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EDITORIAL

Special issue on long-term ecotoxicological effects: an introduction

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Two years after the Special Issue published in Journal *Ecotoxicology* on Evolutionary Process in Ecotoxicology (vol 20 (3); see Coutellec and Barata 2011), the present issue proposes a timely update on long-term biological impacts of pollutants, including delayed, trans-generational, and evolutionary effects. The number of articles contributed in the present issue confirms that this question is gaining ground in the field of ecotoxicology. Despite the awareness of the scientific community that evolutionary change can occur rapidly (e.g., Carroll et al. 2007), and that addressing the long term ecotoxicological impact is relevant to improve the significance of ecological risk assessment of chemicals (e.g., Bickham 2011), this research field has been poorly explored until recent time. Most often, long term trans-generational or evolutionary responses to pollution have been limited to experimental studies based on model species appropriate to multi-generation exposure (primarily waterflea or other short-lived invertebrates) and to molecules with some knowledge or expectation on their mode of action (e.g., metals, candidate genes involved in tolerance and adaptation). While these remain undeniably sound models, current advances in molecular technology allow tackling long term trans-generational or evolutionary effects under more realistic conditions, involving natural ecosystems, presence of multistressors in the field, and using species of particular ecological relevance (including longer-lived species), or by tracing back evolutionary processes in wild populations (see van Straalen and Feder 2012).

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Long-term effects of pollutants: inferring causative relationships

One recurrent problem in assessing the evolutionary impact of a given chemical is that cause-effect relationships are still extremely difficult to determine in the field, as many factors can be responsible for an observed pattern of neutral or selectable genetic variation. Nevertheless, we simply cannot ignore such impact just because it is difficult to assess. The current endeavor of scientists towards filling this gap is further motivated by the fact that the environment needs to be considered in its changing nature (i.e., with regard to climate or biological invasions). The ever-increasing demand imposed by humans on natural resources also requires that some trade-off can be reached between human impact and conservation (see Bijlsma and Loeschcke 2012). Reliable tools are needed to enable decision makers to apply conditions under which this trade-off could be optimized in order to ensure a sustainable use and management of ecosystems and resources. This is clearly a new challenge, and in this respect, long-term and evolutionary ecotoxicology represents a building block of such a strategy, providing concepts and tools with both indicative and predictive value, that could be implemented in environmental risk assessment (see also Breitholtz et al. 2006; Bickham 2011).

In the present issue, long-term effects of pollutants are either documented under natural situations or tested using experimental designs, and some contributions combined

both approaches to decipher adaptive and acclimation processes. Selected studies focused on levels ranging from the organism and its progeny (delayed and trans-generational effects) to populations, communities, and phylogenetic scales. Refinements are proposed to improve toxicity testing and by extension, ecological risk assessment.

Evolutionary responses above the species level: lineage, community

The role of the genetic background and the phylogenetic component of population and species sensitivity are illustrated through a review on invertebrate metal tolerance (Dallinger and Höckner). These authors summarize a set of key features such as genome size, population genetic structure, size and diversity, heritability, epigenetic effects, selection pressure and macroevolutionary processes, that can potentially affect the distribution of species sensitivity to pollutants. In particular, the authors illustrate differential micro- and macroevolutionary mechanisms of tolerance to Cd across invertebrate species, as linked to a single gene or to gene networks, functional diversification of metallothionein isoform families, point mutations in exonic sequences or in regions involved in transcription regulation.

Evolutionary patterns above the species level were also inferred at the community level, using multigeneration exposure of natural phytoplanktonic communities to triclosan (Pomati and Nizzetto). These authors used high-throughput phenotypic trait analysis to track effects at the individual, population and community levels, across generations. Results suggest that phytoplankton acclimates to a pulse of triclosan stress by increasing average size and biovolume. Besides functional effects, observed changes in the rate of phenotypic evolution suggest the occurrence of micro-evolutionary responses. The evolutionary component of community responses to triclosan was further investigated using a partition of total community change into ecological and physiological contributions, and residual component.

Evolutionary cost of tolerance

A new framework is also proposed to estimate evolutionary risk, by including fitness impairment and fitness cost associated to adaptation (Tanaka and Tatsuta). The method builds on the hypothesis that evolution optimizes fitness on the basis of a trade-off on tolerance, and uses laboratory estimates of the cost of tolerance (reduction of population growth rate under uncontaminated conditions). A case-study focused on insecticide tolerance in *Daphnia galeata* suggests that tolerance may have a substantial contribution to population fitness decline, persisting after the toxic pressure is relaxed.

Delayed impact of pollutants: within- versus trans-generation effects

Pollutants may have various delayed effects. A series of articles addressed these issues, and illustrated a diversity of mechanisms through which early exposure or exposure during previous generations can impact performances at later stages or generations. Dormant eggs or ephippia are sexually produced by water fleas under harsh conditions, which can accumulate over years in the sediment. These egg banks represent a reservoir of species and genetic diversity. They may also potentially integrate repeated episodes of environmental contamination. Navis and colleagues showed that pesticide exposure during dormancy could be responsible for embryo developmental defects, and for effects on survival and reproduction. Therefore, effects on dormant eggs, as cohort effects (see Beckerman et al. 2002), deserve a particular attention in ecotoxicity testing, especially because many sources of pollution can accumulate in sediments and affect population and community adaptability to further environmental change or stress.

The long-term consequences of genotoxic and mutagenic effects of pollutants have been a controversial issue, as these effects may not be passed on to next generations. However, if mutagenesis affects the germline or if genotoxicity triggers some repair mechanisms that may in turn generate DNA modifications (e.g., error prone polymerases), these molecular impairments are then expected to have profound evolutionary consequences. Two studies included in the present Issue addressed this question in fishes. Santos and colleagues showed that developmental impairment of *Gasterosteus aculeatus* embryos was induced by parental exposure to the model alkylating compound methyl methane sulfonate. The experimental design allowed to show that such effects were related to the male germline, in consistency with the noticeably weak DNA repair ability of spermatozoa. Using the same compound, Fassbender and Braunbeck described similar effects on *Danio rerio* embryos. However, by further monitoring the progeny life history, including survival of their own offspring, the authors also showed that in the zebrafish, trans-generational effects of methyl methane sulfonate may not extend beyond F1 early stages. This means that effects were directly related to a poor embryo quality and were not genetically inherited.

Sobral et al. used a cytotoxic assay to investigate the mutagenicity and genotoxicity of an acide mine drainage

effluent. According to the authors, results could explain the absence of sensitive clones in *Daphnia longispina* populations inhabiting a reservoir near the entrance of the effluent. Mutagenesis of acide mine drainage effluents were also related to the unusual high levels of neutral genetic diversity reported in *D. longispina* populations inhabiting the affected reservoir.

The relative contribution of delayed and parental effects were also tackled by Plautz and Salice, using cadmium and the freshwater snail *Physa pomilia*. While both parent exposure and exposure from the egg stage increased early juvenile tolerance to Cadmium, the study also showed that in this system, developmental plasticity has a predominant effect relative to parentally transmitted tolerance.

The interplay between biotic factors and toxicants can also extend over generations, as shown in the freshwater snail *Biomphalaria glabrata* by Plautz and colleagues. In this work, exposure to predator cues in one generation increased survival in the next generation when this one was exposed to Cadmium. These results also highlight the importance of controlling for parental origin in ecotoxicity testing, especially when tests are based on integrative traits such as life history and fitness related traits.

Experimental evolution under toxic conditions

Experimental exposure is a powerful approach to test hypotheses related to toxicity and adaptive stress response. Two studies included in the present issue addressed the evolutionary impact of Uranium on *Caenorhabditis elegans* experimental lines. In the work reported by Dutilleul and colleagues, contrasted responses were obtained through generations, reflecting a possible role of acclimation at low concentrations, whereas evolutionary adaptive responses might be necessary to cope with higher concentrations. However, no evidence for adaptation to U could be found by Goussen and colleagues after a 16 generation exposure experiment. While a low evolvability of the traits measured might be responsible for this outcome, a design involving replicate populations as well as final relaxation of U pressure for several generations would be necessary to limit stochastic effects and to rule out maternal environmental effects.

Adaptation does not necessarily occur in chronically exposed populations, even in the presence of genetic variation. There may be constraints on selection at a given trait (physiological, developmental or phylogenetic constraints, selection on other traits leading to negative genetic correlations), or other forces may prevent selection from arising (gene flow, random genetic drift). Furthermore, pollutants may simply not act as a selective force, especially when they are present as mixtures of compounds with different modes of action. Under such conditions, chronic or repeated exposure is more likely to inflate the effect of random genetic drift, through recurrent population demographic bottlenecks, and possibly through reduced gene flow. This hypothesis was tested using experimental populations of the freshwater snail *Lymnaea stagnalis* exposed to cocktails of pesticides. After three generations, both the level and distribution of genetic diversity were consistent to some extent with expectations based on demographic impairment. However, although the design implemented replicate populations initiated with the same gene pool, results emphasized a critical influence of sample size, and more generally call for high caution when interpreting population genetic parameters in an ecotoxicological context.

Hypothetical evolutionary outcomes expected under different ecotoxicological scenarios were proposed by Ribeiro and Lopes.

Adaptive genetic divergence versus environmental acclimation

While the detection of population adaptive divergence associated to natural selection is not an easy task *per se*, it becomes still more complicated to attribute a pattern of local adaptation to a putative factor such as pollution. Nevertheless, methods currently developed in population genetics and population genomics are most promising in this respect (Beaumont and Balding 2004; Nosil et al. 2009; see also examples of application in link with pollution: Lind and Grahn 2011; Orsini et al. 2012). In the present issue, Jansen and colleagues used cDNA microarray in *Daphnia magna* to investigate the modes of action of natural and chemical stressors known to have evolutionary effects in this species, Navarro and colleagues compared variation at genetic, transcriptomic and physiological markers in populations of the invasive mussel *Dreissena polymorpha*, as a function of industrial pollution. While they found no evidence for genetic neutral differentiation of the studied populations, gene expression profiles correlated with physiological performances and heavy metal load in the tissues of wild-caught individuals, suggesting a predominantly environmental role of pollution in the measured phenotypic responses (acclimation). Nevertheless, it is to be noted that the lack of neutral genetic divergence at the studied markers did not prove that divergent selection does not operate on other regions of the genome, either directly under selection, or indirectly through hitch-hiking effect (selective sweep).

Acclimation was also invoked as primary cause of increased tolerance to Copper in populations of the barnacle *Amphibalanus variegatus* occupying contaminated

coastal sites in Australia. Using a genome scan approach, Gall and colleagues did not find evidence for selection driven differentiation, i.e., no loci with higher divergence than expected under neutrality. Although true patterns might not have been detected due to the method applied and to the number of loci studied, results were consistent with high gene flow counteracting local selection, as expected in highly dispersing marine species.

Bellanger-Deschênes and colleagues developed a omic-based approach in the yellow perch specifically designed to detect molecular adaptive evolution due to metal contamination. A set of neutral and candidate markers (potentially under directional selection) were identified from an annotated transcriptome scan, and used to genotype individuals from populations historically exposed to a range of Cd and Cu concentrations. By linking allele frequencies at outlier loci to metal concentration in liver, they were able to identify three candidate genes, whose functional characterization was further performed with high-throughput sequencing and using closest available reference genomes. Compared to traditional ecotoxicological approaches which rely on sets of candidate genes chosen on an a priori knowledge (or expectation) about the mode of action, this innovative methodology significantly increases the power to detect pollutant driven selection patterns among natural populations. The fact that the approach can be applied to species without a reference genome is an additional advantage for environmental risk assessment, since the choice of model species can be based on criteria of ecotoxicological significance (e.g., chemical exposure, ecology).

The set of publications organized in the present issue addressed various aspects of long term pollutant impact, based on empirical description of effects as well as on methodological developments. Beyond basic results and explanatory hypotheses put forward in each article, we hope that the work presented will contribute as a whole to pave the way to the incorporation of trans-generational and evolutionary effects of pollutants on natural systems into ecological risk assessment.

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References

Beaumont MA, Balding DJ (2004) Identifying adaptive genetic divergence among populations from genome scans. Mol Ecol 13:969–980

Beckerman A, Benton TG, Ranta E, Kaitala V, Lundberg P (2002) Population dynamic consequences of delayed life-history effects. Trends Ecol Evol 17:263–269

Bickham J (2011) The four cornerstones of evolutionary toxicology. Ecotoxicology 20:497–502

Bijlsma R, Loeschcke V (2012) Genetic erosion impdes adaptive responses to stressful environments. Evol Appl 5:117–129

Breitholtz M, Ruden C, Hansson SO, Bengtsson BE (2006) Ten challenges for improved cotoxicological testing in environmental risk assessment. Ecotoxicol Environ Saf 63:324–335

Carroll SP, Hendry AP, Reznick DN, Fox CW (2007) Evolution on ecological time-scales. Funct Ecol 21:387–393

Coutellec MA, Barata C (2011) An introduction to evolutionary processes in ecotoxicology. Ecotoxicology 20:493–496

Lind E, Grahn M (2011) Directional genetic selection by pulp mill effluent on multiple natural populations of three-spined stickleback (*Gasterosteus aculeatus*). Ecotoxicology 20:503–512

Nosil P, Funk DJ, Ortiz-Barrientos D (2009) Divergent selection and heterogeneous genomic divergence. Mol Ecol 18:375–402

Orsini L, Spanier KI, De Meester L (2012) Genomic signature of natural and anthropogenic stress in wild populations of the waterflea *Daphnia magna*: validation in space, time and experimental evolution. Mol Ecol 21:2160–2175

van Straalen NM, Feder ME (2012) Ecological and evolutionary functional genomics: how can it contribute to the risk assessment of chemicals? Environ Sci Technol 46:3–9