genetic variation in standardized toxicity testing could represent a relevant issue for reflection towards this endeavor (see e.g., Côte et al. 2015). Next, the coupling of laboratory approaches and field population monitoring in ecologically relevant species might provide a way to complement *a priori* ERA, once a substance has been authorized. Last, the current corpus of results acquired on evolutionary impacts of PPPs could be implemented in a rational approach of knowledge sharing allowing the development of extrapolation strategies, for example, through the use of phylogenetic components of species sensitivity (Brady et al. 2017b ; Lalone et al. 2018), and through the search of mechanistic links between organism responses and evolutionary « modes of action » of PPPs. This type of approach could fit in with an evolutionary extension of the currently expanding concept of adverse outcome pathway (Ankley et al. 2010). Moreover, by using in particular tools offered today by environmental omics, we can quickly hope for new gains in mechanistic knowledge on the eco-evolutionary effects of contaminants based on the coupling of multigenerational experiments in the laboratory and field studies in historically exposed populations (in gammarids, see e.g., Gouveia et al. 2018). This knowledge on the genetic, epigenetic, or plastic nature of tolerance, on the physiological costs potentially induced by these adaptive or maladaptive phenomena should thus advance our understanding of the spatial and temporal dynamics of evolutionary impacts of environmental contamination. From this, we could be able to better decide on the relevance and way of inclusion of the examination of evolutionary processes in the predictive risk assessment schemes for PPPs, but also in our analysis of the causes of the ongoing decline of aquatic biodiversity in agricultural landscapes.

372

373 **Acknowledgements** The authors thank Stéphane Pesce, Laure Mamy, and Wilfried Sanchez for
374 inviting us to participate to the collective scientific assessment on PPP effects to biodiversity and
375 ecosystem services, Sophie Leenhardt for the coordination of our work, and Sophie LePerchec for her
376 bibliographical assistance.

377

378 **Author contribution** Marie-Agnès Coutellec, Arnaud Chaumot and Elliott Sucré realized the
379 scientific collective assessment. MAC drafted the first version of the present manuscript. AC and ES
380 revised and drafted the final version. All authors contributed to the writing of the manuscript,
381 revisions, and approved the final submitted version.

382

383

384 **Funding** This work was funded by the French Office for Biodiversity (OFB) through the national
385 Ecophyto plan.

386

387 **Declarations**

388 **Ethics approval and consent to participate** Not applicable

389 **Competing interests** The authors declare no competing interests.

390 **Consent to publish** Not applicable

391 **Availability of data and materials** Not applicable

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393 **References**

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