Conséquences de la pollution environnementale sur l'évolution des traits d'histoire de vie : l'exemple de la Daphnie

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Sommaire

INTRODUCTION

La pollution d'origine anthropique est classée parmi les menaces majeures pesant sur la biodiversité (Salafsky *et al.*, 2008). En effet, dans des cas extrêmes, elle peut causer l'extinction locale de populations (Helmuth *et al.*, 2002). Cette pollution peut avoir des conséquences évolutives et affecter les quatre mécanismes de l'évolution que sont la sélection naturelle, les flux de gènes, la dérive et la mutation (Bickham, 2011). Par exemple elle peut imposer une pression de sélection et favoriser les individus résistants lorsqu'elle génère des effets délétères réduisant la survie et le succès reproducteur des individus qui y sont exposés de manière chronique (sur plusieurs générations). Les individus résistants possèdent des adaptations avantageuses et héritables réduisant les effets délétères de la pollution. Cette sélection pourra se traduire par une divergence phénotypique entre les individus provenant de populations exposées à la pollution et ceux provenant de populations non exposées. Si ces populations se trouvent dans un environnement hétérogène pour la pollution et ne sont pas homogénéisées par des flux de gènes ou que la sélection n'est pas contrebalancée par mutation ou par dérive génétique, elles pourront s'adapter localement (Kawecki & Ebert, 2004). Outre ces adaptations qui peuvent modifier le comportement, la physiologie, la morphologie ou encore les traits d'histoire de vie des individus, la sélection induite par la pollution peut également générer une « érosion » de la diversité génétique des populations (van Straalen & Timmermans, 2002). De plus, l'évolution de ces résistances aux polluants peut générer un coût rendant les populations résistantes moins performantes en absence de la pollution à laquelle elles sont adaptées en comparaison de populations non résistantes/adaptées (Agra, Soares, & Barata, 2011). Les conséquences évolutives potentielles de la pollution sur les populations semblent donc majeures et suggèrent l'importance d'en tenir compte dans les études écotoxicologiques (Coutellec & Barata, 2011).

La pollution aux métaux traces comme pression de sélection…

Les métaux traces ou éléments traces métalliques (ETM) regroupent les éléments métalliques présents à des concentrations inférieures à 1g.kg^{-1} dans la croute terrestre. Ils peuvent être confondus avec les « métaux lourds », une appellation désuète qui catégorisait les métaux selon leur masse volumique. Certains métaux traces sont des oligoéléments essentiels au fonctionnement métabolique (e.g. le Cuivre, le Chrome, le Nickel ou le Zinc) comme dans les métalloprotéines impliquées dans la respiration que sont l'hémoglobine (Fer) ou l'hémocyanine (Cuivre). Néanmoins, les ETM deviennent toxiques au-delà d'une certaine concentration dépendante de l'ETM, de l'espèce (biologique) et de la population considéré-e-s. En effet, les éléments trace métalliques sont peu stables et se trouvent majoritairement sous forme ionique positivement chargée (cation, e.g. Cu^{+} ou Cu^{2+}, Ni^{2+} …) et ont une affinité forte pour les atomes de soufre et d'azote (Nieboer & Richardson, 1980) qui composent de nombreuses protéines, ils peuvent ainsi se lier à ces protéines et molécules, dont l'ADN (Patlolla *et al.*, 2009), modifier leur conformation et les rendre non fonctionnelles (Rainbow, 1997, 2002). De plus, les ETM peuvent augmenter la production des dérivés réactifs de l'oxygène générant le dysfonctionnement de protéines, lipides et molécules: appelé stress oxydatif (Nuran Ercal, Hande Gurer-Orhan, & Nukhet Aykin-Burns, 2001; Tchounwou *et al.*, 2012).

Les sources des métaux traces sont diverses et peuvent être naturelles et anthropiques. Bien que les sources naturelles soient multiples comme les poussières et les gaz provenant des sols, des volcans, des embruns marins, des feux de forêts… les activités humaines sont les principales responsables de la pollution aux métaux traces (Nriagu & Pacyna, 1988; Azimi *et al.*, 2003, 2005b; Hill, 2010). En effet, les sources anthropiques des métaux traces sont nombreuses: les activités minières, la combustion

fossile (industrielle et par le trafic routier), les stations d'épurations, le chauffage domestique, l'utilisation de certains produits phytosanitaires, les industries chimique et métallurgique… (Nriagu & Pacyna, 1988; Azimi *et al.*, 2005b; Hill, 2010; DRIEE, 2013). Les métaux traces se retrouvent ainsi dans l'air, dans le sol et dans l'eau.

Les écosystèmes dulçaquicoles comme les mares et les lacs sont impactés par ces apports en polluants car ils présentent des volumes relativement peu importants et ils ne possèdent pas ou peu de connexions permettant la dilution ou le déplacement de la pollution (Hill, 2010). En Île-de-France, huit métaux traces sont considérés comme des micropolluants métalliques majeurs des écosystèmes dulçaquicoles (DRIEE, 2013): l'Arsenic (Arsenic - As), le Cadmium (Cadmium - Cd), le Chrome (Chromium - Cr), le Cuivre (Copper - Cu), le Nickel (Nickel - Ni), le Plomb (Lead - Pb), le Zinc (Zinc - Zn), le Mercure (Mercury - Hg).

Bien que leur leurs utilisations soient encadrées (e.g. DRIEE, 2013) et leurs émissions réduites (Azimi *et al.*, 2005a), les métaux traces, via leur toxicité, la multiplicité de leurs sources, leur ubiquité et leur persistance dans les milieux (Hill, 2010) restent une problématique importante dans l'évaluation des risques écotoxicologiques.

Les écosystèmes dulçaquicoles présentant des quantités élevées d'éléments traces métalliques, comme par exemple au niveau des sites de drainage minier, ont fait l'objet de nombreuses études sur les impacts écotoxicologiques de cette pollution (e.g. Lefcort *et al.*, 2002; Lopes, Baird, & Ribeiro, 2005; Agra, Soares, & Barata, 2011). Par ailleurs, les ETM sont généralement plus présents dans les milieux urbains, qui concentrent les activités humaines et les pollutions qui y sont associées, que dans les milieux ruraux (e.g. Azimi 2005, Bilos 2001, Maas *et al.*, 2010). Les conséquences écologique et évolutive de l'urbanisation sont largement étudiées (Voir Box 1: L'habitat urbain) néanmoins les effets de la pollution aux métaux traces à des concentrations urbaines ont fait l'objet de moins

d'intérêt (mais voir les études sur le pigeon biset de Chatelain *et al.*, 2014 - 2018) et restent méconnus chez les espèces d'eau douce.

Box 1 : L'habitat urbain

Les humains modifient drastiquement leur environnement ce qui génère des effets souvent néfastes sur les populations naturelles. Les changements d'utilisation et d'occupation des sols, considérés comme la modification de la nature physique et/ou biotique d'un site, font partie des changements globaux imposés par les humains (Vitousek, 1997; Grimm *et al.*, 2008). Une des causes de ces changements d'utilisation et d'occupations des sols est l'urbanisation et son importante expansion au cours du 20ème siècle et qui ne devrait pas ralentir pendant les 50 années à venir (Grimm *et al.*, 2008).

Le milieu urbain est un milieu perturbé par une perte rapide et la fragmentation de l'habitat natif, la présence d'espèces introduites (e.g. McKinney 2002), de la pollution chimiques, sonore (e.g. Nemeth & Brumm, 2009) et lumineuse, des températures plus élevées comparé au milieu rural, phénomène appelé « îlot de chaleur urbains » (Oke, 1973; Arnfield, 2003)… Ces perturbations affectent la biodiversité ainsi que la morphologie, la physiologie et le comportement des populations urbaines et impactent leurs dynamiques éco-évolutives (Donihue & Lambert, 2014; Alberti, 2015).

… et les adaptations attendues pour leurs avantages sélectifs.

De manière intéressante, les populations de Daphnies montrent des capacités d'adaptations rapides en réponse à la pollution et notamment aux métaux traces (e.g Medina, Correa, & Barata, 2007; Agra *et al.*, 2011; Messiaen *et al.*, 2012; Hochmuth *et al.*, 2015; Turko *et al.*, 2016). La pression de sélection potentiellement exercée par la présence de polluants peut favoriser les génotypes capables de réduire leurs effets

délétères par un « évitement » du polluant qui peut être déterminé (fixe) ou conditionnel (plasticité phénotypique) : chez les Daphnies, il peut s'agir d'évitement comportemental (Lopes, Baird, & Ribeiro, 2004), physiologique via la détoxication (Haap, Schwarz, & Köhler, 2016), ou encore d'évitement temporel ou spatial grâce à des stratégies de dormance et de dispersion. L'environnement urbain peut présenter une hétérogénéité spatiale et temporelle de la pollution aux métaux traces (Azimi *et al.*, 2003, 2005b; spatiale Chapitre 1, Figure 3) et cette hétérogénéité peut favoriser les formes de dormance et de dispersion permettant d'échapper respectivement temporellement et spatialement à la détérioration de l'habitat (Wiener & Tuljapurkar, 1994; Hairston & Cáceres, 1996; Bowler & Benton, 2005; Gerber & Kokko, 2018).

Il est intéressant de constater que, chez les Daphnies, la reproduction sexuée donne lieu à la production d'éphippie permettant à la fois d'entrer en dormance, de disperser et d'accumuler les métaux et de potentiellement les détoxiquer.

Les prodigieuses éphippies et l'hypothèse en 3D: Dormance, Dispersion et Détoxication

Les Daphnies se reproduisent par parthénogenèse cyclique et peuvent produire sexuellement des oeufs de diapause (également appelés oeufs de durée) encapsulés dans une éphippie (Voir Box 2 : Cycle de vie de la Daphnie). Ces éphippies présentent des capacités de dispersion à la fois dans le temps et dans l'espace ainsi que d'accumulation des métaux :

La **D**ormance

La dormance, considérée comme une diminution/un arrêt métabolique et/ou développemental, permet une persistance dans le temps lorsque les conditions environnementales ne sont pas favorables à la survie des individus actifs (« dispersion

dans le temps » (Pietrzak & Slusarczyk, 2006)). Elle est divisée entre phénomènes de quiescence et de diapause. Chez la Daphnie, il s'agit de diapause car la sortie de dormance n'est pas permise par la disparition des indices environnementaux ayant induit l'entrée en dormance mais nécessite la perception de nouveaux indices environnementaux (Cáceres, 1997).

Les éphippies peuvent rester en dormance durant une saison ou peuvent éclore jusqu'à plusieurs dizaines d'années après leur ponte ((Hairston, 1996) ainsi que toutes les études utilisant des éphippies pour des approches paléolimnologiques et d'écologie de la résurrection dont Rogalski, 2015, 2017; Turko *et al.*, 2016). Durant ce temps elles peuvent être exposées à l'anoxie dans les sédiments, à la dessiccation et au gel. Il a également été montré que les éphippies résistent au passage dans le tube digestif de poissons et oiseaux (et même de rats de laboratoire) et que le taux d'éclosion de ces éphippies était d'autant plus important lorsque les éphippies étaient mélanisées (Mellors, 1975). Certaines éphippies tombent rapidement dans les sédiments et se retrouvent dans la banque d'oeufs dormants, tandis que d'autres flottent, facilitant leur dispersion spatiale et suggérant l'existence de stratégies de dispersion différentes chez les Daphnies (Pietrzak & Slusarczyk, 2006; Bernatowicz *et al.*, 2018).

La **D**ispersion

Bien que très bonnes dormeuses, les éphippies sont capables de disperser efficacement dans l'espace et de coloniser rapidement de nouveaux environnements (Cáceres & Soluk, 2002; Louette & Luc, 2005). Les éphippies peuvent disperser grâce aux déplacements d'autres animaux (phorésie) sur lesquelles elles peuvent adhérer (comme sur les pattes des Notonectes ; van de Meutter, Stoks, & de Meester, 2008), ou qui les auraient mangées (Mellors, 1975). Il a été observé que les plus petites éphippies adhéraient mieux sur les Notonectes et étaient donc mieux dispersées par ce vecteur (van

de Meutter, Stoks, & de Meester, 2008). Néanmoins, le facteur majeur permettant la dispersion des éphippies semble être le vent. En effet, même lorsque couverts par des filets empêchant aux animaux d'y accéder, les mésocosmes des études de Cáceres & Soluk (2002) et Cohen & Shurin (2003), pouvaient tout de même être colonisés par les oeufs de diapause des Daphnies.

Box 2 : Cycle de vie de la Daphnie

Les Daphnies sont des crustacés planctoniques d'eau douce, elles sont protégées par une armure (carapace chitineuse) et sont connues sous le nom de « puces d'eau » car elles nagent, à l'aide de leur seconde paire d'antennes larges et bi-ramées, en faisant de petits bonds. Leur première paire d'antennes quant à elle est réduite et est munie de sensilles. Les cinq à six paires d'appendices thoraciques permettent à la respiration et au nourrissage par filtration en créant un courant guidant l'eau et son contenu jusqu'aux maxilles, aux mandibules et à la bouche (Fryer, 1991; Ebert, 2005).

Le cycle de vie des Daphnies est complexe et variable (Figure 1). Certaines espèces se reproduisent par parthénogenèse obligatoire (reproduction asexuée continue ; e.g. Hebert & Crease, 1983). D'autres se reproduisent par parthénogenèse cyclique : lorsque les conditions sont optimales pour la population considérée, les femelles parthénogénétiques produisent de nombreuses filles clonales et maximisent leur fécondité, en effet les couvées parthénogénétiques peuvent aller jusqu'à 100 oeufs (Ebert, 2005). En réponse à certains indices environnementaux, les femelles, sous contrôle hormonal (LeBlanc & Medlock, 2015) produisent d'un part des mâles, et d'autre part une enveloppe résistante, chitineuse et mélanisée, nommée l'**éphippie** (Hiruta & Tochinai, 2014), qui pourra recevoir les oeufs fécondés et diapausants. Les femelles Daphnies peuvent ainsi alterner entre reproduction sexuée et asexuée au cours de leur vie. Au niveau de l'individu, mâle et éphippie sont produits à des moments différents évitant la

fécondation entre mère et fils tandis qu'au niveau de la population, les productions de mâles et d'éphippies sont synchronisées maximisant la probabilité de rencontre et de fécondation entre les partenaires (Yampolsky, 1992; De Meester & Vanoverbeke, 1999). Les éphippies seront libérées avec la mue et peuvent être vides ou contenir un ou deux oeufs fécondés selon le succès de rencontre/de fécondation.

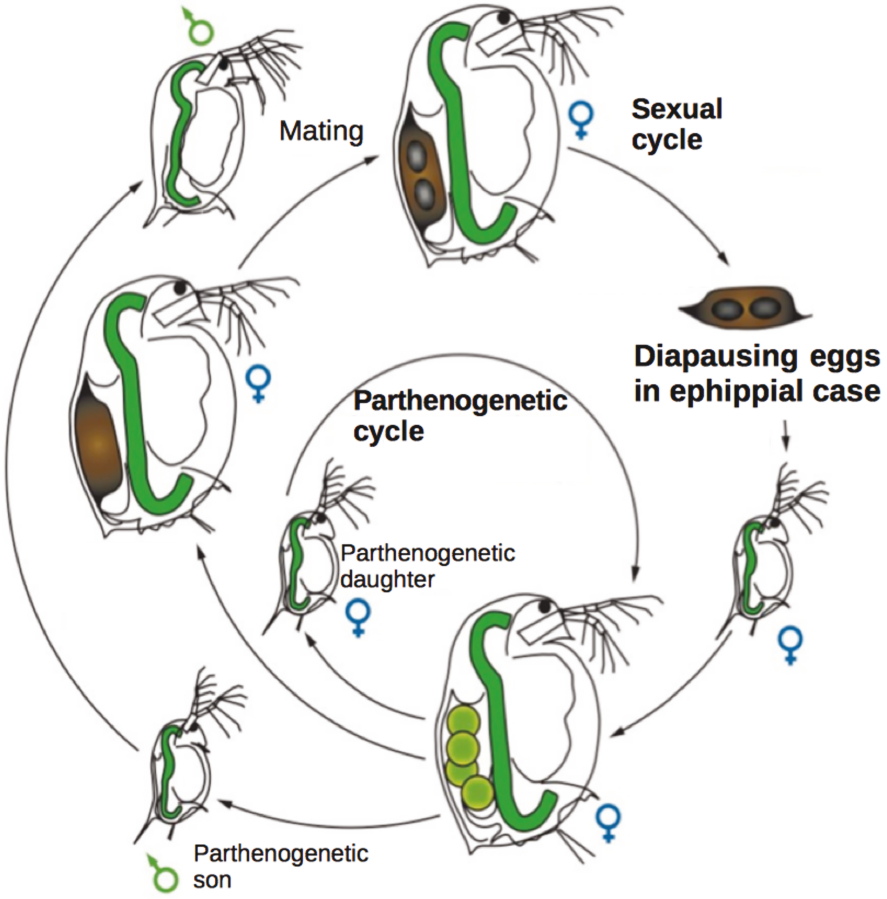


Figure 1: Cycle de vie des Daphnies, d'après Ebert, 2005

Les indices environnementaux pouvant modifier l'investissement individuel ou populationnel dans la reproduction sexuée chez les Daphnies sont nombreux, il peut s'agir d'un changement de photopériode, de la température, de la disponibilité en ressources, de la temporalité de l'habitat, de la présence potentielle de prédateur, de certains parasites, de certains polluants, de la surpopulation, et bien souvent de l'interaction entre ces facteurs et avec le génotype/la population considéré-e (e.g. Stross & Hill, 1968; Hairston &

Olds, 1984; Kleiven, Larsson, & Hobek, 1992; Deng, 1996; Zhang & Baer, 2000; Haeba *et al.*, 2008; Baer, McCoole, & Overturf, 2009; Roulin *et al.*, 2013; Conde-Porcuna, Ramos-Rodríguez, & Pérez-Martínez, 2014).

La **D**étoxication

La détoxication est un processus physiologique permettant d'inactiver et/ou d'excréter les éléments toxiques pour l'organisme. Pour fixer et/ou excréter un élément toxique pour l'organisme, un ligand spécifique de cette substance toxique est souvent nécessaire. Par exemple, chez les Daphnies, les métallothionéines (Klaassen, Liu, & Choudhuri, 1999) sont des protéines connues pour réduire la disponibilité des métaux dans les tissus et il a été observé qu'elles sont plus exprimées chez les individus exposés au Cadmium (Haap, Schwarz, & Köhler, 2016).

Une comparaison entre les concentrations en métaux traces mesurées dans les éphippies et dans les sédiments qui les contiennent a montré que les concentrations en Cadmium, en Chrome et en Molybdène étaient plus élevées dans les éphippies que dans les sédiments (Wyn *et al.*, 2007). Ces résultats indiquent des capacités d'accumulation dans les éphippies et peuvent suggérer des propriétés de détoxication de ces dernières. Ces capacités d'accumulation des métaux traces par l'éphippie pourraient détoxiquer la Daphnie l'ayant produite lui conférant une fitness plus élevée dans un habitat pollué en comparaison d'une Daphnie ne produisant pas d'éphippie dans le même habitat. De plus, produire une éphippie plus grande pourrait être avantageux car elle pourrait stocker plus de métaux.

La mélanine est connue pour ses capacités de chélation des ions métalliques permettant de les inactiver et de les séquestrer dans des parties inertes et mélanisées de l'organisme (voir Box 3 : Métaux & Mélanine). L'oeil composé (issu de la fusion des deux yeux au cours de l'ontogenèse), l'ocelle (reliquat d'oeil de la larve nauplius) et les

éphippies (enveloppe des oeufs de durée) sont généralement les seules parties mélanisées chez la Daphnie. Néanmoins, chez les populations alpines et arctiques, la carapace des individus peut également être mélanisée ce qui les protègerait des radiations UV (McWalter, 1983; Hebert & Emery, 1990; Rautio & Korhola, 2002). Les éphippies accumulent donc les métaux et sont mélanisées. Chez la Daphnie, la variation de pigmentation mélanique de l'éphippie est principalement d'origine génétique (héritabilité de 0.77 ± 0.04 (moyenne ± erreur standard) dans Gerrish & Cáceres, 2003) mais certains facteurs environnementaux peuvent également l'influencer, comme l'investissement populationnel dans la production d'éphippies, la température ou la morphométrie (aire et profondeur) du point d'eau d'origine (Gerrish & Cáceres, 2003). On peut faire l'hypothèse que les Daphnies produisant des éphippies plus mélanisées pourraient détoxiquer les ETM dans cette enveloppe et seraient ainsi favorisées dans les milieux pollués aux métaux traces par rapport aux Daphnies produisant des ephippies plus claires.

La production d'éphippie fécondée représente une étape clef dans le cycle de vie des Daphnies qui permet d'échapper à des conditions défavorables par la dispersion à la fois dans le temps et dans l'espace (Gerber & Kokko, 2018) mais également de potentiellement détoxiquer les métaux traces grâce notamment, à la mélanine. La production d'éphippie pourrait être avantageuse dans un milieu pollué aux métaux traces et perturbé comme le milieu urbain. On peut donc supposer que les Daphnies sexuées et produisant des éphippies plus mélanisées soient sélectionnées dans ce milieu.

Box 3 : Mélanine & Métaux

La mélanine, pigment le plus répandu dans le monde animal, est responsable de la coloration rousse et brune dont l'intensité dépend de sa concentration. En plus d'être impliquée dans les mécanismes de défense immunitaire majoritaires chez les invertébrés (système prophénoloxydase et encapsulation; Ebert, 2005) la mélanine est connue pour ses capacités de chélations de cations métalliques grâce à ses groupements négativement chargés (carboxyles, hydroxyles et amines) (MacGraw, 2003). Chez différents taxons, il a été observé que les individus présentant une pigmentation mélanique élevée étaient plus présents que les individus plus clairs dans des milieux pollués aux ETM (Loumbourdis & Vogiatzis, 2002; Fenoglio *et al.*, 2005; Marques, Gonçalves, & Pereira, 2008; Goiran, Bustamante, & Shine, 2017). Aussi, chez les simocéphalus (Crustacés, cladocères, comme les Daphnies) exposés au Cuivre et au Chrome, un assombrissement de la carapace a été observé (Mishra, Shukla, & Chopra, 2018).

La production de mélanine permettrait de stocker les métaux dans des parties inertes de l'organisme (comme les plumes ou la carapace) et les individus les plus mélanisés subiraient moins les effets délétères causés par les ETM, auraient une meilleure fitness que les individus moins mélanisés dans les environnements pollués aux métaux traces et seraient ainsi plus représentés dans ces environnements. C'est le cas chez le pigeon biset chez qui, les individus plus pigmentés sont présents plus fréquemment dans les milieux plus urbanisés (Jacquin *et al.*, 2013) et potentiellement plus pollués et la pigmentation mélanique des plumes est positivement corrélée aux concentrations en Zinc et en Plomb dans ces plumes (Chatelain *et al.*, 2014, 2016). De plus les juvéniles présentant le plumage le plus foncé avaient un meilleur taux de survie suite à une exposition au Plomb suggérant une fonction adaptative de ce plumage mélanique en milieu pollué (Chatelain *et al.*, 2016).

Détection & évitement comportemental

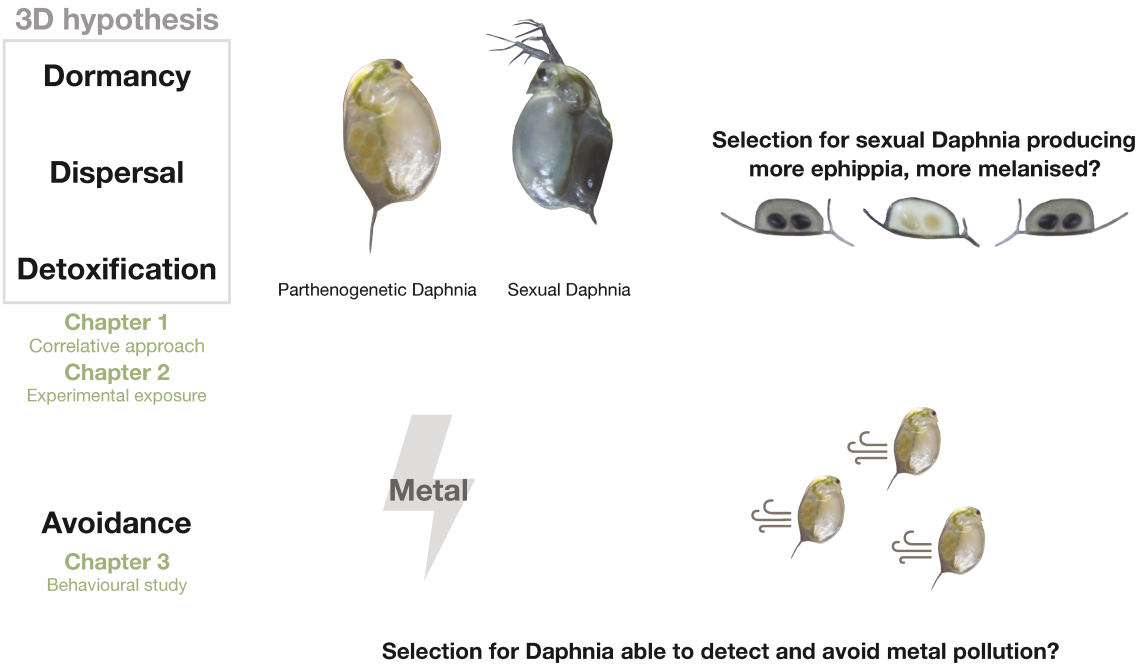
Une fois exposé à un polluant, la détoxication semble être une stratégie adaptative limitant les effets délétères de ce polluant. Une autre stratégie pourrait être de réduire son exposition au polluant, ce qui nécessite des capacités de détection de ce polluant et une réponse comportementale d'évitement de la zone polluée. Cette adaptation pourrait être sélectionnée dans les environnements présentant une pollution hétérogène spatialement.

La détection et l'évitement comportemental de la pollution aux métaux traces ont été observés au sein de plusieurs taxons et en particulier chez des invertébrés aquatiques (Wentsel *et al.*, 1977; Oakden, Oliver, & Flegal, 1984; Lefcort *et al.*, 2004; Lopes *et al.*, 2004; Eriksson Wiklund, Börjesson, & Wiklund, 2006; Ward, Simpson, & Jolley, 2013a,b; Araújo *et al.*, 2016). Il s'agit le plus souvent de populations exposées à une pollution importante et de source localisée (e.g. site de drainage minier (Lefcort *et al.*, 2004)) ou de populations de laboratoire (Ward *et al.*, 2013b) et il semblerait que la détection et l'évitement des ETM n'aient jamais été observés chez des populations urbaines.

La capacité à détecter et à éviter les métaux traces pourrait être avantageuse dans un milieu pollué aux métaux traces comme le milieu urbain. On peut donc supposer que les Daphnies capables de détecter et d'éviter les métaux soient sélectionnées dans ce milieu.

Problématique & Hypothèses

- **Les Daphnies sont-elles capables d'affronter la pollution aux métaux traces en milieu urbain ?**
- **Par quels moyens ?**



CHAPITRE UN

This chapter presents our correlative field approach along a gradient for pollution by metals in Parisian region that has resulted in a publication in *Biological Journal of the Linnean Society* in February 2018 (doi:10.1093/biolinnean/bly026/4956956).

Since Mercury is one of the primary freshwater micropollutants in this region, we planned to measure it. Nevertheless, Mercury was below the detection threshold in our samples, thus it could not be included in our study.

As urbanisation concentrates human activities and resulting disturbances and pollutions, we took the degree of urbanisation into account in our study. One reviewer suggested that urbanisation is not important for the hypothesis that we aimed to test, and it could be a confounding factor. Thus, this environmental factor have been removed from the publication, but results related to the degree of urbanisation can be found in Annex 1.

More and smaller resting eggs along a gradient for pollution by metals: dispersal, dormancy and detoxification strategies in Daphnia?

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Trace metals are bioavailable, persistent and potentially harmful chemicals commonly found in cities. In metal-polluted habitats, natural populations may evolve adaptations and may be composed of individuals exhibiting detoxification mechanisms, in particular through melanization, dispersal or dormancy abilities. Interestingly, Daphnia cyclically produce chitinous melanized envelopes called 'ephippia' encasing the resting eggs, which allow dispersal in space and in time (dormancy). Moreover, the success of dispersal decreases with increasing ephippial size. We hypothesized that populations living in polluted ponds produce more, darker and smaller ephippia than populations from unpolluted ponds. We sampled 51 ponds distributed in the Paris region and investigated the link between concentrations of seven trace metals and the presence of Daphnia and ephippia, and the size and pigmentation of ephippia. First, the presence of Daphnia was not linked to local metal concentrations, which ranged gradually from low to high values. Second, the probability of the presence of ephippia in sediments increased with metal concentrations, suggesting a selective advantage of Daphnia in producing dormancy stages in polluted habitats. Third, although ephippial pigmentation was not linked to metal concentrations, ephippial size decreased with increasing metal concentrations, suggesting a selection for increased dispersal in polluted habitats. Overall, our results show that anthropogenic pollution may have important microevolutionary consequences in urban populations, which are generally overlooked.

Keywords: ephippia – melanin – microevolution – size – trace metals.

Introduction

Urban areas are environments that concentrate human activities and associated forms of pollution, such as trace metals (Azimi et al., 2003). These chemical pollutants are bioavailable, bioaccumulative and persistent and have various and often toxic effects on reproductive output and survival (Møller, Forbes & Depledge, 1996; Žaltauskaitė & Sodienė, 2014; Rogalski, 2015). It is thus likely that they will affect the functioning and eco-evolutionary dynamics of ecosystems and that they may exert selective pressures leading to adaptations by organisms living in polluted environments (Klerks & Weis, 1987; Medina, Correa & Barata, 2007; Chatelain, Gasparini & Frantz, 2016).

Among potential adaptive phenotypic traits, melanin pigmentation may present an advantage owing to its detoxification properties. The chelation of metal ions by the several negative charges of melanin (McGraw, 2003) may promote organism detoxification by sequestering trace metals in melanized inert parts. Accordingly, a positive link between melanin pigmentation and trace metal pollution has been documented in several taxa (Loumbourdis & Vogiatzis, 2002; Fenoglio et al., 2005; Marques, Gonçalves & Pereira, 2008; Chatelain et al., 2014, 2016; Goiran, Bustamante & Shine, 2017). Therefore, more melanized individuals would have greater fitness than paler ones in the presence of toxic trace metals. Accordingly, previous studies in feral pigeons have shown that darker pigeons sequester more metals in their feathers and have higher juvenile survival when exposed to metals than less melanic ones (Chatelain et al., 2014, 2016). Thus, darker phenotypes may be more prevalent in habitats polluted by trace metals.

Adaptations to metal pollution may also include the ability to invest in the production of dispersal forms and/or dormancy stages. Dispersal is known to be selected when environmental variability in habitat quality is mainly spatial (Wiener & Tuljapurkar, 1994; Bowler & Benton, 2005) and would make it possible to leave polluted habitats in favour of less polluted ones. Dormancy is favoured when temporal variability in habitat quality

prevails (Hairston & Cáceres, 1996) and would make it possible to avoid pronounced pollution events until the return of optimal living conditions. Although the precise relative investment in dispersal or dormancy would thus depend on the spatial vs. temporal variability in pollution, the general feature of spatio-temporal heterogeneity of environmental pollution may therefore favour high dispersal and/or dormancy in polluted habitats.

In this study, we investigated whether metal concentrations were positively associated with the following: (1) the presence of dispersing and dormant stages; and (2) the intensity of melanin-based coloration of these stages in Daphnia. During its life cycle, this crustacean switches from parthenogenesis (clonal female reproduction) to sexual reproduction involving sexual females and males and resulting in up to two eggs. These eggs are protected by a chitinous envelope called the 'ephippium', which exhibits several interesting characteristics, such as variation in dispersal propensity, dormancy ability and melanin-based coloration. Indeed, ephippia can be dispersed by wind and by animals ('dispersal in space'; Maguire, 1963; Cáceres & Soluk, 2002; van de Meutter, Stoks & de Meester, 2008), and dispersal propensity decreases with ephippium size and buoyancy (van de Meutter et al., 2008). They can also remain dormant for several years or even decades ('dispersal in time'; Hairston et al., 1995; Cáceres, 1997, 1998). Finally, the ephippium is the only melanized stage in Daphnia (except in alpine and arctic populations, where active individuals are also melanic; Hebert & Emery, 1990), and this pigmentation is variable and heritable (Gerrish & Cáceres, 2003). Altogether, this set of traits offers a unique opportunity to test our hypotheses.

To this end, we sampled 51 ponds in the Paris region, measured the concentrations of seven trace metals (arsenic, cadmium, chromium, copper, nickel, lead and zinc) and correlated them with the presence of both Daphnia individuals and ephippia, and with the size and melanin pigmentation of the ephippia. According to the 'dispersal in space' and

'dispersal in time' hypotheses (Pietrzak & Ślusarczyk, 2006) and to the melanin 'detoxification' hypothesis (Chatelain et al., 2014, 2016), we predicted a higher production of ephippia and smaller, more melanized ephippia in more heavily polluted ponds.

Material & Methods

Sampling design

Fifty-one non-interconnected freshwater ponds and lakes were sampled on one occasion each in the Île- de-France (Paris region) between November 2015 and April 2016. Natural and artificial ponds were randomly chosen, according to their position and distance from the centre of Paris (Point Zéro des routes de France, 48° 51′ 12,24845′′ N, 2° 20′ 55,62563′′ E), to the elevation and to their depth and volume (calculated as half of an ellipsoid). Although we do not have information on either zooplankton assemblages or sedimentation rates, the high number of ponds (N = 51) sampled ensures that these factors are randomized and not confounded with the factors of interest in our study, making their influence on the variables very unlikely. For each pond, the presence of Daphnia individuals was recorded by vertically passing a net with a mesh size of 570 µm in the deepest part of the pond up to 50 cm. Regardless of the presence of Daphnia individuals in the water and at the same pond locations, we collected the top 3 cm of sediment in a circular area of 12 cm in diameter from each pond, representing ~3 years of egg accumulation in sediments (Hairston et al., 1995; Francis, 1997). To retrieve ephippia from the sediments, we used the sugar centrifugal flotation method (Onbé, 1978; Cromar & Williams, 1991), which allows > 90% of the sampled eggs to be retrieved with one treatment (Onbé, 1978) without affecting egg via- bility (Onbé, 1978; Cromar & Williams, 1991), making it useful for future studies on these eggs.

Trace metal measurements

A sample of water was taken from each pond and filtered (0.45 µm mesh) in order to measure concentrations in seven trace metals [arsenic (As), cadmium (Cd), chromium (Cr), copper (Cu), nickel (Ni), lead (Pb) and zinc (Zn)] considered to be primary freshwater micropollutants in the Île-de-France region (Nriagu & Pacyna, 1988; DRIEE, 2013). These metals originate both from industrial and urban direct effluent discharges and from total (wet and dry) atmospheric deposition (Nriagu & Pacyna, 1988; Azimi et al., 2003, 2005; DRIEE, 2013). This was a first-time sampling; therefore, we do not have historical data on metal pollution in the ponds, but we sampled ponds at increasing distance from the centre of Paris in order to obtain a pollution gradient that represents the variability of the Île-de-France region. We assumed that, despite their temporal variation, concentrations in water reflect recent exposure, including the period of release of ephippia. Concentrations were determined by mass spectrometry (ICP-MS Agilent 7500 cx) as described by Chatelain et al., 2016. All materials were cleaned and sanitized in 10% nitric acid baths to avoid trace metal contamination.

Characteristics of ephippia

For each ephippium, the area and the intensity score of melanin pigmentation (grey level of the ephippial surface) were measured via photographic analysis with ImageJ software (Gerrish & Cáceres, 2003). Photographs were taken with a motorized microscope (SteREO Discovery.V12 Zeiss). Each ephippium was assigned to one of two Daphnia species groups, according to its shape and size characteristics: the *Daphnia galeata* and *Daphnia pulex* group (larger ephippia and ‘narrowing sharply at posteroventral side’) or the *Daphnia ambigua* and *Daphnia parvula* group (smaller ephippia and ‘more or less symmetrical’; Vandekerkhove et al., 2004).

Statistical analyses

We investigated associations between pond characteristics (metal concentrations, longitude, latitude, elevation, distance from the centre of Paris, and pond volume) with a Pearson correlation matrix. Given that the concentrations of several metals appeared to be highly correlated, we performed all further statistics for each metal separately in order to avoid collinearity issues. We first used generalized linear models for binomial data to investigate the effects of metal concentration presence of Daphnia individuals and ephippia. Second, we used linear mixed models to test the effect of the metal concentration on ephippial size and melanin coloration, adding groups of species as a fixed effect and pond as a random effect to take pseudo-replication issues into account. For all analyses, metal concentration, ephippial size and melanin coloration were log10-transformed. Statistical analyses were performed with R version 3.3.1. We did not correct P-values for multiple testing, as suggested by García (2004), Moran (2003) and Nakagawa (2004).

Results

Environmental variables linked to metal concentrations

There was an important variability in concentrations of trace metals among ponds (Table 1). Concentrations of these seven trace metals in the ponds were positively and significantly correlated (all $P \leq 0.03$, $0.31 \leq \rho \leq 0.76$), except between Zn and As ($P = 0.17$; Table 2). We found interesting correlations between environmental variables and metal concentrations. First, concentrations of Cr, Ni, Cu, Zn and Cd in water were lower when pond volume was high (Table 2). Second, concentrations of Cd, Cr and Ni were higher at higher elevation (Table 2).

Probability of the presence of Daphnia and ephippia

The probability of the presence of ephippia in pond sediments increased significantly with the concentration of Pb (logistic regression (LR) $\chi21 = 7.55$, d.f. = 1, P = 0.006; Fig. 1A), Cd (LR $\chi21 = 6.59$, d.f. = 1, P = 0.01; Fig. 1B) and Ni (LR $\chi21 = 4.50$, d.f. = 1, P = 0.03; Fig. 1C) but was not related to the concentrations of other metals (all P > 0.32). There appeared to be threshold concentrations in Ni, Cd and Pb (Ni, 3.61 µg/L; Cd, 0.056 µg/L; Pb, 1.45 µg/L) above which ephippia were systematically found in the ponds. The presence of Daphnia individuals was not dependent on trace metal concentrations (all P > 0.29). Pond size characteristics were not linked to the presence of ephippia and Daphnia individuals in ponds (all P > 0.11).

Ephippial characteristics

We did not find any statistical link between ephippial pigmentation and any of the metal concentrations (all P > 0.07). However, as the species group effect was significant for all metals (all P ≤ 0.0008) we analysed ephippial size separately for each group species. In the *D. galeata* and *D. pulex* group, ephippia size decreased significantly with increasing concentrations of Pb ($\chi21 = 12.35$, d.f. = 1, P = 0.0004), Zn ($\chi21 = 5.96$, d.f. = 1, P=0.015),Cu($\chi21 = 5.63$,d.f.=1,P=0.018)andCr ($\chi21 = 4.32$, d.f. = 1, P = 0.038; Fig. 2B–E), and such a tendency was observed for As ($\chi21 = 3.28$, d.f. = 1, P = 0.070) and Cd ($\chi21 = 2.83$, d.f. = 1, P = 0.093; Fig. 2A, F). In the *D. ambigua* and *D. parvula* species group, a significant decrease in ephippial size with increasing concentrations of As ($\chi21 = 5.44$, d.f. = 1, P = 0.019) and Pb ($\chi21 = 5.65$, d.f. = 1, P = 0.017) was observed (Fig. 2A, B). Pond size characteristics were not linked to the size and pigmentation of ephippia (all P > 0.14).

Discussion

First, we found that concentrations of metals in ponds were variable at a local scale (Parisian region; Table 1, Fig. 3) and were positively correlated with one another (Table 2), as previously observed in various other environmental compartments [e.g. urban atmospheric deposition (Azimi et al., 2005), street dust (Charlesworth et al., 2003; Yongming et al., 2006)], suggesting common sources of these metals. Furthermore, these concentrations depended on the geographical and abiotic environmental factors measured (i.e. longitude, latitude, elevation, distance from the centre of Paris, and pond volume). This high heterogeneity in environmental pollution is likely to impact on populations and ecosystems.

In this context, we investigated whether the presence of both Daphnia individuals and ephippia was related to the levels of metallic pollution in ponds. Daphnia individuals were present all along the pollution gradient, even at the highest measured concentrations of metals (higher than the no observed effect concentration; Table 1). This is surprising given that Daphnia are considered to be highly sensitive to pollutants and are used as bioindicators (Tomasiks & Warren, 1996). This result suggests that Daphnia might have evolved the ability to survive sustainably in elevated concentrations (higher than the no observed effect concentration; Table 1) of metallic pollution; accordingly, cases of natural Daphnia populations adapted to elevated concentrations of metals have been documented previously (Barata et al., 2002; Lopes, Baird & Ribeiro, 2006; Coors et al., 2009; Agra, Soares & Barata, 2011). Moreover, experimental evolution approaches have shown that Daphnia are able to adapt rapidly to elevated concentrations of metals (LeBlanc, 1982; Bodar et al., 1990; Hochmuth et al., 2015; Haap, Schwarz & Köhler, 2016). For instance, adaptation could involve the production of metallothionein, which binds metal ions and thus detoxifies the organism (Klaassen, Liu & Choudhuri, 1999; Haap et al., 2016).

However, the precise phenotypic adaptations to elevated concentrations of trace metals remain unknown, and future studies should focus on their identification.

According to our predictions, we expected Daphnia to invest more in the production of diapausing ('dispersal in time' hypothesis) and dispersing stages, i.e. smaller ephippia ('dispersal in space' hypothesis), and in more melanized ephippia ('detoxification' hypothesis).

In agreement with our first prediction, the presence of ephippia in sediments was positively linked with the concentrations of three of the seven metals measured (Pb, Ni and Cd; Fig. 1). This result could be explained by various causes. First, as we hypothesized, higher production of ephippia could itself constitute an adaptation to adverse environments. Ephippia represent a diapausing stage that make it potentially possible to escape unfavourable conditions, such as temporarily dry (Roulin et al., 2013) or polluted ponds, until the return of optimal life conditions ('dispersal in time' hypothesis) and to maximize fitness in local habitats where the mortality rate of Daphnia individuals is high. This advantage of producing a dormancy stage in harsh environments has been proposed to account for a higher production of resting frost-resistant eggs by aphids in regions where low winter temperatures do not allow adult survival (Rispe et al., 1998). Second, the higher production of ephippia may allow them to leave a polluted patch, because the ephippium is the only dispersal stage in Daphnia ('dispersal in space' hypothesis; Wiener & Tuljapurkar, 1994; Bowler & Benton, 2005). Alternatively, the higher production of ephippia in metal-polluted habitats could be adaptive, because the ability of ephippia to accumulate some metals (Wyn et al., 2007) could participate in pollutant sequestration out of the living tissues. However, our study is correlative, and we cannot exclude confounding effects in this positive association between ephippia and metal concentrations. Indeed, smaller ponds are more polluted (Table 2) but are also more temporal ponds that favour ephippial production (Roulin et al., 2013). Moreover, other non-tested factors are known to

favour and/or initiate sexual reproduction in Daphnia, including exposure to solvents or endocrine disruptive compounds, pond temporality, parasite infection or predation (Hairston & Olds, 1984; Ślusarczyk, 1999; Zhang & Baer, 2000; Haeba et al., 2008; Roth et al., 2008; Baer, McCoole & Overturf, 2009; Roulin et al., 2013). Additionally, more ephippia could be found in more polluted habitats owing to the metal toxicity that would lower the hatching rate (Rogalski, 2015). Experimental approaches involving manipulation of metal concentrations are now called for.

Contrary to our second prediction, there was no relationship between the melanin coloration of ephippia and the concentrations of metals. However, we found that ephippia were smaller in more polluted ponds. This relationship could simply reflect the negative direct ecotoxicological effects of metals on the body length of adult Daphnia (van Leeuwen, Luttmer & Griffioen, 1985; Münzinger, 1990; Ward & Robinson, 2005; Agra et al., 2011) or an indirect ecotoxicological effect (e.g. through the food chain; Yan & Pan, 2002; Prosnier, Loreau & Hulot, 2015) and, subsequently, on the size of the ephippia they produce. More interestingly, as smaller ephippia have better success in dispersal (van de Meutter et al., 2008), the production of smaller ephippia could be selected in polluted habitats because it allows greater dispersal success. The differences we observed between metals in their relationships with life-history traits of Daphnia might suggest that not all trace metals are in a toxic range of concentrations in these localities.

Altogether, our correlative work suggests that urban metal pollution may constitute an important environmental factor influencing natural populations, with regard to both ecotoxicological and microevolutionary aspects that need to be confirmed and investigated further through experimental approaches.

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Table 1. Trace metal concentrations in the sampled ponds of Ile-de-France, No-Observed Effect Concentrations (NOEC) and references.

Element	Range	Mean (µg/L)	SE[a]	NOEC[b] (µg/L)	References
Arsenic (As)	0.109 - 9.466	1.612	0.248	260	Biesinger and Christensen, 1972
Cadmium (Cd)	0.005 - 0.124	0.027	0.004	0.6	Kühn et al., 1989
Chromium (Cr)	0.063 - 3.095	0.623	0.099	0.00006	van Leeuwen et al., 1987
Copper (Cu)	0.041 - 56.560	4.136	1.182	11	Biesinger and Christensen, 1972
Nickel (Ni)	0.214 - 10.450	2.496	0.271	13	Kszos et al., 1992
Lead (Pb)	0.053 - 7.904	1.183	0.269	15	Biesinger and Christensen, 1972
Zinc (Zn)	0.658- 147.360	25.050	4.780	74	Biesinger et al., 1986

[a] SE indicates the standard error

[b] NOEC (21 days) determined on *Daphnia magna*

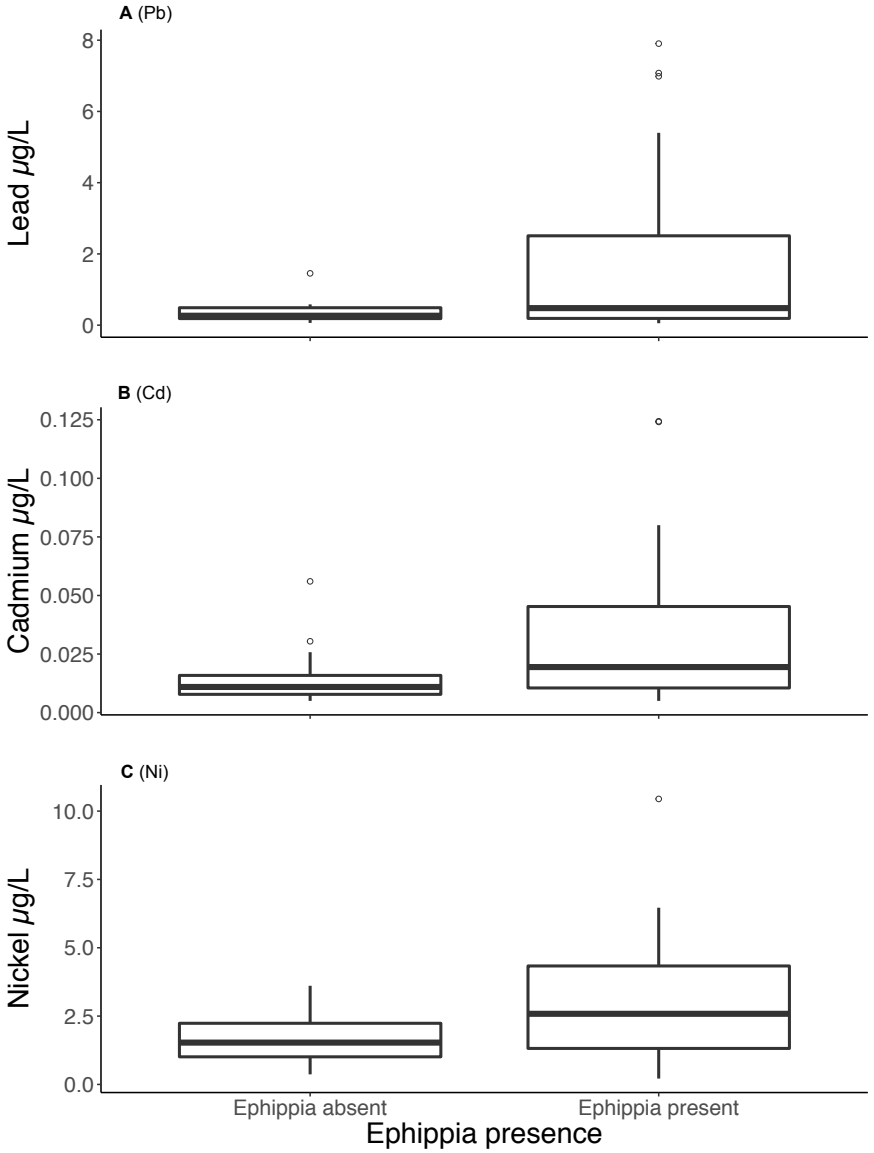

Fig. 1 Concentrations of metals in water ponds (µg/L) for (A) Lead (Pb), (B) Cadmium (Cd) and (C) Nickel (Ni) and ephippia presence.

Although relationships between ephippia presence probability and concentration of metals were tested using GLM with binary distribution, we present boxplots of metals concentrations among ponds according to ephippia presence/absence for better illustration purposes. Central bars represent median, boxes represent interquartile range, dots are outliers (> 1.5 × interquartile range).

Table 2. Pearson correlation matrix for environmental factors and trace metals concentrations of sampled ponds of Ile-de-France.

	Zn	Cd	Cr	Ni	Cu	As	Pb	Alt	Lat	Long	Dist	Vol
Zn		< 0.001	0.001	0.017	< 0.001	0.168	< 0.001	0.261	0.442	0.969	0.797	0.013
Cd	**0.68*** **		< 0.001	0.001	< 0.001	0.007	< 0.001	0.041	0.087	0.509	0.253	0.006
Cr	**0.45** **	**0.58*** **		< 0.001	< 0.001	0.018	< 0.001	0.001	0.144	0.774	0.084	0.016
Ni	**0.33***	**0.47** **	**0.56*** **		0.013	< 0.001	0.002	0.046	0.038	0.130	0.006	0.020
Cu	**0.71*** **	**0.65*** **	**0.48*** **	**0.35***		0.027	< 0.001	0.774	0.989	0.164	0.129	0.013
As	0.20	**0.37** **	**0.33***	**0.57*** **	**0.31***		< 0.001	0.255	0.746	0.225	0.534	0.222
Pb	**0.57*** **	**0.76*** **	**0.66*** **	**0.43** **	**0.59*** **	**0.52*** **		0.009	0.050	0.388	0.337	0.052
Alt	0.16	**0.29***	**0.43** **	**0.28***	0.04	0.16	**0.36** **		0.123	0.703	< 0.001	0.008
Lat	-0.11	-0.24	-0.21	**-0.29***	0.00	0.05	**-0.28***	-0.22		0.263	0.046	0.687
Long	0.01	-0.09	-0.04	-0.21	-0.20	-0.17	-0.12	0.05	0.16		0.336	0.777
Dist	-0.04	0.16	0.24	**0.38** **	-0.22	0.09	0.14	**0.68*** **	**-0.28***	0.14		0.804
Vol	**-0.35***	**-0.38** **	**-0.34***	**-0.32***	**-0.34***	-0.17	-0.27	**-0.37** **	0.06	-0.04	-0.04	

Given are correlation coefficients rho (below diagonal) and associated P-values (above diagonal). Alt: altitude, Lat: latitude, Long: longitude, Dist: distance from Paris, Vol: volume.

Significant correlations are in bold. * P<0.05; ** P<0.01; *** P<0.001

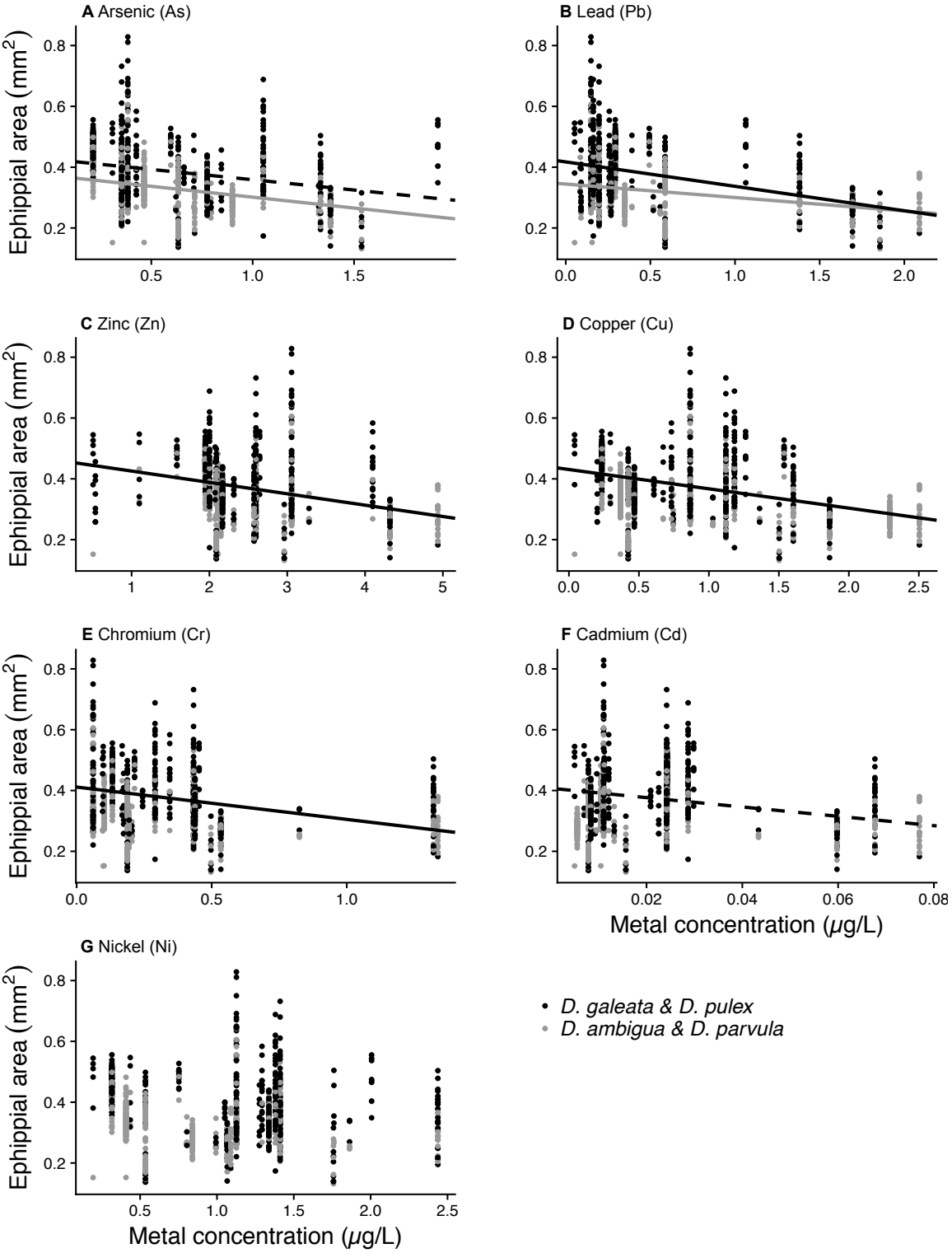

Fig. 2 Ephippial area (mm²) according to metals concentrations (µg/L) in water ponds.

Continuous lines show significant relationships; dashed lines show trend. Data for *D. ambigua & D. parvula* species group are in grey; data for *D. galeata & D. pulex* species group are in black.

Note that axes scales are log-transformed and that X axis has different scales for each metal.

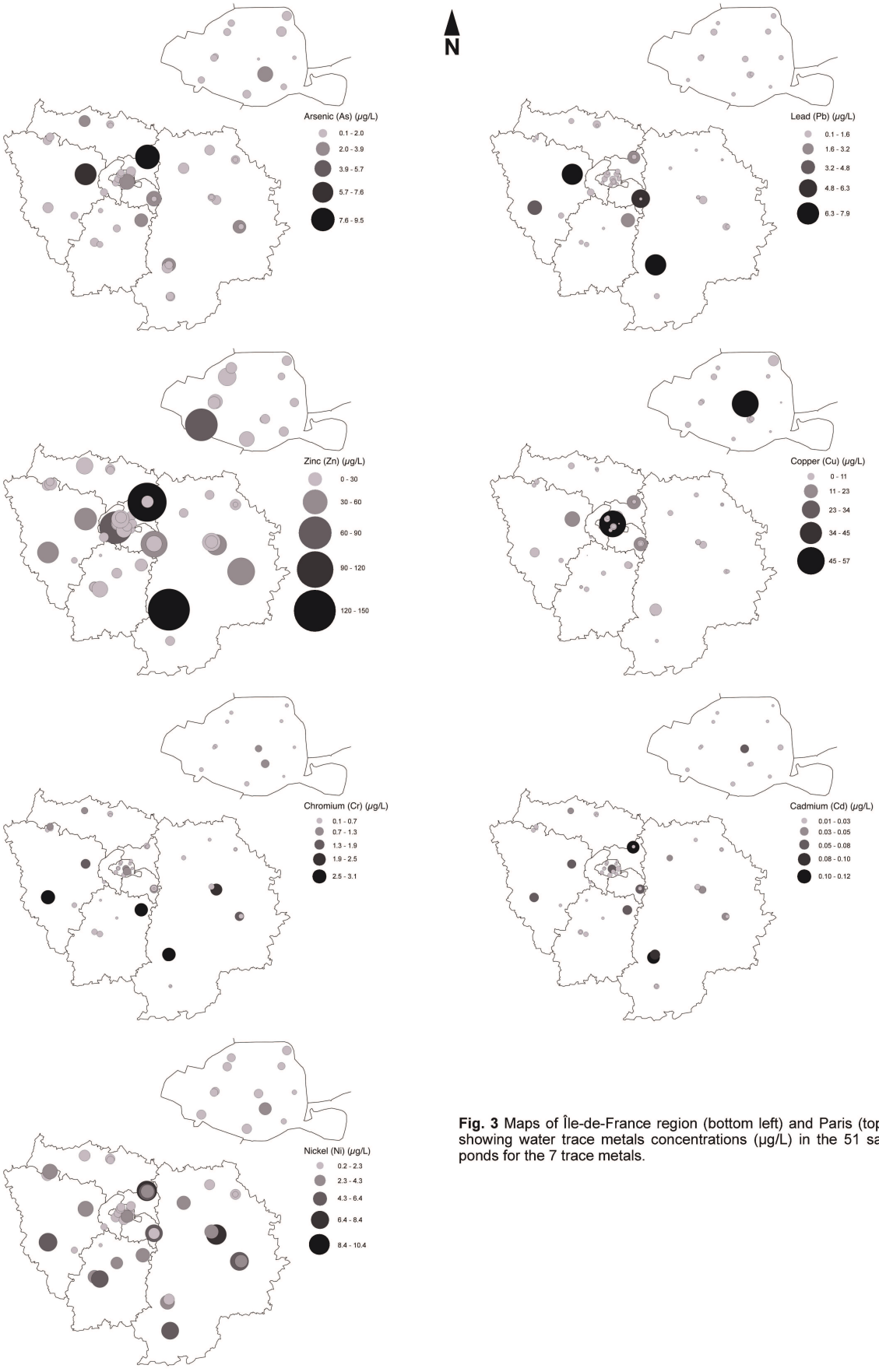

Fig. 3 Maps of Île-de-France region (bottom left) and Paris (top right) showing water trace metals concentrations (µg/L) in the 51 sampled ponds for the 7 trace metals.

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CHAPITRE DEUX

This chapter presents my experimental approach with trace metal exposure that could not be submitted, as it did not include some unanalysed data that will be discussed in Annex 2.

Dormancy, dispersal and detoxification in Daphnia: the 3D hypothesis in response to an experimental exposure to trace metal at increased temperature

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Trace metal pollution and raising temperature concentrate in urban environment in which their effects could be synergised. As elevation of temperature raises metabolism rates, warming could enhance pollutants uptake and increase their toxicity. This temperature-toxicity interaction could lead to the evolution of adaptations among urban populations, such as dispersal, dormancy or detoxification strategies. We tested this hypothesis in an urban population of *Daphnia magna*, a ubiquitous freshwater crustacea and cyclically parthenogenetic. Daphnia can switch to sexual reproduction and produce ephippia, a chitinous envelope, containing sexually produced diapausing-eggs. Ephippia may be empty or contain up to two eggs depending on the fertilisation success. Interestingly, ephippia display dormancy, dispersal, and trace metals accumulation abilities, thus their production may represent an adaptive strategy in response to metal-polluted environment (the 3D hypothesis, Dormancy-Dispersal-Detoxification). We experimentally exposed an urban population of *Daphnia magna* to a Nickel-polluted medium at three different temperatures 20, 22 and 25°C. When exposed to Nickel Daphnia tended to produce more ephippia at 25°C and exhibited higher fertilisation success at 20°C and 22°C. Altogether, our study outlines the possibility that urban metal pollution generates rapid evolutionary responses of populations.

Keywords: Nickel, Daphnia magna, urban, sex, ephippia, stress

Introduction

Global change is a worldwide phenomenon comprising various environmental modifications such as altered biogeochemical cycles, land use and cover change and anthropogenic activities (Vitousek, 1997; Grimm *et al.*, 2015). Among such disturbed habitats, cities are exposed to extreme rapid changes and allow to study populations that survive and/or thrive towards these new conditions (Donihue & Lambert, 2014). Consequently, such modifications might drive populations to adapt to urban life (Johnson & Munshi-South 2017). Especially, chemical pollution (e.g. trace metals, hydrocarbons (Azimi *et al.*, 2003, 2005)) and raising temperature (including through urban « heat island » effect (Oke, 1973; Arnfield, 2003)) concentrate in cities. They may have important impact on eco-evolutionary dynamics (Alberti, 2015) and their interaction could synergise their effects on urban populations.

The toxicity of metals is now well documented. Through their affinities for proteins, metals can alter proteins functioning and disturb some metabolic processes leading to reduction of survival and reproductive output above a certain concentration (e.g Bertram & Hart, 1979; Langdon et al., 2001; Notten et al., 2006). For instance, Lefcort et al., (1998) observed that metals exposure reduce survival and growth and alter metamorphosis in the frog *Rana luteiventris*. As elevation of temperature raises metabolism rates, warming could enhance pollutants uptake through respiration and feeding rate and increase their toxicity (Sokolova & Lannig, 2008; Noyes *et al.*, 2009). This temperature-toxicity interaction could lead to the evolution of adaptations (either plastic or not) among urban populations, such as dispersal, dormancy or detoxification strategies (Ringot *et al.*, 2018). We tested this hypothesis in an urban population of the common freshwater organism, *Daphnia magna*.

Daphnia, as many ectotherm filter feeders inhabiting freshwater ecosystems, are particularly sensitive to chemical pollutants and warming (Sokolova & Lannig, 2008; Messiaen *et al.*, 2012). In addition, elevation of temperature increases the uptake of

Mercury, the accumulation of Cadmium and the toxicity (induced-mortality) of Mercury, Chromium and Cadmium in Daphnia (Heugens *et al.*, 2003; Martínez-Jerónimo, Martínez-Jerónimo, & Espinosa-Chávez, 2006; Tsui & Wang, 2006).

Daphnia are ubiquitous crustacea in freshwater ponds and can be found in urban environment including in highly polluted urban habitats (Ringot *et al.*, 2018). During the growing season, cyclically parthenogenetic females produce numerous clonal daughters. In response to several environmental cues, as photoperiod (Stross & Hill, 1968; Conde-Porcuna, Ramos-Rodríguez, & Pérez-Martínez, 2014), crowding, food deprivation (Kleiven, Larsson, & Hobek, 1992), parasite infection (Roth *et al.*, 2008) or presence of chemical stressors (as predators cues (Ślusarczyk, 1999) or chemical pollutants (Zhang & Baer, 2000; Haeba *et al.*, 2008; Baer, McCoole, & Overturf, 2009)), Daphnia switch to sexual reproduction and start producing sons. Sexual females deposit resting-eggs in chitinous resistant envelope, called « ephippium », derived from their own carapace and released with the next molt. Ephippia may be empty or contain up to two eggs depending on the mating/fertilisation success.

These ephippia represent a key stage of Daphnia life cycle, potentially adaptive in certain conditions (Roulin *et al.*, 2013, 2015). Ephippia allow to temporally (dormancy) and spatially disperse through wind (Cáceres & Soluk, 2002) and phoresy (Mellors, 1975; van de Meutter, Stoks, & de Meester, 2008). These resting-eggs also show higher metal accumulation than sediments which contain them (Wyn *et al.*, 2007) implying potential detoxification property. Owing to these dormancy, dispersal and potentially detoxification abilities of these sexually-produced ephippia, their production may represent an adaptive strategy in response to metal-polluted environment (the 3D hypothesis, Dormancy-Dispersal-Detoxification) (Ringot *et al.*, 2018; Gerber & Kokko, 2018) that could be exaggerated by increased temperature. We experimentally exposed an urban population of *Daphnia magna* to a Nickel-polluted medium at three different temperatures 20, 22 and

25°C (corresponding to the elevation of temperature of some urban ponds, (Brans *et al.*, 2017)Brans et al. 2017, 2018) over ten weeks. Our 3D hypothesis predicted higher ephippia production, larger ephippia (allowing higher metals sequestration and therefore detoxification), more melanic ephippia (as melanin could sequester metal pollutants in inert body parts (Marques, Gonçalves, & Pereira, 2008; Chatelain, Gasparini, & Frantz, 2016; Goiran, Bustamante, & Shine, 2017; Mishra, Shukla, & Chopra, 2018)), with higher fertilisation success (ie: presence and number of embryos in ephippia ensuring effective temporal and spatial dispersal).

Material & Methods

Origin and culture of organisms

Daphnia magna individuals originating from “Bassin de la Géode” in the “Parc de la Villette”, Paris (GPS coordinates: Latitude: 48.8944, Longitude 2.38861). Sampled individuals were randomly split to place 30 individuals in each of 60 jars (500 mL). During the 10 weeks of the experiment, Daphnia populations were maintained under 12L:12D photoperiod, in mineral water (Volvic®) and were fed on *Scenedesmus obliquus* (strain ALCP 349) every two days. Jars were distributed in three thermostatic chambers at different temperatures: 20°C, 22°C and 25°C. For each temperature, half of the jars were exposed to Nickel (Nickel(II) acetate tetrahydrate 98%) (Table 1) at 10 µg/L corresponding to the higher range of urban concentrations in this region (Ringot *et al.*, 2018), the other half of jars were used as control. Medium was renewed every week.

Table 1: Experimental design (number of jars)

Treatments	20°C	22°C	25°C
Ni	10	10	10
Control	10	10	10

Data collection of ephippia and Daphnia

Ephippia presence was checked every other day before the feeding session; all ephippia present were collected from jars and laying date was recorded for each ephippium. From the forth week, for half of the experimental populations weekly randomly chosen within each of the six treatments (Table 1), Daphnia individuals were counted.

2091 ephippia were collected in total and were immediately kept at 4°C in the dark during at least 14 weeks following usual advices for ephippia conservation (e.g. Barata et al., 2000, Schwartz & Hebert, 1997). 1349 ephippia were photographed to assess size and pigmentation score of each ephippia with ImageJ (Gerrish & Cáceres, 2003; Ringot *et al.*, 2018). Size of the ephippia is linked to offspring fitness, in terms of hatching success, adult size and future reproduction (Boersma, 1997). A score of 0 corresponds to white and 250 to black. These ephippia were manually decapsulated to record embryo presence (fertilisation success) and embryo number (one or two). Fertilisation was considered as successful if ephippium contained at least one embryo (failure: 0 embryo, success: 1 or 2 embryos).

Statistical analyses

We investigated the combined effects of Nickel treatment and temperature (20°C, 22°C and 25°C) and time (ten weeks) on Daphnia density, number of ephippia produced, fertilisation success, number of embryos (one or two) in fertilised ephippia and ephippial size and pigmentation using six separated mixed models in R (version 3.4.3). For daphnia density, we ran a generalized linear model (glmer) for data with Poisson distribution. Since we placed the same number of Daphnia in each jar at the beginning of the experiment and started counting Daphnia at week 4, our density models were performed on data from weeks 4 to 10. For the number of ephippia produced, we conducted generalized linear models (glmmPQL) for data with quasipoisson distribution to take overdispersion issues of count data with repeated zeros into account. For fertilisation success and the number of

embryos in fertilised ephippia, we ran generalized linear models (glmer) for binomial data. Ephippial size and pigmentation were analysed with linear models (lmer) for normally distributed data. We simplified our models by removing step-by-step non-significant interactions. When the interaction time*Nickel*temperature was significant, we performed one separate model for each temperature treatment to assess the effect of Nickel treatment at each temperature. Jar, representing the experimental population level, was systematically added into the models as random effect to take pseudo-replication into account.

Results

Density

Density of Daphnia significantly depended on the T°C*Ni*time interaction (X^2_{12} = 315.6; P-value < 0.0001). The interaction Nickel*time was significant at 20, 22 and 25°C (Table 2). At 20°C, this significant interaction did not reveal any difference between densities in Nickel treatment and in control whatever the week (Figure 1). In contrast, at 22°C the density of Daphnia was higher in Nickel treatment than control at week 7 (X^2_1 = 5.68 ; P.value = 0.02 ; Figure 1). Similarly, this higher density in Nickel treatment was also found at weeks 4 (X^2_1 = 12.59 ; P.value = 0.0004), 9 (X^2_1 = 3.92 ; P.value = 0.05) and 10 (Chisq = 3.39 ; P.value = 0.06) at 25°C (Figure 1).

Number of ephippia produced by jar

The number of ephippia produced by jar significantly depended on the T°C*Ni*time interaction (X^2_{18} = 41.6; P-value = 0.001). The interaction Nickel*time was significant at 20°C and 25°C and not at 22°C (Table 2). Indeed, at 20°C, the number of ephippia produced by jar was higher in Nickel treatment than in control at weeks 3 (X^2_1 = 220.03 ; P.value < 0.0001), 4 (X^2_1 = 2.72 ; P.value = 0.09) and 9 (X^2_1 = 2.76 ; P.value = 0.09) but was lower at week 10 (X^2_1 = 10.41 ; P.value = 0.001; Figure 2). At 25°C, the number of ephippia produced by jar was lower in Nickel treatment than in control at week 8 (X^2_1 =

14.45 ; P.value = 0.0001) but higher at weeks 9 (X^2_1 = 3.65 ; P.value = 0.06) and 10 (X^2_1 = 10.26 ; P.value = 0.001). At 22°C, the number of ephippia significantly varied among weeks, but not between Nickel treatments.

Fertilisation success

Fertilisation success tended to depend on the T°C*Ni*time interaction X^2_{11} = 18.2; P-value = 0.076). The interaction Nickel*time was not significant at 20°C and 22°C but was significant at 25°C (Table 2). At 20°C and 22°C, fertilisation success varied significantly among weeks; and was marginally higher in Nickel treatment than in control at 20°C and significantly higher at 22°C (Table 2; Figure 3). At 25°C, the effect of Nickel tended to differ among weeks: in particular at week 7, fertilisation success was lower in Nickel treatment than in control (X^2_1 = 3.25 ; P.value = 0.07), while no difference was detected at any other week (P-value > 0.1 ; Figure 3).

Number of embryos in fertilised ephippia

The number of embryos (one or two) in fertilised ephippia did not depend on the Nickel treatment nor on time (alone or in interaction; all P-values > 0.1) and the simplification of the model only retained a significant effect of temperature: ephippia contained significantly less often two embryos at 22°C as compared to 20°C or 25°C (Table 2 and Figure 4).

Ephippial pigmentation

The ephippial pigmentation significantly depended on the T°C*Ni*time interaction (X^2_{11} = 46 ; P-value < 0.0001). At 20°C, only time significantly affected the ephippial pigmentation (Table 2). In addition, at 22°C, the pigmentation significantly varied among weeks and ephippia were significantly paler in Nickel treatment than in control (Table 2). At 25°C, we detected a significant interaction between Nickel and time (table 2): ephippia were darker in Nickel treatment than control at weeks 7 (X^2_1 = 3.8 ; P.value = 0.05) and 9 (X^2_1 = 5.35 ; P.value = 0.02).

Ephippial size

The ephippial size significantly depended on the T°C*Ni*time interaction (X^2_{11} = 65.6; P-value < 0.0001). Dynamic of ephippial size significantly differed according to Nickel treatment at all temperatures (significant Nickel*time interaction, table 2). At 20°C, ephippia from Nickel treatment were significantly larger than from control at week 4 (X^2_1 = 6.59 ; P.value = 0.01) and smaller at weeks 6 (X^2_1 = 4.72 ; P.value = 0.03) and 8 (X^2_1 = 6.74 ; P.value = 0.009). At 22°C, ephippia were smaller in Nickel treatment than in control for week 7 (X^2_1 = 7.55 ; P.value = 0.006).

Discussion

Some of our results followed our predictions. Concerning the number of ephippia produced, we found more ephippia produced at the end of the experiment at 25°C, but this trend was less observed at 20°C and 22°C. This is in line with our hypothesis of adaptive dormancy, dispersal and potential detoxification strategies (the 3D hypothesis) through ephippia production. However, we cannot exclude a simple effect of Daphnia density (Gerber *et al.*, 2018) which was higher in Nickel treatment at 25°C (Figure 1). This counterintuitive result of higher Daphnia density in Nickel treatment at 25°C raises question about underlying mechanisms as we expected lower density in such stressful environmental treatments (Sokolova & Lannig, 2008; Messiaen *et al.*, 2010). This might be the result the evolutionary rescue process in which evolution by natural selection limits demographic decline (Gomulkiewicz & Holt, 1995) in environments combining both high temperature and metals. Testing this hypothesis would require genetic analyses and phenotypic and fitness measurements of initial and potentially evolved experimental populations (Bell & Gonzalez, 2009.

Also in agreement with our 3D hypothesis, we found a higher fertilisation success in Nickel treatment at 20°C and 22°C. Daphnia density increases the presence of sexual

forms (Gerber *et al.*, 2018) and mating probability. However, these differences can not be attributed to density effect, as densities were similar between Nickel treatment and control at these temperatures (Figure 1). Curiously, this difference was not found at 25°C despite the higher density observed in Nickel treatment at this temperature, suggesting toxicity affecting any step of fertilisation (e.g. male production, male/female efficiency, gamete quality). Contrary to our expectations, Nickel treatment did not affect the number of embryos (one versus two) in fertilised ephippia (Table 2). However, the number of embryos in fertilised ephippia was lower at 22°C as compared to 20°C and 25°C (Figure 4). As this variable has (to the best of our knowledge) never been investigated in Daphnia before, further studies are required to understand this phenomenon and its biological significance.

We found contrasting results for ephippial pigmentation. As predicted, at 25°C we observed production of darker ephippia in Nickel treatment for some weeks (Figure 5), suggesting detoxification adaptation through metal sequestration by melanin (Marques *et al.*, 2008; Chatelain *et al.*, 2016; Goiran *et al.*, 2017; Mishra *et al.*, 2018). However, at 22°C Daphnia produced paler ephippia in Nickel treatment throughout the experiment, suggesting that the production of darker ephippia is counteradaptive. No difference was observed at 20°C (Figure 5). Overall, our results fail to show a clear evidence of detoxification role of melanin in our experiment. Future studies should now try to identify the adaptive role of melanin pigmentation in ephippia (but see Gerrish & Cáceres, 2003).

Contrary to our 3D hypothesis, ephippia were overall not larger in Nickel treatment as compared to control whatever temperature (Figure 6).

In conclusion, our 3D hypothesis is partly supported by our results; especially, when exposed to Nickel Daphnia tended to produce more ephippia at 25°C and exhibited higher fertilisation success at 20°C and 22°C. Our design could not disentangle between ultimate factors (adaptive plasticity versus the effect of natural selection during our experiment) and proximal causes (toxic, positive or hormetic effect of Nickel). These alternative

mechanisms could be distinguished by measuring fitness and phenotypic traits of final experimental populations in the six different experimental conditions (Ni*Temperature, Table 1). Overall our study outlines the possibility that global change generates rapid eco-evolutionary responses of populations.

Table 2 : Results of the separate models fitted to test the effect of Nickel treatment and time on Daphnia density, number of ephippia produced, fertilisation success, number of embryos (one or two) in fertilised ephippia and ephippial size and pigmentation at three temperatures treatments.

	Density of Daphnia				Ephippia produced/jar				Probability of fecundation success			
	X^2	Df	P.value		X^2	Df	P.value		X^2	Df	P.value	
20°C												
Ni	0.071	1	NS	0.79	0	1	NS	1	3.75	1	.	0.05
time	74.62	6	***	< 0.0001	42.23	9	***	< 0.0001	27.27	7	***	0.0002
Ni:time	49.92	6	***	< 0.0001	19.37	9	*	0.02	5.86	5	NS	0.32
22°C												
Ni	2.97	1	.	0.08	2.43	1	NS	0.11	4.39	1	*	0.03
time	82.79	6	***	< 0.0001	29.13	9	***	0.0006	21.42	8	**	0.006
Ni:time	147.81	6	***	< 0.0001	12.97	9	NS	0.16	2.09	6	NS	0.91
25°C												
Ni	52.79	1	***	< 0.0001	0	1	NS	1	0.79	1	NS	0.16
time	313.82	6	***	< 0.0001	31.79	9	***	0.0002	69.18	8	***	< 0.0001
Ni:time	247.38	6	***	< 0.0001	18.95	9	*	0.03	14.04	8	.	0.08

	Ephippia pigmentation				Ephippia size			
20°C								
Ni	0.58	1	NS	0.44	2.18	1	NS	0.14
time	76.86	7	***	< 0.0001	112.86	7	***	< 0.0001
Ni:time	4.08	5	NS	0.54	78.62	5	***	< 0.0001
22°C								
Ni	4.69	1	*	0.03	0.73	1	NS	0.39
time	129.85	8	***	< 0.0001	30.80	8	***	0.0002
Ni:time	7.62	6	NS	0.27	27.46	6	***	0.0001
25°C								
Ni	1.56	1	NS	0.21	NS	1	NS	0.42
time	467.25	8	***	< 0.0001	71.58	8	***	< 0.0001
Ni:time	59.99	8	***	< 0.0001	22.49	8	**	0.004

	Number of embryos in ephippia			
T°C	8.66	2	*	0.01
Ni	2.16	1	NS	0.14
time	8.83	8	NS	0.36

Significance levels * P.value < 0.05; ** P.value < 0.01; *** P.value < 0.001; NS = non significant

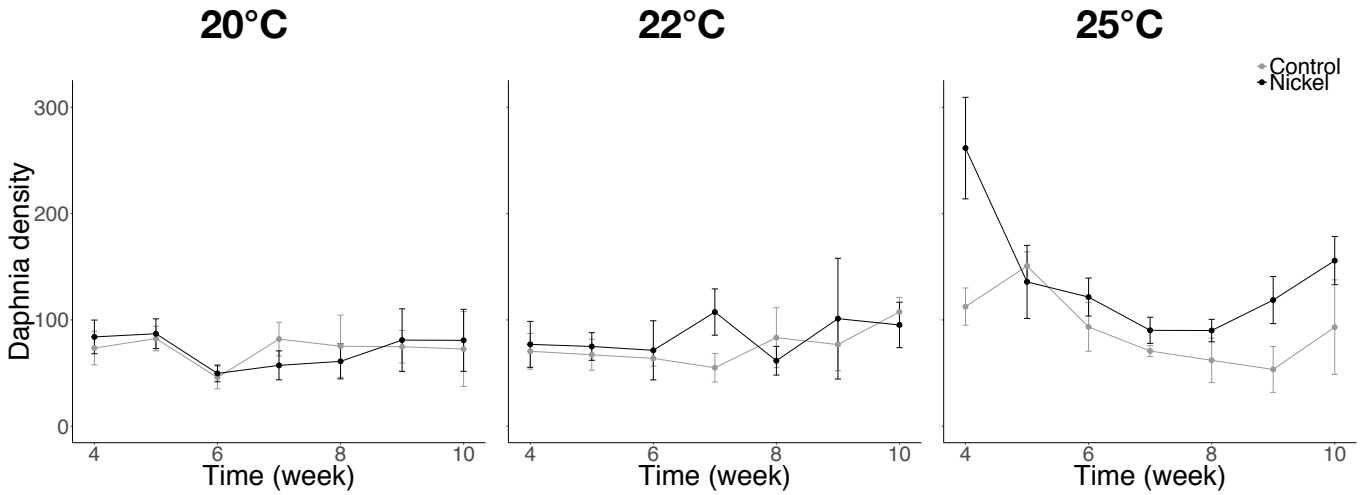

Fig. 1 Average Daphnia density and standard errors among weeks. Black dots and line correspond to Nickel treatment and grey dots and line correspond to control.

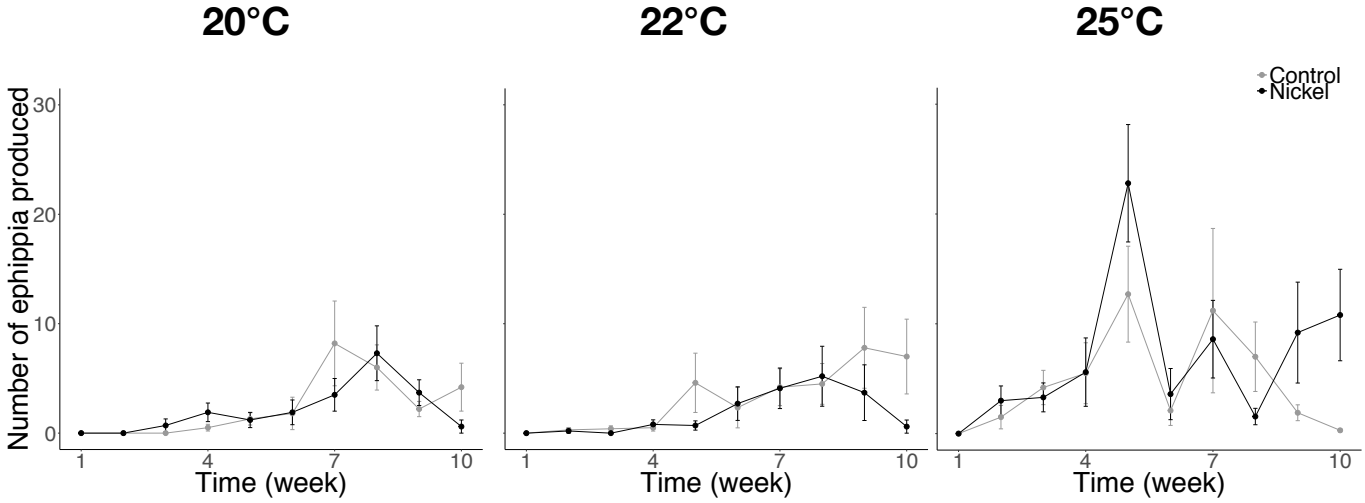

Fig. 2 Average number of ephippia produced and standard errors among weeks. Black dots and line correspond to Nickel treatment and grey dots and line correspond to control.

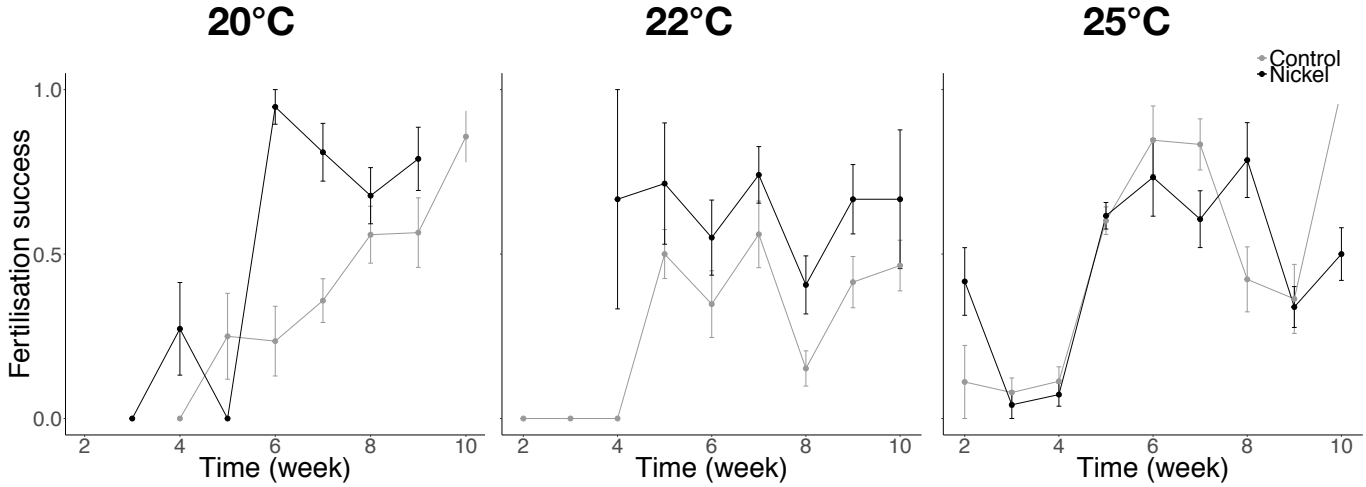

Fig. 3 Average fertilisation success (at 0 ephippia are empty. at 1 ephippia contain 1 or 2 eggs) and standard errors among weeks. Black dots and line correspond to Nickel treatment and grey dots and line correspond to control.

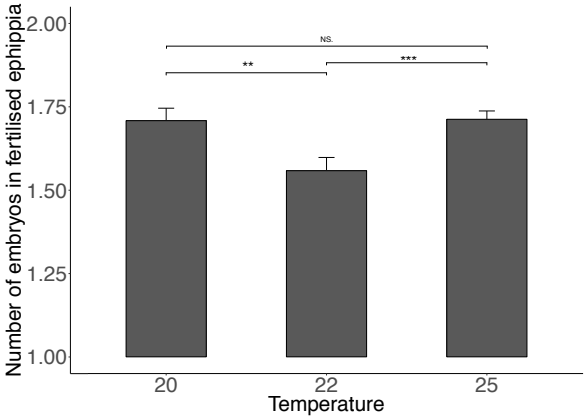

Fig. 4 Average number of embryos in fertilised ephippia and standard errors according to temperature treatments. Tukey post-hoc tests were performed to detect significant differences between temperatures (** is for p.value < 0.01. *** is for p.value < 0.001. NS = non significant).

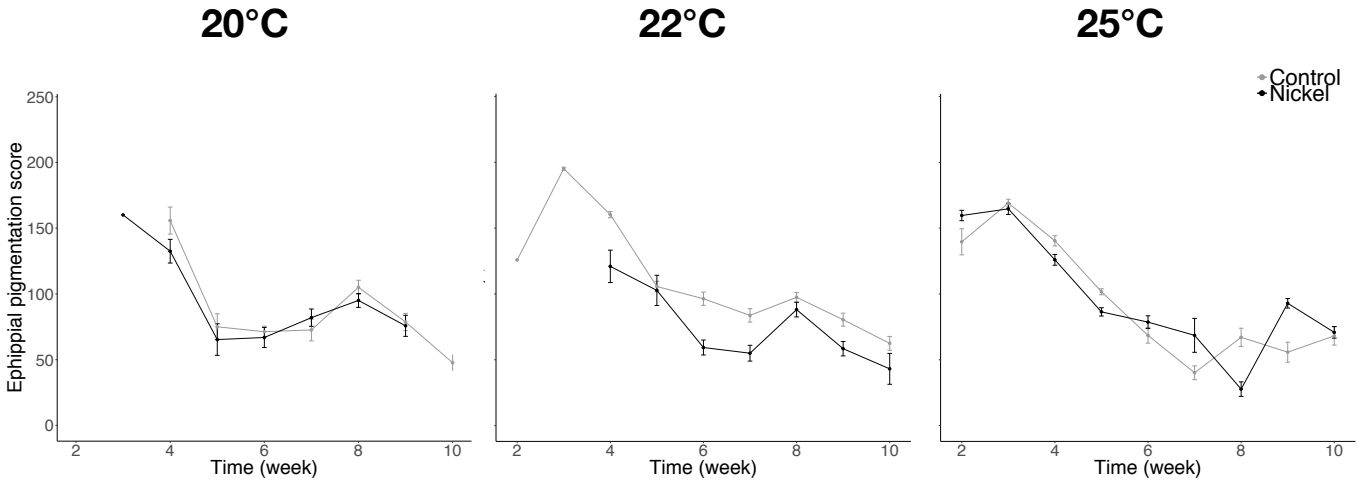

Fig. 5 Average ephippial pigmentation score and standard errors among weeks. Black dots and line correspond to Nickel treatment and grey dots and line correspond to control.

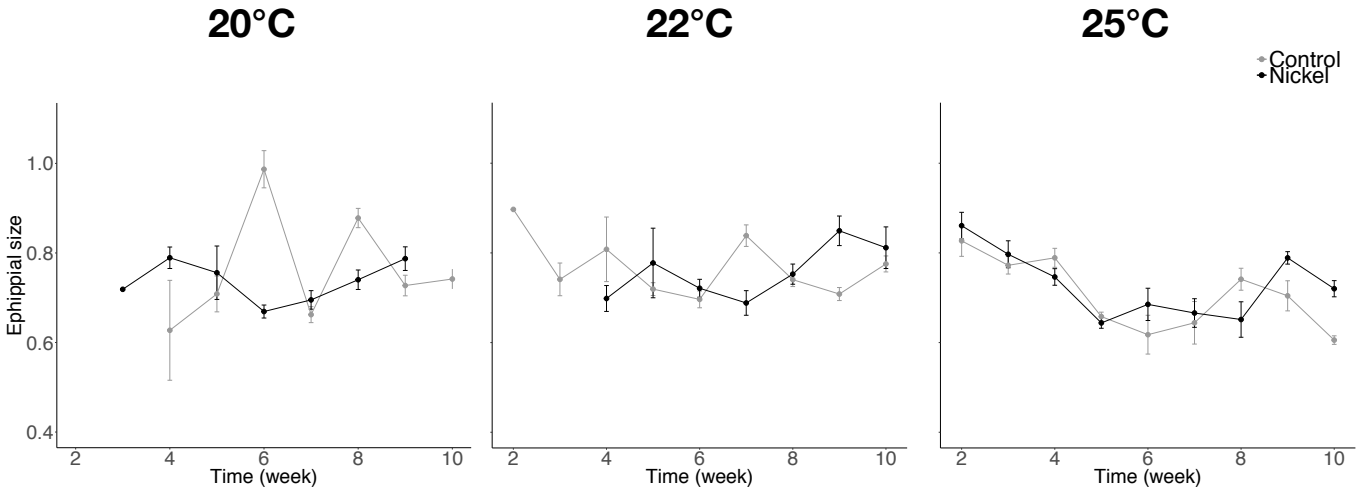

Fig. 6 Average ephippial size and standard errors among weeks. Black dots and line correspond to Nickel treatment and grey dots and line correspond to control.

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CHAPITRE TROIS

This chapter presents our study of behavioural avoidance of Copper in an urban population of *Daphnia magna* that has been submitted in *Aquatic Toxicology*.

Behavioural avoidance of Lead was also planned to be included in this study, but we did not succeed to calculate Lead EC_{50} even after several trials suggesting manipulation errors (including problem with metal used (Lead (II) acetate trihydrate)) or higher tolerance for Lead than expected for this population.

Behavioural avoidance of a trace metal in an urban population of *Daphnia magna*

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Trace metals, naturally present in ecosystems or originating from anthropogenic activities, are known to negatively impact survival and reproductive success. These metals may thus exert selective pressures on populations inhabiting polluted environments as urban areas. In habitats with spatial heterogeneity for metal pollution, detection and behavioural avoidance of metals are expected to be selected to reduce exposure to pollutants. To test whether Daphnia individuals from an urban pond were able to avoid metals and whether this potential ability could be modulated by the presence of an attractive stimulus (a local source of food), we used small-sized experimental aquaria with possibility to choose between unpolluted side and Copper-polluted side, with or without algae (food source). We first found that Daphnia individuals spent more time in the Copper-polluted area, possibly reflecting the reduction of mobility caused by metal pollution. However, at the end of the experiment, we observed a progressive vanishing of deviation between treatment and control, suggesting that urban Daphnia were able to avoid metals at a very small spatial scale. Local food source did not modulate the response to metal pollution of non-starved Daphnia. These results stress the need to investigate the effects of metals on vertical migration in Daphnia and support the importance to consider metal pollutants as new selective forces in urban environment and of using behavioural avoidance for ecotoxicological test reliability.

Keywords: avoidance; Copper; adaptation; urban; crustacea

Introduction

In addition to their natural presence in ecosystems, trace metals are emitted by anthropogenic activities and could be concentrated in urban areas (Azimi et al., 2003). At the molecular level, trace metals can alter the function of several proteins because of their affinities for sulphur and nitrogen that compose many amino acids (Rainbow, 1997, 2002). This toxicity translates into impairment of survival and reproduction, which may thus exert selective pressures on populations living in polluted habitats (e.g.Lefcort et al., 2002; Rogalski, 2017; Skaldina et al., 2018). Avoidance of trace metals, either through metabolic pathways or behaviour, could confer a selective advantage for individuals facing metal exposure. Indeed, the ability to reduce metal availability in living tissues owing to the expression of metallothionein or to the production of insoluble metaliferous granules with a high affinity to metals has been documented in several taxa ((Klaassen, Liu, & Choudhuri, 1999); e.g. (Nassiri et al., 2000) in amphipods, (Haap, Schwarz, & Köhler, 2016) in Daphnia). In addition, detection and behavioural avoidance of polluted areas are expected to be associated with a selective advantage because they would participate in reducing exposure to pollutants, and may evolve in habitats with spatial heterogeneity for metal pollution.

Pollutant avoidance has been regularly studied, often to optimize ecotoxicological tests reliability, considering that constant pollutant exposure in usual ecotoxicological studies is not representative of field exposure if organisms are able to detect and avoid pollutants (Araújo, Moreira-Santos, & Ribeiro, 2016a). Hence, behavioural avoidance of metal pollution has been observed in several taxa, particularly in aquatic invertebrates as amphipods, cladocerans, copepods, decapods, insect larvae or snails (Wentsel et al., 1977; Oakden, Oliver, & Flegal, 1984; Lefcort et al., 2004; Lopes, Baird, & Ribeiro, 2004; Eriksson Wiklund, Börjesson, & Wiklund, 2006; Araújo, Blasco, & Moreno-Garrido, 2012; Araújo et al., 2016b; Ward, Simpson, & Jolley, 2013a,b).

Documented effects of trace metals on Daphnia include reduced survival, fecundity (Biesinger & Christensen, 1972; Bertram & Hart, 1979; Ingersoll & Winner, 1982; Biesinger, Christensen, & Fiandt, 1986) and adult body size (van Leeuwen, Luttmer, & Griffioen, 1985; Münzinger, 1990; Baillieul & Blust, 1999; Ward & Robinson, 2005; Agra, Soares, & Barata, 2011). Reduction in Daphnia mobility and velocity has also been observed in the presence of metal pollution (Wolf, Scheunders, & Selens, 1998; Baillieul & Blust, 1999; Untersteiner, Kahapka, & Kaiser, 2003; Clément & Zaid, 2004). Hence, Daphnia mobility is used for ecological risk assessment to rapidly measure ecotoxicological thresholds (EC50, the half maximal effective concentration, (Organisation for Economic Co-operation and Development: Paris, 2004)). Daphnia mobility could be motivated by food foraging (Beklioglu et al., 2008; Muller et al., 2013), predation avoidance (Dodson, 1988; Demeester, Weider, & Tollrian, 1995) and for diel vertical migration. This latter daily synchronised movement of Daphnia and in zooplankton in general has important implications in ecosystem dynamics (Hays, 2003).

The presence of Daphnia in habitats with elevated concentrations of metals has been previously described (Barata et al., 2002; Lopes, Baird, & Ribeiro, 2006; Coors et al., 2009; Agra et al., 2011; Ringot et al., 2018), even in ponds with concentrations higher than No Observed Effect Concentrations (NOEC) (Ringot et al., 2018). This observation raises questions about potential adaptations allowing Daphnia to survive in such polluted habitats. We hypothesized that the ability to detect and avoid metal pollution may have evolved in populations from polluted ponds because it would reduce the level of exposure to pollutants. The possibility for this ability to evolve would be greatly favoured if there is a spatial heterogeneity of metal pollution within a habitat, which has indeed been previously documented (Stockdale, Davison, & Zhang, 2009). Natural Daphnia populations face several environmental conditions which may respectively act as repulsive or attractive

stimuli and may interact in shaping individual mobility (Beklioglu et al., 2008). For instance, the ability to avoid metals may be affected by the local presence of food.

In this study, we tested whether Daphnia individuals were able to avoid metals at a very small spatial scale, and whether this potential ability could be modulated by the presence of an attractive stimulus (food). In the laboratory, we followed individual movement of hundreds of isolated Daphnia originating from an urban population, in an experimental design with possible choice between Copper-polluted water and unpolluted water, with or without local food source. Since Daphnia are able to actively avoid several pollutants (pulp mille effluent: (Rosa et al., 2008); atrazine: (Rosa et al., 2012); Copper: (Lopes et al., 2004)) and toxic metabolites (cyanotoxine: (Muller et al., 2013)), we expected Daphnia to spend more time in unpolluted area in our experimental design. Alternatively, due to the reduction of mobility caused by metals (Baillieul and Blust, 1999; Clément and Zaid, 2004; Untersteiner et al., 2003; Wolf et al., 1998) Daphnia could be trapped in the polluted area of our experimental design. Since local food source could attract Daphnia ((Muller et al., 2013) we expected the presence of algae to interact with the behavioural response of Daphnia to Copper pollution in our experiment.

Materials & Methods

Origin and culture of organisms

We collected hundreds of Daphnia magna individuals from “Bassin de la Géode” in the “Parc de la Villette”, Paris (GPS coordinates: Latitude: 48.8944, Longitude 2.38861), an artificial urban pond of 40 square meters and a depth of 50 centimeters. Daphnia were then maintained in the laboratory at 20°C under 16L:8D photoperiod in medium composed of half origin water and half mineral water (Volvic®) and fed on Scenedesmus obliquus (strain ALCP 349). The 48h EC50 acute immobilisation tests were realized on this population for Copper ($CuSO_4$) following OECD 202 guidelines (Organisation for Economic Co-operation and Development: Paris, 2004) giving a Copper EC50 of 0.055

mg/L. We used Copper because it is one of the major toxic trace metal in aquatic ecosystems (DRIEE, 2013).

Experimental system

Experimental systems (Figure 1) were composed of 5 glass sheets (10 cm*23 cm) on each of which 3 glass aquaria (15cm*2.5cm) were directly built. Each aquarium was filled with 50 mL of mineral water (Volvic®). Aquaria were placed in thermostatic cabinets, at 20°C with homogenised light. To record position tracking of each Daphnia individual, we distinguished four zones in each aquarium (Z1 to Z4; see Figure 1).

A total of 207 individuals were used in the experiment (Table 1). For Copper treatment (N=75), 0.110mL of 0.25mg/L Copper solution was deposited in Z1 to reach previously measured EC50 if Copper solution was fully homogenised in the entire aquarium. For algae treatment (N=100), 0.5 mL of a solution containing Scenedesmus obliquus (strain ALCP 349) of approximately 2.2 106 cells/mL was added in the aquarium in zone Z1; in treatments containing both algae and Copper (N=70), algae were added before Copper deposition to avoid physical perturbation and metal spread. These additional volumes respectively corresponded to 0.22% (Copper solution) and 1% (algae solution) of the total water volume in the aquarium, and most likely did not have any effect by themselves on Daphnia locomotion activity. Each individual was first introduced in the centre of each aquarium (at the limit between Z2 and Z3; see Figure 1), left for a 5-minutes phase of acclimation, then filmed during 30 seconds every 5 minutes (6 sequences in total). The time spent by each Daphnia in each zone was recorded during the 6 sequences. Before every test session, aquaria were sanitised in 10% nitric acid baths to remove Copper contamination and their position and orientation were randomised in thermostatic cabinets. Six to eight aquaria were tested during the same test session.

For each individual in each sequence, we calculated the spatial position along the aquarium by multiplying the central coordinate of each zone by the time spent in that zone.

For Copper treatment, Z1 was the zone where Copper was placed. For Control aquaria, we arbitrarily named Z1 the zone out of which water was drained.

Statistical analyses

We tested the effects of sequence, metal treatment, algae, and their interaction on mean spatial position of each individual through a generalized mixed model (function lmer in package lme4 (Bates et al., 2015). Nesting individual within aquarium and aquarium within glass sheet allowed to take both the temporal replication and the non-independence of data from aquaria constructed on a same given glass sheet into account. We compared models with and without random slope for time sequence (anova function) and retained the model with lower AIC. The complete model was progressively simplified by stepwise backward elimination of all non-significant terms. To further investigate the difference in mean Daphnia position between control and metal treatment, we performed a separate generalized mixed model for each time sequence with aquarium nested within glass sheet as random effect. Normality of the residuals was visually checked to conform to statistical assumptions of the models. Statistical analyses were performed with R version 3.5.0.

Results

We retained the model with random slope which improved the model significantly (Akaike Information Criterion : AIC = 6427.6 and AIC = 6499.1 with and without random slope respectively; $\chi^2 6 = 83.49$, P = 7e-16***).

The triple interaction Copper*Algae*Sequence was not significant ($\chi^2 1 = 0.0014$, P = 0.97) and was thus removed from the model, as were Algae*Sequence ($\chi^2 1 = 0.0004$, P = 0.98), Copper*Algae ($\chi^2 1 = 0.063$, P = 0.80), Copper*Sequence ($\chi^2 1 = 0.122$, P = 0.73) and Algae ($\chi^2 1 = 0.65$, P = 0.42). The final model showed a significant influence of Copper treatment ($\chi^2 1 = 5.44$, P = 0.020) and time sequence ($\chi^2 1 = 3.96$, P = 0.047) on Daphnia position in the aquarium. Mean position of Daphnia was significantly lower in the Copper

treatment (i.e. closer to the side where Copper was placed in that treatment) than in the Control treatment.

The Copper treatment limited the movement of Daphnia and tended to reduce their avoidance of zone Z1 (Figure 1).

Even though the Copper*Sequence interaction was not significant, the difference in mean position significantly differed between Copper and Control treatments at 5, 10 and 20 minutes ($P < 0.05$; Table 2; Fig. 2). The non-significant difference between Copper and Control treatments at 15, 25 and 30 minutes ($P > 0.05$; Table 2; Fig. 2) suggested that avoidance is taking place at the end of experiment.

Discussion

Avoidance of trace metals has often been studied in sites exhibiting historically important sources of pollution such as acid mine drainage sites (Lopes et al., 2004) or Superfund sites (Lefcort et al., 2004), or with laboratory cultures population for ecotoxicological purpose (Ward et al., 2013b). To our knowledge, this behaviour has never been studied in an urban context.

Daphnia individual mean position was closer to Z1 zone in Copper treatment (polluted zone) than in Control treatment (zone where water was drained). We did not expect mean position to differ from 7.5 cm (predicted from random distribution) in the control treatment; however, taking control as reference, Daphnia spent significantly more time near zone Z1. This suggests that copper has a negative impact on mobility and velocity of Daphnia, as previously documented (Copper: (Untersteiner et al., 2003; Clément & Zaid, 2004); Cadmium: (Wolf et al., 1998; Baillieul & Blust, 1999)). Daphnia could thus be trapped in the copper-polluted side.

The progressive vanishing of deviation between treatment and control indicates the avoidance of copper at the end of the experiment. Since habitat choice is expected to evolve in spatially heterogeneous environments, our results suggest a spatial

heterogeneity of metal pollution in the urban and artificial small-sized pond without thick layer of sediments where Daphnia were collected. We expect that expression of this ability of detection and avoidance is costly and be lost if the habitat does not exhibit this spatial heterogeneity of metal pollution. Accordingly, reference populations originating from unpolluted locations were less sensitive and avoided less metal pollution than populations from polluted sites (Lefcort et al., 2004). This ability of Daphnia to avoid copper should be taken into account in ecotoxicological tests involving metals, as already suggested by (Lopes et al., 2004; Araújo et al., 2016a).

Contrary to our prediction, we did not observe any effect of algae on the mean position, whatever the presence of copper. We had hypothesized that the presence of food resource (algae) might act as attractor (Muller et al., 2013). Daphnia were not starved before our experimental tests, potentially explaining the absence of algae effect by reducing attractiveness of the food resource.

In our study, we were interested in horizontal movements of Daphnia. However, zooplankton exhibits vertical diel migration which could also be affected by metal presence and remains to be investigated. The effects of metals on Daphnia movements might have consequences on their interactions with predators. In particular, fish are able to avoid metals (Hansen et al., 1999). Therefore, metals may modulate this prey-predator interaction and more generally the ecosystem functioning.

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Table 1: Number of Daphnia in each treatment

	No algae	Algae	Total
Control	32	30	62
Copper	75	70	145
Total	107	100	207

Table 2: Results of the separate models fitted to test the effect of Copper on individual position in the aquarium for each time

Time (minutes)	Df	Chisq	*P-value*
5	1	$\chi^2 = 4.63$	P = 0.031*
10	1	$\chi^2 = 5.56$	P = 0.018 *
15	1	$\chi^2 = 1.61$	P = 0.204 NS
20	1	$\chi^2 = 5.09$	P = 0.024 *
25	1	$\chi^2 = 2.23$	P = 0.136 NS
30	1	$\chi^2 = 0.43$	P = 0.512 NS

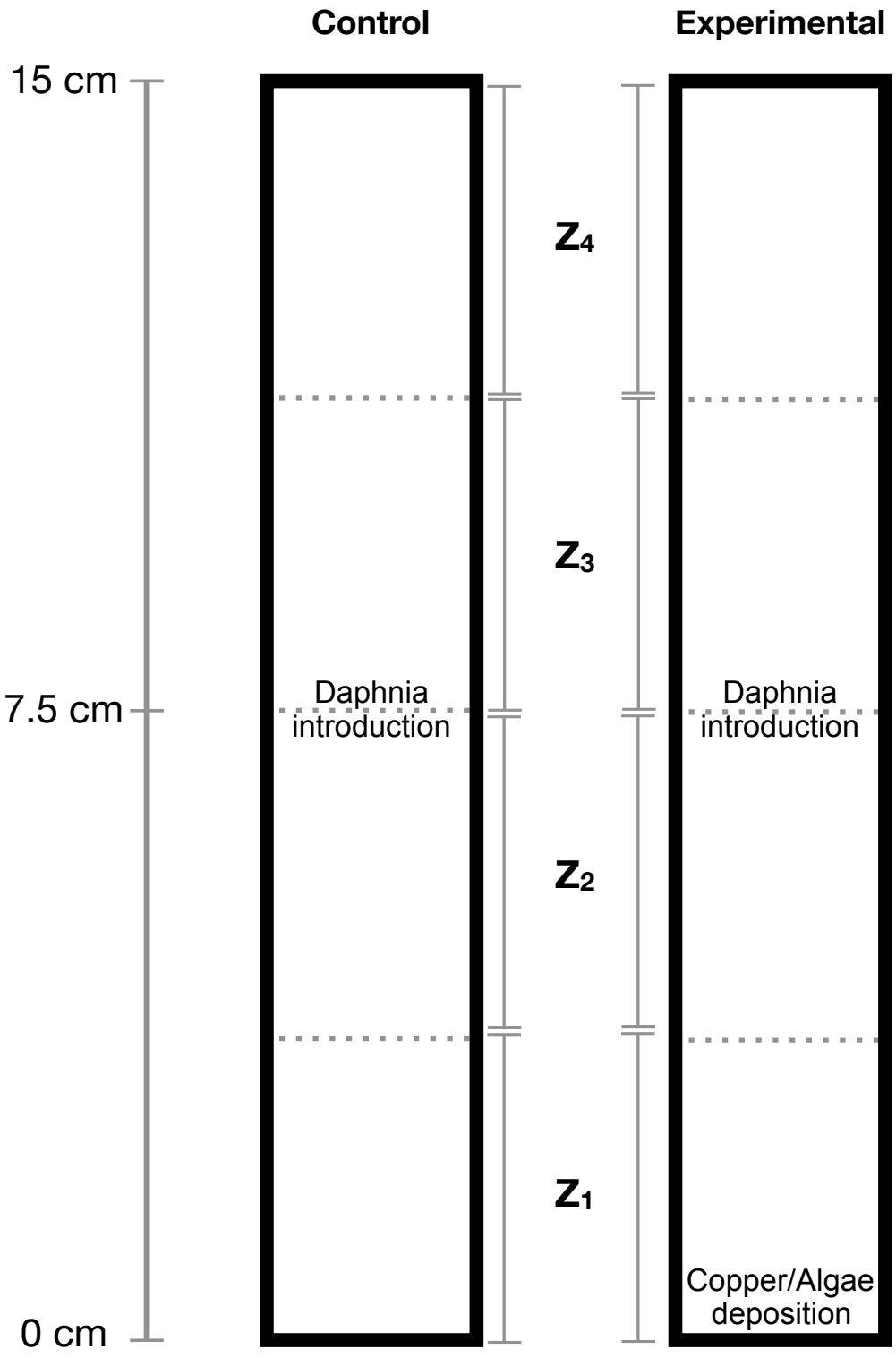

Figure 1: Two aquaria of the experimental system

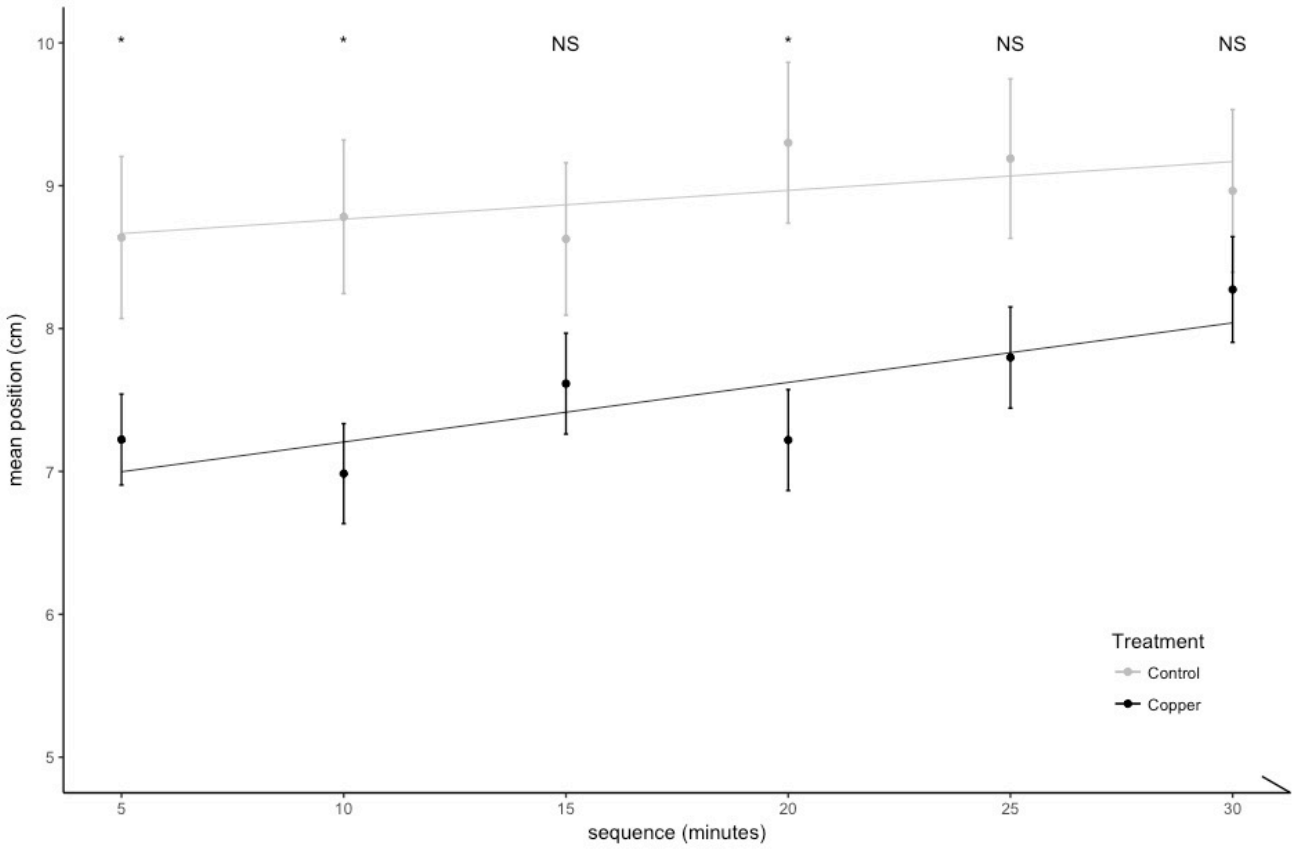

Figure 2: Mean Daphnia position along the aquarium over time for Control (light grey dots) and Copper (black dots) treatments. Z1 zone is located at the bottom.

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CONCLUSION GÉNÉRALE & PERSPECTIVES

- **Les Daphnies sont-elles capables d'affronter la pollution aux métaux traces en milieu urbain ?**
- **Par quels moyens ?**

Les résultats principaux mis en exergue au cours de cette thèse sont :

1. Les Daphnies sont présentes tout au long du gradient de pollution.
2. Les éphippies sont trouvées plus souvent dans les mares les plus polluées.
3. Les éphippies des mares les plus polluées sont plus petites.
4. Aucun lien n'a été observé entre pigmentation mélanique des éphippies et exposition aux métaux.
5. L'exposition au Nickel pendant plusieurs générations augmente le succès de fécondation des éphippies chez une population urbaine.
6. Les individus de cette même population urbaine semblent capables de détecter et d'éviter le Cuivre dans leur milieu.

Notre résultat 1. indiquant que les Daphnies sont présentes dans les mares les plus polluées, suggère que les Daphnies urbaines sont capables d'affronter la pollution aux métaux traces. Ces résultats semblent confirmer notre hypothèse en 3D, selon laquelle dans un milieu pollué, les Daphnies sexuées pourraient être sélectionnées pour les capacités de dormance, de dispersion et de détoxication des éphippies qu'elles produisent. Les résultats de 2. à 5. soutiennent cette hypothèse. L'hypothèse de l'évitement comportemental est, quant à elle, soutenue par le résultat 6.

Les Daphnies sont présentes tout le long du gradient de pollution aux métaux traces mesurés en région parisienne (Résultat 1.), il semble donc que certaines populations soient adaptées à la pollution de cette région urbanisée. De plus les éphippies, qui sont produites par reproduction sexuée, sont plus fréquemment trouvées dans les mares de la région les plus polluées aux métaux traces (Résultat 2.), pouvant suggérer une sélection des Daphnies sexuées dans les milieux pollués.

Par ailleurs, il existe une relation négative entre la taille de ces éphippies et la concentration en métaux (Résultat 3.), les mares les plus pollués contiennent donc plus souvent des éphippies mais il s'agit d'éphippies de petites tailles en comparaison de celles produites dans les mares moins polluées. Or, chez *Daphnia magna*, il a été observé que plus l'éphippie est grande plus son succès d'éclosion est élevé (Boersma, Boriss, & Mitchell, 2000), de plus, des éphippies provenant de sédiments plus pollués aux métaux présentaient des taux d'éclosion plus faibles que celles provenant de sédiments contenant moins de métaux (Rogalski, 2015). Néanmoins, ces deux mesures ont été réalisées dans des conditions de laboratoires non polluées et il est possible qu'ils ne soient pas généralisables dans les milieux tout au long du gradient de pollution. Il s'agirait, maintenant, de tester le succès d'éclosion et la fitness des juvéniles issus des éphippies dans des conditions croisées représentatives de ce gradient. Ceci permettrait d'estimer le rôle adaptatif potentiel de la production de petites éphippies observée dans nos milieux pollués et d'éliminer l'hypothèse alternative d'effets toxiques directs.

Dans cette perspective, les éphippies du jeu de données du Chapitre 1 ont été conservées pour chercher un lien entre degré de pollution métallique du milieu d'origine, caractéristiques morphologiques de ces éphippies et (résultats non présentés) :

(i) le succès de fécondation des éphippies (i.e. leur contenu en oeuf(s) fertilisé(s)) : aucune relation n'a été établie entre le succès de fécondation et la teneur en métaux du milieu d'origine. Cela suggère, premièrement, que les métaux n'ont pas d'effet

toxique direct sur ce succès de fécondation ou que cet effet est masqué par une réponse adaptative des populations étudiées. Néanmoins, cette absence de relation contraste avec celle observée dans le Chapitre 2, dans lequel le succès de fécondation était supérieur dans les populations expérimentales maintenues en présence de Nickel. Ce contraste pourrait être expliqué par la différence d'approche entre les deux chapitres (Chapitre 1 : corrélatif ; Chapitre 2 : expérimental), le succès de fécondation pouvant probablement dépendre de multiples paramètres environnementaux (autres que la présence de métaux) qu'il s'agirait d'identifier dans des études futures.

(ii) le taux d'éclosion chez ces populations : aucune des éphippies n'est parvenue à éclore (après décapsulation mécanique) au cours de cette étude (Chapitre 1), néanmoins les éphippies obtenues au cours de l'expérience du Chapitre 2 ont été mises à éclore dans des conditions croisées d'exposition au Nickel et ces résultats sont en cours d'analyse (voir Annexe 2).

Outre le succès de fécondation et d'éclosion, l'avantage adaptatif des petites éphippies en milieu pollué pourrait être leur capacité plus élevée à disperser via des insectes (van de Meutter *et al.*, 2008). Néanmoins, un déclin de l'abondance de certains arthropodes est observé en milieu urbain (Vergnes *et al.*, 2014) ce qui pourrait limiter leur implication dans la phorésie des éphippies dans ce milieu. En perspective, il serait intéressant d'identifier les mécanismes de dispersion des éphippies (vent, insectes, oiseaux) en milieu urbain pouvant dépendre de la taille de l'éphippie.

L'étude corrélative ainsi que l'exposition expérimentale n'ont pas permis de mettre en évidence de lien entre coloration mélanique des éphippies et exposition des individus aux métaux traces (Résultat 4.). La coloration mélanique en général peut avoir plusieurs autres rôles adaptatifs comme son rôle antibiotique (Mackintosh, 2001) et pourrait être sous contrainte d'autres facteurs environnementaux (Gerrish & Cáceres, 2003) pouvant

expliquer cette absence de lien observée. Par ailleurs, indépendamment de leur contenu en mélanine, les éphippies peuvent stocker les métaux (Wyn *et al.*, 2007) et potentiellement de participer à la détoxication de la Daphnie produisant l'éphippie. Afin de tester cette hypothèse, il serait intéressant de comparer les concentrations en métaux chez la mère, avant et après libération de l'éphippie en s'attendant à trouver moins de métaux après libération de l'éphippie. De plus, on s'attendrait à ce que les Daphnies produisant des éphippies contiennent moins de métaux et aient donc une meilleure fitness que les Daphnies ne produisant pas d'éphippie en milieu pollué.

De manière générale, l'identification du rôle adaptatif de la production d'éphippie (hypothèse en 3D) devrait intégrer des expérimentations de transplantations réciproques entre populations urbaines et populations rurales, avec suivi phénotypique, de taux de croissance et de mode de reproduction des populations qui permettraient de mettre en lumière l'adaptation locale de ces populations (Kawecki & Ebert, 2004), adaptation locale pouvant être accélérée par l'investissement dans la reproduction sexuée et la dispersion (North *et al.*, 2011).

Un des résultats intéressants de mon étude est l'augmentation du succès de fécondation des éphippies chez une population urbaine expérimentalement exposée au Nickel (Résultat 5.) suggérant un investissement dans la production de mâles plus important. Cette expérience ne permet pas de distinguer les effets proximaux (comme des effets toxiques des métaux) des effets ultimes (de la nature plastique de la réponse aux métaux ou de sélection d'individus résistants au cours de l'expérience). Des mesures phénotypiques et de fitness des individus de fin d'expérience et en transplantations réciproques dans les différentes conditions pourraient permettre de distinguer les mécanismes mis en oeuvre.

Tout comme le résultat 2. (Chapitre 1), le résultat 5. suggère un investissement plus important dans la reproduction sexuée induit par la pollution à ce métal trace. La

production répétée de ces éphippies (Chapitre 1) pouvant mieux résister aux métaux toxiques et leur succès de fécondation supérieur (Chapitre 2) pourraient être favorisés dans les milieux pollués. Ainsi, la sélection de la production d'éphippies pourrait induire une sélection conjointe forte sur le mode de reproduction sexué, indépendamment des coûts et avantages génétiques associés (cf. reproduction sexuée en températures basses et environnement perturbé chez les pucerons (Simon, Rispe, & Sunnucks, 2002; Frantz, Plantegenest, & Simon, 2006), parce qu'il produit un stade supplémentaire dormant, dispersant et détoxiquant (hypothèse en 3D). De plus, des approches de génétiques (e.g. Innes, Schwartz, & Hebert, 1986; Halkett, Simon, & Balloux, 2005) sur les populations le long du gradient de pollution pourrait permettre de mesurer quantitativement l'investissement dans le sexe de ces différentes populations et pourraient permettre de confirmer notre hypothèse sur la sélection des Daphnies sexuées en milieu pollué aux métaux traces.

Chez la même population urbaine que celle étudiée lors du Chapitre 2, les individus semblent capables de détecter et d'éviter le Cuivre dans le milieu (Résultat 6. Chapitre 3). Ce résultat suggère une adaptation à un milieu hétérogène spatialement pour la pollution et à une toxicité supérieure des métaux en comparaison au coût de cette adaptation dans cette population. Comme la balance entre le coût de l'évitement d'une part et de la toxicité des métaux d'autre part doit probablement dépendre du niveau local de pollution et de l'hétérogénéité spatiale de la pollution, on pourrait s'attendre à des expressions différentes du comportement d'évitement selon les populations (Lefcort *et al.*, 2004; Lopes *et al.*, 2004) le long de notre gradient de pollution.

En conclusion, mes travaux de doctorat indiquent que les Daphnies urbaines sont capables d'affronter la pollution aux métaux traces en milieu urbain :

- par la production d'éphippie leur permettant d'entrer en dormance, de disperser et potentiellement de détoxiquer les métaux (hypothèse en 3D)
- et par le développement des capacités de détection et d'évitement comportementaux des métaux dans leur milieu.

Cette thèse permet de remettre en cause le statut de bioindicateur de la Daphnie. De plus, elle apporte de nouvelles preuves soulignant l'importance de prendre en compte les effets sur le long terme de l'exposition à la pollution et ses conséquences évolutives dans les études écotoxicologiques. Enfin, ces résultats mettent en évidence la possibilité que les changements liés à l'urbanisation entrainent des réponses micro-évolutives des populations.

Annexe 1

Chapter 1 and urbanisation

Methods for measure the degree of urbanisation: The degree of urbanisation in each site was determined on land use maps (data from "Institut d'Aménagement et d'Urbanisme", Île-de-France, 2012) with ArcGIS (10.3.1). A 2500 meters buffer area was drawn around each sampling point. Items were considered as urbanised (individual housings, collective housings, activities spaces, transport and quarry equipments, building sites and landfills) or non-urbanised (forests, semi-natural environments, agricultural lands, water and artificialised open spaces) and the degree of urbanization was calculated as (urbanised surface) / (urbanised surface + non-urbanised surface).

Results: The degree of urbanisation were negatively related to the concentrations of Cr, and Ni (Table 2) but were not linked with the presence of Daphnia and ephippia in ponds or with ephippial characteristics (all P-values > 0.11).

Table 2 Pearson correlation matrix for environmental factors and trace metals concentrations of sampled ponds of Ile-de-France

	Zn	Cd	Cr	Ni	Cu	As	Pb	Alt	Lat	Long	Dist	Vol	Urb
Zn		> 0.001	0.001	0.017	> 0.001	0.168	> 0.001	0.261	0.442	0.969	0.797	0.013	0.635
Cd	**0.68*** **		> 0.001	0.001	> 0.001	0.007	> 0.001	0.041	0.087	0.509	0.253	0.006	0.111
Cr	**0.45** **	**0.58*** **		> 0.001	> 0.001	0.018	> 0.001	0.001	0.144	0.774	0.084	0.016	0.006
Ni	**0.33***	**0.47** **	**0.56*** **		0.013	> 0.001	0.002	0.046	0.038	0.130	0.006	0.020	0.003
Cu	**0.71*** **	**0.65*** **	**0.48*** **	**0.35***		0.027	> 0.001	0.774	0.989	0.164	0.129	0.013	0.540
As	0.20	**0.37** **	**0.33***	**0.57*** **	**0.31***		> 0.001	0.255	0.746	0.225	0.534	0.222	0.999
Pb	**0.57*** **	**0.76*** **	**0.66*** **	**0.43** **	**0.59*** **	**0.52*** **		0.009	0.050	0.388	0.337	0.052	0.159
Alt	0.16	**0.29***	**0.43** **	**0.28***	0.04	0.16	**0.36** **		0.123	0.703	> 0.001	0.008	> 0.001
Lat	-0.11	-0.24	-0.21	**-0.29***	0.00	0.05	**-0.28***	-0.22		0.263	0.046	0.687	0.020
Long	0.01	-0.09	-0.04	-0.21	-0.20	-0.17	-0.12	0.05	0.16		0.336	0.777	0.320
Dist	-0.04	0.16	0.24	**0.38** **	-0.22	0.09	0.14	**0.68*** **	**-0.28***	0.14		0.804	> 0.001
Vol	**-0.35***	**-0.38** **	**-0.34***	**-0.32***	**-0.34***	-0.17	-0.27	**-0.37** **	0.06	-0.04	-0.04		0.241
Urb	-0.07	-0.23	**-0.38** **	**-0.41** **	0.09	0.00	-0.20	**-0.72*** **	**0.32***	-0.14	**-0.92*** **	0.17	

The below diagonal part is rho (correlation coefficient); the above diagonal part is associated P-value. Alt: altitude, Lat: latitude, Long: longitude, Dist: distance from Paris, Vol: volume, Urb: urbanization.

NS: non significant; * P<0.05; ** P<0.01; *** P<0.001

Annexe 2

Chapter 2 and analysed data

Urban conditions could impact fate of ephippia not only through their effects on adult individuals that carry them but even during the storage of this resting-eggs bank (Rogalski, 2015). As ephippia disperse through time (dormancy) and space, the timing of their production and hatching activation affect populations dynamics and could have evolutionary and ecological consequences at populations to ecosystem level. We thus record hatching success of ephippia produced during experimental exposure to metal under reciprocal transplant.

Since the size of the ephippia could be linked to their hatching success and to the fitness of the « emergent » offsprings, in term of adult size and future reproduction (Boersma, Boriss, & Mitchell, 2000), we also surveyed morphologic characteristics of Daphnia and resting-eggs produced.

Data collection of ephippia and Daphnia

From the forth week, for half of the experimental populations weekly randomly chosen within each of the six treatments (Table 1), Daphnia individuals were counted and 10 Daphnia were photographed and placed back in the jars. We analysed these photographs with Image J software (version 1.51m9) to extract individual size, spine and eye size, clutch-size, the presence of male and the presence of Daphnia carrying ephippia.

Hatching success

Embryos (one or two originating from the same ephippia) were put in individual jar in thermostatic chamber at 20°C under 12L:12D to stimulate hatching (Haghparast *et al.,* 2012). 10 embryo(s) by each Ni*Temperature treatment were tested where possible. Among these 10 embryo(s), 5 were put in pure Volvic® water and 5 in Nickel-water treatment.

Publication

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Communications

2018	Montpellier	*More and smaller resting eggs along a gradient for pollution by metals: dispersal, dormancy and detoxification strategies in Daphnia?* – Poster **II Joint Congress on Evolutionary Biology**
2018	Versailles	*Are Daphnia able to cope with metal pollution?* - Oral **Journées Scientifiques de l'iEES - Paris** **Prix du meilleur oral**
2017	Ghent	*More and smaller resting eggs along a gradient for pollution by metals: dispersal, dormancy and detoxification strategies in Daphnia?* – Oral **Ecology Across Borders: Joint Annual Meeting**
2017	Paris-Saclay	*Production of melanized diapausing eggs as a way to survive in metal polluted habitat?* – Oral **Le Petit Pois Déridé - Groupe de Biologie et de Génétique des Populations**
2016	Marseille	*Numerous and melanized diapausing eggs as a way to survive in metal polluted habitat?* – Poster **Congrès de la Société Française d'Ecologie**
2016	Paris	*Effects of urbanization on communities and behaviors of earthworms.* - Poster **Young Natural History scientist Meeting**

Enseignements

2015 - 2018	Biologie cellulaire Licence 1 (TP et TD) : 48 heures / an Encadrement d'étudiant-e-s en stage de Master 1 et de Licence 3
2016 - 2018	Atelier de Recherche Encadrée Licence 1 (TD et Jury) : 16 heures /an
2015 - 2016	Enseignante référente en Licence 1 : 16 heures
2017	Examinatrice dans un jury de Master 2 Écologie–Biodiversité–Évolution

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Ringot Gabrielle - Thèse De Doctorat - 2018

Résumé :
Les métaux traces sont biodisponibles, persistants et concentrés dans les milieux urbanisés. Via leur affinité pour les protéines, les métaux traces peuvent perturber les processus métaboliques et réduire la survie et le succès reproducteur. En réponse à ces nouvelles pressions de sélection imposées par ce milieu perturbé, les populations urbaines peuvent développer des adaptations telles que des stratégies de dormance, de dispersion, de détoxication ou encore de détection et d'évitement des métaux dans le milieu. Au cours de leur vie, les Daphnies peuvent switcher de reproduction parthénogénétique, lorsque les femelles produisent de nombreuses filles clonales, à reproduction sexuée. En réponse à différents stimuli environnementaux, les femelles commencent à produire des males et à fabriquer une enveloppe chitineuse et résistante: l'éphippie qui recevra les oeufs de durée après fécondation. De manière intéressante, ces éphippies produites sexuellement peuvent disperser à la fois dans le temps (dormance) et dans l'espace et peuvent accumuler les métaux traces suggérant des propriétés de détoxication. Cette thèse montre tout d'abord, qu'il existe une relation positive entre la concentration en métaux dans le milieu et la présence d'éphippie, suggérant une sélection pour les Daphnies sexuées dans les milieux pollués aux métaux traces. De plus, suite à une exposition expérimentale à un métal chez les Daphnies urbaines, le succès de fécondation des éphippies est plus élevé chez les Daphnies exposées à la pollution comparée aux Daphnies non exposées. De plus, les individus provenant de cette même population urbaine semblent capables de détecter et d'éviter les polluants métalliques dans leur milieu. Ces résultats suggèrent que la pollution anthropique a des conséquences évolutives sur les populations urbaines et souligne la nécessité de tenir compte des aspects évolutifs dans les études écotoxicologiques.

Mots clés : écotoxicologie - adaptation - métaux traces - écologie urbaine - éphippie

Consequences of environmental pollution on evolution of life history traits in Daphnia

Abstract :
Trace metals are bioavailable, persistent and concentrate in urban areas. Because of their affinity for proteins, trace metals can disturb metabolic processes and impair survival and reproductive output. In response to these new selective pressures created by such disturbed habitats, urban populations could evolve adaptations as dormancy, dispersal, detoxification or pollutant detection and avoidance abilities. Daphnia, can switch to parthenogenetic reproduction, when females produce numerous clonal daughters, to sexual reproduction. In response to several environmental cues, females start to produce males and a chitinous and resistant envelop: the ephippium that receives diapausing eggs after fertilisation. Interestingly, these sexually produced ephippia can disperse both in time (dormancy) and space and exhibit metal accumulation ability suggesting detoxification property. First, this study shows that a positive relationship exists between the environmental concentration of metals and probability of ephippia presence suggesting selection for sexual Daphnia in metal polluted habitats. Moreover, after an experimental exposure to a trace metal in urban Daphnia, ephippial fertilisation success was higher in Daphnia exposed to metal pollution compared to non-exposed Daphnia. Additionally, Daphnia individuals originating from the same population are able to detect and avoid metal pollution. These results suggest that anthropogenic pollution has evolutionary consequences in urban population and stress the need to take into account evolutionary aspects in ecotoxicological studies.

Keywords : ecotoxicology - adaptation - trace metals - urban ecology - ephippia