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Approche holistique des facteurs favorisant le développement du moustique tigre en milieu urbain

Pénélope Duval

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Pénélope Duval. Approche holistique des facteurs favorisant le développement du moustique tigre en milieu urbain. Ecosystèmes. Université Claude Bernard - Lyon I, 2023. Français. NNT : 2023LYO10217 . tel-04786558

HAL Id: tel-04786558

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**THESE de DOCTORAT DE
L'UNIVERSITE CLAUDE BERNARD LYON 1**

Ecole Doctorale N° 341

Evolution, Ecosystèmes, Microbiologie, Modélisation

Discipline : Biologie / Sciences humaines et sociales

Soutenue publiquement le 15/11/2023, par :

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**Approche holistique des facteurs favorisant le développement
du moustique tigre en milieu urbain**

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Remerciements

Tout d'abord, je souhaite remercier ma directrice de thèse, Claire Valiente Moro, et ma co-directrice de thèse, Christina Aschan Leygonie, pour m'avoir proposé ce sujet de thèse et m'avoir guidée et soutenue tout au long de ces trois années. Je vous suis reconnaissante à toutes les deux pour la confiance que vous m'avez accordée, vos précieux conseils et votre soutien. Claire, je te remercie tout particulièrement pour ta bienveillance, ta bonne humeur, ta pédagogie et tes encouragements au quotidien. Christina, je te suis reconnaissante de m'avoir fait découvrir le monde des sciences humaines, qui m'ont permis d'avoir une vision nouvelle sur les problématiques autour du moustique, ce qui m'a particulièrement plu.

J'adresse également mes remerciements à l'ensemble des membres de l'École Urbaine de Lyon pour leur confiance et leurs précieux accompagnements. Je souhaite exprimer ma reconnaissance particulière envers Jérémy Cheval pour sa bienveillance et nos échanges enrichissants. Mes remerciements vont également à Thomas Balestrieri, qui m'a initiée à l'univers de la radio et m'a donné l'opportunité de participer à des émissions qui m'ont profondément captivée. Je souhaite également à remercier les autres doctorants de l'École Urbaine de Lyon pour nos échanges fructueux, même lorsque nos domaines de recherche étaient très éloignés. J'ai particulièrement apprécié de découvrir d'autres disciplines grâce à vous.

Je remercie Jocelyn Raude et Thomas Pollet d'avoir accepté d'être rapporteur de ma thèse, ainsi que les examinateurs, Nathalie Mondy et Frédéric Simard pour l'intérêt et les retours sur mon travail.

Je tiens aussi à remercier ma tutrice, Laurence Mouton, et tous les membres de mon comité de suivi de thèse, Anne-Emmanuelle Hay de Bettignies et Julien Viremont, pour leur bienveillance et leurs conseils.

Je remercie tous les collaborateurs de ce projet de thèse qui m'ont aidée et permis de mener à bien mes multiples analyses.

Merci à tous les membres de l'équipe Dynamique Microbienne et Transmission Virale qui m'ont soutenue (et supportée) pendant ces trois années. Je vous remercie tous pour votre aide et notamment lors de ma campagne d'échantillonnages dans les jardins.

Je tiens à remercier Edwige, la pile électrique de l'équipe, la reine des escape game mais surtout notre AP préférée. Je n'oublierai pas nos semaines d'extraction d'ADN, nos moments peinture et nos longues sessions de pêche à l'insectarium ! Merci pour ta gentillesse, ton aide, ta bonne humeur à toute épreuve, et tes encouragements !

Merci à Laurent, le footballeur et le JCVD de l'équipe, d'avoir pris le temps de m'épauler dans les manip mais aussi de m'avoir soutenue dans les moments où j'en avais besoin.

Merci à Patricia de m'avoir aidée en particulier pendant mes interminables après-midi d'isolement et de mise en culture. Merci de m'avoir accompagnée dans la plupart de mes prélèvements et d'avoir été ma traductrice franco-portugaise dans les jardins collectifs. Merci de m'avoir fait rire tous les jours avec ton amour pour les étudiants.... Je te remercie aussi de m'avoir fait monter dans ta mustang !!

Merci à Guillaume pour ta patience et pour avoir pris le temps de m'aider pour les analyses statistiques. Ça n'a pas dû être une tâche facile pour toi, « je te jure ».

Merci à Aurélien, le petit nouveau de l'équipe, j'ai été contente de pouvoir passer ces derniers mois de thèse en ta présence, merci pour tes conseils et ton humour.

Merci à mes trois supers colocs de bureau pour tous les bons moments passés ensemble. Pierre, merci de m'avoir supportée en tutrice de stage et qui, malgré cela, a eu le courage de continuer en thèse avec nous. Maxime, merci pour ton aide infallible en stat. Merci d'avoir décoré mon bureau avec tous tes petits dessins et surtout de m'avoir bien fait rire et de m'avoir fournie en bonbecs ! Simon, notre historien géographe et l'autre dalleux de l'équipe, je tiens à te remercier d'avoir toujours été le premier à me rejoindre aux buffets. Nos échanges de recettes me manquent déjà ! Un grand merci à toi pour m'avoir initiée à GeoGuessr, je commence à devenir incollable en géographie grâce à toi.

Je tiens aussi à remercier Mathilde, notre coloc de bureau à mi-temps, pour m'avoir soutenue tout au long de cette thèse que ce soit sur le plan professionnel ou personnel !

Merci à Thibault, notre breton d'adoption, pour les petits skills en GeoGuessr et en Squiz et ton délicieux cidre.

Un très grand merci à ma famille pour m'avoir soutenue et surtout supportée pendant ces trois longues années. Je tiens particulièrement à remercier mes trois petites sœurs, Anna,

Lou et Gabie de m'avoir entourée de tout leur amour. Je remercie ma mère pour son soutien sans faille et son amour. Je remercie en particulier mon oncle, François, et mes grands-parents de m'avoir épaulée et avoir cru en moi tout au long de ma thèse.

Un immense merci à mon compagnon, Éric, pour m'avoir accompagnée pendant cette dernière année de thèse qui n'aurait pas été la même sans toi. Merci d'avoir toujours cru en moi, de m'avoir soutenue tous les jours et d'avoir bien pris soin de moi au quotidien. J'adresse également mes remerciements à ses parents, Carla et Hervé, qui m'ont accueillie pendant la canicule pour me permettre de rédiger au frais mais aussi de nous avoir prêté leur Vito, ce qui m'a permis de faire une pause bienvenue à un moment crucial de l'été, alors que j'en avais grandement besoin. Un merci particulier à Carla pour avoir consacré du temps à corriger et relire mon manuscrit à plusieurs reprises, tu es désormais incollable sur le moustique tigre !

J'ai également une pensée pour tous mes amis de Montpellier en particulier Lolo, Paul et Célia pour leur soutien. Mais aussi pour mes voisins, Flore et Axel, qui m'ont toujours accueillie lors des moments plus compliqués.

Pour finir, je tiens à remercier ma meilleure amie, Joséfine qui, même à l'autre bout du monde, a toujours été là dans les moments difficiles.

Résumé

La colonisation en cours de la France métropolitaine par le moustique tigre, *Aedes albopictus*, en fait dorénavant un problème sanitaire au niveau national. L'identification des facteurs favorisant sa prolifération est devenue un enjeu majeur pour prévenir le risque d'émergence de maladies vectorielles. Ce moustique affectionne les milieux urbains où de nombreux gîtes larvaires artificiels permettent son développement. En ville, ces habitats aquatiques sont directement exposés à des pollutions liées aux activités humaines. Pourtant, l'impact de cette exposition sur la composition physico-chimique et microbienne de l'eau et la colonisation des gîtes reste peu étudié. De plus, une meilleure compréhension des pratiques et des connaissances de la population s'avère également nécessaire pour améliorer la gestion de cette espèce. Dans ce contexte, l'objectif de ces travaux était d'élaborer une approche interdisciplinaire afin d'identifier les facteurs favorisant la propagation du moustique tigre dans les jardins collectifs, des initiatives qui connaissent une popularité croissante en milieu urbain et où le moustique prolifère. Pour cela, nous avons réalisé une enquête sur les connaissances, les attitudes et les pratiques vis-à-vis du moustique tigre auprès des adhérents à ces jardins (désignés ci-après comme des "jardiniers" ou "jardiniers amateurs") dans la métropole de Lyon. Les résultats de cette étude ont montré que les jardiniers percevaient les moustiques comme une nuisance en raison des désagréments provoqués par ses piqûres, plutôt que comme un vecteur de maladies. De plus, ils ont permis d'identifier que les attitudes, plus que les connaissances, influençaient la bonne gestion de cette espèce. Nous avons ensuite sélectionné un échantillon de ces jardins, associés à différents contextes de pollution, dans lesquels nous avons réalisé des prélèvements dans des récupérateurs d'eau de pluie, colonisés ou non par le moustique. L'analyse de la composition physico-chimique et microbiologique de l'eau a montré de plus fortes concentrations en protoxyde d'azote et en méthane dans les eaux non colonisées. En outre, ces résultats ont révélé un impact différentiel des gradients de pollution sur la diversité microbienne de l'eau selon le statut de colonisation. Dans un contexte marqué par un désir croissant des citoyens de renouer avec la nature, ces résultats soulignent l'importance de poursuivre les efforts de recherche pour mieux caractériser l'incidence des facteurs humains (attitudes, représentations mais également pollutions anthropiques) sur l'expansion du moustique en milieu urbain. Ces connaissances pourraient permettre, à terme, d'offrir de nouvelles pistes de recommandations pour diversifier les outils de lutte antivectorielle et adapter les campagnes de sensibilisation.

Mots-clés : moustique tigre, gîtes larvaires, pollution, pratiques humaines, communautés microbiennes, jardins collectifs

Abstract

The progressive colonization of mainland France by the Asian tiger mosquito, *Aedes albopictus* is of major public health concern. Identifying the factors promoting its proliferation has become a high priority to prevent the emergence of vector-borne diseases. This mosquito thrives in urban environments where numerous artificial larval habitats facilitate its development. In cities, these aquatic habitats are directly exposed to pollution associated with human activities. However, its impact on the water physicochemical and microbial composition as well as on the colonization of breeding sites remains poorly understood. Furthermore, a better understanding of the practices and knowledge of the citizens is also necessary to improve the management of this species. In this context, the objective of this study was to develop an interdisciplinary approach to identify the factors that promote the spread of the tiger mosquito in community gardens, which are initiatives becoming increasingly popular in urban areas and where mosquitoes proliferate. To that end, we conducted a survey on the knowledge, attitudes and practices related to the Asian tiger mosquito among members of community gardens in the Lyon metropolis. The results of this study showed that gardeners were concerned by the tiger mosquito due to the nuisance caused by its bites rather than its role as a disease vector. This study also allowed to identify that attitudes, rather than knowledge, influence the effective management of this vector species. We then selected a sample of these gardens, associated with different pollution contexts, in which we took samples from rainwater collectors, colonized or not by the mosquito. Analysis of the water physicochemical and microbiological composition of breeding sites showed higher concentrations of nitrous oxide and methane in non-colonized waters. Moreover, these results also revealed a differential impact of pollution gradients on water microbial diversity depending on the colonization status. In a context characterized by an increased desire of citizens to reconnect with nature, these results highlight the importance of continuing and concerted research efforts to better characterize the impact of human factors (attitudes, representations but also anthropogenic activities) on the spread of this mosquito species in urban areas. This knowledge could ultimately make it possible to offer new recommendations for diversifying vector control tools and adapting awareness campaigns.

Key words: *Aedes albopictus*, larval breeding sites, pollution, human practices, microbial communities, community gardens

Table des matières

I. Introduction.....	10
II. Synthèse bibliographique	20
1. Impact de l’urbanisation sur la prolifération des moustiques en milieu urbain... 21	
1.1 Publication n°1: “Impact of Human Activities on Disease-Spreading Mosquitoes in Urban Areas” 22	
2. Les jardins collectifs en France : historique, diversité et fonctions..... 44	
2.1 Historique	46
2.2 Diversité de fonctionnement.....	49
2.3 Bienfaits et inconvénients.....	51
3. Impact des comportements humains sur la prolifération des moustiques en régions non endémiques	55
3.1 Publication n°2: “Knowledge, attitudes and practices regarding mosquitoes and mosquito-borne infectious diseases in nonendemic regions”	55
III. Mémoire expérimental.....	81
1. Évaluation des Connaissances, Attitudes et Pratiques (CAP) des adhérents aux jardins collectifs de la métropole de Lyon vis-à-vis du moustique tigre....	Erreur ! Signet non défini.
1.1 Contexte et objectif général de l’étude	82
1.2 Publication n°3: “How do attitudes shape protective practices against the Asian tiger mosquito in community gardens in a nonendemic country?”	82
1.3 Discussion et conclusion	99
2. Influence des caractéristiques physico-chimiques et microbiennes de l’eau sur la colonisation des habitats aquatiques par le moustique tigre	103
2.1 Contexte et objectif général de l’étude	103
2.2 Publication n°4 “Pollution gradients contribute to the microbial and physicochemical composition of <i>Ae. albopictus</i> larval habitats in urban community gardens”	104
2.3 Discussion et conclusion	159
IV. Discussion générale	163
1. Impact des connaissances et de la perception du risque sur les bonnes pratiques de gestion du moustique tigre dans les jardins collectifs	165

2. Vers une meilleure caractérisation de l'influence des activités humaines sur les interactions biotiques et abiotiques dans les habitats larvaires	170
3. Mobilisation sociale et recherche scientifique : un levier pour ralentir la progression du moustique tigre en milieu urbain ?	176
4. Conclusion et perspectives	179
V. Références	183

Liste des abréviations :

ANSES : Agence nationale chargée de la sécurité sanitaire de l'alimentation, de l'environnement et du travail

Bti : *Bacillus thuringiensis* variant *israelensis*

CAP : Connaissances, Attitudes, Pratiques

COV : Composés organiques volatiles

COVAR : Comité de Veille et d'Anticipation des Risques Sanitaires

EID : Entente interdépartementale de démoustication

HAP : Hydrocarbures aromatiques polycycliques

ICU : Îlot de chaleur urbain

INRAE : Institut national de recherche pour l'agriculture, l'alimentation et l'environnement

JTSE : Jardins dans Tous Ses Etats

NPK : Azote, phosphore et potassium

ONU : Organisation des Nations unies

OMS : Organisation mondiale de la santé

SNCF : Société Nationale des Chemins de fer Français

UTO : Unité taxonomique opérationnelle

Liste des figures

- Figure 1.** Distribution géographique mondiale simulée d'*Ae. aegypti* (A) et d'*Ae. albopictus* (B) en 2050. D'après Ding et al. (2018). 12
- Figure 2.** Évolution du nombre de départements métropolitains où le moustique tigre est installé et actif en 2012 (A), 2017 (B) et 2022 (C). D'après les données du ministère de la Santé et de la Prévention (<https://sante.gouv.fr/>). 12
- Figure 3.** Exemples d'habitats larvaires du moustique tigre en milieu urbain. (A) conteneur en métal, (B) terrarium, (C) trous dans des pierres, (D) vases en céramique, (E) conteneur en plastique, (F) gouttière, (G) pneus, (H) surfaces accumulant de l'eau, (I) gobelet jetable, (J) dalles percées, (K) pots de fleurs, (L) réservoir en métal. D'après Bonizzoni et al. 2013. 13
- Figure 4.** Cycle de vie du moustique tigre. Les différentes formes de lutte antivectorielle sont indiquées pour chaque phase, aquatique et aérienne. (Adapté du compte rendu n°6 publié en 2020, de la commission d'enquête de l'Assemblée Nationale chargée d'évaluer les recherches, la prévention et les politiques publiques à mener contre la propagation des moustiques *Aedes* et des maladies vectorielles). En marron sont représentés les moyens de lutte physique, en vert les moyens de lutte biologique et en rouge les moyens de lutte chimique. 15
- Figure 5.** Influence du gîte larvaire sur les traits phénotypiques des moustiques à l'âge adulte. Les facteurs abiotiques (partie bleue) et les facteurs biotiques (partie verte) du gîte ont des effets directs sur le développement des larves de moustiques et influencent indirectement les caractéristiques physiologiques et morphologiques du moustique adulte. D'après Héry *et al.* 2021. 17
- Figure 6.** Exemples de bienfaits liés à la présence d'espaces verts en ville. D'après Déoux, 2020..... 45
- Figure 7.** Historique des jardins collectifs en France. D'après Asaad 2016. 47
- Figure 8.** Différentes phases d'émergence d'un jardin collectif. D'après Vandebroucke et al. 2017. 50
- Figure 9.** Impact des jardins collectifs sur la santé et le bien-être des adhérents, l'environnement et l'économie (Tharrey et al. 2019). 52
- Figure 10.** Exemple de la gestion de l'eau de pluie dans 4 jardins collectifs de la métropole de Lyon. Les photographies **A** et **B** montrent des réservoirs d'eau stagnante non couverts permettant la colonisation par le moustique tigre. Les photographies **C** et **D** illustrent une gestion de l'eau de pluie exemplaire empêchant ainsi la colonisation par ce moustique. Photographies prises sur le terrain en 2021 lors des prélèvements. 100

Liste des tableaux

- Tableau 1.** Nombre de cas importés et autochtones en France métropolitaine depuis 2014 (d'après les données de Santé publique France). 15

I. Introduction

Les maladies vectorielles représentent plus de 17% des maladies infectieuses au niveau mondial et provoquent plus d'un million de décès chaque année (OMS 2020). Ces maladies sont transmises par des arthropodes vecteurs, essentiellement insectes et acariens hématophages. Parmi eux, les moustiques du genre *Aedes* sont responsables d'épidémies comme la dengue, le Zika ou encore la fièvre jaune (Lee et al. 2018). La dengue, à elle seule, est à l'origine d'environ 50 millions de cas par an, dont 500 000 cas de dengue hémorragique, qui sont mortels dans plus de 2,5% des cas (OMS 2023). Aujourd'hui, cette maladie est endémique dans plus de 100 pays mais son incidence progresse de manière importante, en particulier due à l'extension des zones géographiques des espèces de moustiques *Aedes aegypti* et *Aedes albopictus*. Désormais présents sur tous les continents à l'exception de l'Antarctique, ces moustiques représentent un risque sanitaire majeur dans les zones nouvellement colonisées (Bonizzoni et al. 2013). Ce risque est d'autant plus élevé que l'aire de répartition de ces deux espèces continue de s'étendre (**Figure 1**) (Ding et al. 2018).

Originaire d'Asie du Sud et de l'Est, *Aedes albopictus*, communément appelé moustique tigre, a été introduit sur le continent européen en 1970 notamment via le commerce de pneus usagés en provenance de Chine (Sherpa et al. 2019). Aujourd'hui installé dans 71 des 96 départements de France métropolitaine, sa première détection en 2004 a été constatée à Menton dans le Sud de la France (**Figure 2**). Sa dispersion sur le territoire national, via les axes autoroutiers, a été favorisée par sa capacité à coloniser l'habitacle des véhicules (Roche et al. 2015; Eritja et al. 2017). Plus généralement, sa dissémination et son établissement durable sur de nouveaux territoires dépendent de deux facteurs intrinsèques. D'une part, sa forte plasticité écologique lui a permis de coloniser des habitats très diversifiés, avec un développement préférentiel dans des environnements péri-urbains et des zones urbaines très denses. En effet, la mosaïque urbaine offre au moustique une multitude de gîtes larvaires artificiels très diversifiés comme par exemple des avaloirs, des bouches d'égouts, des gouttières et plus globalement, tous types de réservoirs pouvant accumuler de l'eau stagnante (Bonizzoni et al. 2013; Li et al. 2014; Wilke et al. 2019) (**Figure 3**). D'autre part, la capacité de ses œufs à entrer en diapause lui a permis de s'implanter durablement dans des zones tempérées (Armbruster 2016). En effet, les œufs de ce moustique sont capables, sous l'effet de certains stimuli, de rentrer en diapause hivernale (absence d'éclosion), permettant la survie de l'espèce pendant l'hiver et la génération de nouvelles populations au printemps quand les conditions de température redeviennent favorables au cycle de développement.

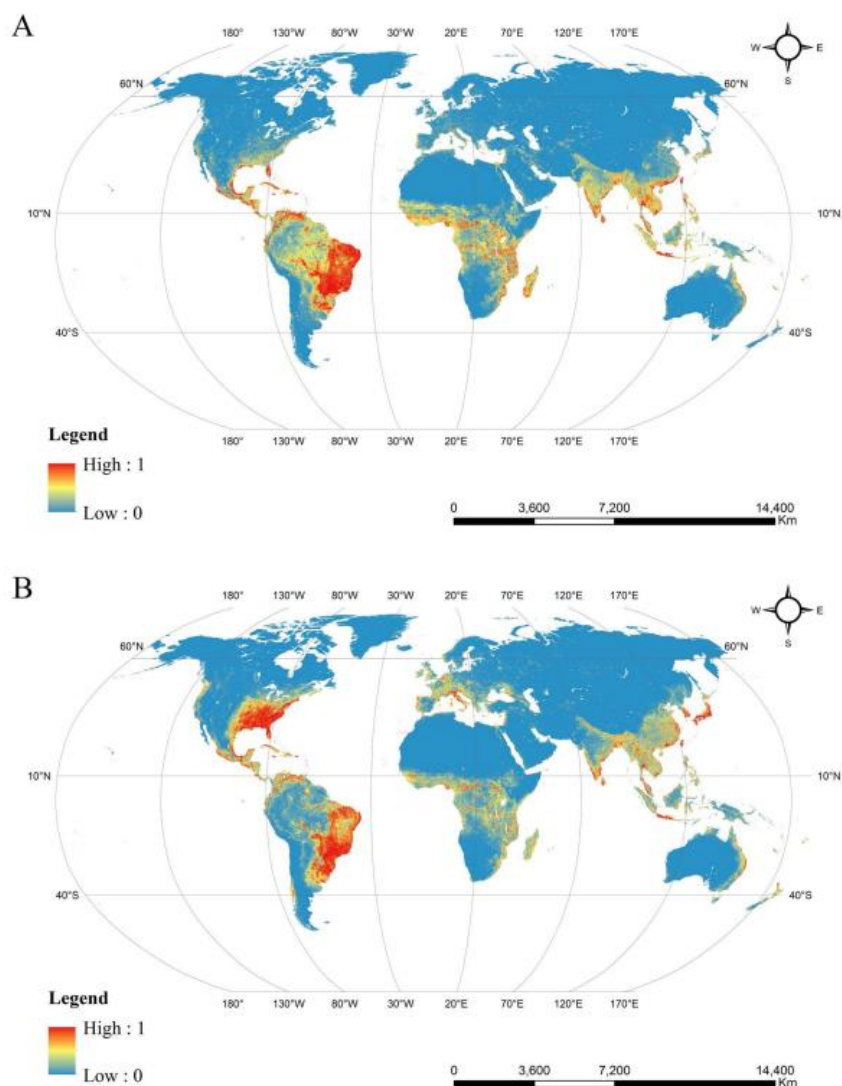


Figure 1. Distribution géographique mondiale simulée d'*Ae. aegypti* (A) et d'*Ae. albopictus* (B) en 2050. D'après Ding et al. (2018).



Figure 2. Évolution du nombre de départements métropolitains où le moustique tigre est installé et actif en 2012 (A), 2017 (B) et 2022 (C). D'après les données du ministère de la Santé et de la Prévention (<https://sante.gouv.fr/>).

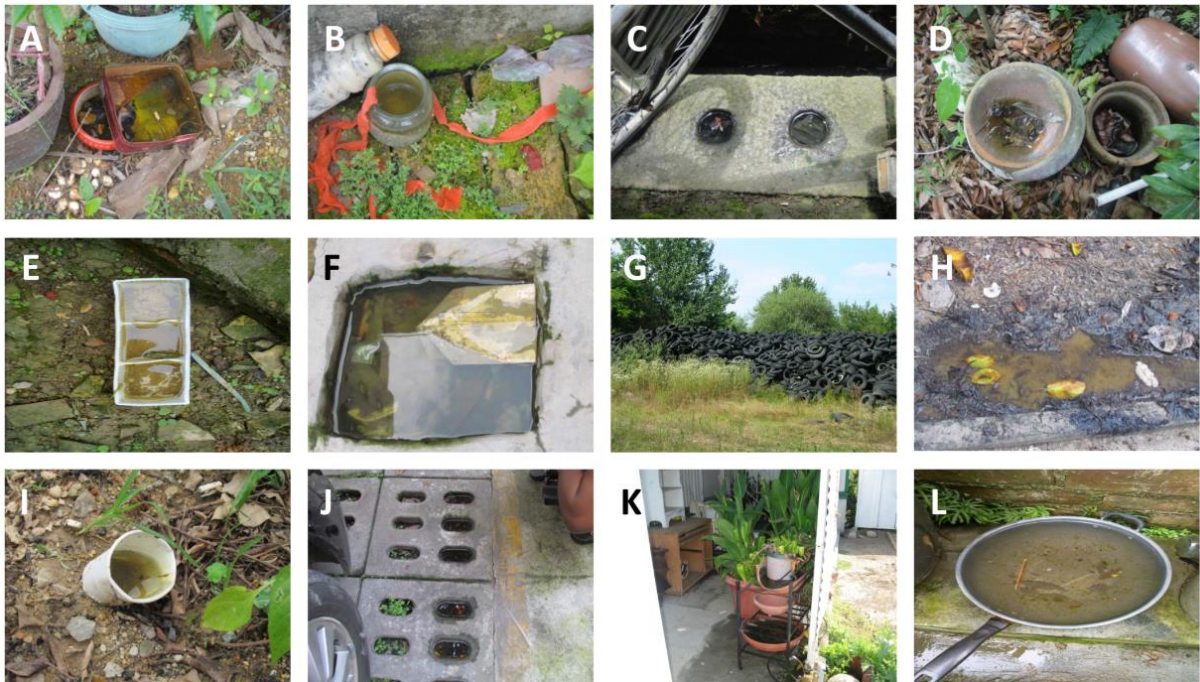


Figure 3. Exemples d'habitats larvaires du moustique tigre en milieu urbain. (A) conteneur en métal, (B) terrarium, (C) trous dans des pierres, (D) vases en céramique, (E) conteneur en plastique, (F) gouttière, (G) pneus, (H) surfaces accumulant de l'eau, (I) gobelet jetable, (J) dalles percées, (K) pots de fleurs, (L) réservoir en métal. D'après Bonizzoni *et al.* 2013.

Ses œufs ont également la capacité de résister à la dessiccation (sécheresse) pendant plusieurs mois et par conséquent de survivre lors de transports de longue durée (Juliano et al. 2002). Ainsi, de par son fort pouvoir invasif et sa capacité à transmettre des pathogènes, *Aedes albopictus*, suscite des inquiétudes grandissantes dans de nombreux pays (Benedict et al. 2007). En juillet 2020, une commission d'enquête de l'Assemblée Nationale a estimé que le moustique tigre pourrait représenter un risque sanitaire majeur en France métropolitaine lors des prochaines décennies (Naillet 2020). Depuis la première identification de cas autochtones de dengue en 2010, le nombre de cas d'arboviroses transmises par les moustiques augmente chaque année. En 2022, 65 cas autochtones de dengue ont été identifiés contre 48 cas en 2021 (Santé Publique France 2022). Même si le vecteur à l'origine de la transmission de ces cas autochtones n'a pas été formellement identifié, il est fort probable qu'il s'agisse du moustique tigre, l'espèce *Ae. aegypti* étant absente en métropole. Concernant les cas importés, 272 cas importés de Dengue, 22 cas importés de chikungunya et 3 cas importés de maladie à virus Zika ont été déclarés en 2022 (Santé publique France 2022) (**Table 1**).

La présence accrue du moustique dans les zones urbaines, où la population y est dense et où la probabilité de transmission des maladies est importante, représente un facteur de risque majeur pour l'émergence de maladies vectorielles. En l'absence de prophylaxie et de vaccins efficaces contre la plupart de ces maladies, la lutte antivectorielle, c'est-à-dire la lutte dirigée directement contre le moustique, apparaît comme la principale solution pour enrayer ce risque (**Figure 4**). Les méthodes actuelles de lutte antivectorielle sont classées selon les techniques (biologiques, physiques, chimiques) ou les objectifs recherchés (limitation du contact hôte/vecteur ou action sur la densité/longévité). Pour enrayer la propagation du moustique tigre en milieu urbain, la lutte mécanique, par la suppression des gîtes larvaires, reste le moyen le plus efficace (Shaukat et al. 2019). Malgré tout, la multiplicité des gîtes larvaires reste importante dans l'espace public et privé et ils ne sont pas toujours accessibles aux services spécialisés des collectivités (Meunier and Foussadier 2020). Ainsi, la mise en place d'une véritable politique de prévention et de sensibilisation des Français s'impose comme une évidence et une nécessité (Naillet 2020).

Tableau 1. Nombre de cas importés et autochtones en France métropolitaine depuis 2014 (d'après les données de Santé publique France).

Année	Nombre de cas importés			Nombre de cas autochtones	
	Dengue	Chikungunya	Zika	Dengue	Chikungunya
2014	163	443	0	4	11
2016	167	18	8	0	0
2018	189	6	8	5	0
2020	834	6	1	13	0
2022	272	22	3	65	0

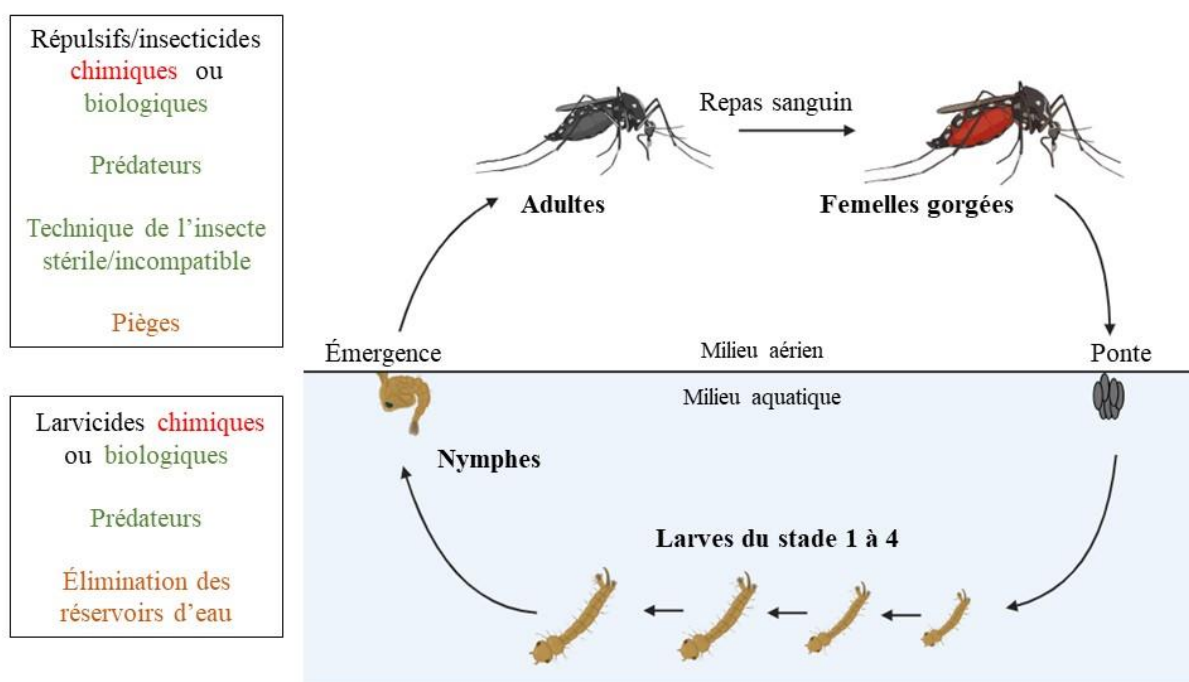


Figure 4. Cycle de vie du moustique tigre. Les différentes formes de lutte antivectorielle sont indiquées pour chaque phase, aquatique et aérienne. (Adapté du compte rendu n°6 publié en 2020, de la commission d'enquête de l'Assemblée Nationale chargée d'évaluer les recherches, la prévention et les politiques publiques à mener contre la propagation des moustiques *Aedes* et des maladies vectorielles). En marron sont représentés les moyens de lutte physique, en vert les moyens de lutte biologique et en rouge les moyens de lutte chimique.

La forte attractivité du milieu urbain pour le moustique tigre nécessite que les autorités locales intègrent la gestion des risques liés aux arboviroses dans la planification urbaine et l'entretien des infrastructures aptes à retenir l'eau (Li et al. 2014; Wilke et al. 2019). En ce sens, il est nécessaire d'encadrer l'aménagement d'espaces verts, susceptibles d'accroître le nombre de réservoirs d'eaux stagnantes dans l'espace urbain. En France, de nombreuses initiatives ont vu le jour pour réintégrer la nature en ville (Den Hartigh 2012). C'est le cas des jardins collectifs dont le nombre est en constante augmentation depuis leur première apparition en 1998 (Mathis and Pépy 2017). Cependant le développement de ces jardins n'est pas sans conséquence sur la prolifération du moustique tigre. En effet, ils offrent des environnements propices à son développement au travers des nombreux équipements de jardinage servant de gîtes larvaires (coupelles de pots de fleurs, arrosoirs ou encore récupérateurs d'eau de pluie...). Assurer une bonne protection de ces réservoirs est indispensable pour éviter leur colonisation par le moustique. Pour cela, il est nécessaire de sensibiliser la population de jardiniers amateurs aux bonnes pratiques de gestion de cette espèce et plus largement amener les pouvoirs publics à se mobiliser par la mise en place de campagnes de surveillance et de prévention dans ces environnements. En parallèle, améliorer les recherches scientifiques sur les facteurs susceptibles d'influencer la colonisation de l'eau par le moustique apparaît également essentiel pour adapter ces actions de sensibilisation.

Des études récentes témoignent du rôle important des propriétés biotiques et abiotiques des habitats aquatiques des moustiques sur leur comportement de ponte, ainsi que le développement et la survie des larves (Darriet and Corbel 2008; Medeiros-Sousa et al. 2020; Früh et al. 2020). Par exemple, certaines propriétés physico-chimiques telles que la température, le pH ou encore la turbidité de l'eau semblent jouer un rôle attractif vis-à-vis des femelles gravides (**Figure 5**) (Hery et al. 2021a). La présence de larves conspécifiques (c'est-à-dire de la même espèce) ou de certains microorganismes semble également moduler le comportement de pré-oviposition (Shragai et al. 2019). Parmi les microorganismes présents dans l'eau des gîtes, une fraction est conservée chez la larve et peut influencer certains traits phénotypiques à l'âge adulte (**Figure 5**) (Dickson et al. 2017). En milieu urbain, ces gîtes artificiels sont directement exposés à différentes sources de pollution d'origine industrielle ou domestique.

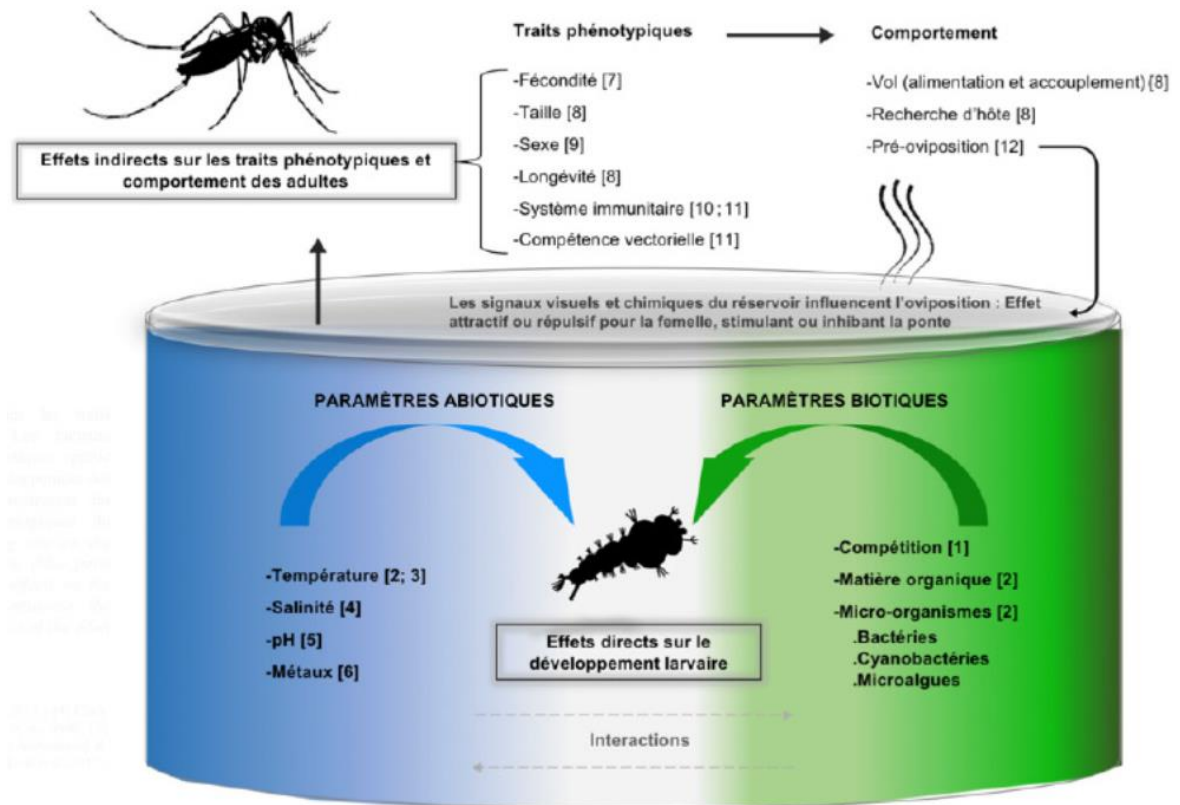


Figure 5. Influence du gîte larvaire sur les traits phénotypiques des moustiques à l'âge adulte. Les facteurs abiotiques (partie bleue) et les facteurs biotiques (partie verte) du gîte ont des effets directs sur le développement des larves de moustiques et influencent indirectement les caractéristiques physiologiques et morphologiques du moustique adulte. D'après Héry *et al.* 2021.

Par exemple, plusieurs études ont démontré que la présence d'engrais (azote, phosphore et potassium) dans l'eau stagnante augmentait l'attractivité pour femelles gravides de plusieurs espèces de moustiques, à savoir *Ae. albopictus*, *Ae. aegypti*, *Culex quinquefasciatus* et *Anopheles coluzzi* (Darriet and Corbel 2008; Young et al. 2014; Darriet 2018b; Borel et al. 2021). Ces composés favorisaient à leur tour le développement des microorganismes dont se nourrissent les larves. En plus de leurs effets directs, les polluants pourraient également moduler les communautés microbiennes présentes dans l'eau des gîtes et ainsi impacter le développement et la survie des larves (Muturi et al. 2016). Alors même que le moustique continue de proliférer en zone urbaine, l'incidence de l'émission de polluants sur les propriétés biotiques et abiotiques des gîtes larvaires du moustique tigre reste encore peu étudiée, hormis ces quelques exemples. Améliorer les connaissances des facteurs favorisant la prolifération du moustique tigre en milieu urbain pourrait permettre un meilleur accompagnement des collectivités et la mise en place de politiques publiques ciblées.

Nous avons choisi comme terrain d'étude les jardins collectifs, présents en grand nombre dans la Métropole de Lyon. Plusieurs raisons ont motivé ce choix. Tout d'abord, le moustique tigre est implanté et actif dans cette collectivité territoriale depuis 2012. De ce fait, dès 2013, le département du Rhône a été classé en niveau 1 du plan national de lutte contre la dissémination du chikungunya, de la dengue et du Zika. De plus, la région Auvergne-Rhône-Alpes enregistre chaque année plusieurs cas autochtones de ces maladies. Enfin, le nombre important d'adhérents à ces jardins collectifs et les activités de jardinage sont des facteurs propices à la prolifération du moustique.

Dans ce contexte, l'objectif général de ma thèse a été d'évaluer les impacts de deux ensembles de facteurs, (i) les connaissances, attitudes et pratiques individuelles des adhérents aux jardins collectifs et (ii) les activités anthropiques (comme sources de pollution) et leurs conséquences sur la composition physico-chimique et microbienne des gîtes larvaires.

Le premier chapitre porte sur une synthèse bibliographique déclinée en trois parties. La première s'articule autour d'une revue et porte sur l'impact de l'urbanisation sur la prolifération des moustiques vecteurs en milieu urbain. La deuxième partie porte sur l'historique, la diversité et les enjeux liés au déploiement des jardins collectifs urbains en France. La troisième partie porte à travers une revue sur une synthèse des enquêtes CAP (Connaissances, Attitudes, Pratiques) sur le moustique tigre réalisé auprès de la population

locale des régions non endémiques, c'est-à-dire des régions où les maladies ne sont pas constamment présentes au sein d'une population généralement associée à une zone géographique spécifique. Le deuxième chapitre de la thèse se concentre sur les résultats du volet expérimental. La première partie présente les résultats d'une enquête quantitative CAP sur le moustique tigre et la lutte biologique auprès des adhérents aux jardins collectifs de la métropole de Lyon. La deuxième partie décrit les résultats de l'étude de terrain dans les jardins collectifs qui visait à caractériser l'impact d'un gradient de pollution sur les propriétés biotiques et abiotiques d'habitats aquatiques colonisés ou non par le moustique. Le dernier chapitre de ce manuscrit est une discussion générale des résultats obtenus et des perspectives de poursuite de ces travaux.

II. Synthèse bibliographique

1. Impact de l'urbanisation sur la prolifération des moustiques en milieu urbain

Selon l'Organisation des Nations unies (ONU), les villes du monde entier s'agrandissent et se multiplient. Aujourd'hui, plus de la moitié de la population mondiale vit en ville (United Nations 2018). L'urbanisation est un phénomène se caractérisant par une concentration croissante de la population autour des agglomérations urbaines existantes. Les espaces urbains s'implantent sur des zones anciennement naturelles et entraînent la destruction d'habitats naturels tels que des forêts, des prairies, etc. La densification des villes induit des modifications sociologiques, économiques mais également écologiques. En effet, les villes sont les lieux où résident de nombreuses inégalités sociales comprenant plusieurs types : de richesse, de revenus, d'accès aux loisirs, d'accès aux services de santé ou encore de logement (Lawrence 2002). Les zones urbaines se caractérisent par une augmentation de la température plus importante par rapport aux zones rurales, un taux de pollution plus élevé, ainsi qu'une densité de population et d'infrastructures considérable (Vlahov 2002; Helbling and Meierrieks 2023).

Cette première partie décrit, au travers d'une revue bibliographique, l'impact de ces différents facteurs sur le cycle de vie, le comportement et la compétence vectorielle des moustiques. Pour cela, nous avons compilé les résultats de 76 études et mis en évidence l'influence exercée par le type d'infrastructure urbaine, la hausse des températures et la pollution urbaine sur le cycle de vie des moustiques, leur comportement et leur compétence vectorielle (aptitude intrinsèque du moustique à transmettre un pathogène). L'originalité de cette revue est qu'elle prend également en compte l'impact potentiel des activités humaines sur les communautés microbiennes associées au moustique. En effet, un nombre croissant d'études témoigne du rôle important du microbiote pour la physiologie, le développement mais aussi la capacité des moustiques à transmettre des pathogènes (Guégan et al. 2018; Zheng et al. 2023). Les communautés microbiennes étant principalement acquises de l'eau des gîtes larvaires ou du nectar de fleurs (Minard et al. 2013; Zouache et al. 2022), il nous semblait important d'inclure dans notre analyse la composante microbienne en étudiant notamment les répercussions possibles des activités humaines sur les communautés microbiennes colonisant les gîtes larvaires et le microbiote associé au moustique tout au long de son cycle de vie.

1.1 Publication n°1: “Impact of Human Activities on Disease-Spreading Mosquitoes in Urban Areas”

Cette revue a été publiée en avril 2023 dans *Journal of Urban Health*.



Impact of Human Activities on Disease-Spreading Mosquitoes in Urban Areas

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Accepted: 25 April 2023
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Abstract Urbanization is one of the leading global trends of the twenty-first century that has a significant impact on health. Among health challenges caused by urbanization, the relationship of urbanization between emergence and the spread of mosquito-borne infectious diseases (MBIDs) is a great public health concern. Urbanization processes encompass social, economic, and environmental changes that directly impact the biology of mosquito species. In particular, urbanized areas experience higher temperatures and pollution levels than outlying areas but also favor the development of infrastructures and objects that are favorable to mosquito development. All these modifications may influence mosquito life history traits and their ability to transmit diseases. This review aimed to summarize the impact of urbanization on mosquito spreading in urban areas and the risk associated with the emergence of MBIDs. Moreover, mosquitoes are considered as holobionts, as evidenced by numerous studies highlighting the role of mosquito–microbiota interactions in mosquito biology. Taking into account

this new paradigm, this review also represents an initial synthesis on how human-driven transformations impact microbial communities in larval habitats and further interfere with mosquito behavior and life cycle in urban areas.

Keywords Urban heat island · Pollution exposure · Mosquito-borne infectious diseases · Infrastructures · Microbiota · Urban health

Introduction

According to the World Health Organization, by 2050, more than 70% of the world's population will live in cities [1, 2]. Cities generate wealth but also concentrate poverty and inequality, as evidenced with the overcrowded slums in the developing world [3]. Meanwhile, anthropogenic activities are increasingly impacting life quality in cities. Through urbanization processes, urban areas encompass various modifications such as changes in population density, reinforcement of environmental stressors, and habitat modification and fragmentation [4–8]. Urban areas are also important contributors to global warming by generating carbon emissions, accounting for up to 76 % of global carbon dioxide (CO₂) emissions. Undoubtedly, rapid urbanization around the world will result in more public health issues within urban areas. This has led to the new concept of “urban health” defined

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as the study of urban environment characteristics that can affect health and diseases [9].

Out of the 3500 mosquito species worldwide, only a few are able to transmit pathogens that can cause diseases for both humans and animals. However, this results in nearly 700 million people that contract infections each year. Among them, Malaria remains the most serious mosquito-borne infectious disease (MBID) with an at-risk population of around 3.4 billion and 409,000 deaths recorded worldwide in 2019 [10]. Arboviruses such as dengue virus (DENV), Zika virus (ZIKV), and chikungunya virus (CHIKV) are transmitted to humans through the highly competent mosquitoes *Aedes aegypti* and *Aedes albopictus* [11]. Several species of *Culex* mosquitoes are also involved in the transmission of pathogens such as West Nile virus (WNV), Japanese encephalitis virus (JEV), and St. Louis encephalitis virus (SLEV) as well as filarial nematodes that cause diseases in birds, humans, and other animals [12]. Notably, transmission of mosquito-borne infectious diseases has increased in urban areas in recent decades [13, 14]. Even though they are native from natural areas with little human-driven landscape transformation, many mosquito vector species had the ability to be introduced into urban areas [15]. Ecological disturbances produced by anthropogenic landscape transformation on vector mosquito species, growing urban population, migration of native populations at risk, poor waste management, or improper water management have greatly favored the spread of mosquito species vectors in urban areas causing health and economic issues [16–19]. In addition, urbanization processes create ideal habitats for many mosquito species that ultimately increase the risk associated with the occurrence of MBIDs [20]. This is particularly the case for the highly invasive and anthropophilic Asian tiger mosquito, *Ae. albopictus*, which prefers very dense urban areas [21, 22]. Interestingly, a recent study showed that the more urbanized a given area the fewer mosquito species were found, and those were primary vectors of arboviruses, *Ae. aegypti* and *Cx. quinquefasciatus* [23]. It seems that the genus *Anopheles* would be correlated with low levels of urbanization, while the genera *Culex*, *Culiseta*, and *Aedes* would be correlated with moderate or high urbanization levels [23–27]. More specifically, specific factors such as high population

density, urban pollution, habitat modification, and temperature increase strongly influence interactions between mosquito vectors and human population but also select mosquito species that are more adapted and favor the diversification and/or the development of artificial mosquito habitats [28–33].

In this context, the aim of this review is to summarize the impact of urbanization on mosquito spreading in urban areas and thus the risk of development and emergence of MBIDs. In particular, this study emphasizes the following issues: (i) the influence of certain types of infrastructures and objects in urban areas on mosquito life cycle; (ii) the impact of temperature increase on mosquito life history traits, behavior and vector competence; and (iii) the impact of urban pollution on mosquito bio-ecology. Because of the importance of the microbiota in mosquito biology, this review also aims to assess consequences of urbanization on mosquito-associated microbiota and its contribution to mosquito spreading in urban areas.

Methods

Searches were conducted on Google Scholar from February to March 2023. Briefly, we searched for empirical research from reviewed or peer-reviewed articles with no time restrictions that investigated mosquitoes in relation to any urbanization process. After a previous screen, we decided to focus on specific search terms in line to urban mosquito, temperature, fertilizer, pesticide, bisphenol, microplastic, heavy metal, glyphosate, and light pollution. We limited our search to terms appearing in the title of articles only. We also consulted forward/backward citation tracking of the included articles to select other appropriate articles including those related to mosquito microbiota and urban contaminants. Duplicate studies were removed, and the remaining studies were then screened for inclusion. Articles were included if the research topic involved laboratory experiments or field experiments performed in urban areas only. We excluded studies dealing with predictive models, urban biodiversity, mosquito-borne diseases, and mosquito control only that are not in the scope of our review and our primary objectives. We also excluded studies such as commentaries and studies written in languages other than English.

Results

The PRISMA flowchart depicts the results of our review process (Fig. 1). Our search yielded a total of 670 studies. After title/abstract review and remove of duplicates, we found 76 studies that matched our inclusion criteria.

Impact of Infrastructures and Objects on the Abundance and Diversity of Urban Mosquitoes

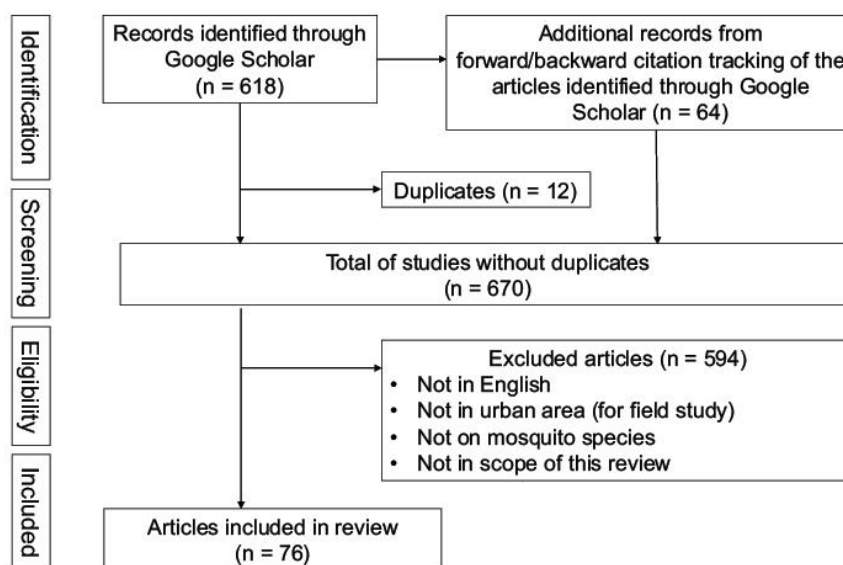
A great variety of infrastructures and objects exist in the urban space and often hosts artificial sites that are favorable to mosquito development and breeding. Some of them such as buildings and construction sites create artificial recipients suitable for mosquito habitats [34]. Artificial breeding sites are often related to small-volume containers of standing water, which are more colonized than larger ones [35]. This has led anthropophilic mosquito species such as *Ae. aegypti* and *Ae. albopictus* to become better adapted to artificial habitats than natural habitats [36]. A great diversity of *Aedes* manmade container habitats, in which garden water and/or rainwater is naturally accumulated, was identified as the most productive mosquito aquatic habitats in many residential areas of metropolitan areas worldwide (i.e., buckets, ornamental bromeliads and flower pots, drains, discarded receptacles, ornamental containers, gully traps, canvas/plastic sheet,

and gutters) [34, 37, 38]. They are widely present in urban areas and difficult to manage in order to limit mosquito proliferation. Previous studies also showed that *Ae. aegypti* and *Cx. quinquefasciatus* develop in high numbers in construction sites frequently equipped with elevator shafts, jersey plastic barriers, flooded floors, and stair shafts and are almost exclusively the only species present [39, 40]. Moreover, *Ae. albopictus*, *Ae. aegypti*, and *Cx. quinquefasciatus* were shown to successfully colonize water habitats in the cemeteries [36].

Meanwhile, the integration of urban green infrastructure is increasing in cities. Urban vegetation provides undeniable benefits to urban climate, health, thermal comfort, and environmental quality of cities [41]. However, management of standing water is difficult in urban green infrastructure and these areas or installations represent potential habitats and breeding sites for mosquitoes [42]. One of the most striking examples of the impact of urban greening on mosquitoes is illustrated by the excessive mosquito proliferation on vegetated exterior facades of buildings described as a “vertical forest” in Chengdu, capital of the Sichuan province in western China. This uncontrolled phenomenon led the inhabitants to leave their apartments (unpublished data).

While many studies evidenced the diversity of artificial breeding sites in urban areas, studies focused on urban infrastructures and objects in the

Fig. 1 PRISMA chart for the identification and selection of articles



development of mosquito habitats are still scarce, and more studies would be necessary to integrate the emergence of MBIDs as a risk factor in subsequent urban planning.

Impact of Temperature Increase on Mosquito Life Cycle

It is a well-established fact that urban areas are associated with increased surface temperatures, also called urban heat islands (UHI) [43, 44], and the ongoing climate change seems to reinforce this phenomena [45]. Cities are often 2 to 3 °C hotter than their rural surroundings [46]. However, mosquitoes are poikilotherms, i.e., the body temperature corresponds closely to the external temperature. Therefore, the temperature strongly influences their physiological functions as well as their ecology and dispersion [47, 48]. This means that increases in temperature are expected to have major impacts on geographical dispersion, life history traits, behavior, and vectorial capacity of urban mosquitoes (Table 1).

Temperature and Life History Traits

Sensitivity of mosquitoes to temperature differs from species to species [49, 50] and has differential consequences in term of performance of larval and adult stages. Temperature of larval breeding sites is mainly influenced by the ambient temperature which itself depends on weather variations [51]. To a lesser extent, other factors such as types of containers (geometry, matter, or colors), biotic composition, turbidity, and type of land covers can also modulate the water temperature of larval habitats [51–54]. Previous studies reported that the average optimal water temperature of larval habitats is around 25–30°C [55–58]. However, many studies conducted in laboratory conditions highlighted differential impacts of the water temperature on the duration of larval development according to mosquito species. Ciota et al. (2014) found that larval development time decreased with rise in water temperature for *Culex* species, ranging from 29 days at 16°C to 9 days at 28°C [59]. Similar observations were reported for *An. gambiae* and *Ae. albopictus* mosquitoes [56, 60, 61] even though some variations occur along development time. For example, raise in water temperature increased the developmental rates of first instar larvae and pupae of *An. gambiae* but

decreased the developmental rates of fourth instar larvae [61]. Interestingly, some authors observed carry-over effects and significant interactions between larval density and temperature in *Ae. albopictus* [62, 63]. The smallest females took the longest to develop in the highest temperature, and this effect was accentuated with density. As for immature stages, many studies evaluated the correlation between increasing temperature and longevity of adult mosquitoes, with contrasted effects depending on the mosquito species [64]. For instance, *Ae. albopictus* and *Culex* sp. live longer at 15°C compared to 30°C [59]. *Ae. aegypti* optimal air temperature is between 24 and 25°C compared to 20°C for *Ae. vexans* and *Cx. pipiens* [65]. Regarding survival rates, higher temperature significantly increases mosquito larval and adult mortality [59, 61, 66–68]. For instance, Murdock et al. (2017) conducted a field experiment and demonstrated a significant decrease in survival of *Ae. albopictus* larvae in urban areas characterized by warmer microclimate compared to suburban and rural [69]. Similarly, the development rate and survival of *An. stephensi* larvae were significantly reduced at higher temperature [49, 66].

To our knowledge, only two studies have investigated the impact of temperature increase on microbial communities associated with larval habitats and mosquitoes. It was shown that higher temperatures led to a reduction of *Wolbachia* abundance in *Cx. pipiens/restuans* mosquitoes, which in turn, resulted in higher susceptibility to WNV in the subsequent mosquito generations [70]. In addition, increase in temperature of 2 °C for *Ae. aegypti* in the field was shown to favor specific bacterial taxa in the gut microbiota that could alter mosquito fitness, adaptation, and vector–pathogen interactions [71, 72]. Another study showed that microbial richness increased with warmer temperatures (30–32°C) [73].

Altogether, these results tend to show that temperature increase reduces the time duration of larval development as well as larval and adult survival rates. Even though warmer temperature would accelerate the larval cycle, it would also increase mortality of larvae and adults leading to counterbalancing effects on mosquito proliferation. Mortality increase could be explained by an acceleration of diverse metabolic processes or lead to the overproduction of reactive oxygen species that in turn could impact key metabolic pathways.

Table 1 Influence of temperature on mosquitoes and mosquito-borne infectious disease risks

Conditions	Mosquito genus	Mosquito species	Temperatures	Results	References
Field	<i>Aedes</i>	<i>Ae. albopictus</i>	15.6, 26°C	Decreased larval and adult mortality at the highest temperature Decreased adult body size at the highest temperature Decreased growth rate at the highest temperature	[69] Murdock et al., 2017
	<i>Aedes/Culex/Anopheles</i>	<i>Ae. aegypti/Ae. albopictus/Ae. vexans/Cx. quinquefasciatus/Cx. pipiens</i>	15, 20, 25°C	<i>Ae. aegypti</i> and <i>Ae. albopictus</i> optimal air temperatures is around 25°C compared to 20°C for <i>Ae. Vexans</i> , <i>Cx. quinquefasciatus</i> and <i>Cx. Piptens</i>	[65] Blagrove et al., 2017
	<i>Culex/Mansonia/Ochlerotatus/Psorophora/Aedes/Ura notenia</i>	23 species	[4–10.9°C], [11–18.9°C], [19–25.9°C], [26–32.9°C]	Differential effects of temperature increase on flight activity according to mosquito species	[78] Freire et Schweigmann, 2009
	<i>Culex</i>	<i>Cx. pipiens</i>	Field temperatures	Higher density of larvae at 24.82°C Higher fecundity rate at 24.82°C Optimal temperature for mosquito development is 21.66–22.22 °C	[63] Nagy et al., 2022
		<i>Cx. pipiens/Cx. restuans</i>	Field temperatures	<i>Wolbachia</i> abundance decreases with higher temperature	[70] Novakova et al., 2017
Laboratory	<i>Aedes</i>	<i>Ae. aegypti</i>	10, 15, 18, 21, 27, 32, 35°C	The optimal temperature for flight was 21°C Flight performance was greater below 27°C	[76] Rowley et Graham, 1968
			Prediction based on peer-reviewed literature	Dengue epidemic potential was higher at 29.3°C Biting rates increased linearly with temperature	[81] Liu-Helmersson et al., 2014

Table 1 (continued)

Conditions	Mosquito genus	Mosquito species	Temperatures	Results	References
			25, 30, and 35 °C	The fecundity is reduced as temperature increased Egg production, oviposition time, and patterns are reduced as temperature increased	[83] Costa et al., 2010
			8, 21, 26, and 30°C	Higher transmission competence of CHIKV at the highest temperature	[92] Winokur et al., 2020
	<i>Ae. albopictus</i>		18, 23, 28, and 32°C (constant) and 28, 23, 18°C (fluctuating) 18, 28°C	Higher transmission competence of DENV at the highest temperature Higher transmission competence of CHIKV at the highest temperature	[89] Liu et al., 2017
			20, 28°C	Higher transmission competence of CHIKV at the highest temperature	[94] Zouache et al., 2014
			20, 28°C	Increased competence to transmit DENV at the highest temperature nor CHIKV	[95] Mercier et al., 2022
			5, 10, 15, 20, 25, 30, 35, 40°C	The optimal temperature for larval development was 29.7°C The shortest gonotrophic cycle was 30°C	[60] Delatte et al., 2009
			21, 27, 34 °C	The highest temperature decreased larval development time The highest temperature increased larval and adult mortality	[62] Ezeakacha et Yee, 2019

Table 1 (continued)

Conditions	Mosquito genus	Mosquito species	Temperatures	Results	References
	<i>Anopheles</i>	<i>An. dirus</i> / <i>An. sawadwongporni</i>	23, 30°C	Larger body size at 23°C Decreased development time at the highest temperature No temperature effect on sex ratio and egg hatching rate	[75] Phasomkusolsil et al., 2011
		<i>An. gambiae</i>	Temperature increase by 2°C from 10 to 40°C	Decreased larval development time as temperature increased (optimal temperature 28 to 32°C) Optimal adult development between 28 and 32°C Higher adult emergence between 22 and 26°C No adult emergence below 18°C or above 34°C	[55] Bayoh et Lindsay, 2003
			23, 27, 31, 35 °C	Decreased larval development time as temperature increased Increased larval and adult mortality as temperature increased Highest number of eggs laid at 27°C and lowest at 31°C No influence on blood meal as temperature increased	[61] Christiansen-Jucht et al., 2015
		<i>An. pseudopunctipennis</i>	15, 20, 22, 25, 27, 30, 33, 35, 37°C	Increased mosquito mortality as temperature increased Decreased gonotrophic cycle time as temperature increased	[85] Lardeux et al., 2008
		<i>An. stephensi</i>	18, 26, 32°C	Increased pre-blood meal period at lower temperature	[66] Paaijmans et al., 2013

Table 1 (continued)

Conditions	Mosquito genus	Mosquito species	Temperatures	Results	References
			21, 24, 27, 30, 32, 34°C	Increased larval and adult mortality as temperature increased Increased biting rates as temperature increased Decreased gonotrophic cycle time as temperature increased The optimal temperature for malaria transmission was 29°C	[68] Shapiro et al., 2017
			Prediction based on peer-reviewed literature	Malaria transmission decreased between 25 and 28°C	[93] Mordecai et al., 2013
		NA	16, 20, 24, 28, 32°C	Decreased larval development time from 29 days to 9 days as temperature increased (16°C to 28°C) Decreased longevity as temperature increased Increased larval and adult mortality as temperature increased Increased blood feeding as temperature increased Reduced the fecundity as temperature increased	[59] Ciota et al., 2014
	<i>Culex</i>	<i>Cx. pipiens</i>	16, 20, 24, 27, 31, 35°C	Decreased larval development time as temperature increased Increased larval and adult mortality as temperature increased	[67] Ruybal et al., 2016
			7, 10, 15, 20, 25, 30, 33°C	The highest survival was found at 25°C Wing length decreased as temperature increased	[58] Loetti et al., 2011
			18, 20, 26, 30°C	Higher transmission competence of CHIKV at 30°C	[90] Dohm et al., 2002

Table 1 (continued)

Conditions	Mosquito genus	Mosquito species	Temperatures	Results	References
		<i>Cx. quinquefasciatus</i>	35, 37, 38, 39, 40, 41 °C	Increased insecticide resistance as temperature increased	[100] Swain et al., 2009
	<i>Culiseta</i>	<i>C. incidens</i>	20, 25, 30°C	The higher biting rates are observed at 20°C The peak blood feeding is observed at 25°C	[82] Su et Mulla, 2001

Temperature and Mosquito Behavior

Temperature also influences different traits of mosquito behavior including flight activity and biting rates as well as reproduction. Wing length is used as a proxy for the mosquito's body size and is positively correlated with flight activity and dispersion [74]. Many studies showed that increasing temperatures reduce body size [49, 57, 58, 74]. For instance, *An. dirus* and *An. sawadwongporni* exhibited higher body size when rearing at 23°C compared to 30°C [75]. In *Ae. aegypti*, both males and females exhibit increased flight activity at 30°C compared to 20°C [76, 77]. Freire et al. (2009) characterized the flight activity of different mosquito species in relation to the average daily temperature in Buenos Aires (Argentina) and identified two groups [78]. The first one consisted of four species (*Cx. eduardoi*, *Cx. chidesteri*, *Cx. pipiens*, *Oc. albifasciatus*) that were active throughout the year in all thermal range (4–30.8°C), while the second group (*Ae. aegypti*, *Ochlerotatus crinifer*, and *Cx. renatoi*) displayed a seasonal activity pattern with no activity below 11°C. It was previously shown that the seasonal component may affect whole-organism metabolic rate which explains why some mosquito species reduce their metabolic rate to allow them to survive during the dry or cold season [79]. As temperature increases, the rate of metabolism increases and then rapidly declines at higher temperature [49]. Interestingly, higher metabolic rates were also shown to increase mosquito flight duration [80]. In addition, temperature may also have a significant effect on mosquito biting rates. It was shown that the biting rates of laboratory-reared *An. stephensi* and *Ae. aegypti* almost doubled and increased by 25% with increasing temperature from 21 to 32°C, respectively [68, 81]. Blood feeding habits also include the duration of the blood meal. For instance, it was shown that *Aedes* sp. females fed faster between 26 and 35°C than at lower temperature [77]. Su et al. (2001) found that *Culiseta incidens* exhibited peak blood feeding at 25°C [82]. In *Cx. pipiens*, the cumulative feeding rates were significantly higher at 28°C and 32°C compared to lower temperatures [59]. On the opposite, many studies highlighted the negative impact of increasing temperatures on mosquito reproductive traits. In *Ae. aegypti*, Costa et al. (2010) demonstrated a reduction of egg production and oviposition time as well as changing in oviposition patterns with increased temperatures

[83]. Similarly, the fecundity, i.e., numbers of eggs, of other mosquito species such as *Ae. krombeini*, *Cx. pipiens*, *Cx. quinquefasciatus*, *An. dirus*, *An. stephensi*, and *An. sawadwongporni* was reduced with increasing temperatures [59, 61, 63, 64, 75]. However, a positive impact on fecundity was observed in *An. arabiensis* [84]. Regarding the gonotrophic cycle (i.e., blood feeding, egg maturation, and oviposition, which are repeated several times throughout adult life), many studies demonstrated that it was shorter at higher temperatures [49, 60, 68, 85, 86]. For instance, Shapiro et al. (2017) found that the average length of the gonotrophic cycle of *An. stephensi* was 5 days at 21°C, compared to approximately 3 days at 34°C [68]. In addition, *An. pseudopunctipennis* may lay eggs during 2–3 consecutive nights at high temperature (35°C) and > 10 at low temperature (12°C) [85].

Altogether, these results are in favor on a positive impact of temperature increase on mosquito flight activity, biting rates, and blood feeding habits, thus increasing both the probability of encountering more vertebrate hosts and thus pathogen transmission. However, regarding mosquito oviposition behavior, temperature increase would tend to inhibit reproduction.

Temperature and Vectorial Capacity

Temperature is one of the most significant abiotic factors affecting major components of vectorial capacity, i.e., opportunities for acquisition and transmission of pathogens [87]. Vectorial capacity is partly shaped by mosquito life history traits, mosquito density, and biting rate. As previously described, all these factors can be affected by temperature increase and can thus have major impacts on the vectorial capacity. Vector competence is another important component of vectorial capacity and is defined as the ability of a mosquito species to infect, replicate, and transmit a pathogen to a subsequent host. Moreover, mosquito lifespan and vector competence are strongly linked to the extrinsic incubation period (EIP), i.e., the time it takes for a pathogen to develop within a mosquito, and become transmissible. Previous studies showed that temperature affects both the average and the variability of the duration of EIP [88]. For instance, higher temperatures lead to accelerated DENV replication and induce shorter extrinsic incubation in *Ae. albopictus* [89]. Similar observations were made for other arboviruses such as

ZIKV, CHIKV, or WNV [57], suggesting that reduced extrinsic incubation period promotes the risk of MBID emergence [90–92]. Conversely, opposite effects were also observed. For instance, Mordecai et al. (2013) demonstrated that the optimum temperature for malaria transmission is 25°C and declined above 28°C [93]. Zouache et al. (2014) showed that *Ae. albopictus* from Brazil exhibited higher CHIKV transmission at 28°C compared to 20°C (85% vs 37.5%), whereas no differences were observed for *Ae. albopictus* from Italia or La Reunion [94]. Similarly, Mercier et al. (2022) found that *Ae. albopictus* could transmit CHIKV either at 20°C or 28°C, while DENV transmission only occurs at 28°C [95]. These results highlight the importance to consider genotype-by-genotype-by-environment interactions (mosquito population × virus strain × temperature) to better predict the impact of temperature increase on vector competence and more broadly vectorial capacity. Moreover, it is important to consider that the temperature has also a direct impact on vector-borne pathogens and could modify their virulence (reviewed by [91–93]).

Following previous observations, integrated studies are needed to evaluate as a whole the impact of UHI and temperature increase on mosquito physiology and behavior and which consequences these complex interactions may have on the emergence risk of MBIDs in urban areas. In addition, the duration of the mosquito gonotrophic cycle is a proxy of mosquito density and frequency of host–mosquito contacts [99]. It means that a decrease of this duration time would increase daily biting frequency and thus increase vectorial capacity. Some studies also highlight that temperature increase could also modify mosquito response to insecticide exposure. It was shown that exposition to higher temperature promote insecticide resistance in *Cx. quinquefasciatus* [100]. Conversely, malathion exposure combined with high temperature induced higher thermo-tolerance and survival to adulthood of *Cx. pipiens* and *Ae. albopictus* [101]. More globally, studies established that where urban heat islands (UHI) exist, most likely urban pollution islands coexist (UPI) [102, 103].

Impact of Human Activity–Induced Pollution on Urban Mosquito Spreading

Urban pollution includes diverse sources (atmospheric, agricultural, wastewater, and light) that can

affect mosquito physiology throughout its life cycle (Fig. 2) [104–108]. Notably, ubiquitous environmental pollutants in urban water bodies include polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), heavy metals (Fe, Pb, Cd, Hg...), agricultural chemicals (fertilizers), or human drugs [109–111].

Urban Gardening– and Urban Agricultural–Induced Pollution

Urban gardening is a well-established phenomenon, whereas urban agriculture (or urban farming) has been developing in many countries in the past 10 years. According to the Food and Agriculture Organization (FAO), urban agriculture is defined as the growing of crops and raising of animals in and around cities. It generally includes the spaces cultivated by farmers who distribute the fruits of the land they work in the environs of the zone of production, as well as urban farming in private homes and in

public spaces such as community gardens [105, 112]. Those spaces create a multitude of artificial containers that frequently serve as larval development sites for mosquito reproduction and also become a pollution source through fertilizer and pesticide applications [113, 114]. Previous studies showed that these compounds could influence mosquito fitness, survival, fecundity, and oviposition site selection. Laboratory studies showed that water containing fertilizers attracted significantly more gravid females than water alone [115–118]. Both mosquito species *An. Arabiensis* and *Cx. Quinquefasciatus* had preference to oviposit in nitrogenous fertilizer treatments (diammonium phosphate or ammonium sulfate) relative to pesticide treatments (cypermethrin or glyphosate) or control [118]. Interestingly, *Ae. albopictus* females feeding on a sugar-supplemented substrate contaminated by nitrogen, phosphorous, and potassium-based (NPK) fertilizer laid significantly more eggs compared to sweetened solutions only. Some studies also demonstrated that *Cx. quinquefasciatus* larvae reared

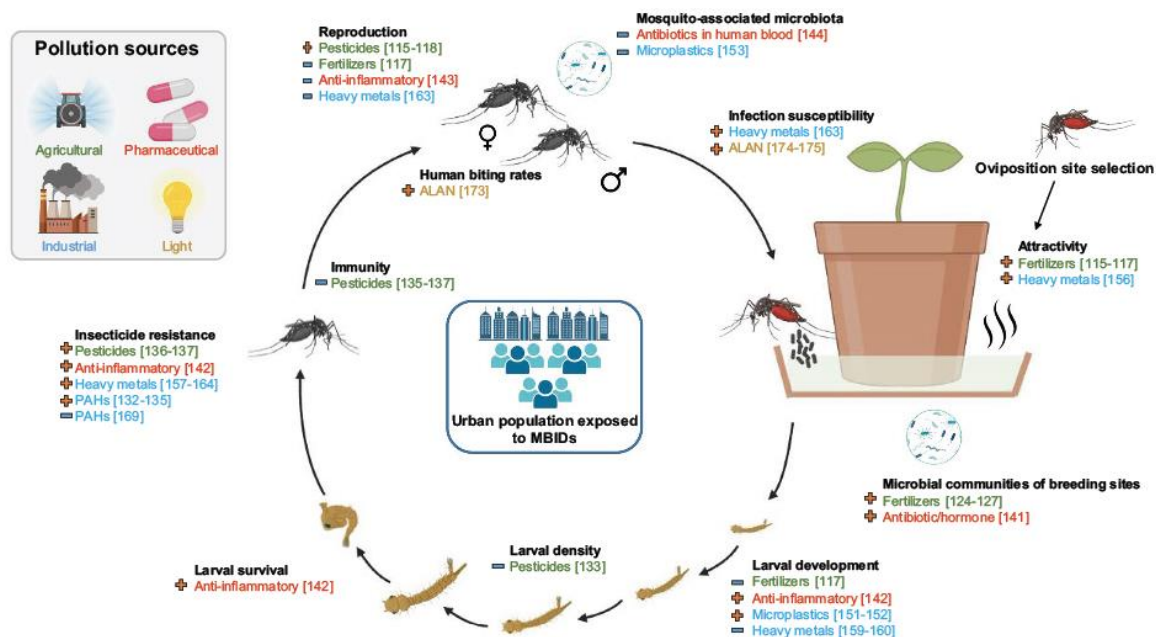


Fig. 2 Influence of urban pollutants on mosquito spreading. Urban pollution includes diverse sources (pharmaceutical, agricultural, industrial, and light) that can affect mosquito physiology throughout its life cycle. Agricultural pollution includes fertilizers such as nitrogen-, phosphorous-, and potassium-based (NPK) fertilizers and pesticides. Pharmaceuticals include anti-inflammatory drugs such as ibuprofen and aspirin

as well as antibiotics. Industrial activities can induce heavy metal (in particular Cu, Fe, Pb, Cd, Hg, Cr, Tl, Ni), microplastics, and polycyclic aromatic hydrocarbons' (PAHs) pollution. Light pollution induces an extensive expansion of artificial light at night (ALAN). The plus sign represents a positive effect, and the minus sign indicates a negative effect. Created by Biorender.com

in water supplemented with NPK fertilizers developed faster compared to larvae reared in water only, i.e., $8 (\pm 2)$ days to reach the pupae vs $10 (\pm 3)$ days [54]. On the opposite, Kibuthu et al. (2016) demonstrated that larval development time of *An. arabiensis* and *Cx. Quinquefasciatus* mosquito species was longer with agrochemical treatments (diammonium phosphate and ammonium sulfate) than control [118]. Although the nitrogen, phosphorus, and potassium supplied by the fertilizer are not directly assimilated by mosquito larvae, it seemed that water enriched with fertilizers would be more attractive for gravid females and favor microbial growth. Interestingly, previous studies highlighted a critical role of environmental microorganisms in the attractiveness of breeding sites for gravid females through the production of volatile organic compounds (VOCs) [119–123]. Fertilizers could thus have a direct impact on larval habitat composition that in turn could have broad implications on water colonization by gravid females [124, 125]. Muturi et al. (2016) showed that fertilizer treatments (ammonium sulfate and potassium chloride) altered bacterial diversity and community structure in *Ae. albopictus* breeding sites [126]. In outdoor experimental mesocosms, Duguma et al. (2017) showed that *Cx. nigripalpus* larval environments supplemented with low- and high-organic-nutrient regimens were preferentially associated with *Clostridiales* and *Burkholderiales*, respectively [127]. All together, these studies seem to show that gravid females deposited offspring in locations that maximize larval survival and growth rate, suggesting that the presence of fertilizers in water would contribute to guide female choice. However, how changes in microbial composition following fertilizer exposure impact oviposition site selection are still an open question and remain to investigate.

Pesticides are also widely used in urban agriculture to control pests and disease infestations. Glyphosate is the most widely used herbicide for urban weed control, and previous studies have already evaluated its impact on mosquitoes [128]. After application, glyphosate and its major metabolite aminomethylphosphonic acid (AMPA) can persist in the soil for weeks, or derivatives can easily be transported by wind or rain into aquatic environments [129]. Glyphosate exposure was shown to influence many important biological traits in mosquitoes such as reproduction, survival rates, development time, and

wing size [118, 130–134]. For instance, Kibuthu et al. (2016) demonstrated that *Cx. quinquefasciatus* and *An. arabiensis* females laid significantly less eggs after glyphosate exposure compared to control [118]. Glyphosate exposure also induced an approximately 2-day increase in larval development time of *Cx. quinquefasciatus*. In addition, glyphosate application in breeding sites instantly decreases the larval density [133]. More recently, it was even shown that glyphosate alters mosquito survival and a mechanism involving melanin inhibition that in turn render mosquitoes more susceptible to parasite infection [135]. Furthermore, one study showed that glyphosate exposure induces higher resistance to insecticides by increasing the expression of detoxification genes such as glutathione S transferase activity [134]. Similarly, exposure to the herbicide atrazine induced increased *Ae. aegypti* larvae tolerance to the organophosphate insecticides temephos and permethrin [136, 137].

To date, we have identified only one study that has investigated the impact of herbicides on mosquito microbiota. Glyphosate was shown to alter the composition of the *A. gambiae* midgut microbiota with a decrease in the relative abundance of *Enterobacteriaceae* and an increase in relative *Asaia* sp. populations [135].

Urban Pharmaceutical-Induced Pollution

Among pharmaceuticals, many compounds are commonly used, and their annual consumption has increased over the past decades [138]. The most used pharmaceuticals are analgesics and antibiotics. In the urban water cycle, hospital wastewater has been identified as a problematic point source due to high concentrations of pharmaceutical contaminants. Most of them cannot be removed and filtered through treatment processes leading to the presence of low concentrations in tap water [139]. Several urban breeding sites are composed of tap water only, or a mix with rainwater, which expose urban mosquitoes to low concentrations of pharmaceutical contaminants [140–143]. Previous studies, under laboratory conditions, showed that human drugs such as ibuprofen and aspirin could influence mosquito biology. Ibuprofen, a commonly used anti-inflammatory, was shown to accelerate *Ae. aegypti* larval development at environmental concentration [142]. When *Ae. aegypti* was exposed, this species had better larval survival and

population growth rates compared to non-exposed generations [142]. Moreover, multigenerational exposure to this drug induced an emergence of tolerance to the bio-insecticide, *Bacillus thuringiensis israelensis* (*Bti*). Molecular mechanisms of mosquito ibuprofen tolerance are still unknown but could be explained either by a multi-generation drug acclimation at low concentrations or the selection of a physiological status allowing tolerance in mosquito population [142]. Aspirin, a nonsteroidal anti-inflammatory drug, is one of the most abundant pharmaceutical found in urban wastewater samples [140]. Aspirin exposure showed an inhibition of mosquito prostaglandin synthesis and an alteration of mosquito reproduction in *Ae. albopictus* [143].

Regarding microbiota, Pennington et al. (2016) evaluated the impact of common pharmaceutical and personal care products (PPCPs) found in wastewater on larval microbiota of *Cx. quinquefasciatus*. They showed that mixture treatments composed of antibiotic and hormone treatments with acetaminophen and caffeine had a greater impact on bacterial species richness and evenness compared to antibiotic treatments alone. The authors suggest a possible impact of other contaminants on facilitating growth of different bacteria. In addition, one study showed that antibiotics in ingested human blood enhance the susceptibility of *An. gambiae* mosquitoes to malaria infection by disturbing their gut microbiota [144].

Due to their increasing presence in urban aquatic habitats, further studies are needed to investigate the impact of pharmaceutical contaminants on mosquito physiology and especially in interaction with other abiotic factors such as temperature. Notably, studies dealing with contraceptive, painkillers, and antidepressants would be of interest given their high prevalence in urban wastewater and effluents [140].

Urban Industrial-Induced Pollution

Microplastics (MPs), heavy metals pollution, and polycyclic aromatic hydrocarbons (PAHs) are prevalent in urban environments. Even though MP contamination is increasing worldwide, knowledge on the sources, fate, and transfer in urban areas remain poorly known [145–147]. Bisphenol A (BPA) is one of the most common chemicals produced by the plastics industry. It is used in combination with other chemicals in manufacturing of various plastics and

resins [148]. Some studies examined the impact of BPA on aquatic invertebrates and found estrogenic activity and other potential disruptions of estrogenic activity for some invertebrates [149]. To our knowledge, only four studies analyzed the effect of BPA or its derivatives on mosquitoes [142, 150–152]. For example, at environmental concentration, these compounds were showed to accelerate the development of *Culex* sp. nor *Aedes* sp. [142, 151, 152]. Gayathri et al. (2021) revealed that eggs deposited in water containing 1–4 ppm BPA hatch in 4 h less time compare to water without BPA. BPA seems also to accelerate mosquito embryonic development [151]. Increased plastic waste-derived BPA concentration (from 1 to 4 mg/L) in breeding sites of *Cx. quinquefasciatus* was shown to decrease the time to emerge as adults from 10 days to 8.5 days [152]. A recent study revealed that plastic waste-derived BPA is a developmental agonist of *Cx. quinquefasciatus* [152]. Conversely, Prud'homme et al. (2017) showed that short- or long-term BPA exposure had no influence on the duration of *Ae. aegypti* larval development [142]. Dose–response effect was observed on mosquito survival with a negative impact at high concentrations. However, Rachel et al. (2011) found no effect of different BPA concentrations on sex ratios, time to adulthood, and mass of *Ae. albopictus* and *Ae. aegypti*. [150].

Regarding their impact on microbiota, Edwards et al. (2023) demonstrated that MP contamination altered the bacterial and fungal composition of *Ae. aegypti* and *Ae. albopictus*, with an increase of *Elizabethkingia* density. Interestingly, such an impact was shown to have potential negative impacts on infection rate of ZIKV in *Ae. albopictus* [153].

Heavy metals are another kind of pollutants widely spread in urban areas. This pollution is mainly due to industrial activities such as foundries and leaching of metals from different sources (automobiles, runoffs, landfills...) [154]. Previous studies showed that heavy metals such as copper (Cu), ferrum (Fe), and lead (Pb) were detected in mosquito larval urban habitats [155, 156]. Arguably, mosquitoes are adapted to a wide range of heavy metals present in breeding sites, and these metals could be attractive to some mosquito species [155–157]. Mireji et al. (2008) demonstrated that copper was positively correlated with the presence of *Ae. aegypti*, *An. gambiae*, and *Ae. aegypti* [156]. Two studies have also shown that

An. gambiae could breed in water polluted with heavy metals and seemed to be well adapted to a wide range of heavy metal pollution [155, 157, 158]. Jeanrenaud et al. (2020) showed that a single exposure to metal pollution (cadmium chloride, copper nitrate, or lead nitrate) could impair mosquito life history traits and induce transgenerational effects [157]. For instance, cadmium (Cd) and mercury (Hg) exposure of *Ae. aegypti* and *An. gambiae* was shown to affect gene expression (associated with metabolism, transport, and protein synthesis) and consequently mosquito physiology [159, 160]. On the contrary, Akthar et al. (2021) demonstrated that consecutive exposure to Hg caused bioaccumulation in *Ae. aegypti* larvae associated with higher mortality and toxicity as well as for their aquatic predator, *Tramea cophysa* [160]. Heavy metals (Hg, Pb, Cr, Cd, Tl, and Ni) were also detected in adult mosquitoes at lower levels than in larvae that can reflect that adults mosquitoes are less exposed to heavy metals at imago stages or less accumulated metals at this stage [160–162]. Interestingly, Maya-Maldonado et al. (2021) evaluated the effects of iron and copper chelation treatment of adult mosquitoes on *Plasmodium berghei* infectivity and mosquito reproduction [163]. They showed that feeding mosquitoes with iron and copper chelators before and after an infectious blood meal protected the mosquito from parasite infection and affected follicular development in the case of iron chelation.

Interestingly, a few examples also demonstrated that exposure to anthropogenic stressors may impact tolerance or resistance to insecticide [157, 164]. PAHs are also widespread air pollutants and are systematically present in urban environments [165, 166]. These pollutants are able to induce metabolic changes on vertebrates and some invertebrates in aquatic environments [167, 168]. To date, no study has yet evaluated their effect on mosquito life history traits, but some studies revealed a modification of insecticide resistance in presence of PAHs. For instance, *Ae. aegypti* larval exposure to the PAHs, benzo[a]pyrene or fluoranthene, leads to an increase of tolerance to the carbamate insecticide propoxur [126, 129]. However, nitropolycyclic aromatic hydrocarbons (nitro-PAHs) which are derivatives of PAHs showed opposite effects in the mosquito species *Cx. quinquefasciatus* with an increase of larval susceptibility to chemical insecticides, i.e., permethrin, imidacloprid, and temephos [169].

Altogether, these observations tend to show that mosquitoes are becoming more tolerant to a wide range of pollutants and thus well adapted to urban areas. This could lead in some cases to increase mosquito tolerance to insecticides. To date, most studies on the impact of microplastics and heavy metals on mosquitoes focus on specific and single molecules. Further studies are needed in order to better understand the complexity of the composition of pollutants in natural breeding sites. Future investigations would also need to test the effect of pollutant mixtures on the mosquito life history traits and its microbiota. Another crucial issue that needs to be dealt with is whether pollutant resistant or tolerant mosquitoes are more likely to transmit vector-borne pathogens than pollutant susceptible mosquitoes.

Impact of Light Pollution

Urbanization and population growth cause an extensive expansion of artificial light at night (ALAN) (i.e., “light pollution”) in terms of both density and spatiotemporal distribution in urban areas [106]. Urban light pollution affects the ecology, behavior, and physiology of plants and animals and is responsible for decline in insect populations [170]. Rund et al. (2020) demonstrated that artificial light at night modify *Ae. aegypti* behavior through increased diurnal biting rates [171]. Conversely, night light suppressed nocturnal biting behavior of nocturnal species such as *An. gambiae* [172]. Fyie et al. (2021) suggested that a high light pollution during the night drives mosquitoes to be actively reproducing and biting later in the season [173]. In addition, recent studies on house sparrow (*Passer domesticus*), an amplifying host for West Nile virus (WNV) and other arboviruses, showed that urban light increased host infectious-to-vector period of 2 days and the risk for WNV outbreak [174, 175]. Interestingly, a very recent review also analyzed how artificial light modulated MBID risks [176]. The authors showed that ALAN alters mosquito biology and could thus have an impact of disease risks.

These observations, i.e., longer reproductive period, increased biting activity, and increased contacts between host reservoir and mosquitoes, are seem to establish that there is a positive impact of light pollution on pathogen transmission risk. Altogether, these results argue the importance to carefully

investigate the impact of urban light on mosquitoes and mosquito-borne diseases.

Conclusions

This review highlights important impacts of urbanization on mosquitoes and their associated risks. Urban mosaic brings ecological and build components that create ideal habitats for many mosquito species. In particular, UHI and UPI can interact with each other, and positively impact mosquito proliferation. In the future, the worlds of urban planning, architecture, and science should work together to provide transdisciplinary responses to urban environmental and health issues. Studies on reciprocal interactions between temperature, anthropogenic stressors, and mosquito microbiota are still scarce and merit further investigations given the key role of mosquitoes as medically important arthropod vectors of diseases [177]. More globally, integrated studies that combine different impacts at different levels of mosquito organization would be necessary to propose risk prediction for predicting MBID emergence.

Acknowledgements PD was financed by Ecole Urbaine de Lyon. Funding for this project was supported by the French National program EC2CO (Ecosphère Continentale et Côtière) and by the Agence Nationale de la Recherche (SERIOUS project). This project has also received financial support from the CNRS through the MITI interdisciplinary programs and its exploratory research program.

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2. Les jardins collectifs en France : historique, diversité et fonctions

Pour pallier aux effets négatifs de l'urbanisation, de nombreuses initiatives ont vu le jour pour réintégrer la nature en ville ces dernières années. Elles impliquent la création de plus d'espaces verts se présentant sous différentes formes : les espaces végétalisés privés ou publics, les espaces naturels, les friches urbaines ou encore des toits ou espaces hors sol végétalisés. Leur apparition a fait émerger de nouveaux concepts comme celui de « services écosystémiques » ou encore de « multifonctionnalité » traduisant la pluralité de leurs fonctions (**Figure 6**) (Selmi et al. 2013; Déoux 2020). Parmi ces nouveaux espaces verts, les jardins collectifs sont en plein essor dans les villes européennes.

Les premiers jardins collectifs (c'est-à-dire tous types de jardins urbains) en Europe sont apparus à la fin du 18^{ème} siècle en Angleterre pour faire face à l'ère industrielle (Bell et al. 2016). Aujourd'hui, ils sont présents dans de nombreux pays européens, en particulier les plus urbanisés mais leur nombre, leur taille et leur visibilité peuvent varier considérablement d'un pays à l'autre. En France, les jardins collectifs sont retrouvés en grand nombre dans de nombreuses villes françaises, avec pour certaines une augmentation croissante observée ces dernières années. Par exemple, en Ile de France, le nombre de jardins est passé de 1064 en 2015 à 1303 en 2019 (Legenne et al. 2020). Bien qu'il n'existe aucun recensement des jardins collectifs à l'échelle nationale, certaines associations ou communes ont réalisé une cartographie des jardins à l'échelle des villes et révélé les tendances à venir pour les prochaines années. Dans cette partie, nous allons traiter de l'historique des jardins collectifs en France, leur diversité de fonctionnement ainsi que des avantages mais aussi des inconvénients potentiels associées à leur présence.

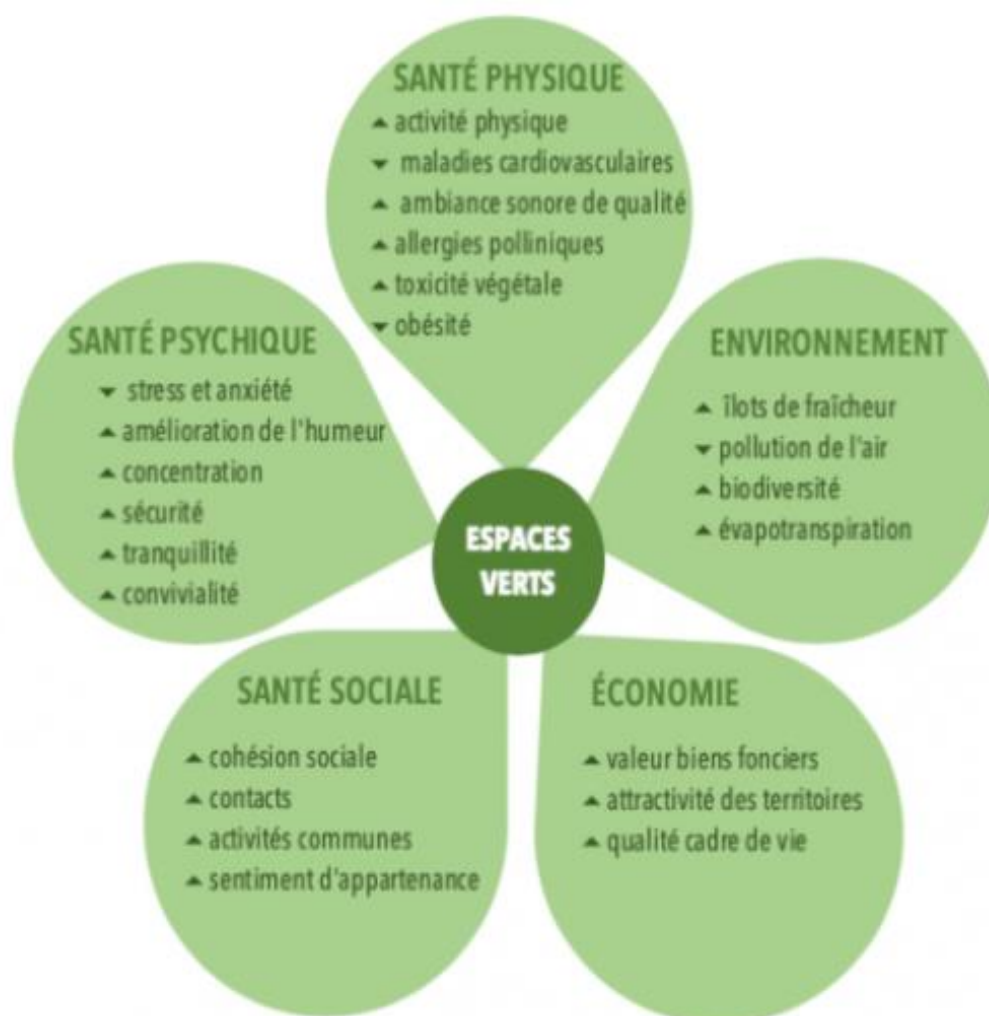


Figure 6. Exemples de bienfaits liés à la présence d'espaces verts en ville. D'après Déoux, 2020

2.1 Historique

Les jardins tels que nous les connaissons aujourd'hui ont pris plusieurs formes au cours de l'histoire en fonction des besoins et demandes en zone urbaine (**Figure 7**). En France, les premiers jardins en ville sont apparus à la fin du XIXe siècle, lors de la révolution industrielle, une période d'urbanisation intense. Ces jardins appelés « jardins ouvriers » ont été créés par l'abbé Lemire en 1896 pour pallier à la pénurie alimentaire et à la pauvreté des ouvriers (Cabedoce 2018). Ces jardins avaient pour missions de donner aux ouvriers la possibilité de cultiver des fruits et des légumes. Parmi les jardins ouvriers, les « jardins du cheminot » visant à subvenir aux besoins des cheminots se sont développés à partir de 1943. Ces jardins étaient généralement localisés à proximité des chemins de fer et réservés uniquement aux employés de la Société Nationale des Chemins de fer Français (SNCF). En fédérant les jardiniers entre eux, les jardins ont progressivement joué un rôle associatif. C'est en 1952 que les jardins ouvriers ont pris officiellement le nom de jardins familiaux grâce à la loi inscrite dans le cadre législatif du Code rural. Les jardins familiaux ont alors eu pour but d'améliorer la qualité de vie des familles et de favoriser une bonne alimentation (Robert-Boeuf 2019). A partir de cette époque, tous les jardins familiaux devaient être gérés par des associations à but non lucratif. Différents types de jardins ont alors vu le jour. C'est le cas par exemple des jardins d'insertion visant à réinsérer des personnes en difficultés sociales ou professionnelles ou encore des jardins thérapeutiques, souvent installés à proximité d'établissements de soin pour prévenir certaines maladies ou favoriser la guérison de personnes atteintes d'affections physiques ou psychologiques et, également, des jardins pédagogiques ayant pour vocation de sensibiliser à l'écologie et l'environnement (Fortier 2003; Rubino 2007).

Ce n'est qu'en 1998 qu'est apparu pour la première fois à Lille le concept de « jardins partagés ». Ce terme, apparu au début des années 1970 à New York, a été proposé par Liz Christy, une artiste voulant revaloriser les terrains vagues présents dans son quartier. Elle avait alors créé un mouvement populaire appelé « Green Guerillas » mettant en œuvre des actions de nettoyage des friches pour installer des espaces agricoles urbains (Reynaud-Desmet 2012). Dès lors, de nombreuses initiatives citoyennes ont vu le jour pour créer des jardins partagés en France. Une proposition de loi a alors été déposée en 2002 par le ministère de l'agriculture et optée par le Sénat pour consolider le régime légal des jardins familiaux et étendre ce régime aux jardins d'insertion (Assemblée Nationale 2002).

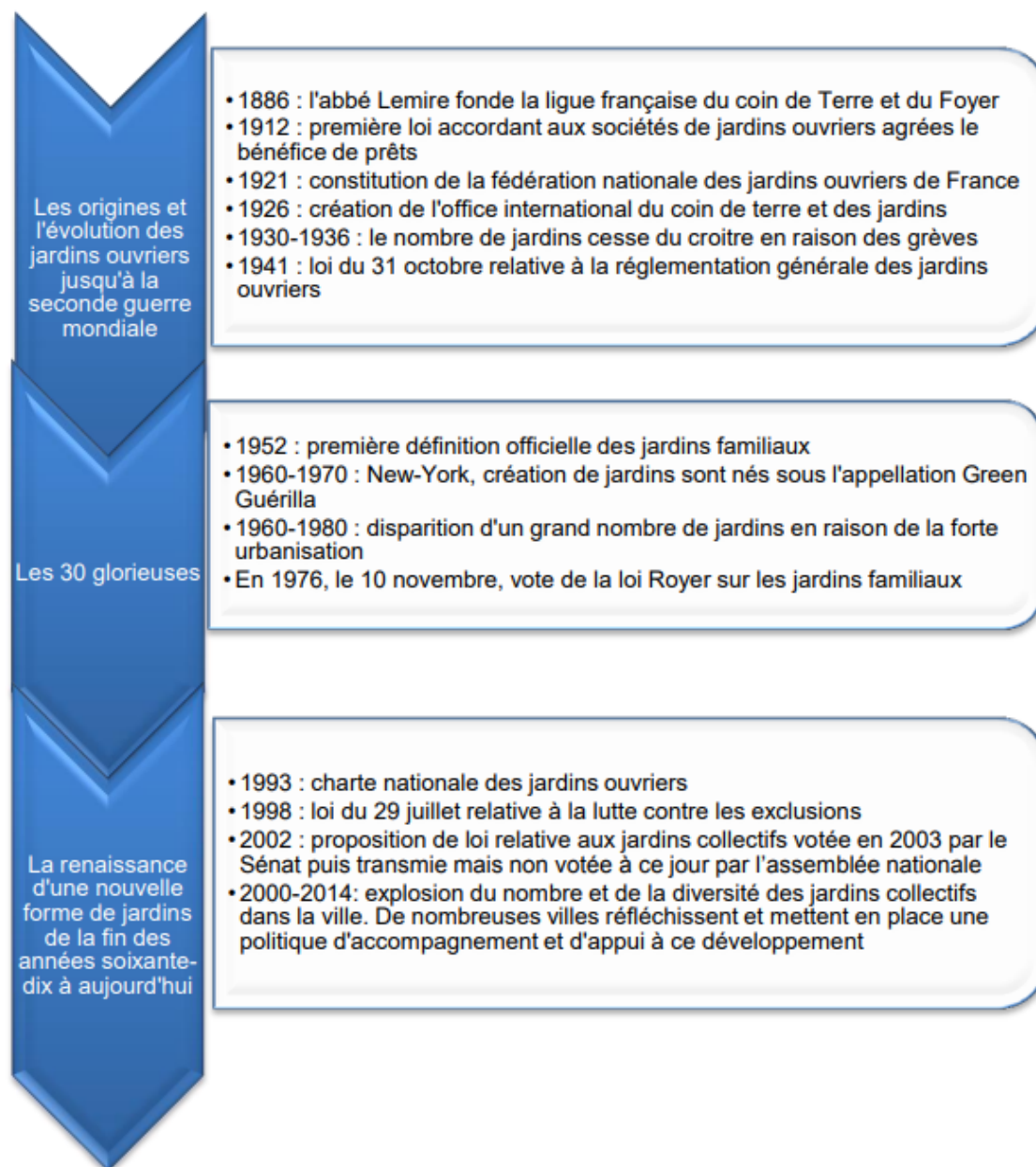


Figure 7. Historique des jardins collectifs en France. D'après Asaad 2016.

Cette loi a ainsi permis de reconnaître officiellement l'existence de jardins partagés au même titre que les jardins familiaux et d'insertion, en les regroupant sous le terme de « jardins collectifs » et de tous les soumettre à un même statut (Den Hartigh 2012).

Aujourd'hui, les jardins collectifs sont de plus en plus populaires en France. Les restrictions sociales causées par la récente pandémie de COVID-19 ont renforcé le besoin de passer du temps en pleine nature pour pratiquer des activités extérieures (Ugolini et al. 2020). Cet engouement encourage les politiques urbaines à créer plus de jardins. Ce contexte particulier laisse présager que le nombre de jardins augmentera de manière significative dans les années à venir, en particulier dans les plus grandes villes. Par exemple, la métropole de Lyon a accompagné la création de 40 jardins sur 22 communes au cours des 20 dernières années (GrandLyon 2021). La ville de Lyon participe également aux financements de ces projets d'aménagement en mettant à disposition 41 jardins (Direction du Développement Territorial 2020). Ces nouveaux espaces attirent de plus en plus de jardiniers amateurs qui s'organisent et fonctionnent de manière très différente.

2.2 Diversité de fonctionnement

L'émergence d'un jardin collectif suit différentes étapes (**Figure 8**) (Vandenbroucke et al. 2017a). La première est celle de la conception d'un projet d'aménagement du jardin et des objectifs partagés par un groupe d'acteurs (association, institution ou groupe d'habitants). La deuxième étape consiste à réaliser le projet paysager afin d'aménager le terrain et son équipement. L'ampleur et les coûts de l'aménagement sont très variables et peuvent être gérés par différents acteurs tels que le service s'occupant des espaces verts des communes concernées, des maîtres d'œuvre et parfois même directement des jardiniers. La dernière étape correspond à la phase de stabilisation, c'est-à-dire au temps nécessaire pour que les jardiniers, les gestionnaires et les partenaires s'adaptent aux contraintes liées à l'environnement du jardin et au mode de fonctionnement afin d'assurer une autonomie financière et matérielle. Le financement pour la création de ces jardins varie également selon différents facteurs. En général, les acteurs publics mettent à disposition des terrains lorsqu'ils sont les propriétaires fonciers, puis des partenariats techniques ou financiers permettent de réaliser des aménagements au sein du jardin.

Le déroulement de ces différentes étapes aboutit à des modes de fonctionnement et d'organisation qui peuvent être très différents d'un jardin à l'autre (Vandenbroucke et al. 2017), que ce soit au niveau du mode de gestion, du type de cultures, de la surface, du nombre d'adhérents, de leur implication, etc. Progressivement, des réseaux informels de jardins se sont mis en place et unis autour de valeurs communes. C'est le cas notamment du réseau « Jardins dans Tous Ses Etats » (JTSE) créé en 1997 par une dizaine de structures régionales, associatives et coopératives. Dès la création de ce groupe, les membres de ce réseau ont rédigé une charte et ont agi pour favoriser la mise en place de jardins collectifs en France. JTSE regroupe des acteurs partageant et soutenant des valeurs et des objectifs communs, par exemple, le renforcement des liens sociaux par l'entraide et le dialogue ou encore la possibilité d'offrir un cadre de vie sans discrimination culturelle, ethnique ou sociale. Globalement, la notion d'écoresponsabilité est aujourd'hui très présente dans les jardins collectifs en milieu urbain ceci pour préserver la biodiversité au sein de ces espaces au même titre que la santé des jardiniers. Certaines associations comme « Jardinot » vont même jusqu'à récompenser les centres de jardins familiaux, qui respectent au mieux la Charte du Jardinage éco responsable et, en particulier, la réduction de l'usage des pesticides.



Figure 8. Différentes phases d'émergence d'un jardin collectif. D'après Vandebroucke et al. 2017.

La configuration des jardins varie également considérablement d'un jardin à l'autre ce qui influe sur la gestion et les pratiques des jardiniers. En particulier, la gestion de l'eau est un point important au sein de ces espaces, et de nombreux jardiniers optent pour l'utilisation de systèmes de récupération d'eau de pluie, soit par soucis d'économie, soit en raison de l'absence de raccordement au réseau d'eau. Cependant, ces dispositifs de récupération d'eau de pluie, s'ils sont mal installés, peuvent permettre le développement de larves de moustiques, et en particulier du moustique tigre, un moustique bien implanté en milieu urbain (Mariappan et al. 2008; Moglia et al. 2016). Cet inconvénient sera explicité dans la partie suivante.

2.3 Bienfaits et inconvénients

Les jardins collectifs présentent de multiples bienfaits sur la santé et le bien-être, l'intégration sociale, l'économie et la biodiversité (**Figure 9**) (Duchemin et al. 2010; Mmako et al. 2019). Par exemple, les activités de jardinage permettent d'entretenir sa forme physique et ainsi de lutter contre les maladies chroniques et non transmissibles (Waliczek et al. 2005; Austin et al. 2006; Sommerfeld et al. 2010; Lovell et al. 2014; Soga et al. 2017). La participation aux jardins collectifs est également bénéfique pour la santé mentale par la diminution de l'anxiété, du stress et des risques de dépression. Elle développe aussi les fonctions cognitives et améliore la qualité de vie (Egli et al. 2016; Tharrey and Darmon 2021). La production de fruits et légumes au sein des jardins favoriserait une alimentation plus saine indispensable à une bonne santé (Duchemin et al. 2010; Ramsey et al. 2012). Les jardins collectifs ont également un impact social positif. En effet, il s'agit de lieux d'échanges et de convivialité permettant non seulement d'éviter l'isolement social mais aussi de favoriser la solidarité et l'insertion sociale. Ils aident à sortir de l'anonymat et à créer un lieu de rencontre entre habitants d'un même quartier quel que soit leur âge, leur culture ou leur milieu social (Agustina and Beilin 2012; Lovell et al. 2014; Whatley et al. 2015). De plus, l'intégration de migrants est grandement facilitée au sein de ces jardins (Agustina and Beilin 2012). Par ailleurs, les jardins collectifs et en particulier les jardins ouvriers sont des lieux de production maraîchère (Hallberg and Stephenson 2009). Ils sont alors d'utilité économique pour des jardiniers en situation de précarité permettant ainsi de limiter les inégalités économiques (Voicu and Been 2008; Lovell et al. 2014). Enfin, l'apparition de ces nouveaux espaces végétalisés a un impact environnemental significatif. D'une part, ils sont source de biodiversité pour la faune et la flore souvent réduites en milieu urbain.

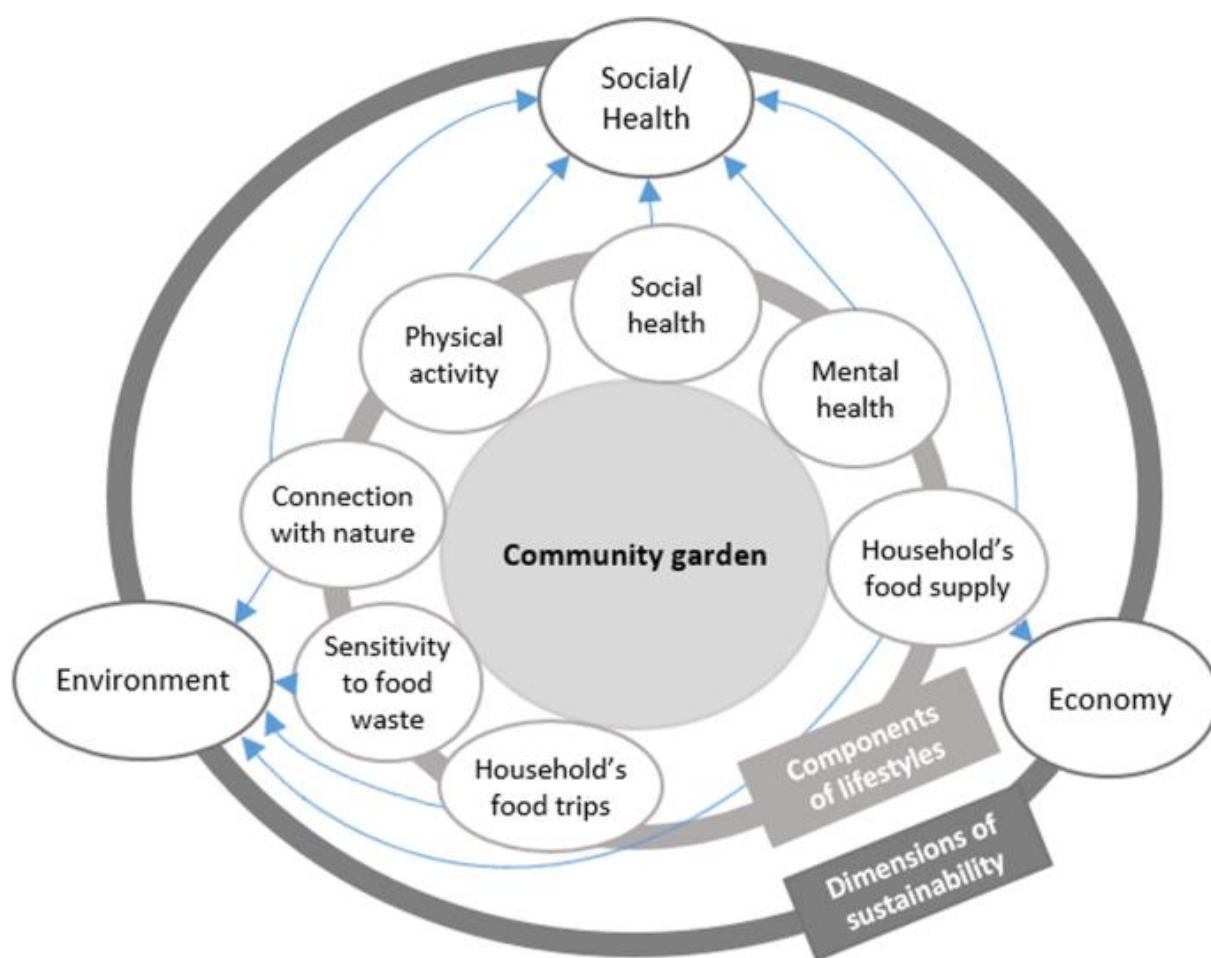


Figure 9. Impact des jardins collectifs sur la santé et le bien-être des adhérents, l'environnement et l'économie. D'après Tharrey et al. 2019.

D'autre part, ils permettent également de sensibiliser et d'éduquer aux questions sur l'écologie et l'environnement (Cabral et al. 2017; Al-Delaimy and Webb 2017). Si les bénéfices de ces jardins en ville sur la qualité de vie des citoyens ne sont plus à démontrer, certaines études témoignent néanmoins d'effets indésirables et invitent à réfléchir à de nouveaux aménagements ou structurations de ces jardins (Al-Delaimy and Webb 2017). Dans certains cas, ces jardins peuvent être utilisés comme des « vitrines » pour des élus ou encore des promoteurs immobiliers. La création du jardin n'est alors plus motivée par des aspirations sociales mais plutôt par une mise en scène pour promouvoir un lieu (Vandenbroucke et al. 2017a).

De plus, certains jardins ont recours à des prestataires, privés ou publics, pouvant amener à une certaine normalisation sociale et paysagère diminuant la diversité et l'inclusion de tous au sein du jardin (Vandenbroucke et al. 2017a). D'après une étude de l'institut national de recherche pour l'agriculture, l'alimentation et l'environnement (INRAE), les adhérents aux jardins collectifs sont généralement des personnes déjà sensibilisées à la cause environnementale. L'adhésion aux jardins ne permettrait donc d'informer qu'un public néophyte sur ces questions environnementales (Tharrey et al. 2020). De plus, une étude récente a montré que 16 néo-jardiniers sur 66 se déclaraient capables de quitter ou abandonner leur jardin en cours d'année s'ils se sentaient dépassés et découragés par leur manque de connaissances pour entretenir un potager (Darmon and Tharrey 2021). En dehors de ces aspects, les jardins représentent également des risques sanitaires. De nombreuses recherches ont prouvé que les légumes cultivés dans les jardins collectifs urbains pouvaient être pollués par la présence de métaux lourds et d'arsenic dans le sol (Kessler 2013; Jean-Soro et al. 2015; Izquierdo et al. 2015; Al-Delaimy and Webb 2017). Ces contaminants représentent des risques pour la santé des jardiniers qui se retrouvent exposés soit par inhalation de poussières contaminées soit par contact direct avec le sol lors de leurs activités de jardinage, soit après l'ingestion de légumes contaminés. Les jardins peuvent également induire la prolifération d'espèces invasives et nuisibles susceptibles de transmettre des maladies et avoir un impact négatif sur l'économie, les écosystèmes et les espèces locales (Al-Delaimy and Webb 2017). Par exemple, une étude récente a révélé la présence d'œufs du parasite *Toxocara* sur des légumes cultivés dans des jardins collectifs en Europe (Healy et al. 2022). Ces parasites résident dans les intestins de chiens ou de chats infectés, souvent présents dans les jardins (Lindsay and Dubey 2007). Les humains peuvent se retrouver infectés accidentellement en ingérant des œufs présents dans le sol contaminé par les selles d'animaux infectés, entraînant la toxocarose (Ma et al. 2018). Les jardins collectifs seraient également propices au développement d'arthropodes vecteurs. Des

tiques sont retrouvées dans les jardins collectifs et peuvent être à l'origine de maladies comme la maladie de Lyme (Ostfeld and Keesing 2000; Pearson and Firth 2012; Borşan et al. 2020). Comme mentionné précédemment, ces jardins disposent de nombreux réservoirs d'eau stagnante (récupérateurs d'eau de pluie, seaux, arrosoirs, coupelles, etc.), propices au développement des larves de moustiques tigre (Hayden et al. 2010). En raison de sa capacité à transmettre des maladies et des désagréments qu'il provoque, certains jardins et collectivités ont mis en place au sein des jardins des affiches explicatives sur le moustique et les risques associés. Par exemple, la ville de Lyon en partenariat avec l'association Le passe jardins (www.lepassejardins.fr) a créé une affiche expliquant comment se protéger du moustique tigre (**Annexe 1**). Certains jardins organisent également des ateliers visant à enseigner des techniques simples pour agir de manière préventive, en se basant sur le cycle de vie du moustique. Par exemple, le réseau « Partageons au jardin » ou encore la ville de Strasbourg ont mis en place des ateliers gratuits et ouverts à tous les adhérents aux jardins pour les informer et les sensibiliser (**Annexe 2**). Les gestionnaires ou directeurs des jardins utilisent également des affiches de sensibilisation et parfois même des pièges installés dans les parcelles pour enrayer la prolifération du moustique.

3. Impact des comportements humains sur la prolifération des moustiques en régions non endémiques

Le chapitre II-2 témoigne de l'augmentation du nombre d'initiatives visant à créer des jardins collectifs en raison de leur rôle essentiel en milieu urbain en Europe. Néanmoins, il paraît crucial de comprendre les pratiques à risque et mettre en place des plans de sensibilisation au sein de ces jardins pour gérer efficacement la prolifération des moustiques. Dans cette optique, nous avons focalisé notre attention, à travers une revue, sur les régions non endémiques, où plusieurs espèces de moustiques prolifèrent ces dernières années.

Bien que le nombre de cas de maladies transmises par les moustiques demeure encore sporadique, la présence de ces insectes représente une menace significative pour la santé publique de ces pays. Cependant, nous disposons encore de peu de données concernant les connaissances, la perception des risques et les pratiques de la population dans ces régions. Dans cette dernière partie, nous avons regroupé et analysé les résultats de 32 enquêtes sur les connaissances, les attitudes et les pratiques (CAP) vis-à-vis des moustiques afin d'identifier les connaissances et attitudes de la population générale qui pourraient influencer les pratiques liées à la gestion de cette espèce et à la prévention du risque vectoriel. Dans ce contexte, nous avons, tout d'abord, évalué 3 points : le niveau de la connaissance générale, les attitudes et les pratiques vis-à-vis du développement des moustiques et des risques associés. Ensuite, nous avons étudié l'impact des niveaux de connaissance et des attitudes mais également des paramètres sociodémographiques sur les pratiques de gestion des gîtes larvaires. Pour finir, les connaissances et les attitudes concernant les méthodes de lutte ont été analysées.

3.1 Publication n°2: “Knowledge, attitudes and practices regarding mosquitoes and mosquito-borne infectious diseases in nonendemic regions”

Cet article est actuellement en révision dans le journal *Frontiers in Public Health* dans la section « Infectious Diseases: Epidemiology and Prevention ».

A review of knowledge, attitudes and practices regarding mosquitoes and mosquito-borne infectious diseases in nonendemic regions

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Abstract

Mosquito-borne infectious diseases (MBIDs) present significant public health risks within tropical and subtropical regions. However, the rapid spread of MBIDs from these areas to temperate regions increase the risk of their emergence in nonendemic regions *i.e.*, regions where diseases are still sporadic and not sustained in the population. Raising awareness about preventive measures and protective behaviors is of primary importance to face the risks of vector-borne diseases. In this context, the number of studies on knowledge, attitude, and practice (KAP) about mosquitoes and MBIDs has grown rapidly in response to the need to identify knowledge and practices in nonendemic countries to fight mosquito proliferation. Building upon the recent developments in this field, we conducted the first-ever literature review to examine KAP studies conducted in nonendemic regions. Our aim was to identify the community's knowledge and attitudes that shape practices concerning the prevention of MBIDs. We used specific keywords regarding the scope of this review and then selected studies that were performed in nonendemic regions for MBIDs, including regions located in European countries, the USA or Asia. We identified 32 KAP studies, the oldest from 2003. The findings in the reviewed studies show that survey participants generally possessed a rather good understanding of mosquito breeding sites. However, there were notable variations in knowledge and perception of MBIDs, primarily linked to the geographic location of the survey and the prevalence of infectious outbreaks related to mosquito transmission. These findings highlight the significant influence of knowledge and awareness in fostering effective mosquito control practices. Moreover, socioeconomic status, particularly educational attainment, and respondents' gender emerged as key determinants in explaining the variability of appropriate practices. The survey results thus show the crucial role of knowledge, emphasizing the need for widespread awareness and information campaigns, encompassing both appropriate practices and efficient mosquito control methods. Understanding the interaction between these factors could provide good guidelines for implementing awareness plans and ultimately motivate the population to actively fight against mosquito proliferation and MBIDs development.

Key words: knowledge, attitude, practice, mosquitoes, mosquito-borne infectious diseases (MBIDs), nonendemic regions, preventive methods

1. Introduction

Global climate change has intensified concerns regarding mosquito-borne infectious diseases (MBIDs) due to the expanding global distribution of arboviruses transmitted by mosquitoes in recent years (1). Nonendemic regions, referring to areas where diseases remain sporadic and are not sustained within the population, are increasingly grappling with this problem, as seen in various European countries and the United States, for instance. The expansion of mosquito vector species in these areas is further compounded by poor mosquito prevention and control practices (2). MBIDs are transmitted to people through the bite of an infected female mosquito, which lays its eggs on water surfaces in various habitats, such as salt marshes, lakes or ponds, polluted water retention systems, or any other location where water accumulates. While all mosquitoes require standing water to reproduce, different mosquito species thrive in different habitats, including temporary water habitats or water sources that remain for long periods (3). For instance, some *Aedes* mosquito species have evolved to lay their eggs only in natural or artificial containers. Urban areas provide mosquitoes with a multitude of man-made breeding sites, such as flowerpots, empty containers, and rain gutters, leading to their rapid colonization in cities (4).

Considering the absence of prophylactic measures or vaccines for the majority of MBIDs, effective control of the mosquito vector stands as a crucial step in curtailing the proliferation of both mosquitoes and these diseases (5). Vector control measures rely on the use of different individual or collective practices. Among them, chemical insecticides are widely used. However, their adverse impacts on nontarget species and their secondary effects due to resistance in mosquito populations have made chemical insecticides a controversial social issue (6). On the other hand, environmental measures such as draining standing water, proper disposal of empty containers or the use of bed nets on standing water containers are considered sustainable methods to control the spread of *Anopheles* mosquitoes in urban areas (7). To be effective, these methods must be used in conjunction with other control methods and involve the local community. Additionally, public health risk awareness, ovitrap surveillance, and door-to-door control measures contribute to reducing the risks of spreading MBIDs (8). The choice between different existing and emerging vector control methods or combinations of techniques must be driven by the epidemiological, environmental, and socioeconomic context (9). One of the most significant explanations for the invasion and proliferation of mosquito vectors in urban areas is the practices of city residents (10). Knowledge, attitude and practices (KAP) questionnaires are frequently used to assess which parameters influence good or bad practices as well as knowledge and attitudes regarding mosquitoes and MBIDs. These surveys focus mainly on the mosquito life cycle, mosquito ecology, mosquito identification, biting times, control methods, and transmitted diseases. These surveys are essential to identifying inappropriate population behaviors and targeting the lack of awareness of a disease and/or vector as well as finding efficient strategies that involve the community in vector control. Given the importance of this issue, a review of studies based on KAP surveys can provide decision-makers with new information to better manage mosquito spreading and health risks. This study is the first to synthesize current knowledge on 32 published KAP studies conducted in nonendemic regions.

2. Material and methods

Searches were carried out on Google Scholar in February and March 2023 from peer-reviewed journal articles and reviews written in English, without period restriction. We first used a list of specific keywords that matched with the scope of our study. This first process of selection allowed us to identify 903 articles. Then, we kept articles that only concerned studies

that were performed in countries that are considered as nonendemic regions (or at least partially, like Vietnam) for MBIDs *i.e.*, regions where diseases are still sporadic and not sustained in the population. It includes studies conducted across various European countries, such as regions in France, Spain, Germany, Greece, and Italy, as well as a diverse array of states in the USA, including California, Florida, Louisiana, Utah, Alabama, and Arizona. Additionally, it encompasses research from regions in Asian countries such as Korea, Vietnam, Bangladesh, India, Malaysia, and Turkey. This second selection process led to the selection of 76 articles. Finally, after removing duplicates or articles that did not match our inclusion criteria (no KAP survey, type of article), our search yielded a total of 32 studies that matched our inclusion criteria (**Table 1**). These studies differed according to several factors such as the country/city, the mosquito species, MBIDs and targeted populations (**Table 2**).

3. Results

3.1 Knowledge, attitude and practices regarding mosquito development and associated health risks

3.1.1 Knowledge of larval breeding sites and MBIDs

The reviewed studies revealed that a major part of the participants in the surveys had very good knowledge of the role of standing water as potential mosquito breeding sites (11–18). For instance, results from campaign prospecting door-to-door visits and interviews in Sant Cugat, Spain, showed that 84.9% of the surveyed persons had good knowledge about *Ae. albopictus* aquatic habitats (13). Similarly, Tuiten et al. (2009) found that 99% of the respondents in New York (USA) knew that *Culex* mosquitoes breed in standing water (12). Morse et al. (2019) obtained similar results in a survey in Alabama (USA) where 98% of the inhabitants identified standing water as sites that are suitable for mosquito breeding (15). In Lyon (France) a survey carried out amongst people participating in community gardens showed that 94.7% of the respondents knew that the tiger mosquito (*Aedes albopictus*) breed in small or medium standing water (18). Thus, overall, respondents in KAP studies recognized standing water containers as potential egg-laying sites.

However, these studies also showed that a significant proportion of the surveyed persons had only a partial knowledge of the type of containers used as breeding sites, or identified other locations that are not suitable as breeding sites (15,18–23). For instance, in Lyon, 81.6% of the respondents believed that tiger mosquitoes breed in vegetation that in fact is suitable resting sites for adults, but cannot serve for larval development (18). Morse et al. (2019) showed that empty containers or rain gutters were not associated with potential breeding sites even though they represent highly suitable larval habitats for mosquito colonization (empty containers and rain gutters were not identified by 49 % and 39%, respectively) . Caputo et al. (2020) also revealed low knowledge about the asian tiger mosquito breeding site characteristics; only 21.3% of the respondents had correct answers regarding knowledge on places where tiger mosquitoes lay eggs and larvae develop . In Greece, a minority of respondents was able to identify correctly the place were mosquitoes lay their eggs and larvae grow (20,22,23). Similar observations were made from a German study where only 11.6% of respondents identified tree holes as potential water-holding larval habitats in their gardens (19).

Although these KAP studies were conducted in nonendemic areas where MBIDs are still sporadic, they reveal different levels of knowledge about these infectious diseases according to places and targeted population in the surveys. Firstly, a good knowledge was evidenced in surveys performed in the South of the United States, where local transmissions occur, *i.e.*, mosquitoes in the area have been infected with the virus and are spreading it to people (12,14,15,17,24–26). For instance, Bohmann et al. (2022) demonstrated that survey participants in South Texas had a high level of knowledge on different diseases transmitted by mosquitoes: 100%, 98% and 79% of respondents having previously heard of West Nile virus (WNV), Zika virus (ZIKV) and Dengue virus (DENV), respectively (17). By contrast, in Europe, MBIDs are still sporadic and epidemic events are mostly dependent on imported cases (*i.e.*, when diseases are contracted abroad). Several studies found that knowledge of diseases transmitted by mosquitoes was limited and that people are generally unaware of the health risks (21,22,27–30). Mouchtouri et al. (2017) found that only 9.6% of the interviewed Greek women answered correctly at all questions concerning Zika virus (28). Constant et al. (2020) found that the use of entomological surveillance of the main mosquito arbovirus vectors in anticipation of the emergence risk of MBIDs generates a significant increase in the population's knowledge of these diseases. Only 51.1% of the respondents residing in French Mediterranean regions knew that tiger mosquitoes could transmit chikungunya in 2012 compared to 88.3% in 2014 (31). In metropolitan France, health risks related to mosquito proliferation have increased significantly since 2011 when only 9 departments, out of 96, were colonized by the tiger mosquito compared to 81 in 2023 (84 % of the departments). During the same period, the total number of imported cases of dengue, zika and chikungunya increased from 15 in 2013 to approximately 1126 cases in 2023 (of which 1099 cases of dengue). This evolution is probably increasing more anxiety in the population toward these emerging diseases (32). For example, Duval et al. (2022) found that a total of 88.5% of community gardeners in Lyon, France, knew that mosquitoes could infect humans and animals (18). In comparison, Caputo et al. (2020) showed that 49% of the Italian residents knew that mosquitoes can transmit diseases but did not know which ones. Only 1% had already heard about dengue, chikungunya or yellow fever (21). Similarly, Stefopoulou et al. (2021) highlighted that only one respondent correctly identified the MBIDs among a list of 9 diseases (22). In 2018, 67.3% of the residents in Attica region in Greece were not able to list diseases that mosquitoes can transmit. Malaria was the most frequently answered response, followed by west nile fever and dengue. It is clear that some viruses are less known by the populations in certain regions (22). For instance, a large part of the population did not know about la crosse encephalitis (LAC) virus while this is the most medically significant California encephalitis virus in the USA (33,34). Only 16.3% of respondents were conscious of the existence of LAC (34).

Travelers and migrants coming from endemic countries could have an impact on the knowledge and practices regarding mosquitoes in nonendemic countries. For instance, in Italy, a questionnaire-based study was conducted to compare the KAP of native populations and two communities originating from the Indian subcontinent where MBIDs are a major public health concern. The results showed higher knowledge and concern about *Aedes*-transmitted diseases in Indian communities associated with a lower level of concern of nuisance caused by mosquitoes (21). This suggests that people who have been exposed to MBIDs are better prepared to face epidemics. More generally, it appears that populations from endemic-mosquito areas have a higher awareness of MBIDs; therefore, it could be expected that migration to nonendemic countries could improve local population knowledge (35,36). To a lesser extent, temporary visitors such as tourists may also affect knowledge of local populations. Interestingly, pregnant women from Greece who traveled abroad (not exclusively in endemic countries) had better knowledge about the risks of MBIDs than women who had not traveled

aboard (OR = 10.47, p value = 0.04) (28). The authors suggested that this difference could be explained by a higher awareness among individuals who have travelled to endemic areas.

To improve knowledge about mosquitoes and MBIDs, educational campaigns are implemented by public services. Among different information sources, participants of KAP surveys are generally informed by mass media (TV, radio, websites and newspaper) (12,18,23,25,28,37). Tuiten et al. (2009) reported that 70% ($n=97$) of the respondents mentioned that newspaper was the main information source about WNV followed by television (36%), radio (26%) and websites (20%) (12). Similar observations were made in the Lyon study in which 85.1% of the respondents were informed about the Asian tiger mosquito by media and internet (18). Surprisingly, information leading to awareness through health care workers seem to be rare. Two studies found only 8-9% of the respondents were informed about mosquitoes and MBIDs by physicians (28,38). This contrasts with endemic countries where between approximately 50 to 80% of the respondents are aware by health professionals that seems to play an important role in prevention about MBIDs and mosquitoes (39,40).

3.1.2 Attitudes toward nuisance and health risk

Studies on MBIDs and nuisances caused by mosquito bites have shown that most respondents are more concerned about the negative impact on their well-being than the related health risks. In the reviewed papers, between 44 and 80% of the respondents considered mosquito bites to be a serious nuisance during their activity periods (14,18,19,21,41,42). For instance, Davidson (2016) found that in St Johns County (Florida, USA) 80% of the respondents were bothered daily or several days per week (14). In Lyon (France) the survey amongst people participating in community gardens showed that the majority (81.3%) of the respondents were highly or moderately concerned by the presence of mosquitoes, with most of them being more preoccupied by nuisance and impacts on their quality of life than by disease transmission (18). An Italian study compared the attitudes of three groups of people: the native population and Italians originating from two endemic regions of India (21). In the group of native Italians 58.3% of the respondents were disturbed by mosquito bites whereas only 0.6% and 14.3% of the Malayalis and Punjabis, respectively, stated that they were concerned with mosquito bites. Some papers show that nuisance due to mosquito bites leads to changing activities, mostly by reducing time outdoors. For instance, in Washington, a large majority of respondents (61%) reported a change in activities (41).

Among other studies that have focused on the health impact of MBIDs and mosquito bites, some were conducted in the south of the USA, where MBIDs are present without being endemic. These studies demonstrated a great variety of risk perception according to the type of groups investigated. For instance, 95.5% of surveyed households in New Orleans stated that mosquitoes represented a major risk of transmitting diseases (37). Curry et al. (2018) found that almost one woman out of three interviewed in the gynecology department was concerned about the spread of ZIKV, particularly when pregnant. This concern was amplified among women who had given birth at least once (82.6%) (25). In contrast, only 23% of surveyed workers in Miami said they were moderately or extremely concerned about contracting a disease transmitted by mosquitoes (42). Similarly, respondents in South Texas had, on average, moderate fear of mosquitoes and the diseases they transmit, although they perceived MBIDs as a serious problem (17). In Greece, Kolimenaskis et al. (2022) showed that 83% of the respondents consider mosquito as a problem for nuisance and health (23).

In contrast to the southern United States, studies conducted in Europe showed relatively lower levels of concern regarding mosquito-borne infectious diseases (MBIDs). In France, vulnerability to MBIDs was found to be low or moderate depending on the populations studied

(ranging from 21.7% to 53.1% for random respondents in south of France and 27.7% for gardeners of community gardens) (18,31). In the Lazio region (Italy), 51% of respondents reported that they felt never or rarely worried about *Aedes*-transmitted diseases compared to 29% who considered themselves very or extremely worried (21). Caputo et al. (2020) found a large difference in attitudes between the investigated categories. Only 15.2% of native Italians reported being worried about diseases transmitted by *Ae. albopictus*, while among Malayali and Punjabi respondents, the proportions were 28.8% and 26.7%, respectively (21).

Overall, these results show that despite concern about MBIDs in nonendemic regions, perceived risks of epidemic-prone diseases are generally rather moderate, and seem to be related mainly to the characteristics of the surveyed group.

3.1.3 Mosquito mitigation practices and personal protection

The respondents in the analyzed surveys adopted different practices to avoid mosquito proliferation or to protect themselves against mosquitoes. Regarding these practices, questions in KAP studies addressed the use of personal protective measures and the management of potential breeding sites.

The majority of respondents in southern Texas (80.5%) declared that they correctly eliminated standing water (17). In a Spanish survey, the researchers observed that 76% of local population in Saint Cugat del Vallès, north of Barcelona, managed standing water to avoid potential breeding sites (13). Richards et al. (2017) reported that 67% of respondents in north Carolina reported removing empty containers such as flowerpots, birdbaths or tires (43). In France, 82.1% of gardeners in community gardens responded that they actively fought against mosquitoes by eliminating standing water containers (18). Despite the efficiency of this mechanical method, some surveys show that it is not systematically used by local residents. Only 40% of respondents in southwest Virginia and 50% in New York reported regularly or occasionally removing standing water containers (12,34). A survey conducted in southern France found that only 17.7% of respondents eliminated standing water and approximately one out of two stated that they did not follow any of the practices recommended by public health authorities (27). Even though construction workers are considered a population at risk for many MBIDs, 80% of surveyed construction industry employees in Miami did not use preventive measures such as removing standing water (44,42).

Several studies also analyzed personal protective measures against mosquitoes. Although personal protective measures are not intended to prevent mosquito proliferation, they contribute to limiting the number of bites and thus slowing the propagation of MBIDs. Personal protection takes many forms such as the use of repellents, wearing covering clothing, using door or bed nets, and limiting outdoor activities especially at hours when mosquitoes are active. In general, the use of repellents was the most frequently cited method by KAP respondents, probably because they are easy to apply and decrease contact between mosquitoes and humans. Overall, between 60% and 90% of the respondents reported using repellents to protect themselves (18,28,37,38,45). However, in the study conducted by Caputo et al. (2020), the majority of surveyed Italians (53%) reported that they used nothing to avoid *Aedes* bites, only 23% used environmental repellents at home, and 15% used personal repellents (21). Some studies have shown that bed nets or covering clothes that offer the advantage of being environmentally friendly and have no impact on human health are commonly used for personal protection (18,22).

3.2 Explanations of urban citizens' practices in the eradication of breeding sites

3.2.1 Influence of knowledge and concern about mosquitoes and MBIDs on mosquito control practices

People's level of knowledge and concern about mosquitoes and MBIDs clearly have an impact on their control practices according to the previous publications that have addressed this issue (10 of the reviewed articles). Some KAP surveys show that a high level of knowledge, for example, of the mosquito life cycle is essential to identify potential larval habitats and implement more appropriate practices (17,18,21,27,41,46). Caputo et al. (2020) found that a low level of Italian citizens' knowledge (21% of correct answers) was associated with a low level of practices to reduce or remove breeding sites (0.7%) (21). Raude et al. (2012) found in their study in French Mediterranean regions that a high ability to identify the *Aedes* species was highly associated with protective behavior by the respondents ($p < 0.001$) and resulted in the elimination of standing water (27). In Washington (USA), Dowling et al. (2013) demonstrated that approximately half of surveyed respondents who recognized breeding sites reported that they removed standing water (41). To our knowledge, only two studies have shown an opposite effect. Duval et al. (2022) revealed no significant relationship between the level of knowledge and type of actions taken against mosquitoes and their breeding sites (18). In this survey focused on the community gardeners in Lyon (France), attitudes, more than knowledge, appear to be an explanatory factor. Gardeners who were very concerned about mosquitoes were overrepresented in the group of gardeners who modified all or some practices because of mosquitoes (76 and 62% of the respondents, respectively). Only 27.9% of respondents who had not changed their habits in the garden due to the presence of mosquitoes were concerned about the presence of mosquitoes. This finding suggests that the more concerned community gardeners are about the presence of mosquitos in their gardens, the more likely they are to take action against this species (18). Moise et al. (2022) observed in New Orleans (USA) that 20.5% of respondents had good practices regarding mosquito control despite a lack of knowledge about potential breeding sites (37).

3.2.2 Impact of socioeconomic status on mosquito management practices

Most studies show that knowledge and practices depend on the socioeconomic status of the respondents, such as employment status, educational level or income (12,17,27,28,37,41,47,48).

Regarding household income, Walker et al. (2018) found that the odds of finding containers colonized by *Ae. aegypti* in outdoor premises were positively associated with low-income households in Arizona (USA) (48). Moise et al. (2022) observed that 31.6% of the New Orleans respondents in high-income households had negative practices against mosquito bites compared to 35.2% of respondents from low-income households. They also showed that employed individuals had significantly higher knowledge regarding mosquito control and practices to prevent mosquito proliferation and mosquito bites than unemployed individuals (OR: 1.00; 95% CI [0.000–0.006]) (37).

Educational level seems to be another key factor that explains good mosquito knowledge and appropriate practices (12,17,27,28,41,47,49). In Washington, respondents without a college degree had significantly more containers that could receive rainwater at their homes than respondents with a college degree (41). However, several studies found no link between the socioeconomic status of respondents and their level of knowledge and practices

regarding mosquitoes and MBIDs (18,19,31,34,37). In a study of nonprofessional gardeners in France, Duval et al. (2022) found no correlation between knowledge, practices and educational level (18). The absence of a significant relationship is probably because the respondents in the survey were all participating in community gardens, where awareness of mosquitoes is generally high. An American study in southwestern Virginia concluded that education and income were not significant predictors of the level of knowledge and practice (34). This study focused on the population of central Appalachia, which is an economically deprived region where financial resources for public health are low. In a study in Germany, educational level did not appear to have a significant effect on knowledge of *Anopheles* breeding sites (19). This could be explained by the fact that 54% of respondents claimed to have no mosquito breeding habitats close to their homes or had no knowledge of them. Following these examples, the lack of relationship between education and income with level of knowledge and practices could be the result of lower education levels in the target population.

Despite the lack of correlation observed in some studies, it seems that the socioeconomic level of residents explains their level of knowledge and behavior in regard to mosquitoes and their potential breeding sites. Numerous publications show that a high living standard and educational level are related to more efficient management of potential larval habitats and reduced frequency of breeding sites in a neighborhood. Similar observations are found in endemic countries (50–52).

3.2.3 Role of demographic factors on protective behavior against mosquitoes

In addition to socioeconomic characteristics, several studies have investigated the effect of demographic variables on knowledge and practices regarding mosquitoes (12–14,17,19,21,27,38,43,53–56). Gender appears to be a key factor explaining the knowledge and behavior of respondents (12,27,31,34,43,49). Overall, with regard to mosquitoes and MBIDs, female respondents show better knowledge and more appropriate practices. Studies in France and China revealed that the percentage of women who adopted protective behavior against mosquito bites (*i.e.*, the use of chemical repellents or covering clothes) was significantly higher than male respondents (27,31). A survey conducted in New York demonstrated that women had overall better knowledge about WNV than men (12). Additionally, in south-western Virginia, women declared themselves to be better informed about MBIDs and more concerned about health risks linked with mosquito bites and contracted diseases than men (34). Higher knowledge and concern in women could be explained by the fact that women are more anxious about health problematics and, in general, exhibit more responsibility than men regarding ‘care’ (57–60). A few publications have reported gender differences in practices regarding mosquito management. For example, two studies revealed that women respondents declared that they adopted better management of breeding sites than men, such as removing standing water and outdoor measures (43,49).

Several studies have revealed that age also influences knowledge and practices regarding mosquitoes. Although the age categories are not similar among studies, most studies have found that older respondents had better knowledge and practices regarding the management of breeding sites (12,41,47,53). For instance, individuals aged 45+ years in Washington reported a better level of mosquito ecology knowledge and were more active regarding standing water management (41). Similarly, Maeda et al. (2017) found that self-reported source reduction was more common for respondents over 50 years than for younger participants in Washington D.C. and Maryland (USA) (47). In Key West and Tucson (USA), respondents aged 35+ years reported that they cleared standing water in their property more often than younger groups (53). Different practices according to age groups could be explained

by the fact that older persons are more likely to garden than younger residents, and thus are more familiar with mosquito larval habitats.

In regards to protective behavior, a few studies have revealed opposite results (31,34,49). Among respondents living in Mediterranean France, Constant et al. (2020) observed that older individuals (≥ 68 years) were negatively correlated with personal protection against mosquitoes (Odd Ratio = 0.34) (31). However, this result should be interpreted with caution because, most of these respondents were retired which could influence specific mosquito protective behavior. In China, younger age was found to be a good predictor of adopting effective outdoor protective measures (49).

3.3 Knowledge and attitudes regarding mosquito control methods

3.3.1 Knowledge of mosquito control

Many types of mosquito control methods exist including physical, chemical, biological, mechanical and environmental methods. Mosquito control implies the local residents have good knowledge of the mosquito life cycle and be aware of their role in prevention. We found only three studies that examined people's knowledge of mosquito control in nonendemic countries (6,55,18). Claeys et al. (2009) investigated social knowledge and perception of mosquitoes and mosquito control methods in France regarding larvicide spraying in wetland (6). This study is based on field surveys conducted between 1995 and 2006 in the Rhone river delta (South of France) and in some Alpine valleys (East of France). In total, the population sample of 639 persons were interrogated via standardized questionnaires or semi-directive interviews. The results showed that in the delta of the Rhone River in France recurring campaigns of larvicide applications were poorly known by the local population (6). More than 40% of the respondents did not know who was responsible for the mosquito control campaigns in the region, although mosquito control has been controversial for more than 10 years. Another French survey in Lyon investigated the level of knowledge about biological control methods among nonprofessional gardeners in community gardens (18). It is interesting to highlight that only 20.5% of the respondents had ever heard about biological control methods. Among them, more than one-third were unable to describe the concept of biological control. Interestingly, only 3.4% of the gardeners were able to explain different biological methods like predation, use of *Bt* toxin, and 2.3% confused these methods with mechanical methods. Despite a general lack of knowledge, the gardeners wanted more information about alternative control methods, as revealed by the average score of 4.16 on a five-point Likert scale on this issue. This study also found that respondents who had already heard about biological control methods also had a better practice regarding mosquito management. Another study on public opinion regarding mosquito control in four locations in Florida, USA, showed low knowledge of mosquito control methods (26,55). The respondents pointed a lack of notifications prior to spraying campaigns and declared they would like to know more on this control method. To our knowledge, no study has yet investigated participants' knowledge of the sterile insect technique (SIT) or the use of the bacterium *Wolbachia* which are both implemented in endemic areas (61). SIT consists in releasing sterilized males in a target area. The systematic and repeated release of unfertile males decreases mosquito population over time (61). Releasing mosquitoes with *Wolbachia* over several months can reduce the number of a specific mosquito species, such as *Ae. aegypti* or compete with viruses within mosquitoes (62).

Knowledge questions in KAP surveys are largely restricted to mosquitoes and MBIDs. To increase the implications for the local population of mosquito control in nonendemic

countries, there is a need for both better information on existing control methods and more surveys dealing with this issue.

3.3.2 Attitudes toward mosquito control methods

Attitudes toward mosquito control methods have been addressed in a larger number of surveys than the level of knowledge regarding those methods (6,27,45,63,29,15,55,22). Understanding the population's perception of mosquito control and its effectiveness is necessary to assess which practices, *e.g.*, protective behaviors or actions against mosquito proliferation would potentially be well accepted and used by the population. Claeys et al. (2009) found two extreme perceptions in two regions of France, with one part of the population in favor of mosquito control regardless of the method and the other part against *Bti* larvicide spreading (6). For instance, in Camargue, 82% of the respondents were favorable to mosquito control. Additionally, the majority of the respondents living in areas where mosquito control had been implemented for 10 years would not want to return to a situation without mosquito control interventions. However, even the respondents who were the most favorable to mosquito control had some reservations; nearly 60% of all respondents underlined the potential damage to the ecosystem (6,64). A Greek study analyzed residents' perception of SIT and highlighted high acceptance by most residents (22). Among the participants, 92.5% thought that SIT was a good idea and on an effectiveness scale of 0 to 5, 70% answered between 4 and 5. In addition, 79.5% of the respondents considered that this method had more advantages than chemical methods. They also expressed concerns regarding the chemicals used and their potential environmental and human health impacts. Moreover, they stated having used most of the recommended at-home mosquito control mechanical methods such as keeping doors and windows shut and eliminating standing water reservoirs (55).

Overall, it is clearly important to understand the public perception and beliefs about mosquito control. Another question that is rarely investigated in the KAP surveys and should be further developed is the perception of individuals' role in mosquito control. To the best of our knowledge, only one KAP survey has analyzed the perception of 126 respondents impact of their own mosquito avoidance behavior may have on mosquito proliferation in their home and neighborhoods (15). More than half of the respondents (53%) considered that removing standing water from containers and flowerpots, have some impact on the local mosquito population. Only 14% thought that their action had a very low impact, while 33% believed their impact very significant around their home and neighborhoods. Convincing people of the impact of their actions represent an educational opportunity to motivate population to contribute to mosquito control.

3.3.3 Attitudes towards awareness plans and effectiveness of citizen sciences

Another important factor that improves effectiveness of control and prevention measures is the willingness of the population to adapt their practices and routines. Awareness plans, information and education are necessary as a complement to vector control campaigns in order to limit mosquito proliferation thus vector-borne diseases. In Washington DC, scientists' interventions to control mosquitoes have significantly reduced the number of breeding sites (41). According to a Greek study, raising awareness through printed educational materials alone has had no effect (20) or the opposite effect due to a decrease of MBID concern after the prevention campaign (65). Several studies highlighted that respondents perceived educational efforts by local authorities too low and asked for better strategies to enhance awareness about mosquito and MBIDs. For instance, Rampold et al. (2020) revealed that all Florida respondents

agreed that education regarding mosquito control efforts and their impact was needed (18,55). Several studies also revealed that respondents identified local governments or public health organisms as responsible for this health issue (15,18,37). Despite this observation, people concerned with mosquitoes in their neighbourhood may have other information channels. For instance, 73.2% of the nonprofessional gardeners in community gardens conducted their own research for information on Internet (18). The opposite was observed in Florida where most of the respondents had not research actively regarding mosquito control (55).

Citizen science is currently considered as a main driver to motivate and involve the public as citizen scientists. Volunteers are commonly involved in data collection, but can also be involved in initiating questions, designing projects, disseminating results, and interpreting data (UNEP 2019). With the aim to facilitate the public participation and collaboration in scientific research to increase scientific knowledge, citizen science could help for mosquito surveillance and mapping. Heym et al. (2017) found that respondents that were involved in a citizen science project were able to recognize potential larval habitats and modified their practices regarding breeding site management (19). Some citizen science projects have already shown their effectiveness such as the “Mosquito Alert” project that aims to make an inventory, via the use of a smartphone app by volunteers, of places where of various mosquito species in Europe are observed. After validation and correction by entomologists, the collected information is used to create early alert for MBIDs risk through predictive models (66,67).

4. Conclusion

This literature review sheds light on the disparities in knowledge, attitudes, and practices pertaining to mosquitoes and MBIDs in nonendemic regions. The understanding and behavior of the local populations have an impact on the proliferation of mosquitoes within urban areas. KAP questionnaires are commonly employed to evaluate the factors influencing both positive and negative behaviors, as well as the knowledge and attitudes concerning MBIDs. These surveys play a crucial role in identifying undesirable behaviors within the population and pinpointing gaps in awareness regarding diseases and vectors. Furthermore, they aid in the development of effective strategies that engage the community in vector control efforts. This review focused in particular on factors explaining what influences people’s practices regarding mosquitoes, as raising awareness about preventive measures and protective behaviors is of primary importance to face the risks of vector-borne diseases. Different explanations were put forward in the surveys. Knowledge about mosquito breeding sites and awareness of mosquito’s role in some MIBDs are key factors that explain more or less appropriate mosquito control practices on the individual level. Moreover, socioeconomic and demographic status also explain the variability of observed practices (**Figure 1**). The reviewed studies revealed that a significant portion of survey participants possessed only partial knowledge of potential mosquito breeding sites. This is an information of utmost importance, as a very good knowledge of these breeding sites is crucial for individuals to adopt effective practices aimed at preventing the spread of mosquitoes. Another important result that we found in these studies is that there are varying levels of awareness and understanding regarding the infectious diseases transmitted by mosquitoes based on geographical locations. These variations are clearly dependent on the frequency and the scale of the sporadic outbreaks of MBIDs in the surveyed regions. Surveys conducted in continents with frequent outbreaks resulting from local transmissions of MBIDs, such as the Southern United States, consistently demonstrated a high level of knowledge. In contrast, in Europe, where MBIDs remain sporadic, and epidemic occurrences are largely reliant on imported cases, awareness regarding health risks associated with mosquitoes tended to be less widespread. Alongside these results on knowledge, several studies have also shown

that most respondents in the surveys are more concerned about the negative impacts on their well-being than the related health risks. Despite a certain knowledge about MBIDs, the perceived risks of epidemic-prone diseases are generally rather moderate in nonendemic regions. We also noticed that multiple studies indicate that a strong understanding of potential mosquito larval habitats and heightened awareness of mosquito presence and MBIDs have a positive impact on the practices. Individuals who are both concerned and well-informed tend to adopt more appropriate measures in combating mosquitoes. There also seems to be a gender disparity, with female respondents that in general are better informed about mosquitoes and MBIDs, which tend to lead to the adoption of more effective practices against mosquito proliferation. Many studies within this review analyze how knowledge and practices are influenced by the socioeconomic status of respondents, and the results show in particular that educational level seems to be a key factor that explains good mosquito knowledge and appropriate practices. This review has also highlighted a scarcity of KAP studies focusing on the population's awareness and perception of different kinds of mosquito control methods and their efficiency. To enhance local community engagement in mosquito control efforts in nonendemic regions, there is a pressing need for improved dissemination of information regarding existing control methods (both individual and control methods used by local authorities), coupled with an increase of the number of surveys addressing this topic. It is evident that further evaluation is necessary to identify sustainable or eco-friendly practices or measures against mosquito proliferation that could be readily embraced and adopted by the population.

All these findings show the crucial role of knowledge in promoting good practices, emphasizing the necessity for widespread awareness and information campaigns. Several studies indicated that respondents perceived local authorities' educational initiatives as insufficient and expressed the need for more effective strategies to enhance awareness regarding mosquitoes and MBIDs. However, there is uncertainty surrounding the definition of an effective awareness and information campaign, when it comes to content, medium and distribution method. Some of the reviewed studies revealed that under specific circumstances, informational and educational efforts may fail to achieve their intended outcomes and, in some cases, could even lead to unintended adverse effects on attitudes and practices concerning mosquitoes and MBIDs. Consequently, there is a great need for additional surveys that evaluate the impact of educational initiatives, information dissemination, and awareness campaigns.

Given the importance of the emerging health risk in nonendemic countries and regions due to mosquito proliferation, and in particular the tiger mosquito, this review of studies based on KAP surveys provide decision-makers with new information to better manage mosquito spreading and mosquito-borne diseases.

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Figure legend

Figure 1. Impact of knowledge, attitudes and sociodemographic parameters on mosquito control practices and dispersal in nonendemic countries. Regarding knowledge, recognizing potential breeding sites and being aware of MBIDs influence the level of mosquito practices. Citizen sciences seems to be a high awareness plan to increase the knowledge of participants. Regarding attitudes, respondents perceived mosquitos more as a nuisance than a vector of disease. Regarding sociodemographic characteristics, women, older people and people with higher incomes and higher levels of education were positively associated with better practices. Regarding practices, respondents used personal protection, especially mosquito repellents, to protect themselves from mosquito bites, and they also took action against mosquitoes by eliminating potential larval habitats. The degree of effect is proportional to the width of the arrow.

Author Contributions

PD wrote the first draft of the manuscript. CAL and CVM reviewed and edited the manuscript. All authors contributed to the manuscript revision, read, and approved the submitted version.

Funding

PD was financed by Ecole Urbaine de Lyon. Funding for this project was supported by the French National program EC2CO (Ecosphère Continentale et Côtière) and by the Agence Nationale de la Recherche (SERIOUS project).

Conflict of interest

The authors declare no conflicts of interest in this scoping review.

Figure 1.

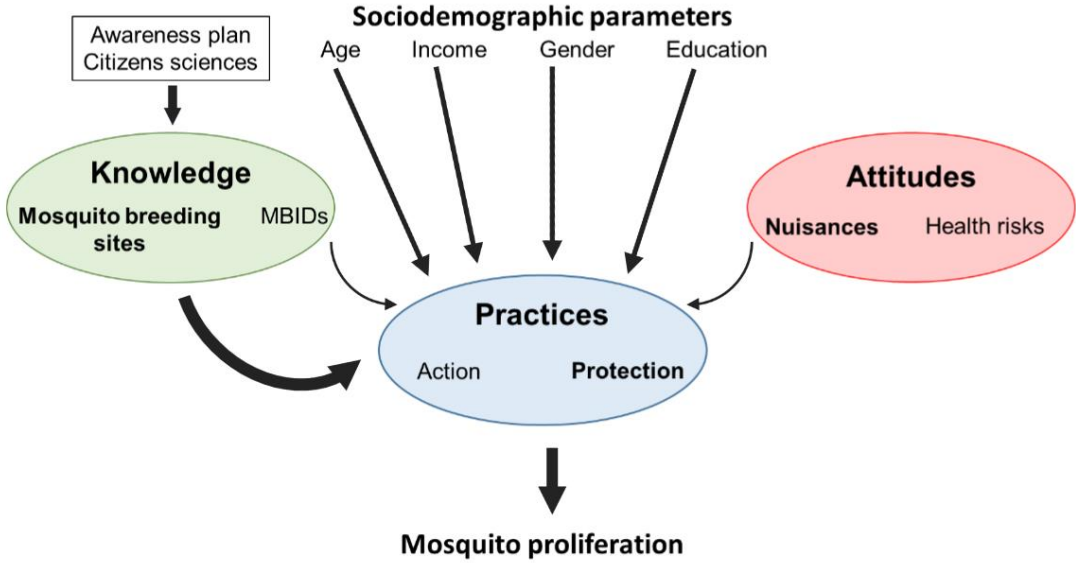


Table 1. Selection criteria used for studies considered for the review

Key words	Number of articles	Filter 1: study in nonendemic countries	Filter 2 : study types
attitude <i>Aedes</i>	13	1	1
attitude <i>Culex</i>	0	0	0
attitude <i>Anopheles</i>	0	0	0
attitude mosquito	45	1	1
attitudes mosquito	26	8	3
education mosquito	32	4	0
KAP mosquito	3	0	0
KAP mosquitoes	3	0	0
survey mosquito	344	8	8
knowledge mosquito	157	20	14
perception <i>Aedes</i>	9	2	2
perception <i>Anopheles</i>	3	0	0
perception <i>Culex</i>	1	0	0
perception mosquito	35	3	1
perceptions mosquito	42	15	13
practice mosquito	42	0	0
practices mosquito	111	9	1
protective behaviors mosquito	2	2	2
questionnaire mosquito	6	2	1
Socioeconomic mosquito	21	2	1
Additional records identified through other sources	8	8	8
Total with duplicates	903	85	56
Total without duplicates	-	-	32

Table 2. Characteristics of selected studies (n = 32).

Continent	Country	City	Mosquito species and/or MBIDs	Participants	Women (%)	Men (%)	Focus group	References
America	USA	NA	<i>Aedes sp. / Zika</i>	59	100	0	Gynecology patients	[25] Curry et al. (2017)
		Key West/Tucson	<i>Aedes aegypti</i>	775	49	51	Randomly selected participants	[53] Haenchen et al. (2016)
		Provo (Utah)	Malaria, Dengue, Zika, Chikungunya and West N	1043	68	32	Participants that traveled outside USA	[63] Omicior et al. (2018)
		Louisiana	<i>Culex sp.</i> and <i>Aedes sp.</i>	260	64	30	Randomly selected participants	[37] Moise et al. (2022)
		six states in the Northeast	<i>Culex sp.</i>	922	44	56	Randomly selected participants	[38] Herrington et al. (2003)
		St. Johns County (Florida)	All mosquito species	24	NA	NA	Randomly selected participants in neighborhoods	[14] Davidson et al. (2016)
		Region of southwestern Virginia	<i>Culex sp.</i> and <i>Aedes sp.</i>	188	NA	NA	Randomly selected participants	[34] Buitenvoort et al. (2010)
		Tennessee/Texas	La Crosse encephalitis	70	69	30	Randomly selected participants	[33] Barnes et al. (2022)
		Miami	<i>Aedes sp.</i>	44	25	75	Outdoor construction workers	[42] Moore et al. (2017)
		Alabama	Malaria, Dengue, Zika, Chikungunya and West N	126	NA	NA	Randomly selected participants	[15] Morse et al. (2019)
		New York	<i>Culex sp.</i>	97	68	32	Randomly selected participants in two neighborhoods (residential areas/suburban area)	[12] Tulien et al. (2009)
		Tucson (Arizona)	<i>Aedes aegypti</i>	355	NA	NA	Randomly selected participants	[48] Walker et al. (2018)
Washington	<i>Culex sp.</i> and <i>Aedes sp.</i>	184	NA	NA	Randomly selected participants in five neighborhoods	[41] Dowling et al. (2013)		
San Antonio (South Texas)	<i>Aedes sp.</i>	31	90	65	Randomly selected participants in five neighborhoods	[17] Bohmann et al. (2022)		
Three cities in North Carolina	All mosquito species	415	49	50	Randomly selected participants in five neighborhoods	[43] Richards et al. (2017)		
Florida	<i>Aedes sp.</i>	413	NA	NA	Physicians	[24] Dobbeck-Lewis et al. (2016)		
Asia	Korea	Seoul, Gyeonggi-do, and Incheon	<i>Aedes sp.</i>	249	88	12	Nursing students	[46] Choi et al. (2018)
Vietnam	Hanoi	Dengue and Japanese encephalitis	513	30	70	Participants in 3 livestock households and 3 non-livestock households	[16] Nguyen-Tien et al. (2021)	
Bangladesh, India, Malaysia, and Turkey	NA	<i>Aedes sp.</i>	223	53	47	Physicians	[30] Koonisey et al. (2021)	
Asia/Europe	Germany	NA	<i>Anopheles plumbeus</i>	118	31	64	Persons involved in citizen sciences project	[19] Heym et al. (2017)
Europe	Spain	Vall d'Areny	<i>Aedes albopictus</i>	309	60	40	Users of the Health Care Center	[11] Curcó et al. (2008)
		Sant Cugat	<i>Aedes albopictus</i>	820	65	35	Randomly selected participants	[13] Abramides et al. (2013)
		Lyon	<i>Aedes albopictus</i>	222	51	49	Community gardeners	[18] Duval et al. (2022)
		South of France	<i>Aedes albopictus</i>	1506	NA	NA	Randomly selected participants	[27] Raude et al. (2012)
		Languedoc-Roussillon, Provence-Alpes-Côte d'Azur, Corsica	<i>Aedes albopictus</i>	574	55	45	Randomly selected participants	[31] Constant et al. (2020)
		Camargue, petit camargue, Rhône alpes	All mosquito species	639	NA	NA	Randomly selected participants	[6] Cléys et al. (2009)
		Athens	<i>Aedes sp.</i>	303	56	44	Participants in camp for migrants and in neighboring residential areas and urban area	[23] Kalimerakis et al. (2022)
		All administrative regions	<i>Aedes sp.</i>	573	100	0	Pregnant women	[28] Mouchtour et al. (2017)
		Vivronna	<i>Aedes albopictus</i>	40	50	48	Randomly selected participants	[22] Stefanou et al. (2021)
		Lazio region	<i>Aedes albopictus</i>	1579	55	45	Native population and resident communities originating from the Indian	[21] Caputo et al. (2020)
Montenegro	Bec'i ci	<i>Aedes japonicus</i>	48	29	69	European participants of EMCA conference	[29] Ibañez-Justicia et al. (2019)	
UK	NA	<i>Anopheles sp.</i>	125	60	44	Participants who plan to visit a malaria-endemic area	[45] Goodyer et al. (2014)	

Dans cette synthèse bibliographique, nous avons mis en avant l'importance de la préservation de la nature en milieu urbain, tout en soulignant que ces espaces verts peuvent également être des environnements favorables à la prolifération des moustiques. De plus, nous avons cherché à comprendre les comportements humains afin de prévenir leur propagation. Dans ce contexte, le mémoire expérimental a pour objectif d'analyser les comportements à risques des adhérents de ces jardins collectifs dans la métropole lyonnaise et d'évaluer l'influence de ce contexte très anthropisé sur la composition biotique et abiotique des eaux stagnantes, colonisées ou non par le moustique tigre. La première étude présente les résultats d'une enquête quantitative CAP sur le moustique tigre et la lutte biologique auprès des adhérents aux jardins collectifs. La deuxième, quant à elle, décrit les résultats de l'étude de terrain visant à caractériser l'impact d'un gradient de pollution sur les propriétés biotiques et abiotiques d'habitats aquatiques colonisés ou non par le moustique.

III. Mémoire expérimental

1. **Évaluation des Connaissances, Attitudes et Pratiques (CAP) des adhérents aux jardins collectifs de la métropole de Lyon vis-à-vis du moustique tigre**

1.1 Contexte et objectif général de l'étude

Comme évoqué précédemment dans la partie 2 de la synthèse bibliographique, le nombre de jardins collectifs a augmenté dans les villes européennes ces dernières années (Ochoa et al. 2019). Ces lieux de nature apportent de nombreux bienfaits et contribuent à améliorer le cadre de vie des citoyens, en luttant notamment contre l'effet d'îlot de chaleur urbain et la pollution de l'air (Norton et al. 2015; Nunho Dos Reis et al. 2022). Pour autant, les observations récentes montrent que ces jardins, de par leur aménagement, peuvent abriter de nombreux gîtes larvaires et devenir des lieux où les moustiques prolifèrent revenant ainsi à enlever tous les bienfaits pour lesquels ces initiatives ont été créés. Cependant, tous les jardins ne semblent pas être impactés de manière égale. Ces différences pourraient notamment s'expliquer par une variabilité dans les pratiques, les connaissances et les attitudes des jardiniers amateurs vis-à-vis de cette espèce de moustique et les lieux où elle se développe. Dans ce contexte, l'objectif principal de cette étude était d'évaluer le niveau de connaissance et des attitudes des adhérents aux jardins collectifs de la Métropole de Lyon à l'égard du moustique tigre et d'identifier les bonnes ou, au contraire les pratiques à risque. De plus, l'étude avait pour but de comprendre quels paramètres influencent ces pratiques, qu'elles soient bonnes ou mauvaises, au sein des jardins collectifs. Pour cela, nous avons réalisé une enquête quantitative diffusée principalement par voie électronique auprès des membres des jardins collectifs de la métropole de Lyon. Au préalable, ce travail a nécessité de remettre à jour la cartographie des jardins collectifs du Grand Lyon. Le questionnaire a ensuite été envoyé aux contacts disponibles au sein de ces jardins, et également par le biais de l'association *Le Passe Jardin* et des municipalités volontaires pour sa distribution (**Annexe 3**). Au total, nous avons obtenu 226 réponses issues de 83 jardins sur les 288 identifiés en 2020.

1.2 Publication n°3: "How do attitudes shape protective practices against the Asian tiger mosquito in community gardens in a nonendemic country?"

Ces travaux ont été publiés en Novembre 2022 dans le journal *Parasites & Vectors* (volume 15, numéro 1).

RESEARCH

Open Access



How do attitudes shape protective practices against the Asian tiger mosquito in community gardens in a nonendemic country?

Pénélope Duval^{1*}, Claire Valiente Moro^{1†} and Christina Aschan-Leygonie^{2†}

Abstract

Background: The Asian tiger mosquito *Aedes albopictus* is responsible for the transmission of many arboviruses worldwide and is well adapted to thrive in urban environments. In mainland France, a nonendemic area, this mosquito is responsible for several autochthonous and imported cases of chikungunya and dengue each year. Better management and prevention of mosquito-borne disease transmission in nonendemic areas is thus of global concern. In this context, the aim of this study was to provide a better understanding of mosquito–human interactions as well as human behavior and beliefs in regard to this mosquito species in urban areas.

Methods: We focused on people who participate in community gardens, which are increasingly popular initiatives in metropolitan France and are conducive to the development of tiger mosquitoes. To evaluate community gardeners' knowledge and practices in relation to mosquito management and control, we conducted a knowledge, attitude, and practice (KAP) survey.

Results: In contrast to previous KAP studies, we showed that attitudes, more than knowledge, influence the practices of community gardeners in relation to mosquitoes. Interestingly, all gardeners who participated in the survey were concerned about the Asian tiger mosquito and were motivated to incorporate mosquito control methods in their gardens. Moreover, mosquitoes were perceived as nuisances rather than disease vector species. A change in community gardeners' perceptions could facilitate more appropriate behavior to control this species.

Conclusions: This survey reveals the lack of knowledge and awareness of good practices for the efficient control of the Asian tiger mosquito in green urban areas.

Keywords: Asian tiger mosquito, KAP survey, Protective practices, Nonendemic country, Green urban area

Background

The Asian tiger mosquito *Aedes albopictus* is one of the world's most invasive species [1]. This mosquito species, which originates in temperate areas in southeastern

Asia, has expanded on all continents except for Antarctica since the second half of the twentieth century [2]. Since its introduction in Europe via the transportation of goods and increased international travel, the presence of this mosquito species has been recorded in an increasing number of European countries, including mainland France, where it was detected for the first time in 2004. Human transportation and land use have been shown to be key factors for *Ae. albopictus* dispersal and the establishment of new mosquito propagules across the territory [3]. Due to its expansion in the French territory, a parliamentary report stated in 2020 that the presence of

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the Asian tiger mosquito constitutes a major health risk in the decades to come [4]. This mosquito species is a competent vector for many arboviruses, such as dengue (DEN), chikungunya (CHIK), and Zika (ZIK) viruses, as well as filarial nematodes [5]. Since 2010, it has been responsible for a growing number of autochthonous cases of CHIK and DEN in mainland France (Calba et al. [6]; Durand et al. [7]) as well as imported cases. For instance, Santé Publique France reported 109 DEN, 12 CHIK, and one ZIK confirmed imported cases from May 1 to June 7, 2019, in mainland France [8]. Beyond these health issues, this mosquito species is an aggressive daytime biter with a social impact because it can significantly alter human behavior and activity [9].

Previous studies have shown that the development and survivorship of *Ae. albopictus* are reinforced in cities compared to rural areas [10, 11]. Urban environments are particularly conducive to mosquito development and survival for several reasons. First, they provide numerous artificial water containers (gutters, tires, flat roofs, flowerpots, cemetery urns, etc.) and storm water management structures (rainwater network, gully or combined sewer) that can be used as larval habitats as *Aedes* mosquito larvae require standing water to complete their life-cycle. Second, densely populated urban areas provide good opportunities for blood feeding [12]. Finally, urban densification generates urban heat islands (UHIs) that are characterized by higher temperatures compared to surrounding areas [13]. The increase in surface temperature in urban areas has a direct impact on mosquitoes as it enhances their chances of survival by stimulating foraging and egg-laying behavior as well as shortening larval development and the duration of the extrinsic incubation period of the pathogen [14–17]. All these factors (human population density, UHIs, larger numbers of artificial water containers) increase the risk of arbovirus exposure in urban environments, a problem due to growth as a result of the urban densification policies that are currently implemented in France.

Numerous studies show that a significant increase in green areas and vegetation in cities is an efficient strategy to combat UHIs and reduce cities' vulnerability to heat and the effects of climate change [18–20]. Consequently, current policies favor nature in the city by developing green spaces as well as roof and wall gardens and, more generally, by avoiding concretization. In recent years, community gardens (e.g., family gardens, shared gardens, and integration gardens) have spread in cities all over Europe [21]. Various stakeholders contribute to the establishment of community gardens, including municipalities, nonprofit organizations, and private companies, and they are often linked with citizens' initiatives. These gardens have multiple

beneficial impacts on health and well-being, such as (i) social impact (e.g., locations for conviviality, tools for reintegration), (ii) political impact (e.g., collective production and discussion), (iii) environmental impact (e.g., awareness of environmental problems, biodiversity, and resources), (iv) economic impact (e.g., food production and exchange), and (v) health impact (e.g., increasing physical activity) [22, 23]. Community gardens are increasingly popular initiatives in France. In the Ile de France region, the number of collective gardens increased from fewer than five in 2000 to approximately 100 in 2021 [24]. Although no database referencing collective gardens in mainland France is available to date, it is assumed that their number will increase significantly in the coming years, especially in the largest cities. An example is the city of Lyon, which has been promoting the development of community gardens for the past 20 years and encourages its residents to green the city. Currently, there is a multiplication of participatory gardening programs [25]. The growing demand for urban gardens is partly explained by the recent COVID-19 pandemic. Lockdowns and social restrictions have changed people's behaviors and attitudes related to urban green spaces [26]. In addition, an increasing interest in urban vegetable gardens and food self-sufficiency has been observed [27]. However, it should be noted that along with the benefits listed above, community gardens increase the number of aquatic containers (watering cans, flower buddies, or rainwater collectors), which are conducive to tiger mosquito development [28].

Due to close links between *Ae. albopictus* and humans, human practices and human knowledge may significantly influence the mosquito life-cycle in urban green areas. Here, we focus on French community gardens in metropolitan Lyon for three main reasons: (i) the *Ae. albopictus* mosquito species has progressively been implemented in this area since 2012, (ii) imported cases of arbovirus infections are reported every year in the Rhône region, with the first autochthonous case of DEN reported in 2019 [29], and (iii) for several years, the Lyon metropolis has encouraged the establishment of collective gardens throughout its territory [30]. By conducting a quantitative survey, we will gain a better understanding of community gardeners' knowledge, attitudes, and practices (KAP) in relation to mosquito management and control for the first time in mainland France to develop an adaptive awareness plan. This survey also allowed us to measure the level of motivation of gardeners regarding their active participation in the fight against the Asian tiger mosquito. The outcomes of the investigation are particularly important as we are currently conducting a study based on the use of biological methods for the control of

this mosquito species in community gardens within the Lyon metropolis. The current survey is part of this larger study.

Methods

Study area

The survey was conducted among community gardeners in the Lyon metropolis, which is the second largest French city located in the region of Auvergne-Rhône-Alpes in southeastern France (Fig. 1). The Lyon metropolis had 1,411,571 inhabitants in 2019 spread over 59 municipalities in an area of 5337 square kilometers. The population density in the metropolitan area is 26,449 inhabitants per square kilometer, but there is high variability between the 59 municipalities. The urban core, with a population density of approximately 10,500 inhabitants per square kilometer, is roughly situated within the two central municipalities (Lyon and Villeurbanne). In the outer periphery, the built environment is less dense, and the share of green areas is increasing (Fig. 1). On the whole, the Lyon metropolis accounts for 40% of green areas. At the beginning of this study, there was no exhaustive inventory of community gardens in the metropolis. The local association of community gardens (*le Passe Jardin*) held the most complete inventory; in 2020, it had 200 registered gardens in its database. This inventory distinguishes between shared gardens, family gardens, and integration gardens but does not account for the number of regular gardeners in each garden. As a first step of the survey, we completed this database by various methods: information requests from municipalities, analysis of satellite images, and internet searches. This painstaking research resulted in a geographical database (using QGIS [31]) with 288 collective gardens located within the metropolitan area of Lyon (Fig. 2). The surveyed respondents were spread over 83 different community gardens located in 25 different municipalities of the Lyon metropolis. The target population in the survey was gardeners in the 288 community gardens that we identified in 2020. Following observations in the field and questioning of gardeners, we made a rough estimate that suggested that there were between 10 and 30 regular gardeners in most gardens.

KAP survey

We implemented a KAP survey to provide representative data on the knowledge, attitudes, and practices of community gardeners in relation to the Asian tiger mosquito. The quantitative survey included 77 questions divided into closed-ended and open-ended questions. For closed-ended questions, survey participants had the option to select several answers. The complete questionnaire is presented in the (Additional file 1: Table S1). The first topic, “frequentation and practices

in collective gardens,” consisted of 34 questions and aimed to identify the garden and the respondent’s role in the specific community garden as well as the gardener’s activities and practices regarding cultivation in the garden (for example, pesticide use, watering, period of presence in the garden). We specifically asked about the layout of the parcels and the watering practices as the type of irrigation directly affects the presence and number of potential mosquito breeding sites in the gardens. In the second topic, 19 questions were related to general “nuisances” and aimed to identify different types of nuisances in gardens. At this stage of the survey, there was no mention of the Asian tiger mosquito to avoid influencing the respondents. In the third topic, namely, “knowledge of the Asian tiger mosquito,” the respondents were given 11 questions related to the mosquito’s physical characteristics, life-cycle, and place in the ecosystem. In the fourth topic, seven questions were related to “biological control methods” and concerned means of mosquito control in community gardens and knowledge of biological control methods. This section also addressed the issue of the respondent’s personal motivation to become an active partner in the implementation of such control methods in the collective garden. The last topic, which included eight questions on “sociodemographic data,” aimed to describe the respondents’ profiles, such as gender, age, employment status, profession, and education. A pilot version of the questionnaire was sent to 10 community gardeners to check its validity (framing of the questions, overall structure, and sequences, etc.) and to ensure that the questionnaire length was compatible with the estimated duration it would take a respondent to complete the questionnaire. The final version was sent by email to all community garden contacts ($n = 164$), to the local association *le passe jardin*, and to all municipalities of the Lyon metropolis ($n = 59$), which agreed to dispatch the questionnaire to the members of the community gardens. We chose an internet-based survey, which is an efficient means to reach a large pool of potential participants who are both geographically dispersed within the metropolitan area and otherwise difficult to access (due to high variability of daily, weekly, and seasonal presence of gardeners in the community gardens). In addition, this type of survey is particularly efficient in regard to data collection and processing. With the advent of the COVID-19 pandemic, this choice proved to be particularly appropriate as the distribution of the questionnaire by mail was performed between March and July 2021. We obtained a total of 265 questionnaires, of which 255 were completed on the internet by the respondents, and 10 were collected directly in the field in one garden.

Ethics statement

The survey was developed using the software Sphinx IQ 2 at the University of Lyon secured server. The anonymity of each participant was respected. Each participant was associated with a code for data collection and analysis.

Data analysis

Statistical descriptive analyses were used to identify the respondents' characteristics, their frequencies and practices in gardens, and their perception of nuisance, biological control, and tiger mosquitoes. Responses are represented as quantities and percentages. We also conducted bivariate analyses based on the chi-square test of independence to determine whether there was a statistically significant relationship between categorical variables. This nonparametric test provides information not only on the significance of the general relationship between two variables but also on which categories account for any differences found between the theoretical and the observed situations. The aim using the chi-square test was, on the one hand, to identify statistical relations within a 95% confidence interval and, on the other hand, to establish which categories were significantly different from the expected values. Explanatory variables included sociodemographic parameters, frequentation and practices in collective gardens, nuisances, and the Asian tiger mosquito.

Results

Sociodemographic characteristics and motivations of the respondents

The sociodemographic characteristics of the surveyed gardeners are shown in Table 1. Overall, 51.4% of the 265 community gardeners who participated in this survey were female, and 48.6% were male. Most participants were in the range of 50–65 years old, and most of the gardeners were either professionally active (51.4%) or retired (39.6%). A large majority of the respondents (64.9%) had a high education level, above a high school diploma. Most respondents joined the garden between 1 and 5 years ago (46%), and in general, they spent more than 2 h (53.1%) each time in the garden (Table 2). A total of 19% of the respondents were activity leaders and/or initiators of the community garden. Gardeners were mainly motivated to join a community garden for the pleasure of gardening (79.6%), to grow fruits and vegetables themselves (71.36%), or to create social links (62.8%).

Knowledge, attitudes, and practices in community gardens

Knowledge of the Asian tiger mosquito. To assess community gardeners' knowledge of mosquitoes and their proliferation control, the participants answered a number of questions about mosquito characteristics (e.g., life-cycle,

Table 1 Sociodemographic characteristics of the survey respondents

Category	No. of respondents	Proportion within each category (%)
Gender		
Female	114	51.4
Male	108	48.6
Age group		
18–35	15	6.8
35–50	65	29.3
50–65	75	33.8
> 65	53	30.2
Profession		
Active	115	51.4
Retired	88	39.6
Looking for a job/not working	9	4.1
In professional training	4	1.9
Other	6	2.7
Education level		
Below high school diploma	32	14.4
High school diploma	43	19.4
Above high school diploma	144	64.9
Other	3	1.4

physical aspect, what mosquitoes feed on) and biological methods that can be used against mosquitoes. Table 3 summarizes mosquito knowledge among the respondents. All respondents had heard about the Asian tiger mosquito, and 85.7% thought that they would be able to identify this unique species among other mosquito species. However, only 32.8% of the respondents knew that the Asian tiger mosquito has a dorsal line that is the distinctive characteristic of the species. In addition, 94.7% of respondents knew that the Asian tiger mosquito breeds in medium and small stagnant water reservoirs, but 81.6% also incorrectly believed that this mosquito breeds in other inappropriate areas, such as vegetation. Only 20.5% of the respondents had ever heard of biological control methods. Among these respondents, 31.3% were unable to explain the significance of biological control, and very few respondents ($n=9$) clearly had knowledge of this type of pest control and could explain different methods, such as mosquito predator reintroduction or the use of *Bacillus thuringiensis* toxin, i.e., a toxin commonly used against mosquitoes. A total of six gardeners were confused about mechanical methods, such as the elimination of standing water containers.

Attitudes toward mosquitoes and mosquito control. Table 4 shows the surveyed gardeners' attitudes toward mosquitoes and means of control within community gardens. The majority of the respondents thought that

Table 2 Responses of gardeners regarding attitudes and practices in the community garden

Category	No. of respondents	Proportion within each category (%)
When did you join a community garden for the first time?		
< 1 year	29	10.9
Between 1 and 5 years	122	46
Between 6 and 10 years	53	20
> 10 years	61	23
How long do you stay on average in community garden?		
Between 30 min and 1 h	33	13.5
Between 1 and 2 h	78	31.8
> 2 h	130	53.1
What motivates you to participate in a community garden? (multiple answers allowed)		
For the pleasure of gardening	199	79.6
Growing your own fruits and vegetables	179	71.6
Enhancing social connections	157	62.8
Participating in outdoor activities	123	49.2
Participating in physical activities	78	31.2
Showing children how to garden	73	29.2
In this garden, what activities do you practice? (multiple answers allowed)		
Growing fruits and/or vegetables	235	91.1
Planting ornamental plants and/or flowers	176	68.2
Participating in workshops	109	42.2
Performing do-it-yourself activities	36	14

the presence of mosquitoes had increased in the past 2 years (56.3%), and only 14.3% had never felt disturbed in the community garden because of the mosquito nuisance. A very large majority of the respondents (81.3%) stated that they were highly or moderately concerned with the presence of mosquitoes in the community garden. Among this group of community gardeners, various reasons for concern were mentioned: bites (62.8%), disease transmission (27.7%), and their beliefs that the majority of mosquitoes in the gardens were Asian tiger mosquitoes (13.8%) and that the growing presence of this species was due to climate change and could cause negative impacts on biodiversity (7.4%). Only 10.5% of the surveyed gardeners believed that the control methods used in their community garden were effective. A five-point Likert scale was used to examine to what extent the gardeners who participated in the survey wanted more information about alternative control methods that could be used against mosquitoes. The average score of 4.16 showed that community gardeners were eager for more knowledge on this issue. Among different sources of information, a majority of participants in the survey answered that they received information about the Asian tiger mosquito through different media, such as newspapers, radio, television, or websites (85.1%). More than half of the participants obtained information through

awareness-raising campaigns organized by the Lyon metropolis or by the municipalities in the metropolitan territory (53.1%).

Practices in the community gardens. Regarding practices, a large majority of the surveyed gardeners (73.2%) conducted their own information research about the Asian tiger mosquito, and in general, this research was carried out online with the use of the internet. Table 5 represents the surveyed gardeners' practices in community gardens located within the Lyon metropolis territory. Regarding the equipment of their garden plots, a majority of respondents had a hut (88.7%), rainwater collection cans (86%), and a composter (73.3%). Concerning their agricultural practices, it appears that most of the responding gardeners used organic farming (65.1%) and permaculture (45%). A quarter of the respondents reported that they practiced conventional farming. However, only a very small portion of respondents said that they used chemical fertilizers and insecticides (3.7 and 2.1%, respectively). Almost every respondent used organic fertilizers in the community garden (>90%). The presence of mosquitoes in the community gardens was responsible for a change in habits and practices for a majority of the gardeners in the survey (69.7%). A large majority declared that they protected themselves by using repellents and/or by wearing covering clothing

Table 3 Mosquito knowledge in the community gardens (multiple answers were allowed for these questions)

Category	No. of respondents	Proportion within each category (%)
Garden pests		
Mosquitoes	172	72.3
Aphids	168	70.6
Slugs	157	66
Snails	123	51.7
Colorado potato beetles (doryphore)	108	45.4
Birds	101	42.4
Rodents	83	34.9
Ticks	7	2.9
The Asian tiger mosquito is		
A mosquito species that infects humans and animals	201	88.5
A pest (that bites)	161	70.9
An invasive species	131	57.7
Very abundant in the French metropolitan area	120	52.9
A useful species in the food web	16	7
What is a characteristic of the Asian tiger mosquito?		
The stripes on the legs	142	76.3
The size	100	53.8
The dorsal line	61	32.8
Diurnal activities	39	21
The Asian tiger mosquito develops in		
Medium and small stagnant water reservoirs	215	94.7
Large water bodies (lakes, for example)	90	39.6
Vegetation	64	28.2
Whitewater (rivers, streams)	18	7.9
Don't know	13	5.9
Mosquitoes feed on		
Human blood	207	91.4
Animal blood	133	58.8
Nectar plants/sweet juices from ripe fruits	62	27.4
Water	47	20.8

(75.7%). Moreover, 82.1% of the respondents actively fought against the presence of mosquitoes by mechanical methods (e.g., elimination of standing water containers) or by using insecticides. Thus, less than a third (30.3%) of the respondents had not changed any of their habits or practices in the community garden due to the presence of mosquitoes. In addition, among the surveyed gardeners, a quarter (24.8%) stated that they had never tried to reduce the mosquito nuisance or actively used any control methods to prevent the proliferation of the mosquito.

How do knowledge, attitudes, and practices influence each other?

In this section, bivariate statistical analysis was used to identify the relationship between gardeners' knowledge,

attitudes, and practices in community gardens. First, we tested the impact of sociodemographic characteristics such as gender, age, or profession on knowledge level, attitude, and practices. No significant relation appeared between gardeners' profiles and KAP. However, it is interesting that 117 of the community gardeners (87.3%) who completed the survey were highly motivated and willing to participate in the current scientific project on mosquito control by using biological methods. We did not find evidence that any sociodemographic characteristics distinguished these volunteers from the rest of the respondents who had no wish to participate in a mosquito control project. Neither specific knowledge or attitudes nor particular practices in the community garden seemed to explain their willingness to strengthen their

Table 4 Mosquito and biological control attitudes in the community gardens

Category	No. of respondents	Proportion within each category (%)
How has the abundance of mosquitoes changed in the past 2 years?		
Increased	134	56.3
Did not change	39	16.4
Decreased	15	6.3
No opinion	50	21
During your activities in the community garden, how frequently have mosquitoes disturbed you?		
Every time	55	23.2
Often	105	44.3
Rarely	43	18.1
Never	34	14.3
Are you concerned about the presence of mosquitoes?		
A lot	110	46.8
A little	81	34.5
Not at all	44	18.7
How do you evaluate the means of control currently used against mosquitoes in the frequented garden?		
Effective	23	10.2
Partially effective	85	37.6
Ineffective	35	15.5
There is no means of control in my community garden	48	21.2
No opinion	35	15.5

contribution to mosquito control. Regarding the statistical relationships between knowledge and practices in the community garden, most of the tested variables showed no significant associations between respondents' knowledge and their practices. The only significant correlation that we found (Table 6) was that gardeners who had already heard about biological control methods against mosquitoes more often had modified various practices in the garden due to the presence of mosquitoes (80%) than gardeners who had never heard about mosquito control methods (53.9%). Interestingly, the level of concern was significantly correlated with the practices of the survey participants in the community garden (Table 7). For instance, gardeners who were very concerned about mosquitoes were overrepresented in the group of gardeners who modified all or some practices because of mosquitoes (76 and 62% of the respondents in these groups, respectively). In contrast, only 27.9% of the respondents who had not changed their habits in the garden due to the presence of mosquitoes were concerned about the presence of mosquitoes. These results suggest that the more concerned community gardeners are about the presence of the mosquito in their gardens, the more likely they are to take action against this species. In addition, gardeners who were concerned about the mosquito's presence were overrepresented in the category of respondents

who "have often taken action against mosquito proliferation" (60.8% of the 97 respondents in this group). In contrast, the chi-square test showed that gardeners who "have never taken action against mosquito proliferation" were underrepresented (22.8%). Interestingly, the reasons why the respondents were concerned with the presence of mosquitoes (e.g., nuisance, disease transmission or ecological impact) had no significant influence on their practices.

Table 8 illustrates the impact of the respondents' perceptions of mosquito proliferation in the last 2 years on their practices and actions taken against mosquitoes. There was a significant correlation between the perceived increase in mosquito presence and changes in practices in the community gardens, the means of control used in the community garden, and the type of actions taken. For example, the respondents who believed that there was an increase in mosquito presence used more repellents than other gardeners did (20%). In contrast, the level of concern of the respondents regarding mosquito presence had no significant influence on the frequency of their actions against mosquitoes or on the mosquito control methods they used.

The perception of existing mosquito control methods in community gardens could lead them to modify their practices (Table 9). Respondents who believed that

Table 5 Practices and effects of mosquitoes on activities in community gardens

Category	No. of respondents	Proportion within each category (%)
Among the following items, your plot contains (multiple answers possible)		
Hut	133	88.7
Rainwater recuperator	129	86
Composter	110	73.3
Flower pot	57	38
No garden equipment	20	13.3
Greenhouse	17	11.3
Planter	41	7.3
Which kind of agricultural practice do you use? (multiple answers allowed)		
Organic farming	157	65.1
Permaculture	109	45.2
Conventional farming	61	25.3
Farming without soil	21	8.7
Don't know	12	5
In the garden you frequent, which do you use? (multiple answers allowed)		
Organic fertilizers (manure, compost, etc.)	223	92.5
Chemical fertilizers	9	3.7
Insecticides	5	2.1
Did you modify any of your practices in the community garden because of the presence of mosquitoes?		
None	61	30.3
Some	119	59.2
All	21	10.4
Did you perform or limit certain practices to avoid the proliferation of mosquitoes?		
Never	57	24.8
Occasionally	136	59.1
Very often	37	16.1

Table 6 Relationship between mosquito biological control methods and modification of gardeners' practices based on the abundance of mosquitoes

Have you changed any of your practices because of the presence of mosquitoes?	Have you ever heard about biological control?		
	None, n (%)	Some, n (%)	All, n (%)
Yes	4 ^a (10)	32 ^a (80)	4 (10)
No	54 ^a (35.1)	83 ^a (53.9)	17 (11)

Chi-square test

Letter indicates statistically significant differences (^a*P* < 0.001)

mosquito control methods used in the garden were "partially effective" were overrepresented in the category of respondents who changed none or only a few of their activities in the community garden due to mosquitoes (42% of the 166 respondents in this group). A significant

Table 7 Relationships between gardeners' concerns due to mosquitoes and their practices in community garden

Are you concerned about the presence of mosquitoes?	Did you modify any of your practices because of the presence of mosquitoes?		
	Not at all, n (%)	A little, n (%)	A lot, n (%)
None	22 ^a (36.1)	22 (36.1)	17 ^a (27.9)
Few	4 ^a (0.3)	40 (33.6)	75 ^a (63)
All	1 (4.8)	4 (19)	16 ^a (76.2)
Have you performed or limited certain practices to avoid the proliferation of mosquitoes?	Never	27 ^a (47.3)	17 (29.8)
	Occasionally	12 (15.8)	28 (36.8)
	Often	4 ^a (4.1)	34 (35.1)

Chi-square test

Letter indicates statistically significant differences (^a*P* < 0.001)

Table 8 Relationship between the perception of the abundance of mosquitoes over 2 years and its impact on gardeners' practices.

How has the abundance of mosquitoes changed over the past 2 years?				
	Increased <i>n</i> (%)	Did not change <i>n</i> (%)	Decreased <i>n</i> (%)	No opinion <i>n</i> (%)
Did you modify any of your practices because of the presence of mosquitoes?				
None	29 ^b (22)	10 (35.7)	3 (20)	19 ^b (73.1)
Few	85 ^b (64.4)	18 (64.3)	9 (60)	7 ^b (26.9)
All	18 ^b (13.6)	0 (0)	3 (20)	0 (0)
How do you evaluate the means of control currently used against mosquitoes in the frequented garden?				
Effective	5 ^b (4.4)	6 (19.4)	6 ^b (42.9)	6 (18.8)
Partially effective	56 (49.1)	14 (45.2)	6 (42.9)	9 ^b (28.1)
Ineffective	28 ^b (24.6)	3 (9.7)	0 (0)	4 (12.5)
There is no means of control	25 (21.9)	8 (25.8)	2 (14.3)	13 ^c (40.6)
Did you take action against the proliferation of mosquitoes?				
Collectively	49 ^a (49.5)	19 ^a (86.4)	5 (50)	
Individually	50 ^a (50.5)	3 ^a (13.6)	5 (50)	10 (37)
Have you performed or limited certain practices to avoid the proliferation of mosquitoes?				
Never	19 ^a (14.7)	15 ^a (39.5)	20 ^a (40.7)	
Occasionally	43 (33.3)	12 (31.6)	4 (26.7)	17 (35.4)
Often	67 ^a (51.9)	11 (28.9)	8 (53.3)	11 ^a (22.9)
What methods have you already used against mosquitoes? (multiple answers allowed)				
Repellents	48 ^a (20)	5 (12.5)	2 ^a (5)	
Chemical insecticides	1 (0.4)	0 (0)	0 (0)	0 (0)
Elimination of standing water tanks	91 (37.9)	21 (52.5)	9 (50)	18 (45)
Biological control	8 (3.3)	1 (2.5)	1 (5.6)	1 (2.5)
Covering clothing	88 (36.7)	12 (30)	6 (33.3)	13 (32.5)
Other	4 ^a (1.7)	1 (2.5)	0 (0)	6 ^a (15)

Chi-square test

Different letters indicate statistically significant differences (^a*P* < 0.05; ^b*P* < 0.001)**Table 9** Influence of the perception of mosquito control methods used in the community garden on gardeners' practices

How do you evaluate the methods of control currently used against mosquitoes in the frequented garden?					
	Effective <i>n</i> (%)	Partially effective <i>n</i> (%)	Ineffective <i>n</i> (%)	There is no means of control in the garden <i>n</i> (%)	No opinion <i>n</i> (%)
Did you modify any of your practices because of the presence of mosquitoes?					
None	4 (7)	16 ^a (28.1)	2 (12.3)	18 ^a (31.6)	12 ^a (21.1)
Some	8 (7)	54 ^a (47.4)	22 (19.3)	20 (17.5)	10 (8.8)
All	4 (19)	9 (42.9)	5 (23.8)	2 (9.5)	1 (4.8)
Have you performed or limited certain practices to avoid the proliferation of mosquitoes?					
Never	6 (10.9)	6 ^b (10.9)	4 ^b (7.3)	22 ^b (40)	17 ^b (30.9)
Occasionally	4 (5.3)	33 (44)	16 (21.3)	15 (20)	7 (9.3)
Often	13 (13.8)	46 ^b (48.9)	15 (16)	11 ^b (11.7)	9 (9.6)
Did you take action against the proliferation of mosquitoes?					
Collectively	12 (12.9)	50 ^b (53.8)	16 (17.2)	6 ^b (6.5)	9 (9.7)
Individually	5 (7.5)	24 ^b (35.8)	14 (20.9)	17 ^b (25.4)	7 (10.4)

Chi-square test

Different letters indicate statistically significant differences (^a*P* < 0.05; ^b*P* < 0.001)

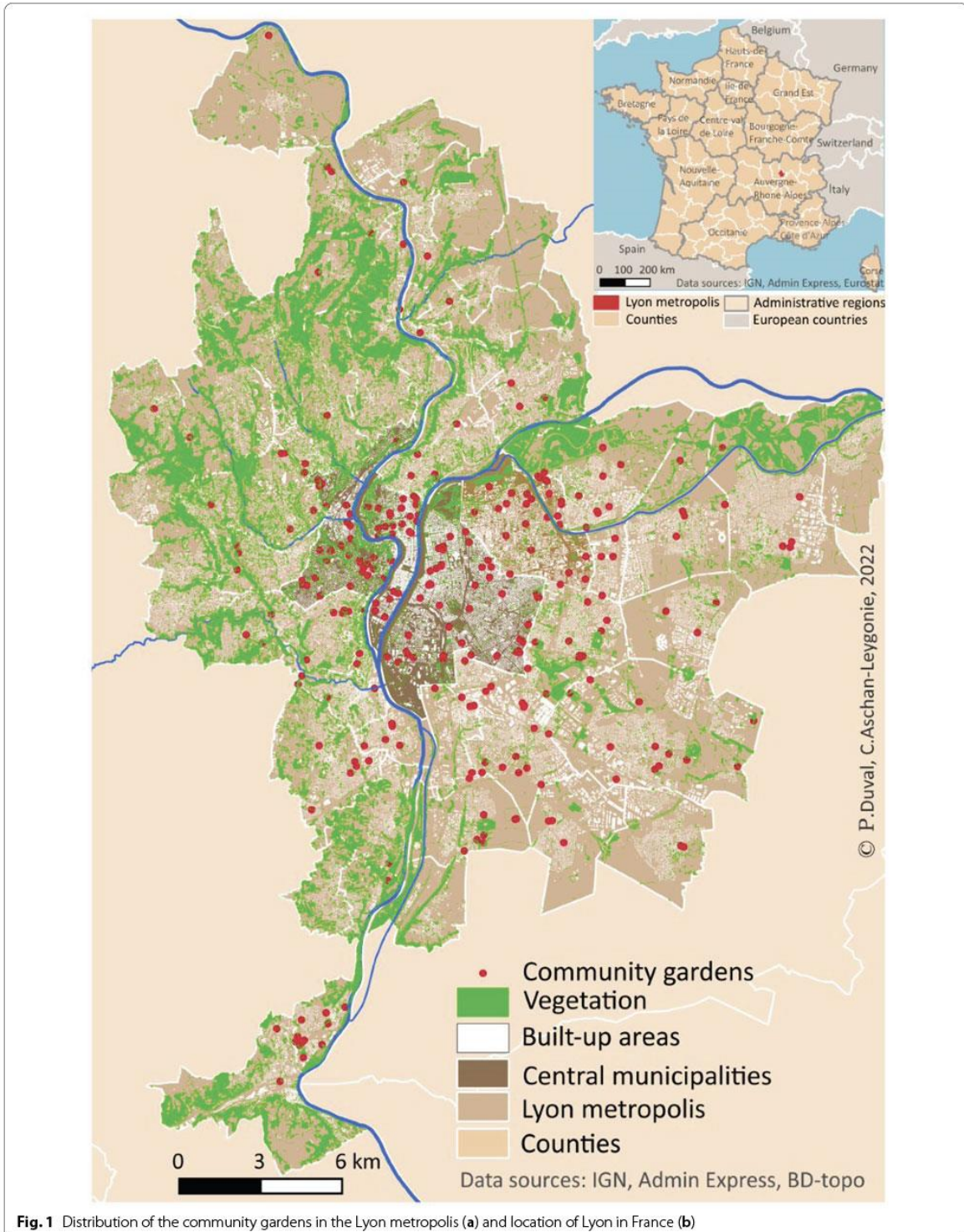


Fig. 1 Distribution of the community gardens in the Lyon metropolis (a) and location of Lyon in France (b)

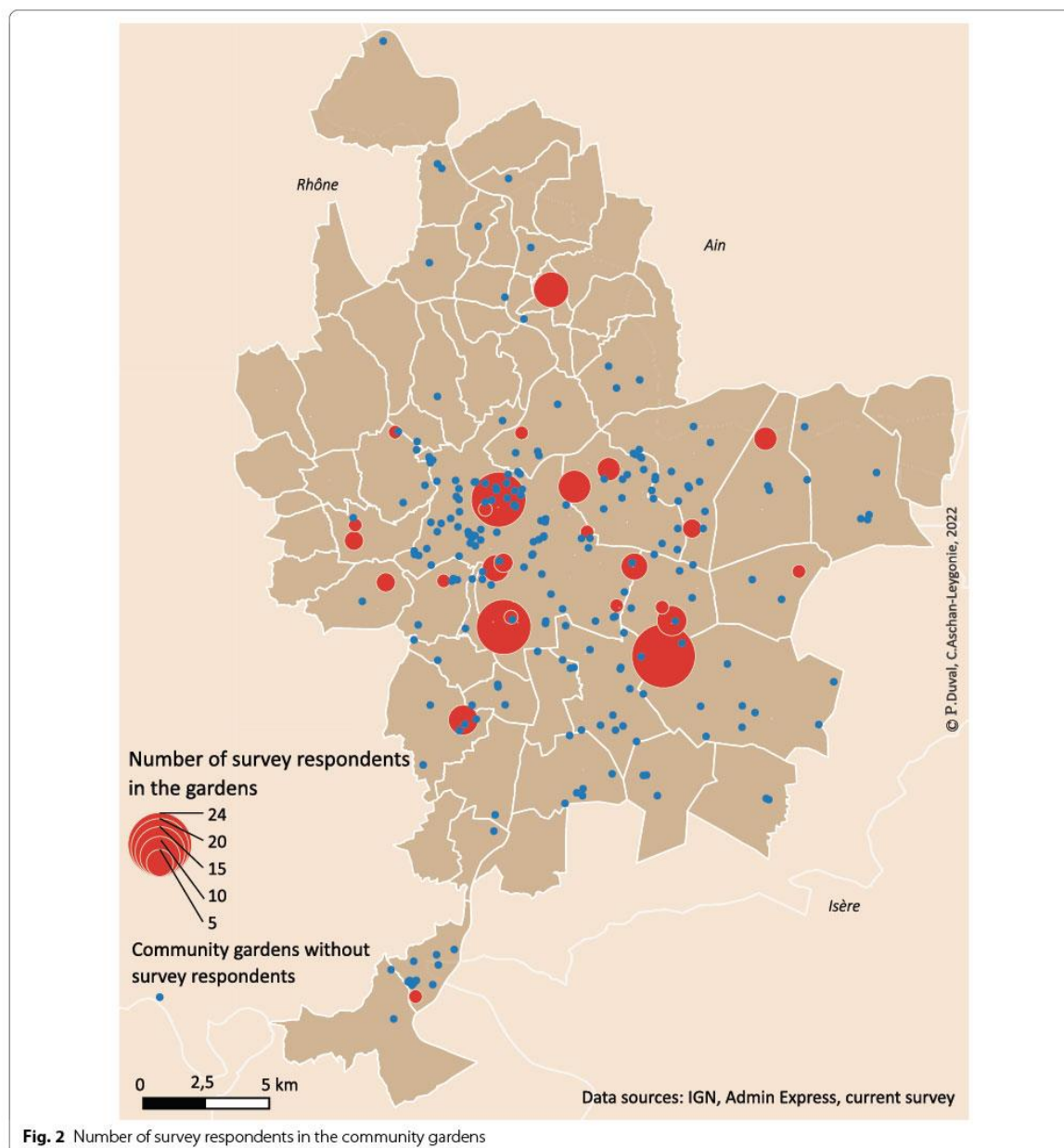


Fig. 2 Number of survey respondents in the community gardens

correlation of this same group was also observed in regard to taking action against mosquitoes. Indeed, 93% of the respondents who believed that mosquito control methods used in the garden were “partially effective” stated that they had taken action against mosquitoes

or limited certain practices to avoid the proliferation of mosquitoes. In addition, surveyed gardeners who used no means of control in their gardens were underrepresented in collective action and individual action against mosquito proliferation.

Discussion

To our knowledge, this study is the first to collect information on nonprofessional gardeners in community gardens concerning their knowledge, attitudes, and practices in relation to the Asian tiger mosquito in Europe.

This survey allowed us to identify some practices of gardeners that may promote the development of Asian tiger mosquitos in gardens. For instance, 86% of the surveyed gardeners had a rainwater collector on their plot. This kind of watering method is commonly used in community gardens and presents a sustainable and alternative water resource [32, 33]. At the same time, these containers are well adapted for mosquito breeding at the larval stage and are often difficult to protect from mosquitoes (Mariappan, Srinivasan, and Jambulingam [34]). Gardeners should be aware of the ability of this material to serve as a potential breeding site in their garden to take action against mosquito proliferation. Regarding the type of agricultural practices, a quarter of the respondents reported that they performed conventional farming. However, only a very small number used chemical fertilizers and insecticides (3.7% and 2.1%, respectively). Three hypotheses might help to explain this contradiction. First, answers may be biased because many community gardens prohibit the utilization of chemical fertilizers in accordance with the national charter, *Jardin dans tous ses états*, which recommends sustainable cultivation techniques. Second, the surveyed gardeners knew that using chemical fertilizers could have a negative impact on the environment. Thus, even if they used chemical fertilizers, they may have provided what they believed was the expected answer. Third, the surveyed gardeners continued to use conventional agriculture techniques, but they tried as much as possible to avoid chemical inputs and insecticides. According to gardeners' willingness to be better informed about alternative methods to chemical insecticides, the last hypothesis seems more plausible. This is supported by the fact that most gardeners who would like to be better informed about alternative methods of mosquito control answered that they would be motivated to become involved in specific campaigns for mosquitoes in their garden. This study concludes that all the gardeners who participated in the survey were aware of the presence of the Asian tiger mosquito in the Lyon metropolis. This is consistent with the fact that this mosquito species began to colonize this territory in 2012. Among the main pests in community gardens, mosquitoes were cited most frequently by the surveyed gardeners (72.3%). Regarding knowledge of mosquito characteristics, less than one third of respondents knew that the main physical characteristic of the Asian tiger mosquito is the white dorsal line. Encouraging awareness of specific recognition of the Asian tiger mosquito is essential because of the role of this species in

outbreak epidemic events compared to other autochthonous mosquito species.

Most of the respondents were concerned about the presence of the Asian tiger mosquito (90%), mainly because of the mosquito's annoying bites during diurnal activity (63.8%) rather than its capacity to transmit infections such as arboviruses (27.7%). This finding shows that the gardeners surveyed were concerned with the *Ae. albopictus* as a pest, because of mosquito bites, rather than its ability to transmit disease. This suggests that people tend to be more motivated by the immediate impacts of biting mosquitoes on their quality of life than they are by the threat of diseases. It is human nature for people to respond more to an immediate and tangible problem than to one they perceive as less than immediate or even as hypothetical. This perception seems to emerge from the fact that autochthonous arboviruses remain sporadic, as well as trust in the French health care system [35]. Currently, diseases transmitted by *Ae. albopictus* in Europe are due to sporadic events and mainly depend on imported cases from endemic countries. However, the expected trend in the coming decades is a larger spread of arboviruses [36, 37]. The spread of mosquitoes and mosquito-borne diseases is driven by many factors, including anthropic activities such as global transportation systems, mosquito adaptation in urban areas, and climate change [38, 39]. Recent studies have examined whether people living in arbovirus-endemic countries have better awareness of the risk of arbovirus emergence than people living in nonendemic countries. In previous studies, the European population was compared with the immigrant population from countries where *Aedes*-transmitted diseases are endemic, and the immigrant population had better knowledge and management of this vector species [40, 41]. The population's awareness is heterogeneous between endemic and nonendemic countries as well as within the country itself due to different ethnic groups and the presence or absence of mosquitoes [41–43]. Changing this perception seems to be an important lever for individual practices that might converge toward mosquito control. Health risk awareness depends on disease susceptibility (which includes the population's exposure to mosquito-borne diseases) and human perception of the severity of those diseases. With regard to disease susceptibility, the perception of being exposed to mosquito-borne diseases is an important factor to enhance protective measures against mosquitos. In addition, the severity of the disease influences protective practices [42, 43]. In the current survey, respondents were concerned about disturbance by the mosquitos rather than about their capacity to transmit infections. However, this is not linked to a lack of knowledge as 88.5% of the surveyed gardeners knew that mosquitoes can cause disease.

A very large proportion of the respondents (89.5%) declared that they modified their practices because of the presence of mosquitoes (such as time spent on outdoor activities or cancellation of garden events). This is consistent with previous observations showing that *Ae. albopictus* deteriorates people's quality of life during outdoor activities [44, 45]. These immediate consequences of the biting mosquito deteriorate their activities and motivated gardeners to fight against this pest. This impact on quality of life in community gardens could be a barrier for future urban residents to join a community garden. These findings suggest that gardeners' community and, to a larger extent, French urban citizens are not prepared to face a potential arbovirus outbreak. Despite the expansion of arboviral diseases in France and on a larger scale in Europe, the European population does not perceive the risk of transmission of these viruses by mosquitoes as a serious problem for the future [35, 40, 46]. Educating people on the dual threat mosquitoes pose in terms biting and disease transmission could have broad implications due to their immediate impact through deterioration of quality of life by bites and the future consequences by proliferation of mosquito-borne diseases.

As expected, better knowledge and protective practices are observed in the general population in endemic areas [40, 47–49]. In this context, knowledge is positively correlated with practices against mosquitoes. In contrast, this study shows that community gardeners' knowledge has a limited impact on their practices. In fact, the only significant relationship between knowledge and practice is that knowledge of biological control methods used against mosquitos influenced the respondents' practices. Thus, better awareness of biological control could improve the means used to manage mosquito proliferation. In contrast, other types of knowledge of mosquitoes (e.g., physical characteristics and life-cycle) did not seem to influence the respondents' practices. This result could be explained by the fact that the study area (mainland France) is not an endemic area. Another plausible explanation is the distribution method of the questionnaires during the survey. Generally, internet surveys lead to a bias of auto-selection (Gingras et al., [50]). Our internet-based survey very likely influenced the characteristics of the respondents according to their educational level and their knowledge of mosquitoes.

In contrast to knowledge, attitudes influence practices. Similar to another KAP survey, this survey highlights significant correlations between attitudes and practices [48]. For instance, the level of concern about mosquito presence modified people's outdoor activities in the community gardens. The respondents used strategies to avoid mosquitoes such as changing their habits of visiting hours in the garden. According to other KAP surveys and

our survey, perceptions of this vector species strongly shape practices, and this link should be considered for future awareness campaigns [48].

The results of this study have some limitations that need to be considered. First, the survey sampling was based on an online distribution that may not properly reflect the community garden population. In this study, all surveyed respondents were included in 30% of the total number of community gardens in the Lyon metropolis, but the profile of the total population of community gardens is still unknown. We can only compare the surveyed population to the Lyon metropolis population estimated in 2018 as no sociodemographic database on community gardens is available [30]. In this study, retired respondents represented 39.6% of the sample population compared to only 21.1% in the Lyon metropolis population. It is very likely that a large part of the garden community participants are retired urban residents; however, we cannot be certain that this group was not overrepresented in this study. A total of 64.9% of the surveyed respondents had a level of education higher than a higher school diploma, compared to 43.2% in the Lyon metropolis population. This could be explained by an overrepresentation of retired people in community gardens, their greater involvement in the associative life of community gardens, or the survey distribution sampling method by email [51]. Second, the questionnaire was written in French, and we know from our fieldwork that non-francophone persons represent a significant proportion in some community gardens. This group of community gardeners might have been underrated in our survey due to language barriers. Third, we compared our study to other surveys in arbovirus-endemic countries, which also differ by socioeconomic context [47, 48, 52, 53]. Such differences can influence the knowledge, attitudes, and practices of the population. Fourth, it is plausible that the surveyed gardeners were more worried about mosquito pests than nonrespondents would be. This is a potential bias linked to the fact that the respondents received information about the study within which the survey took place. Respondents who were concerned about mosquitoes in their community gardens were thus likely to be overrepresented in our study.

This KAP survey focusing on the community gardens of the Lyon metropolis allowed us to identify inappropriate practices that can be used as a basis to correct misinformation and/or create an adapted and effective awareness plan. Improving knowledge of the tiger mosquito could be an objective of education plans. As suggested by our findings, mosquitoes are perceived as nuisance pests rather than disease vectors. Changing the perception of gardeners could induce better control

of their behavior against mosquitoes. In a general context of increasing green areas in cities and a greater risk of outbreaks of *Aedes*-transmitted diseases, this survey shows a significant lack of knowledge and awareness of practices to help control the Asian tiger mosquito proliferation among people who take a particular interest in nature. Pest proliferation in urban green spaces needs to be better understood by society and political stakeholders [54]. This study is the cornerstone to enhance pest integrative management in green urban areas. In addition, community gardens are spaces of racial/ethnic diversity and community sharing [55]. The richness of different cultures in community gardens could contribute to improving awareness and could be the key to an efficient educational plan in gardens related to community engagement (e.g., sharing knowledge, preventive practice). Further studies are needed to implement similar surveys in other cities where *Ae. albopictus* has been present for a longer period to compare perceptions of the Asian tiger mosquito and to determine any awareness-raising operations that have been established.

Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s13071-022-05520-3>.

Additional file 1: Survey of the collective gardens of the metropolis of Lyon.

Acknowledgements

We sincerely acknowledge Julien Virmont from Lyon metropolis, Clara Gaulme from the local association *Le passe jardin*, all municipalities of Lyon metropolis, and community gardeners who helped to diffuse the survey. We thank the anonymous reviewers for helpful comments and Guillaume Minard, Patricia Luis, Edwige Martin, and Laurent Vallon for their proofreading and constructive criticism of the questionnaire.

Author contributions

PD made the maps, built the KAP survey, and wrote the initial draft of the manuscript under the supervision of CVM and CAL. All authors read and approved the final manuscript.

Funding

PD was financed by *Ecole Urbaine de Lyon*. Funding for this project was supported by the French National program EC2CO (*Ecosphère Continentale et Côtière*) and by the *Agence Nationale de la Recherche* (SERIOUS project). Financial aid for English proofreading was provided by UMR EVS (Environnement, Ville Société).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Received: 15 July 2022 Accepted: 28 September 2022

Published online: 22 November 2022

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1.3 Discussion et conclusion

A notre connaissance, cette étude est la première à collecter des informations sur les connaissances, les attitudes et les pratiques des adhérents aux jardins collectifs à l'égard du moustique tigre. Les résultats ont permis de mettre en évidence l'influence mutuelle entre ces trois paramètres et leur modulation en fonction du profil des répondants.

Notre enquête révèle qu'environ 70% des répondants ont modifié leurs habitudes et/ou leurs pratiques au sein des jardins à cause du moustique tigre. La majorité d'entre eux se protège par l'utilisation de répulsifs ou de vêtements couvrants et déclare agir activement contre les moustiques par l'utilisation d'insecticides ou l'élimination de réservoirs d'eau stagnante. Néanmoins, notre enquête a permis d'identifier des aménagements pouvant favoriser le développement du moustique dans les jardins. En effet, ces jardins sont fréquemment équipés de récupérateurs d'eau de pluie (86% des répondants ont affirmé en avoir sur leur parcelle). D'après nos observations sur le terrain et les données de la littérature, nous savons que ce type de conteneur est particulièrement apprécié par le moustique et est difficile à couvrir (Mariappan et al. 2008; Gee et al. 2022) (**Figure 10**). Dans le futur, il serait intéressant d'évaluer si les répondants étaient conscients des mesures nécessaires pour protéger ces réservoirs afin d'empêcher les femelles de venir y pondre leurs œufs. De par leur utilité au sein des jardins, supprimer ces conteneurs n'est pas envisageable, en revanche guider et sensibiliser les jardiniers pour choisir des collecteurs adaptés, c'est-à-dire faciles à couvrir, est indispensable pour limiter la prolifération du moustique tigre.

La majorité des jardiniers (90%) a déclaré se sentir préoccupée par la présence du moustique. Cette préoccupation était cependant plus liée aux nuisances occasionnées par ses piqûres (63,8%) que par sa capacité à transmettre des maladies (27,7%). De par son activité diurne, le moustique tigre gêne les jardiniers amateurs pendant leurs activités dans les jardins. Ce résultat interroge car il semble de prime abord traduire le fait que les jardiniers n'ont pas pris conscience, bien qu'il soit encore minime en France métropolitaine, du danger potentiel que représente ce moustique pour la santé. Or, il est important de souligner que le faible pourcentage de personnes interrogées exprimant des inquiétudes quant au risque de contracter une maladie par la piqûre de ce moustique ne semblait pas être attribuable à un manque de connaissances. Au total, 88,5 % des jardiniers interrogés étaient conscients que les moustiques peuvent être vecteurs de maladies.



Figure 10. Exemple de la gestion de l'eau de pluie dans 4 jardins collectifs de la métropole de Lyon. Les photographies **A** et **B** montrent des réservoirs d'eau stagnante non couverts permettant la colonisation par le moustique tigre. Les photographies **C** et **D** illustrent une gestion de l'eau de pluie exemplaire empêchant ainsi la colonisation par ce moustique. Photographies prises sur le terrain en 2021 lors des prélèvements.

En effet, la faible exposition aux maladies en France induit une faible préoccupation par rapport à celles-ci. Ces résultats confirment les observations déjà faites par ailleurs auprès de résidents sélectionnés aléatoirement (Davidson et al. 2016; Stefopoulou et al. 2018), de résidents choisis en fonction leurs origines (Caputo et al. 2020) ou de personnes ayant signalé la présence de moustique (Heym et al. 2017), tous vivant en zones non endémiques. Il est important de noter que certaines de ces études datent, et il est possible que la perception des risques ait évolué aujourd'hui avec l'augmentation des cas de maladies causées par les moustiques. Il est possible que le risque sanitaire associé au moustique tigre ne soit pas perçu avec gravité ou suscite peu d'inquiétude au sein de la population française, qui a confiance dans le système de santé français (Claeys and Mieulet 2013). En règle générale, les personnes interrogées ont tendance à accorder une importance beaucoup plus marquée à l'impact immédiat sur leur qualité de vie plutôt qu'au risque sanitaire potentiel. Changer la perception des personnes semble alors être une piste pour motiver les adhérents aux jardins à lutter activement contre le moustique. En effet, la perception du risque, la sévérité et la fréquence des maladies, est un facteur déterminant pour motiver les personnes à prendre des mesures de précaution (Ferrer and Klein 2015). Susciter une prise de conscience collective du risque d'émergence de maladies vectorielles pourrait favoriser de meilleures pratiques de lutte.

Notre enquête révèle également que le niveau de connaissance sur le moustique n'influence pas significativement les changements de comportements ni les pratiques des adhérents aux jardins. Cependant, bien que la majorité d'entre eux pensaient savoir l'identifier, moins d'un tiers connaissait ses caractéristiques distinctives. Une étude démontre que la capacité de reconnaissance visuelle de ce moustique joue un rôle crucial en tant que prédicteur du comportement de protection (Raude et al. 2012). Il est donc utile de promouvoir la sensibilisation à l'identification précise du moustique tigre par rapport à d'autres espèces de moustiques autochtones, étant donné son importance dans le risque d'émergence de futures épidémies.

Cette enquête montre aussi que le niveau de préoccupation des jardiniers influence, plus que les connaissances, leurs pratiques pour faire face au moustique tigre. En effet, plus ils sont préoccupés, plus ils modifient leurs pratiques (par exemple, l'heure de venue dans le jardin) et plus ils agissent contre le moustique. Ainsi, près de 60 % des participants se disant fortement inquiétés par le moustique tigre prenaient fréquemment des mesures pour lutter contre sa propagation, alors ceux qui ne s'en préoccupaient pas du tout (seulement 4 % d'entre eux) déclaraient agir de manière similaire. Par ailleurs, les personnes qui ne manifestaient aucune

préoccupation à l'égard du moustique tigre étaient nettement surreprésentées parmi les répondants qui n'avaient modifié aucune de leurs pratiques en réponse à ce dernier. A l'instar de plusieurs études, ces résultats montrent effectivement que l'exposition importante aux moustiques, notamment la gêne occasionnée par les piqûres motive les comportements de protection (Raude et al. 2012; Gaillard et al. 2019; Constant et al. 2020).

Dans le contexte actuel d'augmentation des espaces verts en milieu urbain et d'une menace croissante d'épidémies de maladies transmises par les moustiques du genre *Aedes*, cette enquête, auprès des adhérents aux jardins collectifs de la Métropole de Lyon, a mis en évidence des lacunes dans l'identification des caractéristiques morphologiques du moustique tigre et une faible perception des risques associés à sa présence. Prendre en compte et améliorer plus spécifiquement ces aspects lors de campagnes de sensibilisation serait essentiel pour réduire les nuisances occasionnées par le moustique mais également prévenir l'émergence d'arboviroses. De manière plus générale, la problématique liée à la prolifération des espèces nuisibles dans les espaces verts urbains devrait être mieux encadrée par les pouvoirs publics (Pons et al. 2006). Augmenter la conscience collective face au risque sanitaire représente alors une piste intéressante pour motiver les citoyens à lutter contre le moustique tigre. Ces jardins, où les activités pédagogiques sont très fréquentes, offrent un levier intéressant pour promouvoir la sensibilisation axée sur l'engagement communautaire (par exemple, le partage des connaissances, les pratiques préventives). Nous avons d'ailleurs profité de cette enquête pour évaluer si les adhérents aux jardins seraient motivés pour devenir acteurs dans la lutte contre le moustique en participant à un projet de science participative. Les résultats ont montré que 87,3 % d'entre eux y serait favorable, traduisant une volonté de s'investir collectivement pour atteindre un même objectif.

Enfin, ce travail est le fruit d'une enquête très localisée, ciblée sur les adhérents aux jardins collectifs du Grand Lyon. Il serait intéressant de mettre en place ce type d'enquêtes dans d'autres villes où le moustique est présent depuis plus longtemps. Cela nous permettrait d'évaluer l'influence d'une exposition plus ancienne sur les connaissances, les attitudes et les pratiques de cette population cible.

2. Influence des caractéristiques physico-chimiques et microbiennes de l'eau sur la colonisation des habitats aquatiques par le moustique tigre

2.1 Contexte et objectif général de l'étude

Les jardins collectifs sont devenus au cours des dernières années des initiatives grandissantes pour promouvoir les espaces agricoles urbains et apporter plus de nature en ville (Mathis and Pépy 2017). Malgré leurs bienfaits incontestables pour les citoyens, ces jardins présentent différents réservoirs d'eau stagnante propices au développement des larves de moustiques tigre (Hayden et al. 2010; Duval et al. 2022). Adopter de bonnes pratiques de gestion de cette espèce est un moyen efficace pour lutter efficacement contre sa prolifération. Cela implique de couvrir les réservoirs d'eau potentiels ou bien d'éliminer leur présence. Cependant, ces pratiques ne sont pas toujours mises en place (voir chapitre précédent). Il est donc important de poursuivre les efforts pour comprendre les facteurs qui influent sur la colonisation et le développement des larves dans ces habitats aquatiques (Hery et al. 2021b). Chez de nombreuses espèces de moustiques, il est maintenant admis que la composition biotique et abiotique de l'eau sont des caractéristiques importantes qui impactent fortement le développement des larves et se répercutent sur les caractéristiques physiologiques et morphologiques du moustique à l'âge adulte (Hery et al. 2021b). Ces propriétés peuvent notamment influencer le comportement de ponte des femelles et jouer un rôle important dans le développement, la survie et le succès reproducteur des moustiques (Anderson and Davis 2014; Shragai et al. 2019; Früh et al. 2020; Borel et al. 2021; Hery et al. 2021a). La composition microbienne du gîte influence aussi le microbiote des larves avec dans certains cas des effets reports chez les adultes, pouvant interférer avec leur compétence vectorielle (Coon et al. 2014; Guégan et al. 2018; Zheng et al. 2023). En ville, ces habitats aquatiques sont directement exposés à différentes sources de pollution. Plus spécifiquement, les sols des jardins collectifs peuvent également être pollués par la présence de métaux lourds et d'arsenic (voir chapitre 2.1.3) (Kessler 2013; Jean-Soro et al. 2015; Izquierdo et al. 2015; Al-Delaimy and Webb 2017). Egalement, plusieurs études montrent la présence de fertilisants comme l'ammonium, le potassium et le sulfate dans l'eau des gîtes larvaires (Mutero et al. 2004; Muturi et al. 2016; Darriet 2018b). Pour autant, à notre connaissance, l'influence qu'exerce la pollution urbaine sur les propriétés biotiques et abiotiques des gîtes larvaires n'a encore jamais été étudiée.

Compte tenu de ces informations, une meilleure caractérisation des propriétés biotiques et abiotiques des gîtes colonisés par le moustique tigre en milieu urbain pourrait permettre d'identifier certains facteurs favorisant leur colonisation et leur prolifération.

Dans ce contexte, l'objectif de ce travail, mené en parallèle de l'étude CAP, était d'évaluer l'influence de l'exposition à des pollutions urbaines sur la colonisation des gîtes larvaires par le moustique tigre. Pour cela nous avons tiré profit de la précédente étude CAP dans laquelle nous avons recensé et cartographié les jardins collectifs de la métropole de Lyon. Ceci nous a permis de sélectionner un sous échantillon de ces jardins sur la base de leur proximité à différentes sources de pollution d'origine agricole, industrielle ou atmosphérique. Nous avons ensuite analysé la composition physico-chimique et microbiologique de réservoirs d'eaux colonisées ou non par le moustique ainsi que la composition du microbiote des larves. La composition des communautés bactériennes, fongiques et micro-eucaryotes a été caractérisée par séquençage à haut débit. Les propriétés physico-chimiques de l'eau (pH, température, conductivité, potentiel d'oxydo-réduction, turbidité et teneur en carbone) ont été caractérisées à l'aide d'une sonde multiparamétrique et de chromatographies en phase gazeuse et ionique. La présence de molécules organiques a également été analysée via une approche sans a priori grâce à un analyseur laser couplé à un spectromètre de masse à temps de vol. Par la suite, nous avons caractérisé chaque échantillon d'eau en lui attribuant un score de pollution (prenant en compte leur proximité aux différentes sources de pollution et leurs propriétés physico-chimiques) et un profil basé sur sa composition en molécules organiques. L'effet de ces deux gradients de pollution (caractérisés soit par les propriétés physico-chimiques de l'eau combinées aux variables de pollution soit par la présence de molécules organiques seule) sur la composition microbienne de l'eau en fonction de la présence ou de l'absence des moustiques *Ae. albopictus*.

2.2 Publication n°4 “Pollution gradients contribute to the microbial and physicochemical composition of *Ae. albopictus* larval habitats in urban community gardens”

Cet article a été soumis dans le journal *Microbiome* - Soumission n°7b338735-8ef4-4b7e-9e8d-ab95dd8d8982.

Pollution gradients shape microbial communities associated with *Ae. albopictus* larval habitats in urban community gardens

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Abstract

The Asian tiger mosquito *Aedes albopictus* is well adapted to urban environments and takes advantage of the artificial containers that proliferate in anthropized landscapes. Little is known about the physicochemical, pollutant and microbiota compositions of *Ae. albopictus*-colonized aquatic habitats and whether these properties differ with noncolonized habitats. We specifically addressed this question in French community gardens by investigating whether pollution gradients (characterized either by water physicochemical properties combined with pollution variables or by the presence of organic

molecules in water) influence water microbial composition and then the presence/absence of *Ae. albopictus* mosquitoes. Interestingly, we showed that the physicochemical and microbial compositions of noncolonized and colonized waters did not significantly differ, with the exception of N₂O and CH₄ concentrations, which were higher in noncolonized water samples. Moreover, the microbial composition of larval habitats covaried differentially along the pollution gradients according to colonization status. This study opens new avenues on the impact of pollution on mosquito habitats in urban areas and raises questions on the influence of biotic and abiotic interactions on adult life history traits and their ability to transmit pathogens to humans.

Key words: *Aedes albopictus*, pollution, urbanization, water, microbial communities, organic molecules, physicochemical parameters

1. Introduction

Urban areas exceeded half of the planet for the first time in 2009 (DESA 2010). The rapid rise in the size, density and heterogeneity of urban areas has had deep impacts on urban populations, biodiversity and climate (Vlahov 2002; Li et al. 2022; Khanh et al. 2023). Rapid urban expansion has led to an increase in poverty, social inequality, temperature (through the urban heat island –(UHI) effect), and homogenization of biodiversity and has also generated much waste and pollution (Kalnay and Cai 2003; McKinney 2006; Dociu and Dunarintu 2012; Liddle 2017; Liang et al. 2019). A promising solution for reducing the negative impacts of urbanization is the creation of green areas and addition of vegetation in cities (Susca et al. 2011; Gago et al. 2013; Pascal et al. 2019). Urban green areas are efficient in remedying UHIs by cooling effects in cities (Norton et al. 2015). In addition, vegetation and green spaces have been shown to reduce atmospheric pollution, have revived biodiversity and have had a positive impact on human health by attenuating mortality and improving mental health through increasing physical activities (Lepczyk et al. 2017; Kondo et al. 2018; Nunho Dos Reis et al. 2022; Royer et al. 2023). Green urban spaces include different types ranging from parks and gardens to cemeteries and derelict lands (Catalano et al. 2021). Among them, community gardens are increasingly implemented in European cities (Ochoa et al. 2019). The concept of the family garden (formerly known as workers' gardens) emerged during the Industrial Revolution, in a period of intense urbanization to mitigate the problem of the precariousness of workers (Keshavarz et al. 2016). Currently, diverse types of community gardens exist, e.g., family gardens, shared gardens and integrated gardens. These urban gardens are managed by different organizations, including metropolises, municipalities, private organizations and nonprofit associations (Holland 2004).

Although communal gardening activities and recognition of their perceived benefits have a long history (Schmelzkopf 1995; Draper and Freedman 2010; Al-Delaimy and Webb 2017; Torres et al. 2018), recent studies have also pointed out potential negative impacts on the health and wellbeing of gardeners, such as increasing physical injury and inequitable input and sharing of resources. For instance, some studies highlighted the presence of heavy metals in the soil that accumulate in vegetables

and are then ingested by gardeners (Lovell et al. 2014; Izquierdo et al. 2015; Al-Delaimy and Webb 2017; Malone 2022; Yan et al. 2022). The role of community gardens in promoting the spread of invasive alien species and pests that could transmit human diseases and thus have a negative impact on the economy, ecosystems and/or local species was also recently questioned (Al-Delaimy and Webb 2017). For instance, it was shown that 2.4% of vegetables from European common gardens were colonized by eggs of *Toxocara* (i.e., a roundworm parasite of domestic animals that occasionally infects humans) (Lindsay and Dubey 2007; Ma et al. 2018; Healy et al. 2022). In addition, a high density and diversity of still water containers can be observed in community gardens (e.g., watering cans, flower buddies, or rainwater collectors) that offer suitable habitats for several mosquitoes, particularly the Asian tiger mosquito (Hayden et al. 2010, Duval et al. 2022). The proliferation of this mosquito species constitutes a major public health challenge due to its ability to transmit more than 19 arboviruses, such as dengue, zika and chikungunya, in humans (Paupy et al. 2009; Duval et al. 2023). Previous studies demonstrated that the number of larval habitats and the development and survival rates of *Ae. albopictus* were positively impacted by the urban context (Li et al. 2014; Wilke et al. 2019; Westby et al. 2021). In urban areas, this species breeds in human-made containers, where water could be exposed to various sources of pollutants. The biotic and abiotic characteristics of larval habitats determine the choice by gravid females for oviposition and obviously affect the development of offspring, from larvae to adults (Afify and Galizia 2015; Malassigné et al. 2020; Hery et al. 2021a; Dalpadado et al. 2022). For instance, physicochemical parameters such as pH, temperature, organic matter, phosphate, ammonia and potassium are known to guide females to choose egg-laying sites (Darriet 2019). In addition, microbial communities produce volatile organic compounds that can be attractive or repulsive to mosquitoes (Díaz-Nieto et al. 2016; Weisskopf et al. 2021). More globally, the microbial structure in breeding sites partly shapes the larval microbiota and can have carryover effects on the physiology of adults and their ability to transmit pathogens (Dickson et al. 2017; Guégan et al. 2018; Zheng et al. 2023). However, reciprocal interactions between physicochemical parameters and microbial structure in artificial breeding sites and their impact on the mosquito life cycle are still poorly understood.

In mainland France (Europe), since the first identification of autochthonous cases of dengue in 2010, viral diseases transmitted by mosquitoes have tended to increase each year, as well as the number of regions at risk due to the continuous spread of the Asian tiger mosquito (Cochet et al. 2022). Following previous observations, the aim of this study was to evaluate whether and how human activities, particularly pollution gradients, impact the biotic and abiotic properties of *Ae. albopictus* larval breeding sites in community gardens in the Lyon metropolis, one of the largest and most populous cities in mainland France. For this purpose, we analysed the physicochemical and microbiological composition of water by comparing *Ae. albopictus*-colonized and *Ae. albopictus*-noncolonized waters as well as the microbiota composition of larvae. Microbial communities were analysed by characterizing bacterial and microeukaryotic communities through high-throughput sequencing. Physicochemical water properties (i.e., pH, temperature, conductivity, oxidation–reduction potential, turbidity and carbon content) and organic molecules were also characterized by using multiparameter probes, gas and ion chromatography and liquid chromatography coupled with high-resolution mass spectrometry (LC-HRMS). We then evaluated whether pollution gradients (characterized either by water physicochemical properties combined with pollution variables reflecting proximity to polluted areas or by organic molecule presence in water) influence water microbial composition depending on the presence/absence of *Ae. albopictus* mosquitoes. While microbial and physicochemical compositions were very similar between colonized and noncolonized water samples, our results revealed differential effects of pollution gradients on microbial community structure according to water colonization status.

2. Material and Methods

2.1. Selection and delineation of the study areas

By taking advantage of our recent database, in which we have inventoried a total of 288 community gardens in the metropolitan area of Lyon (Duval et al. 2022), we characterized the gardens on the basis

of different criteria: the proximity to different pollution sources, the surface of nearby agricultural areas and the level of atmospheric pollution. The proximity with highways and agricultural lands were defined thanks to the BD CARTO® of French national mapping agency (www.geoportail.gouv.fr), while that of industrial areas was characterized using an open platform for French public data (www.data.gouv.fr). The surface of agricultural areas was calculated from satellite images of Google Earth using QGIS (www.qgis.org). In addition, air pollution through emission data of NO₂ and particular matter (PM_{2.5} and PM₁₀) was provided by Atmo Auvergne-Rhône-Alpes (www.atmo-auvergnerhonealpes.fr) the local authority for air quality as 1 x 1 grid, in 2021. From this selection process, field sampling was carried in gardens if they met the following conditions: garden closest to one pollution source (atmospheric, industrial or agricultural) (**Table 1**), garden surface area > 1km², access authorization and presence of *Ae. albopictus* colonized and noncolonized waters. Pollution variables reflecting each pollution source i.e. atmospheric (Var_atmo), agricultural (Var_agri) or industrial pollution (Var_indus) were then calculated for each garden using the following formula:

$$Var_atmo = \frac{qNO_2 + qPM_{2.5} + qPM_{10}}{dH}$$

Where ‘qNO₂’, ‘qPM_{2.5}’ and ‘qPM₁₀’ correspond to air pollution scores ranging from 1 (the first quartile of gardens with the lowest pollution rates) to 4 (the last quartile of gardens with the highest pollution rates) and ‘dH’ corresponds to the distance between the garden and the nearest highway.

$$Var_agri = \frac{surface^2}{dA}$$

Where ‘surface’ corresponds to the area of the nearest agricultural area and ‘dA’ corresponds to the distance between the garden and the nearest agricultural area.

$$Var_indus = \frac{1}{dI}$$

Where ‘dI’ corresponds to the distance between the garden and the nearest industrial area.

Atmospheric, industrial and agricultural pollution variables were normalized for each community garden using the following formula:

$$\frac{X - \min}{\max - \min}$$

Where 'X' corresponds to calculated pollution variables and 'min' and 'max' correspond to the minimum and maximum variables for the same pollution context.

2.2. Sample collection

Field sampling was performed in 23 community gardens of Lyon metropolis between June and September 2021. For each garden, *Ae. albopictus* larval habitats were sampled in the morning, by collecting water from a colonized and a noncolonized reservoir (referred hereafter as C and NC, respectively) as well as *Ae. albopictus* 3rd and 4th instar larvae (n=50 per breeding site) (referred hereafter as L). Colonized waters were identified by the presence of both *Ae. albopictus* larvae in water and adults flying around the reservoir. Larvae were identified using morphological identification keys of Darsie and Ward (1981) (Kline et al. 2006) and then verified by COI barcoding by randomly selected 4 larvae per colonized water, as previously described (Raharimalala et al. 2012). Polymerase Chain Reaction (PCR) products were sent to Sanger sequencing at Microsynth France SAS (Vaulx-en-Velin). Regarding noncolonized waters, the entire volume of water in the container was filtered to ensure the absence of larvae. For each selected container (C and NC), water was mixed thoroughly before sampling and the water temperature (°C), pH, dissolved oxygen (mg/L), oxidation-reduction potential (mV) and electrical conductivity (µS/cm) were measured directly in the field with a portable multiparameter water probe (Horiba, U-50, France). Water samples (1 L) were collected and split into one sterile 50 mL Falcon tube (Greiner, Germany) for microbial analysis (40 mL) and three HDPE bottles (VWR, Radnor, USA) for chemical analysis (400 mL, 250 mL and 250 mL), respectively. Third- and fourth-instar larvae were collected with a sterile plastic pipette into a sterile 50 mL Falcon tube (Greiner) and then transferred

individually to the laboratory in sterile 1.5 mL micro-centrifuge tubes (Sarstedt, Germany). Water samples were stored at -20°C and defrosted before each analysis except for ion chromatography analyses where samples were filtered at $0.22\ \mu\text{m}$ before freezing. The process of field water sampling is detailed in **Figure 1**.

2.3. DNA extraction

Water samples were homogenized by vortexing for 1 min and a 2 mL aliquot was centrifuged for 10 min at maximum speed (13 400 rpm) at 4°C . The supernatant was discarded and a new aliquot of 2 mL was added. This step was repeated 3 times for each sample. Pooled pellets were resuspended in $250\ \mu\text{L}$ of CTAB buffer (2% hexadecyltrimethyl ammonium bromide, 1.4 M NaCl, 0.02 M EDTA, 0.1 M Tris pH8, 0.2% 2- β mercaptoethanol). After incubation for 1h at 60°C in a shaker (300 rpm), $4\ \mu\text{L}$ of RNase (100 mg/mL) was added and incubated at 37°C for 5 min. Mixtures of phenol:chloroform:isoamyl alcohol (25:24:1) and chloroform:isoamyl alcohol (24:1) were added and after vortexing for 5 min, samples was centrifuged for 30 min at 13 200 rpm at room temperature, DNA was precipitated using isopropyl alcohol and Glycoblue coprecipitant (15 mg/mL). DNA pellets were rinsed with 75% cold ethanol, air-dried and resuspended in $24\ \mu\text{L}$ of sterile RNase free water. Prior to DNA extraction, larvae were surface-sterilized as previously described (Zouache et al. 2022). Each larva was crushed for 1min using a FastPrep-24TM 5G (MP Biomedical) into a sterile 2 mL screw-cap tube containing 3 beads of 3 mm diameter (Merck) and $250\ \mu\text{L}$ of CTAB buffer. Larvae DNA extractions were then performed as previously described for water samples. DNA extractions from larvae and water were performed in 23 batches (one DNA extraction per garden) and a negative control (i.e. extraction performed without biological matrix) was included in each batch. DNA extracts purity and concentration were estimated using the NanoPhotometer NP80 (Implen) and the Qubit dsDNA High Sensibility kit combined with the Qubit 4 fluorometer (Invitrogen), respectively.

2.4. Library preparation and high throughput sequencing

DNA samples from water and larvae were used as templates to systematically amplify three gene markers. The V5-V6 region of the 16S rRNA genes of Bacteria was amplified using the universal primers 784F/1061R (Andersson et al. 2008), whereas the V1-V2 region of the 18S rRNA genes of Eukaryotes was amplified using the universal primers Euk82F/Euk516R (Thongsripong et al. 2018) (**Supplementary Table S1**). The fungal nuclear ribosomal internal transcribed spacer (ITS2 region) was amplified using the universal primers gITS7 / ITS4 (Ihrmark et al. 2012) (**Supplementary Table S1**). PCR amplifications were also performed on 10 larvae per garden for bacterial and fungal markers. Primers were tagged with the Illumina adapters 5'-GTC TCG TGG GCT CGG AGA TGT GTA TAA GAG ACAG-3' and 5'-TCG TCG GCA GCG TCA GAT GTG TAT AAG AGA CAG-3', enabling a two-step PCR construction of amplicon libraries. PCR amplifications were conducted in duplicate in a Bio-Rad T1000 thermal cycler (Bio-Rad, Hercules, USA) with 5× HOT BioAmp master mix (Biofidal, France) containing 2 µL sample DNA and 1 µM of each primer as previously described (Zouache et al. 2022; Girard et al. 2023) (**Supplementary Table S2**). For recalcitrant samples, PCR additives such as 10× of GC rich Enhancer and 0.5 mg/mL of bovine serum albumin (New England Biolabs, Evry, France) were added to the PCR mix. Amplicons were checked by electrophoresis on 1.5% agarose 20 min at 100 V and UV visualization. The PCR products were then sent to Microsynth sequencing company (Balgach, Switzerland) for purification and second-step PCR, 2x300bp Miseq sequencing (Illumina). DNA matrix that did not amplify during the first PCR reaction was used as templates for quantitative PCR, as previously described (Girard et al. 2023).

2.5. Bioinformatics analysis

A total of 7,325,894; 9,591,794 and 2,311,285 reads were obtained for each dataset (i.e. bacterial 16S rRNA genes, fungal ITS2 and eukaryotic 18S rRNA genes, respectively) and paired-end reads were

demultiplexed in the different samples. R1 and R2 reads were merged using Fastp, based on 5 mismatches in the overlap region. Sequence quality control and analysis of sequence data were carried out using the FROGS pipeline (Escudié et al. 2018). Briefly, denoising was carried out by discarding reads without the expected length of that displayed ambiguous bases (N). Clustering was performed using SWARM (Mahé et al. 2014) based on an aggregation distance of 3 for identification of operational taxonomic units (OTUs). Chimeric OTUs were discarded using VSEARCH (Rognes et al. 2016) and sequences of low abundance were filtered at 0.005% of all sequences (Bokulich et al. 2013). The alignment and OTU affiliation were performed using Silva v.138.1 database for Bacteria and Eukarya (Quast et al. 2012) and the UNITE 8.2 database for fungi (Nilsson et al. 2019). Reads that did not align were filtered out and contaminant OTUs were filtered out using negative control (negative control of extraction and PCR). OTUs were removed if they were detected in the negative control sample and their relative abundance was not at least 10 times greater than that observed in the negative control (Minard et al. 2015). Normalization for sample comparison was performed by randomly resampling down to 4 103, 6 567 and 7 204 sequences in the bacterial, fungal and eukaryotic datasets, respectively.

2.6. Gas and ion chromatography

For gas chromatography analysis, water samples were thawed at room temperature then transferred into internal flask gases and 50 mL of Helium was added. The flasks were incubated at 60°C for 3h at 150rpm. After incubation, gas present in water samples were measured using a gas chromatograph (μ GC R990, SRA Instrument, Marcy L'Etoile, France) with MS5A SS columns TCD detector (10m*0.25mm*30 μ m BF) and PPQ (μ m 10m*0.25mm*8 μ m) and 48 channels custom automatic sample changer (SRA Instrument, Marcy L'Etoile, France). A calibration mix solution was prepared using helium combined with each dissolved gas, with concentrations ranging from 0 to 14 000 ppm for CO₂, 0 to 50 000 ppm for N₂O, 0 to 100 000 ppm for CH₄, 0 to 79 % for N₂ and 0 to 21% for O₂. The ions present in water samples were analyzed by an ionic chromatograph (AQUION) with automatic sample

changer (AS-AP 120). Anion concentrations were measured on pre-column and column (AS9-HC, 4*250 mm) at 30°C and eluted with 9 mM Na₂CO₃ with a flow rate of 1 mL/min and a 4 mm carbonate suppressor (AERS 500). Cation concentrations were evaluated on pre-column and column (CS-19, 4*250 mm) at 40°C and eluted with 30 mM methanesulfonic acid with a flow rate of 1 mL/min and a 4 mm suppressor (CDRS 600). Both ions were detected with a conductimeter (Thermofischer Electron SAS Courtaboeuf 91941). Calibration standards were prepared for each ion with concentrations ranging from 0 (ultra-pure water only) to 500 mg/L. Anions (F⁻, Cl⁻, NO₂⁻, Br⁻, NO₃⁻, PO₄³⁻, SO₄³⁻) were prepared by diluting a more concentrated stock solution (1 g/L) from NaF, KCl, NaNO₂, KBr, KNO₃, Na₃PO₄·12H₂O and K₂SO₄. Cations (Na⁺, NH₄⁺, K⁺, Mg²⁺, Ca²⁺) were prepared by diluting a more concentrated stock solution (1 g/L) from NaCl, (NH₄)₂SO₄, KCl, MgCl₂·6H₂O and CaCl₂·2H₂O.

2.7. UPLC-HRMS analysis

In these analyses, the 23 gardens were classified into three groups according to their proximity to different pollution sources and the presence of atmospheric pollutants (**Table 1**). An aliquot of each water sample (5 mL) was prepared by liquid-liquid extraction with 5 mL acetonitrile acidified with 0.1 % formic acid. Each sample was fortified with a labelled internal standard (diuron-d6), then ultrasonicated, centrifuged and frozen overnight at -18°C. A 2.5 mL aliquot was retrieved. A volume of 500 µL of each sample extract was pooled to prepare a quality control sample (QC). Then the 2 mL left extracts were dried under a slight nitrogen flow at 40°C. Each dry extract was reconstituted in 200 µL of water/methanol 90/10, v/v. Moreover, to ensure the reliability of the results, each sample was extracted and analyzed 3 times. Extracts with ultra-pure water were prepared with the same protocol and considered as blank matrix. Analyses by LC-HRMS were performed on an Ultimate 3000 UHPLC system (Thermo Scientific®, MA, USA) coupled to a quadrupole time-of-flight mass spectrometer (QToF) (Maxis Plus, Bruker Daltonics®, Bremen, Germany) equipped with an electrospray ionization interface (ESI). Analyses were carried out in reverse phase (elution gradient) employing an Acclaim

RSLC 120 C18 column (2.2 μm , 100 \times 2.1 mm, ThermoScientific®), protected with a KrudKatcher Ultra In-Line Filter guard column from Phenomenex (Torrance, CA, USA) and heated at 30°C. The injected volume was 5 μL . Mobile phases consisted of: an aqueous phase (90%/10% ultrapure water/methanol mixture with 5 mM ammonium formate and 0.01% formic acid) and an organic phase (methanol with 5 mM ammonium formate and 0.01% formic acid). All extracts were analyzed in positive electrospray ionization with the following settings: capillary voltage of 3600 V, end plate offset of 500 V, nebulizer pressure of 3 bar (N_2), drying gas of 9 L/min (N_2), and drying temperature of 200 °C. An external calibration of exact masses was systematically performed at the beginning of each run, using a solution of sodium formate and acetate (0.5 mL of 1 M NaOH, 25 μL of formic acid and 75 μL of acetic acid in H_2O /isopropanol 50/50 v/v), generating cluster ions $[\text{M}+\text{H}]^+$ in the range 90.9766–948.8727 Da with high precision calibration (HPC) mode at a search range ± 0.05 m/z. Accepted standard deviations were inferior to 0.5 ppm. QCs were run several times at the beginning of the analytical sequence to equilibrate the column and at regular intervals throughout the sequence to check for instrument drift and to control analytical repeatability and sensitivity. Instrument control, data acquisition, and processing were performed using OTOF Control and Hystar™ (v4.1, Bruker Daltonics®) software. Samples were randomly analyzed in full-MS scan over a m/z range of 50-1000 Da. QCs were also analyzed in DDA (Data Dependent Analysis), a mode with alternative acquisition in MS and MSMS, with predefined parameters. The data preprocessing was performed with W4M and Metaboscape (Giacomini et al. 2015). A feature matrix was generated for each sequence. It contained 1382 features for which the mass to charge ratio m/z in ion adduct form, the retention time RT of each detected peak, the intensity in each sample. Each feature was then normalized to the intensity of diuron-d6. It was discarded from the dataset when the coefficient of variation in the QCs (CVQC) was over 30%. Missing intensities in a replicate were replaced by the mean intensities in the two others replicate or by 0 if not available. Then for each group of gardens (**Table 1**), a detection rate was calculated. If it was less than 100%, the feature wasn't considered afterwards. Signals that were not significantly higher in samples compared to blanks (intensity ratio > 10), and were not significantly different between C and NC (t-test $p > 0.05$) were not considered. Annotation and identification were performed using Metaboscape and MetFrag (Wolf et al. 2010) an *in silico* fragmentation tool, by comparing experimental

MSMS spectra. Using these filters, a list of 147 LC-HRMS signals was kept on the 1382 features. Finally markers were putatively identified according to the level of identification confidence from 1 to 5 (Schymanski et al. 2015) (**Table 2**).

2.8. Total organic carbon analysis and measurement

Total Organic Carbon (TOC) was performed on a Shimadzu TOC 5050 (Kyoto, Japan). From 16 to 54 μL of samples were injected in a vertical combustion tube held at 680°C and half filled with platinum-based catalysis in a flow of 150 mL/min. Total Carbon (TC) was converted into CO_2 and measured on Non-Dispersive Infrared (NDIR) CO_2 detector. Inorganic Carbon (IC) was measured by the same amount of injection in a vessel containing phosphoric acid at a concentration of 25%. Carbon contained in carbonates was converted into CO_2 and quantified on the same infrared detector. TOC concentration was obtained by the difference between TC and IC concentrations.

2.9. Statistical and data analysis

Data analysis and statistical tests were carried out with R Statistical Software (v4.1.1, R Core Team 2018), using the *vegan* package for diversity analyses (Oksanen et al. 2018) and the *ggplot2* package for graphical representations (Wickham 2016). Heatmap graphs were plotted with the *complexHeatmap* package (Gu et al. 2016). Prokaryotic, fungal and eukaryotic communities within-sample diversity also referred as alpha-diversity was estimated with the Shannon index (H') while community dissimilarity also referred as beta diversity was estimated with the Bray-Curtis index. Shannon index was tested using either nonparametric tests (Kruskal-Wallis and paired Wilcoxon signed rank tests with Benjamini-Hochberg correction) for bacteria or parametric tests (ANOVA and Tukey HSD post hoc tests) for fungi and other microeukaryotes. Beta diversity was analyzed using permutational multivariate analysis

(adonis2, R package *vegan*) (Legendre and Anderson 1999). Non-metric multidimensional scaling (NMDS) plots using Bray-Curtis dissimilarity matrices were used to visualize beta diversity of microbial communities. Comparison of physicochemical parameters and organic molecule occurrence between NC and C was performed with partial least squares discriminant analysis (PLS-DA) with *mixOmics*, R package (Rohart et al. 2017). The influence of the breeding site colonization status on the 5 most discriminant physicochemical parameters was tested by linear mixed model (lmm) after correcting for variations between inter-individual garden effect. Considering organic molecules, data were not analyzed with lmm because the residuals of the model were heteroskedasts and did not follow a normal distribution. We therefore tested them using Kruskal-Wallis rank sum test, a non-parametric test. Principal Components Analysis (PCA) were performed for NC and C samples independently in function of either physicochemical combined with pollution variables or organic molecule water occurrence. Monte Carlo test on the sum of the singular values of a procustean rotation was performed to test the concordance of each dataset. To analyze whether the abiotic factors of NC and C (either physicochemical and pollutant variables or organic molecule occurrence) were correlated with the microbial communities, we performed the same analysis. Then, we asked what was the proportion of the microbial communities within NC and C that could be explained by major variations in the abiotic factors. To that end, we have used the two first components (PC1_poll/PC2_poll or PC1_mp/PC2_mp, for physicochemical and pollution variables or organic molecules, respectively) of the abiotic factors' PCA as an explanatory variable for a PERMANOVA analysis of the microbial communities Bray Curtis dissimilarities. Then, we performed distance-based Redundancy Analyses (dbRDA) as constrained multivariate analyses to represent the microbial OTUs that were mostly influenced by PC1_poll/PC2_poll or PC1_mp/PC2_mp. Those OTUs were correlated with PC1_poll/PC2_poll or PC1_mp/PC2_mp using Spearman's rank correlations.

Then, the objective was to develop a method allowing to determine relationships between variables of different natures, including categorical, numerical, and not following normal distributions, and then to visualize them with graph representations, in which nodes represent privileged relationships between those variables. To that end, we used a python code and visualized with the pyvis library. For each numerical variable (OTUs, Water T°, LC-HRMS data, etc.), we converted it into a binary value,

such as exceptionally high values were identified as 1, and average or low values were identified as 0. Given the non-normal distribution of values, we used a robust variant of the z-score to identify exceptionally high values. More precisely, we computed for each variable the so-called modified z-score, computed using the median absolute deviation to the median instead of the standard deviation.

Each value x_i is thus transformed using the following formula:

$$\frac{x_i - \text{med}(x)}{MAD}$$

Where ‘med(x)’ corresponds to the median of variable ‘x’ and ‘MAD’ the median absolute deviation.

We then defined a threshold above which a value is considered exceptional. In the second step, we used association rule scores to identify strong associations. More precisely we used Zhang’s score to assess how strongly related a variable is compared with another one (https://rasbt.github.io/mlxtend/user_guide/frequent_patterns/association_rules/). Zhang’s score is defined between -1 and +1, with 0 meaning no particular association. Here again, we defined a threshold to keep only values above a given threshold, i.e., having a strong enough association. Zhang’s score does not include statistical significance, so we add a statistical test (Binomial test, p-value < 0.05) *a posteriori* to assess the robustness of potential associations. An edge is added to the graph if the strength of the association is above the threshold and statistically significant. We used this method to avoid pitfalls with existing approaches.

3. Results

3.1. Noncolonized and colonized waters exhibit slight differences in physicochemical and organic molecule composition

Following our selection criteria (see the Materials and Methods section), a total of 23 gardens were selected for water sample collection. The characterization of each garden according to the level of atmospheric pollution, the calculated pollution variables and the distance from the garden to each pollution source is given in **Table 1**. The most common containers sampled were plastic rainwater

collectors (87%), followed by plastic buckets (9%) and plastic watering cans (4%) (**Table 3**). Water physicochemical properties, i.e., ionic and gas composition, TOC, temperature, pH, conductivity, redox potential, and dissolved oxygen, are all summarized in **Table 3** and **Table 4**. We conducted a PLS-DA supervised analysis to identify the most discriminant physicochemical parameters between C and NC (**Figure 2A**). Among the physicochemical properties, N₂O and CH₄ were significantly higher in NC than in C according to linear mixed model ($\chi^2 = 4.702$, $p = 0.03$ and $\chi^2 = 4.674$, $p = 0.03$, respectively) (**Figure 2B**). In fact, the average N₂O and CH₄ were two and nearly seven times higher in NC than in C, respectively. In a second analysis, NC and C samples were separated (**Figure 2C-D**) to compare their physicochemical composition through a Procrustean analysis. The abiotic factors (physicochemical factors and pollution variables) had no significant effects on colonization status (NC or C) (Monte Carlo permutation test, observation = 0.345, $p = 0.135$). Regarding organic molecule analysis, the application of a nontargeted approach involving liquid chromatography coupled with quadrupole time-of-flight mass spectrometry (LC-QToF) allowed the detection of compounds that were found to be significantly different between C and NC from the same garden (t-test $p < 0.05$) (**Supplementary Table S3**). Among them, 22 features were putatively identified (**Table 2**). However, PLS-DA performed with all 147 features did not reveal significant differences between C and NC regarding organic molecule presence ($p > 0.05$) (**Figure 3A-B**). When NC and C samples were separated (**Figure 3C-D**) through the Procrustes analysis, no significant effect was found depending on the colonization status (NC or C) (Monte Carlo permutation test, observation = 0.06, $p = 0.985$).

3.2. Fungal composition differs between noncolonized and colonized waters while microbial community structure is similar between larvae and water samples, regardless of colonization status

A total of 1373 bacterial OTUs belonging to 27 phyla and 329 genera were identified from the 23 gardens (23 NC, 23 C and their associated larvae). NC and C exhibited similar bacterial compositions at the phylum level (**Supplementary Figure S1A**). The predominant phylum was Proteobacteria (37%

and 34%), followed by Actinobacteriota (20% and 24%) and Firmicutes (17% and 12%) for NC and C, respectively (**Supplementary Figure S1A**). In larvae, the most abundant bacterial phylum was Proteobacteria (35%), followed by Actinobacteriota (24%) and Bacteroidota (17%) (**Supplementary Figure S1A**). At the genus level, 40%, 34% and 31% of the bacterial genera displayed low abundance (<1%) in NC, C and L, respectively (**Supplementary Figure S1B**). Among the 42 most abundant genera, 7 were common (e.g., *Rhizobium*, *Legionella*, and *Abditibacterium*), and others were specific to NC (6), C (7) and larvae (14) (**Supplementary Figure S1B**). Interestingly, *Actinomycetospora* and *Actinoplanes* were abundant in C and L but in low abundance in NC (<1%). The alpha diversities of the bacterial communities in NC and C were comparable (Wilcoxon pairwise; $p = 0.53$), while that in L was significantly lower than that in C (Wilcoxon pairwise; $p = 0.0012$) and NC (Wilcoxon pairwise; $p = 0.0005$) (**Supplementary Table S4**). Regarding the fungal community (i.e., ITS sequencing), a total of 18 gardens were analysed out of the 23 sampled gardens (with 18 NC, 18 C and their associated larvae). If the ITS sequencing preferentially targeted and revealed the presence of fungi (56.4%, 56.6% and 57.8%), other eukaryotic kingdoms such as Viridiplantae were also detected (15.4%, 16.4% and 15.8%) for NC, C and L, respectively, followed by 3 minority kingdoms (Rhizaria, Protista and Ichthyosporia) (data not shown). Regarding fungal communities, a total of 716 fungal OTUs belonging to 8 phyla and 264 genera were identified. *Ascomycota* was the predominant phylum (47%, 55% and 72% in NC, C and L, respectively), followed by Basidiomycota (27%, 24% and 16%) (**Supplementary Figure S2A**). The phylum Rozellomycota was the third most abundant phylum in NC and C (7% and 6%) but represented only 0.5% of the total fungi in L (**Supplementary Figure S2A**). At the genus level, 40%, 34% and 31% of the fungal genera displayed a low abundance (<1%) in NC, C and L, respectively (**Supplementary Figure S2B**). Only *Dioszegia* was common to NC and C, while *Phoma* was shared among NC, C and L (**Supplementary Figure S2B**). Interestingly, *Dacrymyces* and *Pyrenochaetopsis* were abundant in C and L but very low in abundance in NC (0.18% and 0.04%, respectively) (**Supplementary Figure S2C**). Comparison analysis performed with the Shannon index indicated no significant differences in the alpha diversity of fungal communities between NC and C (Tukey HSD post hoc tests, t ratio = 0.559, $df = 51$, $p = 0.84$) or between C and L (Tukey HSD post hoc tests, t ratio = -1.914, $df = 51$, $p = 0.14$) (**Supplementary Table S4**). Conversely,

the alpha diversity of fungal communities was significantly higher in L than in NC (Tukey HSD post hoc test, t ratio = 2.473, df = 51, p = 0.04) (**Supplementary Table S4**). Regarding eukaryotic communities, a total of 739 eukaryotic OTUs belonging to 38 phyla and 181 genera were identified among 13 gardens (13 NC and 13 C). In contrast to bacteria and fungi, the main eukaryotic phyla differed between NC and C. The predominant phylum was Ciliophora (22.5%), followed by Chlorophyta (18.4%) and Ochrophyta (10.2%), in NC, while the predominant phylum was Bicosoecida (19.5%), followed by Ochrophyta (14.6%) and Chlorophyta (9.8%), in C (**Supplementary Figure S3A**). At the genus level, 10.3% and 14.9% of the eukaryotic genera displayed low abundance (<1%) in NC and C, respectively (**Supplementary Figure S3B**). Among the 28 most abundant genera, three were common (*Sterkiella*, *Scotinosphaera* and *Goniomonas*), and others were specific to NC (13) and C (11) (**Supplementary Figure S3B**). The alpha diversities of the eukaryotic communities in NC and C were comparable (Tukey HSD post hoc test, p = 0.9) (**Supplementary Table S4**). Nonmetric multidimensional scaling based on Bray–Curtis dissimilarity revealed no differences in microbial composition between NC and C (*adonis2*-PERMANOVA, R^2 = 0.02, p = 0.77 for bacteria; R^2 = 0.04, p = 0.7 for eukaryotes) (**Figure 4A, 4B and 4C**). However, significant differences in fungal composition were found between NC and C (*adonis2*-PERMANOVA, R^2 = 0.04, p = 0.03). Moreover, significant differences in bacterial composition were observed between NC and L (*adonis2*-PERMANOVA, R^2 = 0.03, p = 0.001) as well as between C and L (*adonis2*-PERMANOVA, R^2 = 0.03, p = 0.001). Heatmaps showed contrasting patterns with some OTUs unique (specific) to certain categories of samples or shared (OTUs that are common among several categories of samples) (**Figure 4D-F**). Among fungi, OTU 3 (family *Didymellaceae*) was present in all samples. Conversely, the bacterial OTUs 50, 51, and 52 (family *Beijerinckiaceae*), 62 (family *Anaeromyxobacteraceae*), 84 (family *Bacillaceae*), 144 (family *Parachlamydiaceae*) and 202 (family *Clostridiaceae*) as well as the fungal OTU 54 (not identified) were found in substantial proportions in NC and C but absent from L. Two other bacterial OTUs (OTU 13 and OTU 14) belonging to family *Pseudonocardiaceae* were enriched in L compared to C

3.3. Organic molecules and physicochemical properties differentially affect microbial communities in noncolonized and colonized waters

According to previous analyses, coordinates of the two first components of PCA (PC1_poll and PC2_poll from **Figure 2C-2D** and PC1_mp and PC2_mp from **Figure 3C-D**) were extracted and subsequently used to reflect the distribution of samples according to either physicochemical and pollution variables or the occurrence of organic molecules in terms their microbial composition. We then evaluated whether those variables shape the microbial structure of NC and C. For this purpose, distance-based redundancy analyses (db-RDAs) were conducted to maximize the separation of water microbial communities based on differences in either their physicochemical composition and pollution context (PC1_poll and PC2_poll) or organic molecule structure (PC1_mp and PC2_mp) (**Figure 5 and 6**). In colonized water samples, the structure of bacterial communities was significantly correlated with PC1_poll (*adonis2*-PERMANOVA, $R^2 = 0.07$, $P = 0.001$), PC2_poll (*adonis2*-PERMANOVA, $R^2 = 0.06$, $P = 0.015$) and PC2_mp (*adonis2*-PERMANOVA, $R^2 = 0.44$, $P = 0.007$), while that of fungal communities varied only according to PC1_poll (*adonis2*-PERMANOVA, $R^2 = 0.07$, $P = 0.006$). The most important microbial OTUs involved in these separations were bacterial OTU 13 (identified as *Actinomycetospora*) and fungal OTU 11 (unidentified Rozellomycota), which correlated with high values of PC1_poll ($p = 0.017$, $R\hat{\rho} = 0.5$ for OTU 13 and $p = 0.003$, $R\hat{\rho} = 0.66$ for OTU 11), as well as bacterial OTU 39 (identified as Rhizobiaceae) (**Figure 5A-B**) and fungal OTU 44 (identified as *Ascochyta phacae*), which correlated with low and high values of PC2_mp, respectively ($p = 0.008$, $R\hat{\rho} = 0.54$ for OTU 39 and $p = 0.04$, $R\hat{\rho} = 0.48$ for OTU 44) (**Figure 5C-D**). Regarding noncolonized water samples, the structure of microbial communities was significantly influenced by PC1_poll (*adonis2*-PERMANOVA, $R^2 = 0.06$, $P = 0.027$ for bacteria and *adonis2*-PERMANOVA, $R^2 = 0.1$, $P = 0.04$ for eukaryotes) as well as by PC1_mp (*adonis2*-PERMANOVA, $R^2 = 0.08$, $P = 0.025$ for fungi). The most important clusters involved in these separations were OTU 24 (identified as *Haematococcus*), which correlated with PC1_poll ($p = 0.017$, $R\hat{\rho} = 0.6$) (**Figure 6A**), and OTU 30 (unidentified Rozellomycota) and OTU 163 (identified as

Neochloris aquatica), which correlated with PC1_mp ($p = 0.03$, $R\hat{\rho} = 0.5$ and $p = 0.04$, $R\hat{\rho} = 0.48$ for OTU 30 and OTU 163, respectively) (**Figure 6B**).

3.4. Organic molecules and microbial communities are highly connected in colonized water samples

A network analysis was performed on colonized water samples to explore associations between microbial OTUs (i.e., bacterial, fungal and eukaryotic OTUs), physicochemical parameters, pollution variables and organic molecules (**Figure 7**). Two independent networks of positive correlations that contained inter-kingdoms associations and organic molecules were characterized (**Figure 7**). In the first network, microbial co-occurrence patterns included the eukaryotic OTU 9 (identified as Ostracoda), 5 bacterial OTUs (i.e., OTU 77 identified as the genus *Azohydromonas*, OTU 355 identified as the class Acidimicrobiia, unidentified OTU 630, and OTUs 186 and 659 identified as the class Clostridia), 5 fungal OTUs (i.e., OTU 8 identified as the species *Neochloris aquatica*, OTU 188 identified as the species *Humicola grisea* and unidentified OTUs 5, 7, and 105) and one unidentified feature (M104T45) (**Figure 7A**). The second network showed module associations between 9 bacterial OTUs (i.e., 89, 93, 112, 130, 275, 590, 684 and 1018 identified as belonging to the genera *Bacillus*, *Bacteriovorax*, *Brevundimonas*, *Candidatus Methylophilus*, and *Flavobacterium*, the families *Yersiniaceae* and *Rhizobiaceae* and the genus *Tyzzarella*, respectively), 7 fungal OTUs (i.e., OTUs 37, 68, 114, 127, 132 and 298 identified as belonging to the order Pleosporales and the genera *Coniochaeta*, *Vishniacozyma*, *Filobasidium*, *Coniochaeta* and *Coniochaeta*, respectively), 8 eukaryotic OTUs (i.e., OTUs 7, 8 and 736 identified as belonging to the phylum Bicosoecida and OTUs 68, 94, 125, 265 and 423 identified as belonging to the class Klebsormidiophyceae, the genus *Coniochaeta*, the phylum Ascomycota, the genus *Gyrodinium* and the class Chytridiomycetes) and 3 unidentified features (M305T477, M209T480 and M285T707). In this second network, we focused on the eukaryotic OTU 68 that was associated with 2 eukaryotic OTUs (i.e., OTU 94 identified as belonging to the class Sordariomycetes and OTU 125 to

the class Chytridiomycetes), the fungal OTU 132 identified as Sordariomycetes and 3 bacterial OTUs associated with 3 different classes (OTU 89 identified as Bacilli, OTU 590 identified as Gammaproteobacteria and OTU 1018 identified as Clostridia) (**Figure 7B**). Finally, the eukaryotic OTU 7 belonging to the phylum Bicosoecida was associated with 3 features (M305T477, M209T480 and M285T707).

4. Discussion

Whether biotic and abiotic factors directly or indirectly impact the ability of mosquitoes to thrive in aquatic habitats remains poorly investigated. We addressed this question by specifically studying *Ae. albopictus*, one of the most invasive mosquito species in the world. As the type of container was shown to influence water physicochemical parameters (Hery et al. 2021b), our analysis mainly focused on rainwater collectors. Rainwater harvesting is a widely used method to collect and store rainwater for garden water supply and leads to common breeding sites for *Aedes* mosquitoes (Ritchie et al. 2002; Duval et al. 2022). We first investigated whether the presence/absence of *Ae. albopictus* larvae in water habitats could be related to specific biotic and abiotic features. With the exception of N₂O and CH₄ concentrations, our results showed no significant differences in water physicochemical properties and microbial composition between colonized and noncolonized water samples. We showed that the average water temperature and pH values of colonized larval habitats were 21.6 °C and 7.4, respectively. The temperature ranged between 15 °C and 26 °C, while the pH values were between 5.7 and 8.8. Consistent with the findings of other studies, the average electrical conductivity and dissolved oxygen were 0.19 S/m and 6.45 mg/L, respectively (Rao et al. 2011; Garcia-Sánchez et al. 2017; Dalpadado et al. 2022). These values are optimal for the development of *Ae. albopictus* (Delatte et al. 2009; Medeiros-Sousa et al. 2018; Reinhold et al. 2018). When measured as a percentage of the calculated (or expected) amount at saturation, referred to as dissolved saturation (%DO), dissolved oxygen was on average 2.1%. This contrasted with previous measures of %DO of approximately 1-1.5% in *Aedes* breeding sites

(Gopalakrishnan et al. 2013; Medeiros-Sousa et al. 2020). Interestingly, it was previously shown that %DO is positively correlated with mosquito larval density and can influence the forms and toxicity of dissolved metals as well as the concentrations of certain chemicals, such as sulphides and ammonia (Khalid et al. 1978; Gopalakrishnan et al. 2013). Regarding redox potential, higher values were observed in *Ae. aegypti* breeding sites (Garcia-Sánchez et al. 2017) and could be explained by variation in microbial communities or ionic composition (DeLaune and Reddy 2005; Søndergaard 2009). With the exception of oxygen, dissolved gases in larval breeding sites remain poorly studied. Our study is the first to examine the presence and concentration of several gases in *Ae. albopictus* water habitats. Interestingly, it was shown that the presence of methane in the larval habitats of *Culex pipiens* increased oviposition, while that of CO₂ did not seem to affect mosquito colonization or fitness (Bentley and Day 1989) or *Aedes* hatching stimulation (Judson 1960). In addition, methane and dissolved CO₂ exposure could also affect water microbial communities (Brankovits et al. 2017). Nitrate, potassium and chloride ions were detected at higher concentrations than those found in tap water, which could be linked to the use of nitrate fertilizers and the importance of fertilizer inputs in intensive urban agriculture (Amerasinghe et al. 1995; Panno et al. 2006; Wakejo et al. 2022). Although previous studies showed that mosquitoes are able to reproduce over a wide range of ion concentrations (Sehgal and Pillai 1970; Bashar et al. 2016; Garcia-Sánchez et al. 2017), the impact of water ionic composition on mosquito physiology remains unclear. Due to confounding factors in a complex field environment, further studies are required to investigate the impact of ionic and gas composition alone or in combination with other physicochemical parameters on different mosquito traits. For instance, the bioavailability of contaminants in water may be strongly influenced by the chemical environment (in particular ionic composition) and could in turn affect mosquito larvae by either directly modulating their physiology or indirectly modulating the microbial communities of breeding sites (Adams et al. 2020; Hery et al. 2021b).

Consistent with previous reports, the dominant bacterial phyla in colonized water samples were Pseudomonadota (previously named Proteobacteria), Actinobacteriota (previously named Actinobacteria) and Bacteroidota (previously named Bacteroides) (Wang et al. 2018; Scolari et al.

2021), while Ascomycota, Basidiomycota and Rozellomycota (previously named Cryptomycota) were the dominant fungal phyla (Tawidian et al. 2021). The microalga *Picochlorum* and the protozoan flagellate *Spumella* were found to be specific to colonized waters. Interestingly, polysaccharides extracted from *Picochlorum* were shown to exhibit antiviral activity against human enterovirus 71 (Guo et al. 2021). It could be interesting to evaluate whether this effect persists in larvae and adult mosquitoes and could interfere with arbovirus transmission. Surprisingly, Egizi et al. (2014) observed the opposite tendency, with a negative correlation between the density of *Culex quinquefasciatus* larvae and that of *Spumella*. Overall, our results confirmed that a fraction of microorganisms present in larval breeding water are maintained in the larval stage with higher microbial diversity in water breeding sites compared to in larvae (Dada et al. 2014; Coon et al. 2016; Wang et al. 2018; Strand 2018; Scolari et al. 2021). We identified bacteria such as *Rhizobium* and fungi such as *Dacrymyces* and *Pyrenochaetopsis* that were shared between water and larvae. However, whether these taxa play a role in mosquito biology is still unknown. *Rhizobium* was listed as part of the core microbiota in different *Anopheles* mosquito tissues (Tchioffi et al. 2016; Guégan et al. 2018). Another study showed that *Ae. albopictus* females were not attracted to or repelled by *Rhizobium huautlense* isolated from canebrake bamboo, but we cannot exclude a role in larval development (Ponnusamy et al. 2015). Interestingly, noncolonized water exhibited higher fungal diversity and specific fungal composition as well as higher concentrations of N₂O compared to colonized samples. This could be explained by the fact that N₂O production is a widespread trait in fungi (Maeda et al. 2015).

Physicochemical parameters and microbial communities interact with each other in aquatic ecosystems (Bojarczuk et al. 2018; Rahman et al. 2021) and can also be influenced by urban pollution. Physicochemical factors and pollution exposure could directly alter bacterial communities by impairing microbial physiological activities or indirectly by establishing conditions that adversely affect microorganisms. In community gardens, rainwater tanks are mainly filled with rainwater and in some cases may be supplemented with tap water, which can be occasionally contaminated by organic molecules in trace quantities (Kim and Zoh 2016). Although rainwater is considered a contamination-free source, human activities, particularly in the industrial and agricultural sectors, pollute this pure form

of water (Guidotti et al. 2000; Samuel et al. 2012). Over the last decade, a wide range of contaminants have been detected in surface water, including pharmaceutical, industrial and pesticide compounds (Rosen 2007; Verlicchi et al. 2010; Yang et al. 2022). Some of them, such as microplastic particles and antibiotic residues, were shown to alter water microbial communities (Qing et al. 2020; Yang et al. 2022; Edwards et al. 2023). To the best of our knowledge, no study has characterized the presence of organic molecules in breeding sites without *a priori* information. In our study, we identified organic molecules, although uncertainties remain regarding some candidate compounds. Further studies could be conducted using a suspect screening approach to better identify organic molecules (Pinasseau et al. 2023). Among the identified organic molecules, we found pharmaceutical residues such as anti-inflammatories, hormones or antioxidants (3-tert-butyl-4-hydroxyanisole), as well as fertilizers (dimethylenetriurea) and plastics (phthalate derived). Due to their high prevalence in aquatic ecosystems, further experiments are needed to test the influence of these compounds on the physiology of *Ae. albopictus* (Yang et al. 2022). Interestingly, a previous study showed an effect of fertilizer (i.e., ammonium sulphate fertilizer) on *Aedes* and an impact on larval development, survival rates and larval density (Mutero et al. 2004; Muturi et al. 2016; Darriet 2019). Moreover, Prud'homme et al. (2017) also revealed an impact of ibuprofen and benzo[a]pyrene on life history traits and insecticide tolerance in *Ae. aegypti* (Prud'homme et al. 2017). More recently, Edwards et al. (2023) showed that microplastics decreased the bacterial and fungal diversity of the *Ae. albopictus* and *Ae. aegypti* microbiota (Edwards et al. 2023).

We classified the community gardens according to their pollution context, which allowed us to define pollution gradients that combined either the water physicochemical parameters and the pollution variables (e.g., proximity to pollution sources) or water organic molecule occurrence. Our results showed differential effects of pollution gradients according to the water colonization status. While no relationship between physicochemical and pollution variables and bacterial composition was evidenced in noncolonized water samples, the opposite was observed in colonized waters. Industrial pollution and ionic composition were positively correlated with OTUs belonging to the bacterial genus *Actinomyces* and fungal phylum *Rozellomycota*. Similarly, we found correlations between the presence of organic molecules in water-colonized samples and OTUs belonging to the bacterial family

Rhizobiaceae and the phytopathogenic fungus *Ascochyta phaca*. *Actinomycetospora* is common in *Aedes* breeding sites (Zouache et al. 2022) and was found in this study to be highly prevalent in larvae. The Rhizobiaceae family contains phenotypically diverse organisms, including N₂-fixing legume symbionts (known as rhizobia), plant pathogens, bacterial predators, and other soil bacteria (Kuzmanović et al. 2021). Interestingly, members of this family were already shown to be involved in the degradation of bisphenol A and 4-nonylphenol, both ubiquitous pollutants with oestrogenic activity in the aquatic environment (Cai et al. 2016; Gayathri and Da 2021). In noncolonized water samples, *Haematococcus* was positively correlated with physicochemical and pollution variables. Interestingly, *Haematococcus* are known to help to reduce water pollution, which may explain their presence in polluted environments (Krishnamurthi et al. 2023). We also found specific patterns highlighting correlations between the organic molecule gradient and OTUs identified as the green alga *Neochloris aquatica* or as belonging to the fungal phylum Rozellomycota. Interestingly, Rozellomycota was also correlated with pollution related to industrial sources and specific physicochemical profiles in colonized waters. The Rozellomycota form a lineage basal or sister to fungi, the ancestor of Microsporidia (Corsaro et al. 2014). Their biodiversity is very rich but remains poorly characterized. However, we know that they are common in aquatic habitats and on pollen (Wurzbacher et al. 2014). They may play a key role in the decomposition of organic matter (Grossart et al. 2016). The few known species are all parasites, including water moulds and algae (*Rozella*), crustaceans (*Mitosporidium*), and endonuclear parasites of amoebae (*Nucleophaga*, *Paramicrosporidium*) (Corsaro et al. 2020; Chung et al. 2020; Gonçalves et al. 2022). Interestingly, *Neochloris aquatica* was shown to have a negative effect on the larval development of *Cx. quinquefasciatus* mosquitoes (Gil et al. 2021). A study also showed their ability to reduce or remove chemical oxygen demand (used as a measurement of pollutants in water), nitrite, and phosphate from water (Ummalyima et al. 2023). Therefore, we could assume that this species could modulate water physicochemical parameters that could in turn have consequences for colonization by *Ae. albopictus*. Further studies under experimental conditions would help disentangle potential interactions between the microbial taxa found to be correlated and the level of anthropization as well as their impact on mosquito biology.

5. Conclusion

Our study confirmed that larval habitat is an important determinant of microbial community structure. It also revealed that N_2O and CH_4 were significantly higher in noncolonized water samples than in colonized water samples. Then, we found that pollution gradients differentially contributed to the microbial ecology of *Ae. albopictus*-colonized and *Ae. albopictus*-noncolonized water samples. The relationships between microbial communities and water quality presented here are a first and simplified step towards longitudinal sampling in the field and delimitation of the type of larval breeding sites in the urban mosaic to determine their environmental exposome. Further studies are needed to determine the influence of biotic and abiotic factors on the larval productivity of breeding sites in highly anthropized environments and their carry-over impact on adult life history traits and vector competence.

Availability of data and material

The COI gene sequences were deposited in GenBank under the accession numbers OR391757 to OR391848. All FastQ files were deposited in the EMBL European Nucleotide Archive (<https://www.ebi.ac.uk/ena>) under the project accession number PRJEB65881, PRJEB65912, PRJEB65914 for, 16S, 18S and ITS sequencing, respectively. Scripts used for analysis and figure generation are available in the following Github Repository (<https://github.com/penelopeduval/Pollution-gradients-shape-microbial-communities-associated-with-Ae.-albopictus-larval-habitats>). All other relevant data supporting the findings of this study are available within the article and its supplementary information.

Authors' contributions

All authors contributed intellectually to and agreed to this submission. PD and CVM designed the experiments. PA, AS, EM, LV, PL, CVM, GM and PD conducted the experiments and sampling collection. LW, AF, CB and EV performed UPLC–HRMS analysis. PJ and EB performed total organic carbon measure and stable isotopic ratio. RC constructed association networks and analyzed the data. AC and JG conducted ionic and gases chromatography. GM, DA, MG and PD made statistical analyses. PD and CVM wrote the initial draft of the manuscript, while GM and RC provided substantial feedback. The authors read and approved the final manuscript.

Funding

PD was financed by Ecole Urbaine de Lyon. Funding for this project was supported by the French National program EC2CO (Ecosphère Continentale et Côtière), La Mission pour les initiatives transverses et interdisciplinaires (MITI CNRS), IDEX-FAPESP grant program and the Agence Nationale de la Recherche (SERIOUS project).

Notes

The authors declare no competing financial interest.

Acknowledgements

We thank the AME platform (University of Lyon, France) for the use of the gases and ionic chromatography and Ibio platform (University of Lyon, France) for sequence analysis.

Abbreviations

dbRDA: distance-based Redundancy Analyses

C: Colonized water samples

L: Larvae

Lmm: linear mixed model

NC: Noncolonized water samples

OTU : Operational Taxonomic Unit

PLS-DA: partial least squares discriminant analysis

Figure legends

Figure 1. Sample collection overview. (A) Distribution of community gardens in the Lyon metropolis, outlining the sites where water sampling collection was performed. The map of the Lyon metropolis was created using QGIS (www.qgis.org) (B) Sample processing describing physicochemical and microbial analyses of selected samples.

Figure 2. Variability of physicochemical parameters associated with noncolonized (NC) and colonized (C) water samples. (A) Partial least squares-discriminant analysis (PLS-DA) showing physicochemical parameters for NC (red circle) and C (blue circle). (B) Loading plot from the PLS-DA showing the physicochemical parameters with higher values in either the NC or C category for the first component of the PLS-DA (C) and (D) principal coordinate analysis (PCA) plots of the measured physicochemical parameters (black vectors) for NC (C) and C (D). ‘Var_indus’, ‘Var_agri’

and 'Var_atmo' indicate calculated industrial, agricultural and atmospheric pollution variables, respectively. 'C Organ' corresponds to organic carbon content.

Figure 3. Variability of organic molecule occurrence associated with noncolonized (NC) and colonized (C) water samples. (A) Loading plot from the PLS-DA showing organic molecule variables most strongly correlated with latent variables. (B) Score plot showing organic molecule composition for NC (red circle) and C (blue circle). (C) and (D) Principal component analysis (PCA) plots of organic molecules (black vectors) for NC (C) and C (D).

Figure 4. Microbial community composition and structure associated with noncolonized waters (NC), colonized waters (C) and larvae (L). Nonmetric multidimensional scaling (NMDS) ordinations displaying operational taxonomic unit (OTU) composition across NC, C and L are shown for each category of microorganisms: (A) bacteria, (B) fungi and (C) microeukaryotes. Heatmaps with cluster categorization representing the number of reads of the 40 most abundant OTUs among the total (D) bacterial, (E) fungal and (F) microeukaryotic OTUs (columns) for each type of sample (noncolonized, colonized, larvae (average of 10 larvae)) (rows). The intensity of the colour corresponds to the relative abundance of OTUs for each sample.

Figure 5. Effect of pollution gradients on the structure and variability of microbial communities associated with colonized (C) water samples. Microbial community distances among samples (dots) were constrained based on physicochemical and pollution parameters (blue vectors) and are represented by distance-based redundancy analysis (dbRDA) plots. PC1_poll/PC2_poll represents the combination of physicochemical and pollution variables, and PC1_mp/PC2_mp represents the organic molecule occurrence. OTU names significantly correlated ($p < 0.05$) with PC1_poll/PC2_poll or PC1_mp/PC2_mp are highlighted in red. Only significant effects are shown. (A) and (B) PC1_poll/PC2_poll dbRDA was performed for microbial community distances obtained from 16S rRNA and ITS sequencing, respectively. (C) and (D) PC1_mp/PC2_mp dbRDA was performed for microbial community distances obtained from 16S rRNA and ITS sequencing, respectively.

Figure 6. Effect of pollution gradients on the structure and variability of microbial communities associated with noncolonized (NC) water samples. Microbial community distances obtained from ITS sequencing (dots) were constrained based on physicochemical and pollution parameters (blue vectors) and represented by distance-based redundancy analysis (dbRDA) plots. (A) PC1_poll/PC2_poll represents the combination of physicochemical and pollution variables. (B) PC1_mp/PC2_mp represents the organic molecule occurrence. OTU names significantly correlated ($p < 0.05$) with PC1_poll/PC2_poll or PC1_mp/PC2_mp are highlighted in red.

Figure 7. A network analysis showing positive correlations between microbial OTUs, i.e., bacteria (dark blue dots), fungi (orange dots) and eukaryotes (light blue dots), organic molecules (green dots) and physicochemical parameters (light green dots). Each link represents a correlation with $p > 0.25$. For this analysis, we kept the samples with positive PCR amplification for each genetic marker, for a total of 13 colonized waters among the 23 sampled.

Table 1. Characterization of selected community gardens. The level of atmospheric pollution, pollution variables and the distance from the garden to each pollution source (agricultural, industrial and atmospheric pollution) are described for each sample. ‘Var_indus’, ‘Var_agri’ and ‘Var_atmo’ indicate calculated industrial, agricultural and atmospheric pollution variables, respectively.

Table 2. Putative identification of organic molecules in noncolonized and colonized water samples.

Table 3. Characteristics of water samples and extemporaneous measures of physicochemical parameters. For each sample, a description of the colonization status, the type of water habitat and measures of physicochemical properties are given. T(°C), salinity (PPT), pH, O₂ dissolved (mg/L) are given for each sample individually and are also expressed per container as mean \pm SE.

Table 4. Measures of ion and gas composition as well as total organic compounds in noncolonized (NC) and colonized © water samples. The concentrations of each ion (F^- , Cl^- , Br^- , NO_2^- , NO_3^- , PO_4^{3-} , SO_4^{3-} , Na^+ , NH_4^+ , K^+ , Mg^{2+} , Ca^{2+}) and each gas (O_2 , N_2 , CH_4 , CO_2 , N_2O) as well as the total organic carbon (TOC) content are given for each sample individually and are also expressed per container as the mean \pm SE.

Supplementary Table S1. Primers used for high-throughput sequencing.

Supplementary Table S2. PCR cycles used to amplify bacteria (V5-V6 16S rRNA gene), fungi (ITS2 region) and eukaryotes (V1-V2 18S rRNA gene).

Supplementary Table S3. Features (n=147) obtained by UPLC–HRMS analysis.

Supplementary Table S4. Shannon diversity index calculated for noncolonized (NC) and colonized (C) water samples as well as for larvae (L). The Shannon index is given for each sample individually and expressed per sample type (NC, C and L) as the mean \pm SE.

Supplementary Figure 1. Relative abundance of the major bacterial taxa at the phylum level (A) and genus level (B) (>1%) found in noncolonized waters (NC), colonized waters (C) and larvae (L) associated with colonized waters. Unaffiliated phyla and those with multiple affiliations were grouped together in a unique group called ‘unknown’.

Supplementary Figure 2. Relative abundance of the major fungal taxa at the phylum level (A) and genus level (B) (>1%) found in noncolonized water (NC), colonized water (C) and larvae (L) associated with colonized water. Unaffiliated phyla and those with multiple affiliations were grouped together in a unique group called ‘unknown’.

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Fig. 1

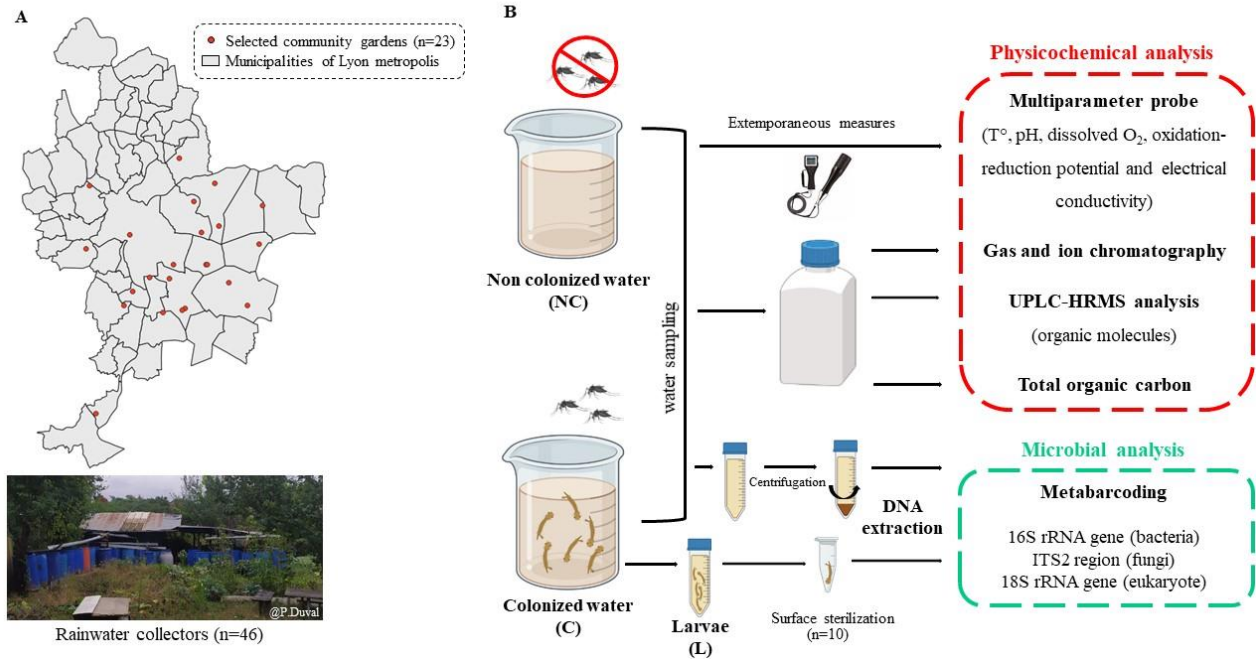


Fig. 2

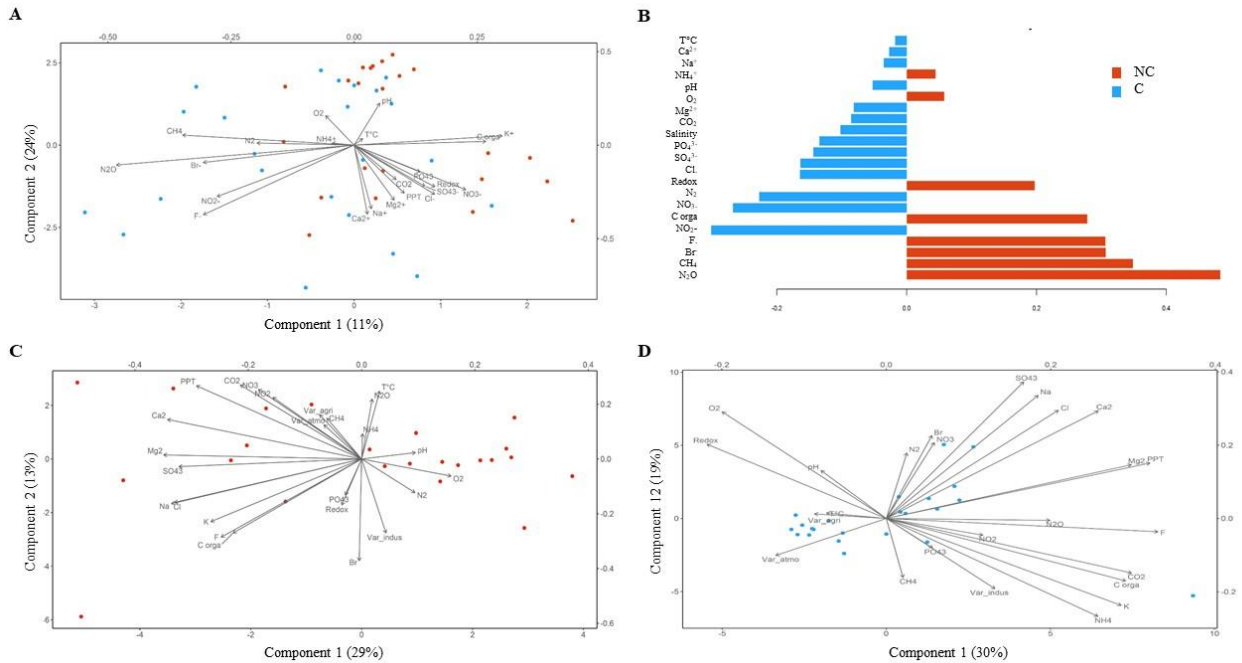


Fig. 3

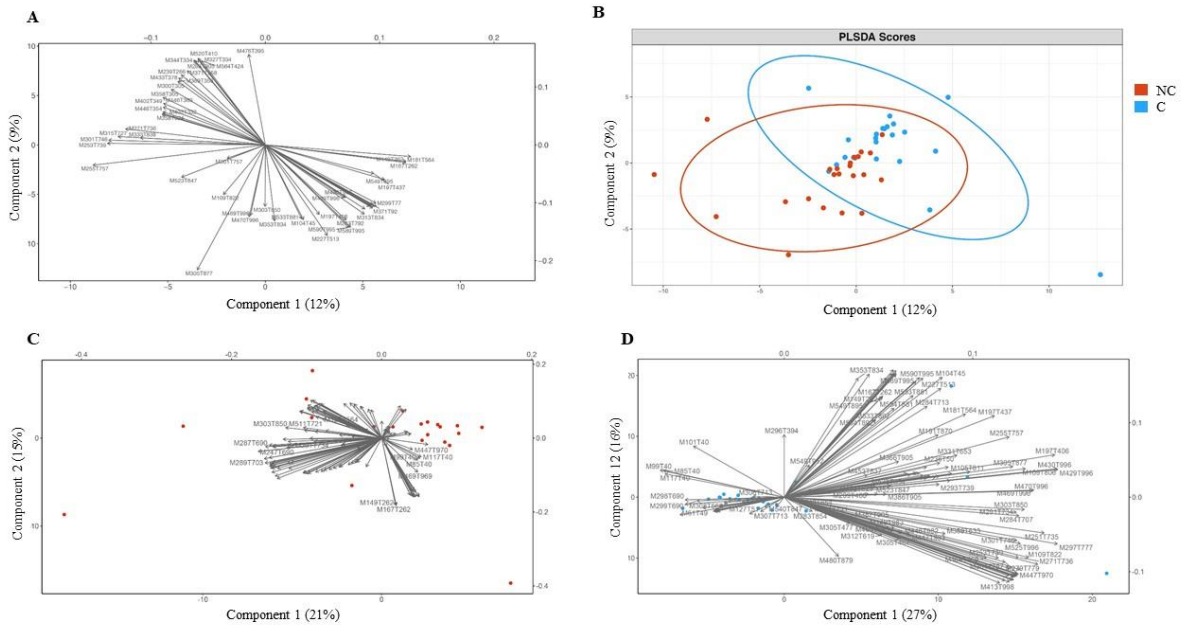


Fig. 4

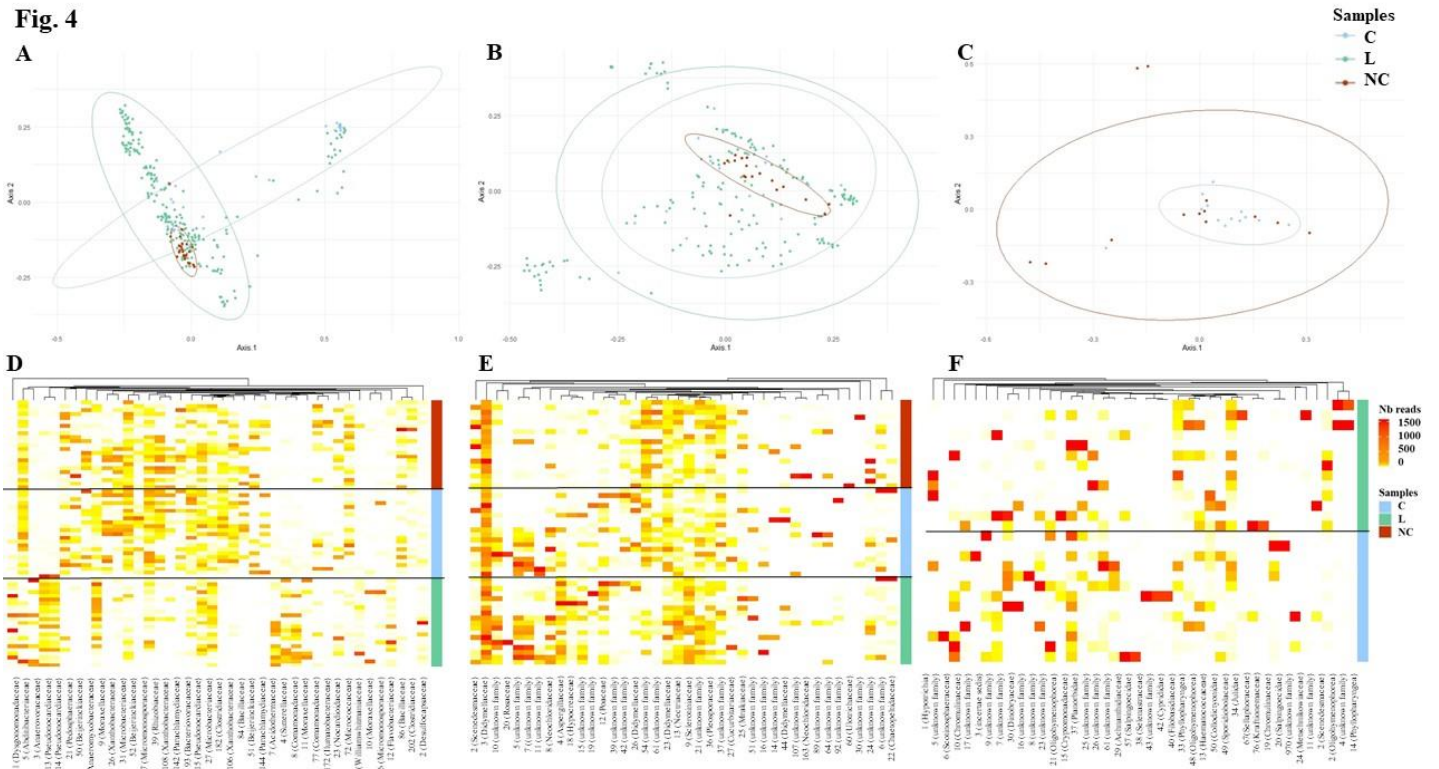


Fig. 5

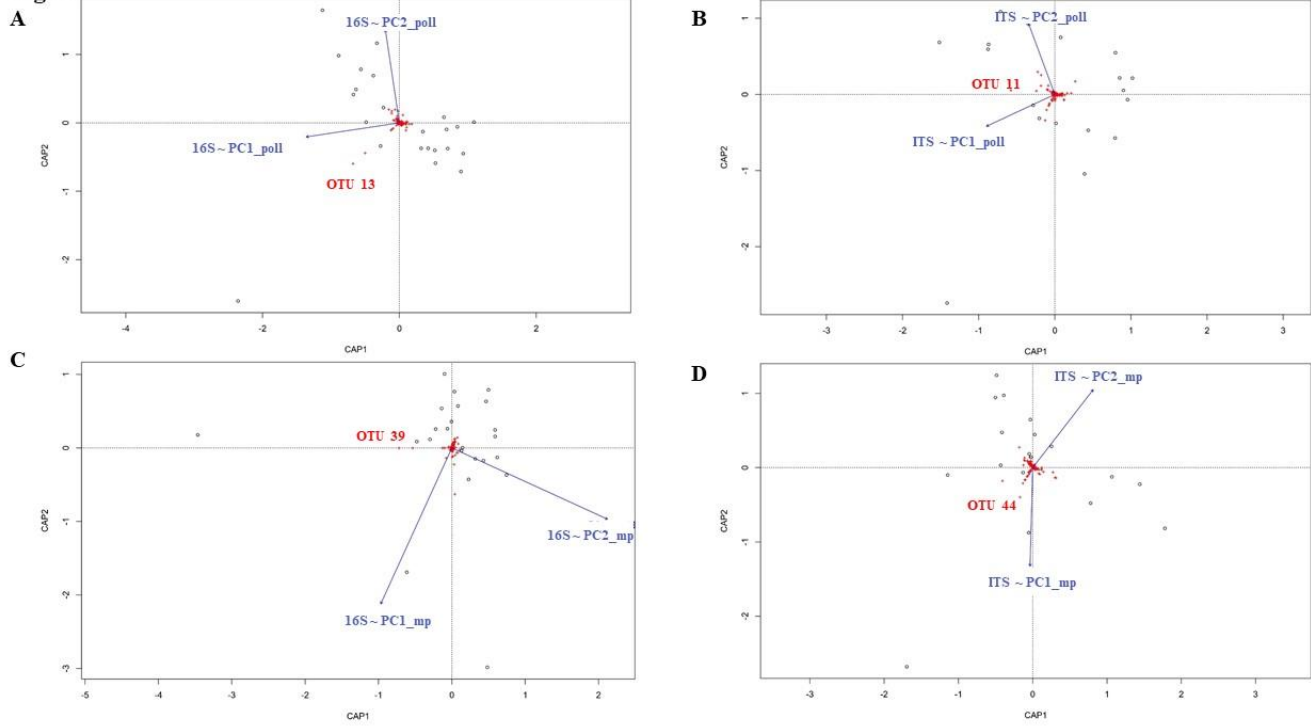


Fig. 6

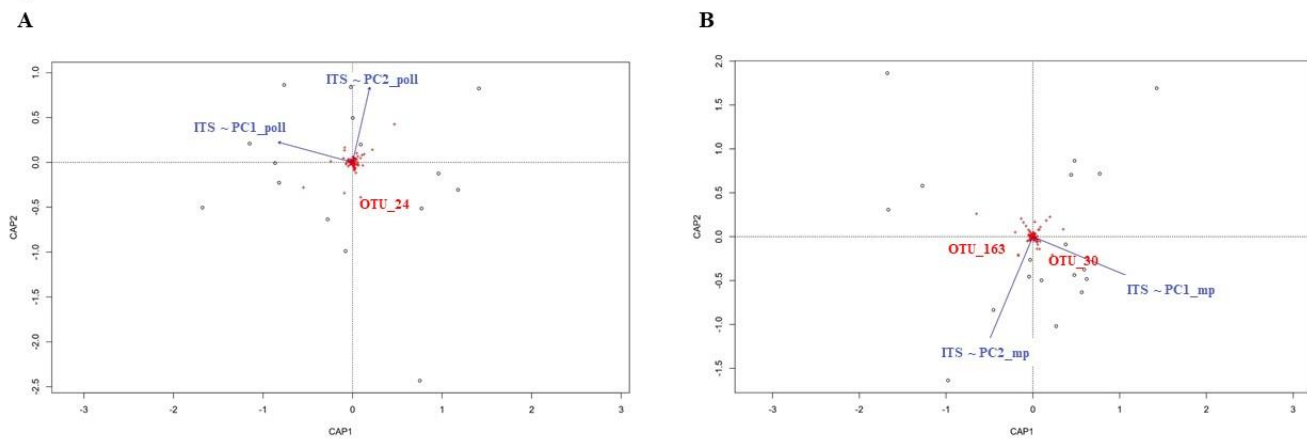


Fig. 7

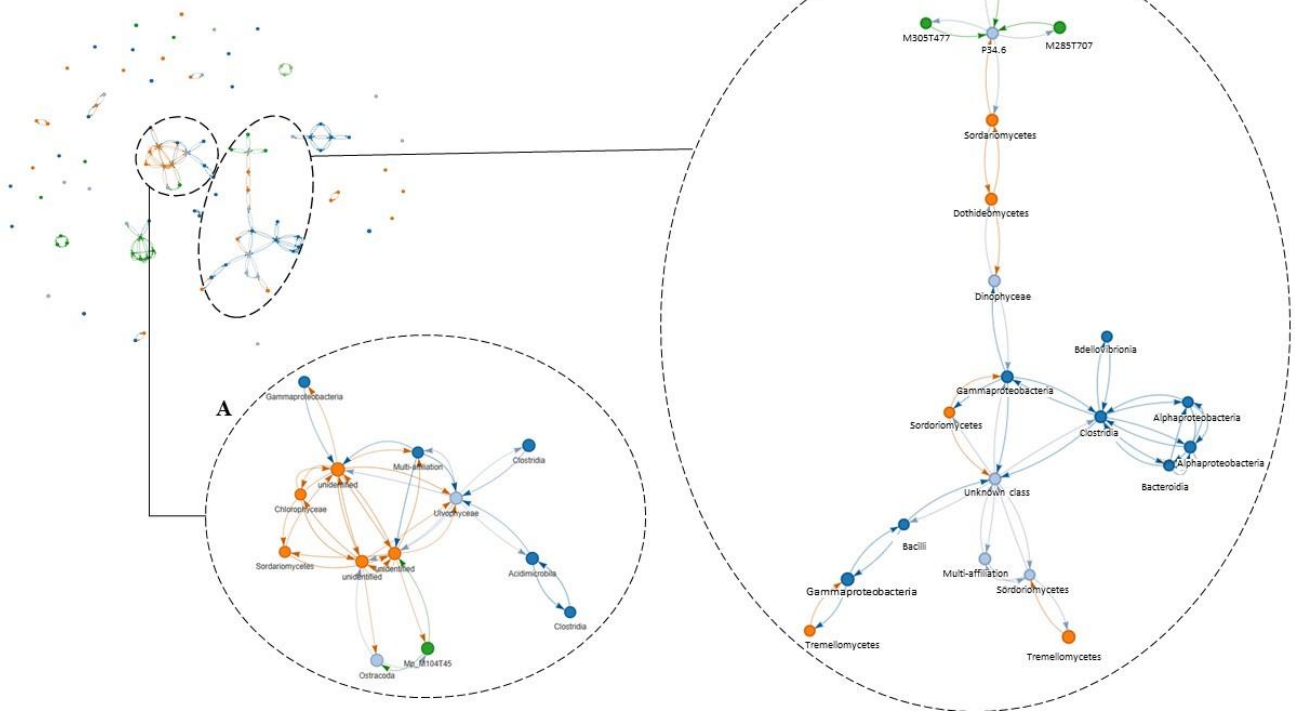


Fig. S1

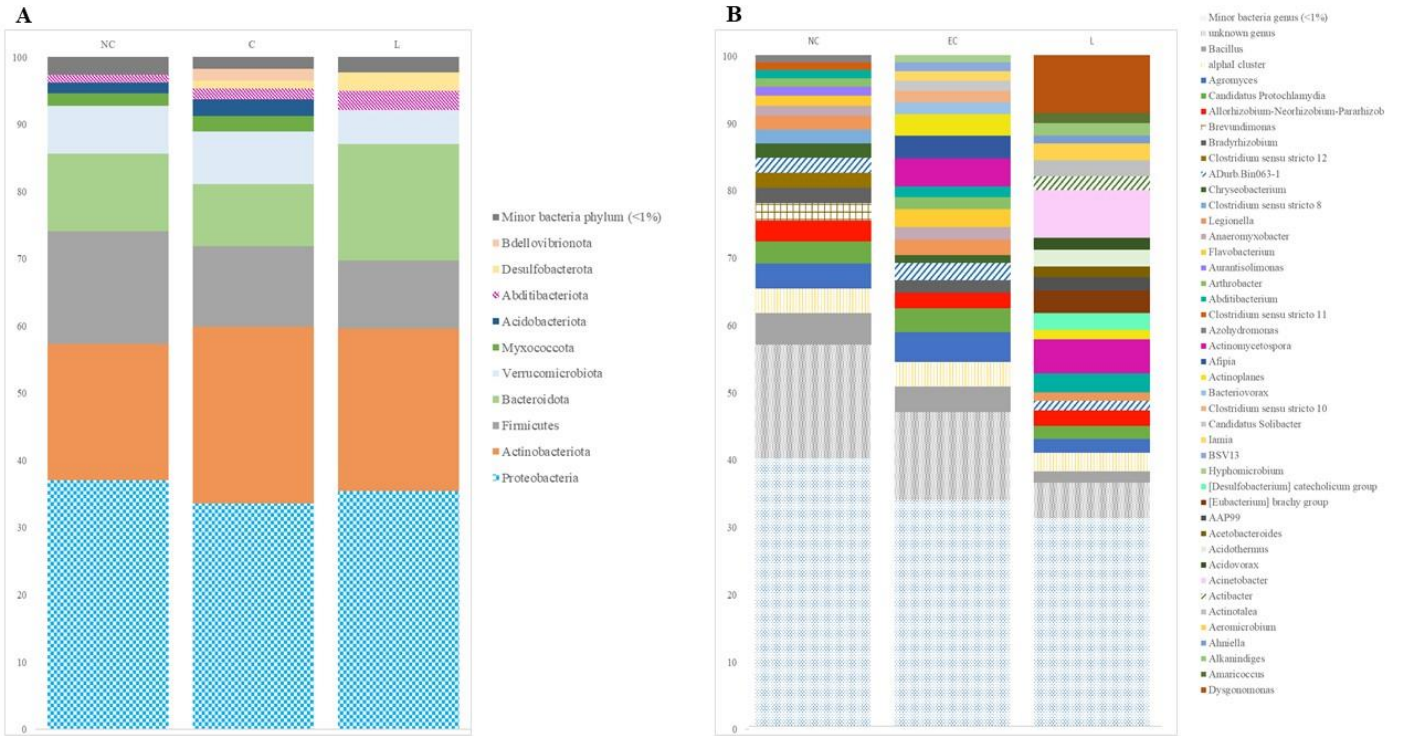


Fig. S3

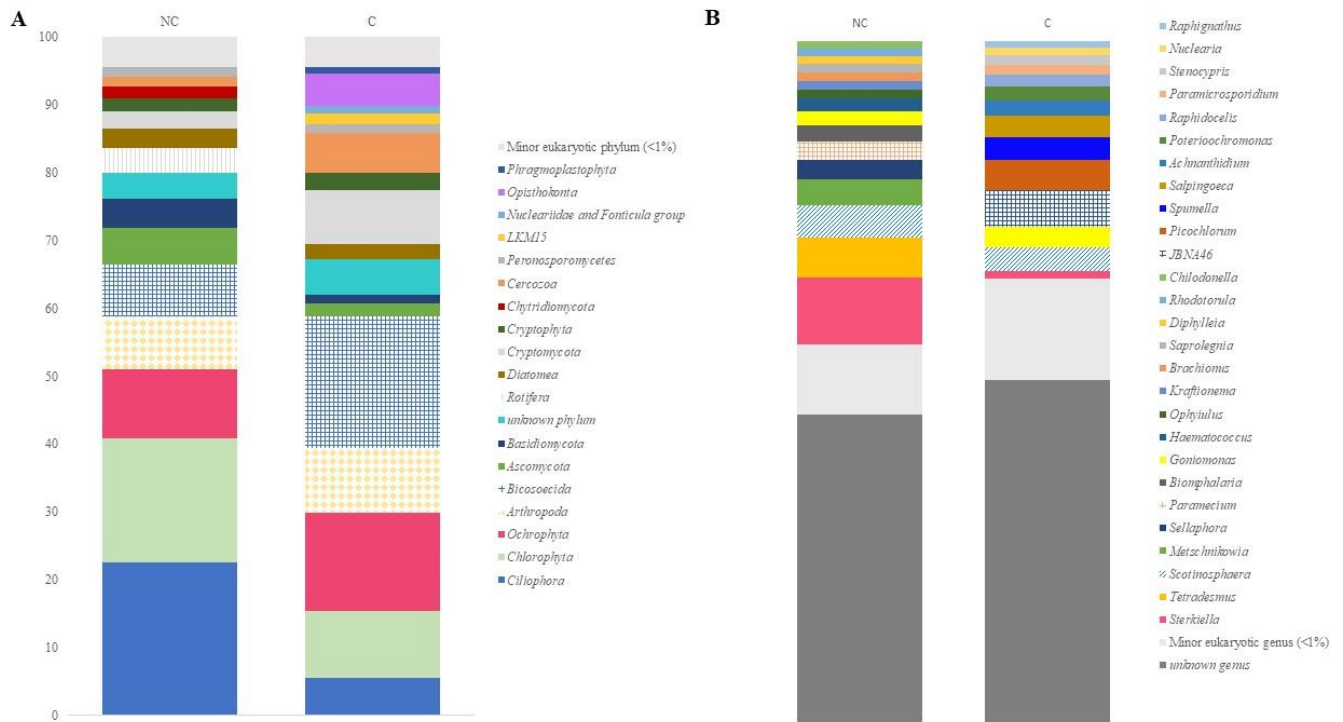


Fig. S2

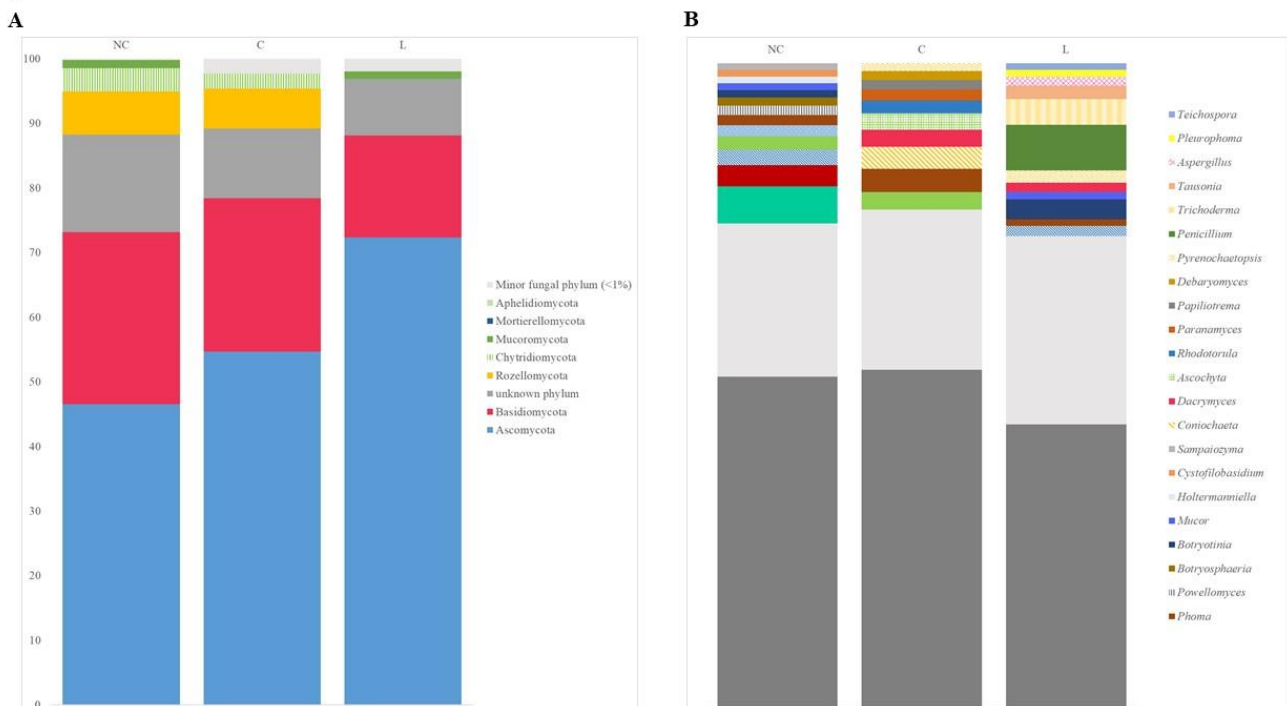


Table 1. Characterization of selected community gardens. For each garden, the level of atmospheric pollution, distance from the garden to each pollution source (agricultural, industrial and atmospheric pollution) and calculated pollution variables (Var_indus, Var_agri and Var_atmo) are given.

Garden	Pollution group	Atmospheric pollution scores	Distance from the garden to each pollution source (m)			Calculated pollution variables		
			Agricultural	Industrial	Atmospheric	Var_indus	Var_agri	Var_atmo
MID	AGRI	8	2624	1167	183	0.0009	4993	0.0437
MOU	INDUS	6	1311	1829	544	0.0005	386	0.011
PER	AGRI	5	158	3442	121	0.0003	228	0.0209
RECU	ATMO	12	1228	4598	4	0.0002	1365	0.1958
REB	ATMO	12	2624	1986	56	0.0005	191	0.2159
BIL	ATMO	12	4066	1818	26	0.0004	92	0.1534
GAR	AGRI	6	327	2209	476	0.0005	1144	0.011
ARK	INDUS	8	2640	528	500	0.0019	192	0.0152
ALST	ATMO	8	1549	3967	464	0.0003	80	0.0172
FRA	INDUS	3	1584	787	3068	0.0013	48	0.0009
TAS	INDUS	6	2044	802	1587	0.0012	923	0.0038
JUST	ATMO	12	3541	2999	24	0.0003	5	0.0587
DEC	AGRI	4	200	3422	59	0.0003	246	0.0382
FOR	ATMO	12	2759	2022	23	0.0005	182	0.2352
SYT	INDUS	11	3317	1974	204	0.0005	113	0.0538
QUA	INDUS	3	1545	590	2495	0.0017	585	0.0012
COR	AGRI	5	164	8643	1430	0.0001	193	0.0034
ESP	AGRI	9	669	1963	814	0.0005	559	0.0111
EDF	INDUS	12	7059	631	14	0.0016	2508	0.0147
BIG	AGRI	3	465	1457	620	0.0007	1099	0.0044
AVIA	AGRI	6	319	1491	298	0.0007	1659	0.0148
BONN	AGRI	6	682	1373	220	0.0007	771	0.0209
VOIL	ATMO	12	3371	2288	16	0.0004	560	0.3004

Table 2. Putative identification of organic molecules in noncolonized and colonized water samples

Id micropollutant	Exact mass m/z (Da)	tR (min)	Formula	Level of identification confidence	Micropollutant affiliation	Detection in water samples		
						C	NC	
M181T564	181,1225	9.4	C11H16O2	2B	3-tert-butyl-4-hydroxyanisole	VOIL, JUST, ARK, FRA, BIL	SYT, FRA, EDF, ALST, TAS	
M197T437	197,1175	7.28	C11H16O3	2A	Loliolide	VOIL, ARK, JUST, FRA, BIL	SYT, QUA, FRA, ALST, VOIL	
M227T64	227,0867	1.07	C8H12N6O3	2B	Dimethylenetriurea	ARK, GAR	PER	
M233T692	233,154	11.53	C15H20O2	2A	Costunolide, Indicanone, Alantolactone, Eremofrullanolide, Glechomanolide, Frullanolide	ARK		
M277T743	277,2165	12.38	NA	5	family of phthalates	VOIL	FOR, QUA, TAS, GAR	
M279T761	279,2321	12.68	C18H32O3	2B	13(S)-HODE, alpha-dimorphophecolic acid laeissaric acid 9(10)-EpOME, (+)-vermolic acid	VOIL	QUA, FRA, FOR, COR, BIL, ALST, GAR	
M284T713/M284T707	284,2225	11.89/11.78	C16H29NO3	2B	N-dodecanoyl-L-homoserine lactone	FOR, JUST	BIG	
M12T751	127,0729	0.85	C3H6N6	2A	Melamine		FOR	
M313T834	313,2741	13.89	C19H36O3	2B	(2E, 18R)-18-hydroxyxnonadec-2-enoic acid	ARK		
M317T759/M317T743	317,2093	12.38/12.66	C20H28O3	2B	9-cis-4-hydroxyretinoic acid inurmatolic acid, 15-deoxy-Delta(12,14)-prostaglandin (A2 or J2)	VOIL	QUA, FRA, GAR, TAS, FOR, ARK	
M331T729	331,1885	12.16	C20H26O4	2B	(7Z)-lipoheleolide, heptylactone D	VOIL		
M289T703	289,1781	11.97	C14H20N6O	4	Unidentified			
M298T690	298,2382	11.51	C17H31NO3	4	Unidentified	ARK	BIG	
M299T90	299,1191	1.49	C14H16F2N2O3	4	Unidentified	VOIL		
M236T50	236,1496	0.83	C10H21NO5	4	Unidentified	VOIL	QUA, FRA	
M371T92	371,1516	1.54	C17H24N4O2S	4	Unidentified	ARK, GAR		
M429T996	429,373	16.6	C29H50O3	2B	13-hydroxy-alpha-tocopherol	FRA, ARK, QUA, VOIL	GAR, ROC	
M533T881	533,2548	14.68/14.87	C33H34N4O3	2B	pyropheophorbide a	ARK, FRA, VOIL, QUA, JUST	FRA, ALST, SYT, EDF	
M301T746	301,2156	12.38/12.43	C16H22O4	2A	Dibutyl phthalate		QUA, FRA	
M419T933	419,316	15.34/15.55	C26H42O4	2A	Diisononyl phthalate	BIL	COR	
M447T983	447,3471	16.17/16.38	C28H46O4	2A	Di-n-decyl phthalate, Diisodecyl phthalate	QUA, BIL, FOR, VOIL	COR	
M167T262	167,0342	4.36	C8H6O4	2A	phthalic acid	ARK, FRA	COR	

Table 3. Characteristics of water samples and extemporaneous measures of physicochemical parameters. For each sample, a description of the colonization status, the type of water habitat and measures of physicochemical properties are given. T(°C), salinity (PPT), pH, O₂ dissolved (mg/L) are given for each sample individually and are also expressed per container as mean ± SE

Sample ID	Garden	Sample type	Date	Type of sample	Matter	Color	T(°C)	Mean ±SE	Salinity	Mean ±SE	pH	Mean ±SE	O ₂ dissolved	Mean ±SE	Redox	Mean ±SE
ALST_NC	ALST		2021-07-22	Rainwater collector	Plastic	Blue	25.17		0		8.49		3.48		49	
ARK_NC	ARK		2021-08-02	Rainwater collector	Plastic	Blue	19.09		0		7.93		8.6		190	
AVIA_NC	AVIA		2021-07-05	Bucket	Plastic	Red	20.42		0		6.95		7.1		305	
BIG_NC	BIG		2021-07-01	Rainwater collector	Plastic	Blue	20.8		0		7.28		5.45		300	
BIL_NC	BIL		2021-07-27	Rainwater collector	Plastic	Blue	25.64		0.2		7.45		4.78		18	
BONN_NC	BONN		2021-07-05	Rainwater collector	Plastic	Green	22.18		0		7.27		7.46		283	
COR_NC	COR		2021-09-22	Rainwater collector	Plastic	White	14.95		0.3		7.35		8.51		250	
DEC_NC	DEC		2021-06-16	Rainwater collector	Plastic	Blue	28.09		0.3		7.29		6.74		265	
EDF_NC	EDF		2021-07-20	Rainwater collector	Plastic	Blue	26.38		0		7.99		2.64		105	
ESP_NC	ESP		2021-07-08	Rainwater collector	Plastic	Blue	20.13		0		8.28		6.91		188	
FOR_NC	FOR		2021-07-19	Watering can	Plastic	White	21.3		0		6.15		6.6		285	
FRA_NC	FRA	Noncolonized	2021-09-29	Rainwater collector	Plastic	Blue	17.7	21.5 (+/- 4)	0	0.15 (+/- 0.14)	6.98	7.4 (+/- 0.6)	7.86	6.4 (+/- 2)	245	198 (+/- 112)
GAR_NC	GAR		2021-08-31	Rainwater collector	Plastic	Blue	17.7		0		7.98		9		206	
JUST_NC	JUST		2021-06-25	Rainwater collector	Plastic	Blue	20.23		0.1		7.59		3.28		70	
MID_NC	MID		2021-08-03	Rainwater collector	Plastic	Black	19.2		0.4		7.04		12.25		271	
MOU_NC	MOU		2021-07-26	Rainwater collector	Plastic	Gray	23.02		0		6.41		7.94		214	
PER_NC	PER		2021-09-07	Rainwater collector	Plastic	Blue	21.8		0.1		7.64		3		257	
QUA_NC	QUA		2021-09-06	Rainwater collector	Plastic	White	24.93		0.1		7.44		6.56		192	
REB_NC	REB		2021-09-01	Rainwater collector	Plastic	Blue	20.72		0.1		6.38		6.14		288	
RECU_NC	RECU		2021-09-13	Bucket	Plastic	White	17.01		0.2		6.58		6.42		254	
SYT_NC	SYT		2021-07-21	Rainwater collector	Metal	Gray	24.94		0.1		7.46		3.91		-158	
TAS_NC	TAS		2021-09-23	Bucket	Plastic	Black	14.82		0.1		8.17		5.96		228	
VOIL_NC	VOIL		2021-06-17	Rainwater collector	Plastic	Blue	29.12		0.2		8.05		5.83		245	
ALST_C	ALST		2021-07-22	Rainwater collector	Plastic	Blue	25.51		0.3		8.19		5.04		230	
ARK_C	ARK		2021-08-02	Rainwater collector	Plastic	Blue	22.25		0.4		6.72		3.12		-64	
AVIA_C	AVIA		2021-07-05	Rainwater collector	Plastic	Green	20.92		0		7.95		7.17		252	
BIG_C	BIG		2021-07-01	Rainwater collector	Plastic	Blue	21.98		0		8.02		8.85		317	
BIL_C	BIL		2021-07-27	Rainwater collector	Plastic	Blue	23.4		0		8.05		3.21		86	
BONN_C	BONN		2021-07-05	Rainwater collector	Plastic	Blue	21.22		0		7.3		6.79		199	
COR_C	COR		2021-09-22	Rainwater collector	Plastic	Blue	15.97		0.3		6.7		8.64		231	
DEC_C	DEC		2022-06-16	Rainwater collector	Plastic	Blue	26.29		0.012		7.63		10.12		194	
EDF_C	EDF		2021-07-20	Rainwater collector	Plastic	Blue	23.81		0		7.8		5.02		248	
ESP_C	ESP		2021-07-08	Rainwater collector	Plastic	Blue	20.29		0		7.99		7.39		165	
FOR_C	FOR		2021-07-19	Rainwater collector	Plastic	Gray	23.71		0		7.85		5.75		296	
FRA_C	FRA	Colonized	2021-09-29	Watering can	Plastic	Green	18.13	21.6 (+/- 3)	0.1	0.19 (+/- 0.19)	5.7	7.4 (+/- 0.8)	10.07	6.5 (+/- 2)	264	215 (+/- 88)
GAR_C	GAR		2021-08-31	Rainwater collector	Plastic	Blue	18.45		0.3		7.97		6.82		160	
JUST_C	JUST		2021-06-25	Rainwater collector	Plastic	Blue	20.83		0.1		7.67		6.91		260	
MID_C	MID		2021-08-03	Rainwater collector	Plastic	Blue	20.43		0		7.28		4.56		284	
MOU_C	MOU		2021-07-26	Rainwater collector	Plastic	Blue	23.68		0.3		7.79		7.02		237	
PER_C	PER		2021-09-07	Rainwater collector	Plastic	Blue	21.5		0.1		7.26		1.95		71	
QUA_C	QUA		2021-09-06	Rainwater collector	Plastic	Blue	21.86		0		7.17		5.09		254	
REB_C	REB		2021-09-01	Rainwater collector	Plastic	Blue	21.21		0		5.72		8.32		328	
RECU_C	RECU		2021-09-13	Rainwater collector	Plastic	Blue	19.91		0.2		6.12		5.03		277	
SYT_C	SYT		2021-07-21	Rainwater collector	Metal	Gray	23.24		0.2		7.63		4.11		207	
TAS_C	TAS		2021-09-23	Bucket	Plastic	White	16.21		0.2		7.78		7.55		256	
VOIL_C	VOIL		2021-06-17	Rainwater collector	Plastic	White	25.97		0		8.76		9.23		199	

De par leurs tailles trop importantes, la table 4 et les tables supplémentaires sont mises en ligne via le lien suivant : https://github.com/penelopeduval/Supplementary_data-of-Pollution-gradients-shape-microbial-communities-associated-with-Ae.-.git

2.3 Discussion et conclusion

Les paramètres biotiques et abiotiques des gîtes larvaires influencent les traits d'histoire de vie des moustiques et leur capacité à transmettre des pathogènes (Guégan et al. 2018; Bellone and Failloux 2020; Ranasinghe and Amarasinghe 2020; Antonelli et al. 2022; Zheng et al. 2023; Duval et al. 2023). (Coon et al. 2014). Cependant, aucune étude n'avait encore exploré la manière dont ces paramètres interagissent avec l'état de colonisation de l'eau par les moustiques, ni comment la pollution peut potentiellement influencer ces paramètres et leurs interactions. Nous nous sommes donc intéressés à cette question en étudiant le moustique tigre, un moustique très bien adapté aux habitats urbains et en choisissant comme terrain d'étude, les jardins collectifs, des lieux propices au développement de cette espèce en ville. Cette étude exploratoire a permis d'obtenir, par une analyse la plus exhaustive possible, une vision d'ensemble des paramètres physico-chimiques et microbiologiques d'eaux colonisées ou non par le moustique tigre prélevées *in situ* en milieu urbain.

Sur l'ensemble des paramètres mesurés, à l'exception du protoxyde d'azote (N_2O) et du méthane (CH_4) qui se sont avérés être présents en plus grande quantité dans les eaux non colonisées, la comparaison des paramètres physico-chimiques et microbiologiques de l'eau n'a montré aucune différence significative selon le statut de colonisation. Les valeurs de température ainsi que la teneur en oxygène, le pH et la conductivité de l'eau étaient comparables aux valeurs précédemment montrées comme étant optimales pour le développement des larves de ce moustique (Delatte et al. 2009; Reinhold et al. 2018; Medeiros-Sousa et al. 2020; Dalpadado et al. 2022). En revanche, la saturation en oxygène dissous (OD %) diffère des données de la littérature. En moyenne, la quantité d' O_2 présente en solution dans l'eau était supérieure à celle précédemment estimée dans les gîtes d'*Aedes* (2,1% contre 1 à 1,5%) (Gopalakrishnan et al. 2013; Medeiros-Sousa et al. 2020). De façon intéressante, l'oxygène dissous a montré des effets sur la forme et la toxicité de certains polluants comme les métaux, les sulfures et l'ammoniac (Khalid et al. 1978; Gopalakrishnan et al. 2013). Nous pourrions supposer que la concentration élevée d'oxygène dissous dans nos échantillons pourrait transformer des sites pollués en des milieux propices à la croissance des larves de moustiques. De plus, notre étude a permis de détecter 1382 molécules organiques dans l'ensemble de nos échantillons. A notre connaissance, aucune étude n'a analysé la présence de molécules organiques via une approche sans a priori dans les gîtes larvaires. Parmi les molécules organiques identifiées dans l'ensemble des prélèvements, nous avons retrouvé plusieurs

composés caractéristiques d'environnements aquatiques pollués, tels que des résidus pharmaceutiques (anti-inflammatoires, hormones, antioxydants) ou encore des fertilisants et des plastiques (dérivés de phtalates). L'impact de ces composés sur le moustique reste encore à déterminer.

Concernant les analyses microbiologiques, nous avons pu montrer que la microalgue *Picochlorum* et le protozoaire flagellé *Spumella*, étaient spécifiques aux eaux colonisées. Peu d'informations existent sur la nature des liens entre ces microorganismes et les moustiques. Les polysaccharides extraits de *Picochlorum* ont montré des effets antiviraux contre des entérovirus (Guo et al. 2021). Il serait intéressant d'évaluer si ce microorganisme persiste dans le microbiote des larves puis dans l'adulte où il pourrait potentiellement interférer avec la transmission d'arbovirus. De façon étonnante, chez *Cx quinquefasciatus*, il a été montré que *Spumella* était corrélé négativement avec la densité larvaire alors qu'il était spécifique des eaux colonisées dans notre étude (Egizi et al. 2014). D'autre part, et en accord avec les résultats d'autres études, nos résultats confirment qu'une partie des microorganismes de l'eau des gîtes est conservée dans la larve avec une diversité moindre (Dada et al. 2014; Coon et al. 2016; Wang et al. 2018; Strand 2018; Scolari et al. 2021).

Un objectif important de cette étude était d'évaluer si l'exposition des gîtes urbains à des contextes différents de pollution pouvait impacter différemment la composition des habitats larvaires. Dans ce but, nous avons classé les jardins collectifs en fonction de leur contexte de pollution. Nous avons tout d'abord examiné l'influence des variables de pollution combinées aux paramètres physico-chimiques sur les communautés microbiennes présentes dans les réservoirs d'eau stagnante. Par la suite, nous avons cherché à déterminer si la composition en molécules organiques pouvait également affecter la composante microbienne de l'eau. Nos résultats ont mis en évidence que ces paramètres (les facteurs de pollution liés aux propriétés physico-chimiques et la composition en molécules organiques) ont affecté différemment les eaux en fonction de leur état de colonisation. Dans les eaux colonisées, la proximité des jardins à des zones industrielles ainsi que la composition ionique de l'eau étaient positivement corrélées à la présence d'UTOs microbiennes appartenant au genre *Actinomycetospora* et au phylum *Rozellomycota*. De même, la composition en molécules organiques semble influencer la présence de certains microorganismes, en particulier les bactéries de la famille *Rhizobiaceae* et le champignon phytopathogène, *Ascochyta phaca*. Des études en laboratoire en systèmes simplifiés seraient nécessaires pour comprendre les interactions entre ces microorganismes, certaines conditions physico-chimiques et la biologie

du moustique. Pour cela, il serait intéressant de voir si ces microorganismes modèleraient la toxicité de certains polluants et s'ils permettraient au moustique de se développer en milieu pollué. De façon intéressante, les *Actinomycetospora* ont déjà été identifiés dans les gîtes de moustiques *Aedes* (Zouache et al. 2022) et nous avons également pu mettre en évidence leur présence dans les larves. Quant à la famille des Rhizobiaceae, elle regroupe des organismes très diversifiés dont certains membres sont impliqués dans la dégradation du bisphénol A et du 4-nonylphénol, deux polluants omniprésents ayant une activité œstrogénique dans l'environnement aquatique (Cai et al. 2016; Gayathri and Da 2021). Dans les eaux non colonisées, le genre *Haematococcus* était positivement corrélé aux variables physico-chimiques et de pollution. Cette algue verte est connue pour contribuer à la réduction de la pollution de l'eau (Krishnamurthi et al. 2023). L'espèce *Haematococcus Pluvialis* peut être utilisée en bioremédiation notamment par sa capacité à réduire significativement la présence de polluants chimiques tels que les nitrates, les fluorures, les sulfates et les phosphates (Radice et al. 2022). Nous avons également observé une influence du gradient de la composition en molécules organiques sur une autre algue verte, *Neochloris aquatica*, et une unité taxonomique opérationnelle (UTO) appartenant au phylum fongique des Rozellomycota. Il est intéressant de noter que *Neochloris aquatica* a été montré pour avoir un effet négatif sur le développement larvaire des moustiques *Cx. quinquefasciatus* (Gil et al. 2021). Une étude a également souligné leur capacité à éliminer la demande chimique en oxygène (utilisée comme mesure de la pollution de l'eau), les nitrites et les phosphates de l'eau (Ummalyima et al. 2023). Par conséquent, nous pourrions supposer que cette espèce pourrait moduler les paramètres physico-chimiques de l'eau, ce qui pourrait avoir en retour des conséquences sur la colonisation de l'eau par *Ae. albopictus*.

Pour finir, l'analyse en réseaux réalisée à partir des échantillons d'eaux colonisées a permis de révéler des associations entre microorganismes de règnes différents (procaryote et eucaryote) mais aussi avec certaines molécules organiques. C'est le cas par exemple d'une UTO identifiée comme étant un ostracode appartenant à l'ordre des Podocopida qui a été retrouvée associée significativement à une molécule organique dont l'identification n'a malheureusement pas été possible (M104T45). Une étude a identifié la forte résistance d'un ostracode issu du même ordre, *Bicornucythere bisanensis*, aux pollutions anthropiques (Yasuhara et al. 2003).. De plus, une UTO identifiée comme appartenant au phylum eucaryote Bicosoecida a également été retrouvée associée à trois molécules organiques non identifiées. De façon intéressante, il a été montré que les Bicosoecida étaient plus prévalents après une contamination de l'eau aux

hydrocarbures. Ces deux eucaryotes, Podocopida et Bicosoecida, pourraient être résistants à des stress anthropiques (Santos et al. 2010). Pour aller plus loin, il serait intéressant d'identifier ces quatre molécules organiques pour pouvoir comprendre comment et pourquoi elles sont plus souvent associées à ces deux microeucaryotes.

Ce travail exploratoire a permis de démontrer des interactions entre les paramètres biotiques et abiotiques dans les eaux colonisées ou non par le moustique tigre. Nos résultats n'ont pas permis de mettre en évidence des paramètres limitant la colonisation par *Ae. albopictus* à l'exception de la concentration élevée en méthane et en protoxyde d'azote mais cette tendance reste à confirmer. Par contre, ces résultats ont souligné l'exceptionnelle capacité d'*Ae. albopictus* à se développer dans une large gamme de conditions physico-chimiques. Cette capacité d'adaptation pourrait être permise grâce aux microorganismes et leur capacité à transformer et diminuer la toxicité de certains polluants. Il serait intéressant d'approfondir les recherches sur ces microorganismes en particulier et leur rôle dans la colonisation de l'eau par les moustiques.

IV. Discussion générale

En raison de sa capacité à transmettre des virus pathogènes aux êtres humains comme celui de la dengue, du chikungunya ou du Zika, le moustique tigre *Ae. albopictus* est au cœur des préoccupations de santé publique. Il a été responsable de plusieurs épidémies virales dans les départements et régions d'Outre-mer (Delatte et al. 2008), et est désormais présent dans 71 des 96 départements métropolitains. Chaque année en métropole, une surveillance renforcée des arboviroses transmises par ce moustique a lieu du 1er mai au 30 novembre. Selon Santé publique France, le nombre de cas de dengue sur le territoire a atteint un record en 2022, avec un nombre de cas supérieur au nombre total de cas identifiés sur l'ensemble de la période 2010-2021 (66 cas versus 48). Ces données (nombre de cas autochtones en augmentation et extension des zones géographiques où le moustique est implanté) laissent planer le risque d'une multiplication des infections par ces arbovirus dans la prochaine décennie. La modification du paysage urbain et en particulier la politique de végétalisation des villes n'est pas sans conséquence sur la biologie et la dynamique de répartition de cette espèce en ville. Les enjeux actuels reposent donc sur une meilleure caractérisation des facteurs propices à son expansion. Au cours de cette thèse qui associe sciences humaines et sociales et sciences du vivant, nous nous sommes attachés à répondre à cette problématique en nous intéressant plus spécifiquement au rôle des êtres humains, au travers de leurs pratiques et des conséquences induites par leurs activités. Mon terrain d'étude s'est porté sur les jardins collectifs, des lieux propices au développement du moustique tigre en ville.

Dans la première partie, nous discuterons tout d'abord des résultats de l'enquête sur les connaissances, attitudes et pratiques vis-à-vis du moustique tigre réalisée auprès des adhérents aux jardins collectifs de la Métropole de Lyon et les recommandations vers lesquelles ce travail a mené. Dans la deuxième partie, nous examinerons les résultats obtenus à partir de l'analyse des échantillons de terrain avec comme objectif initial d'identifier si des caractéristiques physico-chimiques et microbiennes spécifiques de l'eau influencent sa colonisation par *Ae. albopictus* en prenant en compte également la localisation des jardins selon leur exposition à des sources polluantes environnantes. Dans la troisième partie, nous discuterons les résultats de ces deux approches sur le terrain (enquête et mesures) pour proposer de nouvelles pistes de réflexion sur les futurs projets à mener en matière de sciences participatives et lutte antivectorielle. La dernière partie portera sur les conclusions générales et sur les perspectives de potentielles recherche pour poursuivre cette étude.

3. Impact des connaissances et de la perception du risque sur les bonnes pratiques de gestion du moustique tigre dans les jardins collectifs

De nos jours, la lutte antivectorielle est primordiale pour limiter la prolifération du moustique tigre et des maladies qu'il véhicule. Les méthodes de lutte antivectorielle sont très variées et leur choix doit être guidé non seulement par le pathosystème vectoriel (vecteur, pathogène, vertébré) mais aussi par le contexte épidémiologique, environnemental et socio-économique (Aryaprema et al. 2023). Le rapport n°3280 de l'Assemblée Nationale place l'engagement des citoyens comme une priorité dans la lutte dirigée contre les moustiques (Naillet 2020). Ce rapport préconise de repenser la politique de prévention, de la fonder sur une meilleure sensibilisation du public habitant dans des zones où le moustique est présent et sur la modification des comportements inadaptés via la formation, l'information et la mobilisation sociale. Éradiquer ces moustiques n'est pas envisageable aujourd'hui, une perspective plus réaliste serait plutôt de limiter leur présence et de permettre une meilleure cohabitation entre ces deux espèces, l'être humain et le moustique. La mise en place de ces plans de prévention nécessite, en amont, d'évaluer les connaissances, les attitudes et les comportements de la population pour identifier les lacunes, la perception et les mauvaises pratiques. Les enquêtes de type CAP sont des outils permettant de cibler les besoins pédagogiques sur un sujet spécifique relevant généralement du domaine médical. Elles évaluent trois aspects : le niveau de connaissances, les attitudes motivant les comportements et les pratiques d'une population cible (Essi and Njoya 2013). Généralement, ces enquêtes s'intéressent également aux caractéristiques socio-économiques des répondants. Elles ont été fréquemment utilisées dans les zones endémiques (où les maladies dues aux moustiques sont présentes en permanence), pour évaluer les connaissances et comprendre les attitudes et pratiques concernant les moustiques et les maladies qu'ils transmettent (Subramaniam et al. 2021). Le risque d'épidémies étant plus incertain, moins d'études se sont focalisées sur les zones non endémiques.

Dans cette étude, nous avons spécifiquement évalué les connaissances, les attitudes et les pratiques concernant le moustique tigre au sein des jardins collectifs de la métropole de Lyon. Notre enquête a révélé que les jardiniers avaient une bonne compréhension du cycle de vie du moustique. En effet, la majorité des personnes interrogées étaient informées que les moustiques se développent en milieu aquatique lors du stade larvaire. La quasi-totalité des jardiniers ayant répondu à l'enquête a correctement identifié les sites de reproduction, les caractérisant comme

des réservoirs d'eau stagnante de taille petite à moyenne. Pour autant, les adhérents aux jardins n'éliminaient ou ne protégeaient pas systématiquement tous les endroits où l'eau stagne. Par ailleurs, certains réservoirs d'eau ne peuvent tout simplement pas être supprimés au sein des jardins de par leur utilité.

La gestion partielle de ces gîtes potentiels pourrait s'expliquer par une identification incomplète de ces derniers. En effet, les répondants étaient capables d'en identifier certains correctement, cependant beaucoup d'entre eux en désignaient d'autres qui n'en étaient pas, comme par exemple la végétation. Ces résultats pointent des faiblesses dans les connaissances des jardiniers sur le type et la localisation des habitats aquatiques propices au développement larvaire du moustique tigre. Des constatations similaires ont également été faites à partir d'autres études CAP réalisées en zones non endémiques auprès de différents types de population en Europe (Heym et al. 2017; Stefopoulou et al. 2018; Caputo et al. 2020; Kolimenakis et al. 2022). Par exemple, Morse et al (2019) ont démontré que près de la moitié des résidents de Mobile (ville située en Alabama, USA) interrogés ne reconnaissaient pas les gouttières comme des endroits propices au développement des larves (Morse et al. 2019). Par ailleurs, notre étude révèle que la majorité des répondants possède sur leur parcelle un ou plusieurs récupérateurs d'eau de pluie (Mariappan et al. 2008). De par leur intérêt économique et écologique, il n'est pas envisageable de demander aux jardiniers de ne plus les utiliser. Actuellement, la plupart des campagnes de sensibilisation encourage fortement d'adopter certaines pratiques comme tendre une moustiquaire entre la sortie de la gouttière et la surface de l'eau. Une étude portant sur l'inspection de près de 500 réservoirs d'eau de pluie dans l'agglomération de Melbourne montre qu'afin de réduire le risque de reproduction des moustiques dans ces réservoirs, il est impératif de bien sceller toutes les voies d'accès possibles. Néanmoins, 4 % des systèmes munis d'une protection adéquate à l'entrée et au débordement avaient encore des moustiques (Moglia et al. 2016). Une réflexion autour de l'utilisation de ces récupérateurs d'eau dans les jardins devrait être menée pour empêcher ou minimiser leur colonisation par le moustique. Certains jardins collectifs ont déjà entamé cette démarche, c'est le cas par exemple du jardin des Oréades en Savoie qui a remplacé les récupérateurs d'eau (souvent de 200L) par 3 grandes cuves collectives de 1000L reliées à des canalisations. Selon nos observations sur le terrain, ce type d'aménagement serait plus efficace car il limiterait le nombre de réservoirs dans les jardins et leur protection serait plus facile à assurer car l'eau est récupérée via un robinet qui empêche la femelle de pénétrer à l'intérieur. Cet aménagement nécessite par contre une gestion de l'eau collective dans le jardin, pas toujours simple à mettre

en place. L'inaction d'une partie des jardiniers observée au sein des jardins (peu ou pas d'élimination des réservoirs d'eaux stagnantes alors que les jardiniers déclarent savoir que ces réservoirs peuvent servir de sites de reproduction aux moustiques) pourrait également être le reflet de la perception qu'ont les jardiniers de l'efficacité de ces méthodes mécaniques et plus globalement de l'ensemble des méthodes de lutte mises en place dans les jardins. En effet, notre étude a révélé que la plupart des répondants considère les moyens de lutte au sein des jardins comme inefficaces, ce qui est un frein considérable pour permettre leur application étant donné que celle-ci repose avant tout sur une mobilisation et une participation collective (Fontenille et al. 2009).

Dans notre étude, la grande majorité des répondants a indiqué être consciente du fait que le moustique peut transmettre des virus pathogènes. Cependant, ces connaissances ne semblaient pas influencer positivement l'adoption de bonnes pratiques pour limiter la prolifération de cette espèce. Ces observations diffèrent de celles obtenues de deux autres études CAP réalisées auprès de résidents habitant en zones non endémiques où une tendance inverse était plutôt observée (Dowling et al. 2013; Caputo et al. 2020). Cette différence pourrait s'expliquer par la population cible et le niveau de formation des répondants. La participation aux activités collectives des jardiniers favorise l'acquisition de savoirs, qu'il s'agisse de connaissances formelles ou informelles, en particulier dans les domaines de la science et de l'environnement (Datta 2016). D'autre part, les personnes avec un haut niveau d'études sont surreprésentées dans notre étude, pouvant créer un biais en faveur des personnes bien informées. La distribution de l'enquête par courrier électronique auprès des responsables des jardins et des adhérents (lorsque leurs adresses étaient connues) pourrait avoir entraîné une participation accrue des groupes socialement favorisés, en particulier ceux ayant un niveau d'éducation élevé (Lindhjem 2011). Étant donné l'absence de données sur le profil et le statut socio-économique des jardiniers de la métropole de Lyon, il est difficile d'évaluer si notre échantillon est représentatif de l'ensemble des adhérents aux jardins collectifs. Il est également important de noter que les réponses aux questionnaires proviennent d'adhérents d'environ 30% des jardins de la métropole, ce qui peut biaiser les résultats en faveur des personnes motivées plutôt que celles représentatives de l'ensemble des membres des jardins. De plus, il faut également souligner que l'enquête a été diffusée uniquement en français, ce qui pourrait potentiellement biaiser les réponses en faveur de certaines populations. Une campagne d'enquête diffusée dans d'autres langues et via différents canaux serait utile pour pouvoir atteindre une plus grande diversité d'adhérents aux jardins collectifs. De la même manière, il

serait intéressant de confronter les résultats de cette enquête à ceux obtenus d'enquêtes menées dans d'autres grandes villes de métropole afin d'évaluer si les tendances observées ici se confirment à une échelle plus large.

Parmi les répondants ayant indiqué être préoccupés par la présence du moustique tigre, plus de la moitié s'inquiétait de la gêne occasionnée par les piqûres pendant la journée, plutôt que de sa capacité à transmettre des pathogènes. Notre questionnaire n'ayant pas abordé de questions relatives aux connaissances des jardiniers vis-à-vis des cas autochtones d'arboviroses à Lyon ou en France métropolitaine, nous ne pouvons pas conclure si la tendance observée pourrait résulter d'un manque d'informations sur le sujet. Néanmoins, cette tendance pourrait s'expliquer par le fait que le risque sanitaire lié au moustique tigre en France métropolitaine n'est pas encore considéré comme élevé. Un questionnaire réalisé auprès d'une population italienne a montré une tendance similaire, alors même que l'Italie a connu une épidémie de Chikungunya dans la région de Ravenne en 2009 (Angelini et al. 2007). Cette étude avait ciblé des personnes natives d'Italie et des personnes originaires de l'Inde résidant en Italie depuis plus d'un an, et ces derniers avaient déclaré être plus préoccupés par la capacité des *Aedes* à transmettre des maladies (Caputo et al. 2020). La France métropolitaine ayant enregistré un nombre record de cas de dengue sur le territoire en 2022, il serait instructif de voir si la perception des jardiniers a changé depuis notre enquête réalisée en 2021. Néanmoins, une étude indique que la population française a généralement confiance dans le système de soins de santé et dans les capacités des autorités publiques à gérer une éventuelle crise, ce qui réduit leur inquiétude quant à l'arrivée de ces maladies (Claeys and Mieulet 2013). Cependant, bien qu'étant encore perçu positivement, la crise sanitaire du Covid 19 a mis à l'épreuve la confiance dans le système de soin de la population française. En effet, un sondage Cluster 17 pour le journal « Le Point » montre que 80% des français se disent inquiets pour l'avenir du système de santé (Schneegans 2023). Ce changement de perception suggère que la population pourrait se montrer plus préoccupée par les risques de maladies vectorielles dans les années à venir.

Enfin, les résultats de l'enquête ont mis en évidence l'absence de lien entre les caractéristiques sociodémographiques des adhérents des jardins collectifs (sexe, âge, catégorie socioprofessionnelle), leur niveau de connaissance, ainsi qu'avec leurs pratiques. Ces résultats diffèrent largement de ceux de la plupart d'enquêtes CAP qui ont été réalisés dans des régions non endémiques (Tuiten et al. 2009; Raude et al. 2012; Dowling et al. 2013; Maeda 2017; Mouchtouri et al. 2017; Walker et al. 2018; Bohmann et al. 2022; Moise et al. 2022). Une

explication plausible serait que les adhérents des jardins se distinguent de la population générale par leur motivation et intérêt pour le jardinage collectif.

Bien que spécifique à une population, ce travail offre de nouvelles pistes de réflexion pour guider de futurs plans de prévention collective de l'implantation du moustique tigre en milieu urbain. Communiquer et alerter sur l'accroissement du nombre de cas d'arboviroses en France et les risques associés pour les décennies à venir semble être une bonne piste pour augmenter la conscience collective et individuelle de la population locale. Selon les recommandations du Comité de Veille et d'Anticipation des Risques Sanitaires (COVAR), la mise en place de structures de coordination visant à renforcer les interactions entre les acteurs nationaux et régionaux est indispensable pour faire face à l'augmentation de ces maladies vectorielles en France métropolitaine. Ces structures pourraient davantage impliquer les collectivités territoriales, les particuliers et les entreprises privées, mais également accueillir des représentants de plusieurs disciplines, notamment l'épidémiologie, l'entomologie, la virologie et la sociologie, afin d'aborder cette problématique via une démarche plus intégrative (COVAR 2023). De plus, les sciences participatives se révèlent de plus en plus comme un levier décisif pour mobiliser le grand public dans la lutte contre ce moustique (Jordan et al. 2017; Bartumeus et al. 2018). Cette thèse s'inscrit dans un programme de recherche qui, à plus long terme, a pour ambition de retourner dans les jardins et d'instaurer des activités de sciences participatives en collaboration avec les jardiniers (voir chapitre suivant). Dans cette optique, nous avons inclus dans l'enquête des questions pour évaluer leur motivation à s'impliquer dans un projet de recherche collaborative. Il est ressorti que la majorité des participants était intéressée à l'idée de participer à un programme de science participative. Ce programme visait à utiliser des pièges olfactifs capables d'attirer les moustiques. Les adhérents aux jardins seraient alors impliqués pour relever les pièges et dénombrer le nombre de moustiques présents à l'intérieur. Dans ce but, nous souhaitons mettre à profit les observations faites sur le terrain et identifier des composés naturellement présents dans les habitats aquatiques du moustique, capables de guider le comportement de pré-oviposition des femelles. Pour atteindre cet objectif, il est important de caractériser, de la manière la plus exhaustive qui soit, la composition de l'eau où le moustique tigre se développe, et ses facteurs de variation.

4. Vers une meilleure caractérisation de l'influence des activités humaines sur les interactions biotiques et abiotiques dans les habitats larvaires

Les moustiques ont développé plusieurs stratégies pour choisir les gîtes larvaires les plus propices au développement de leur progéniture. Dans le cas du moustique tigre, les femelles fractionnent leur ponte dans le temps et dans l'espace, ceci afin de maximiser les chances de survie de leur progéniture (Fay and Perry 1965). Ce comportement est en faveur d'une stratégie de vie colonisatrice et un facteur important pour expliquer sa dispersion. Le choix du site de ponte dépend également de stimuli visuels, olfactifs et tactiles (Clements 2006; Day 2016). Leurs organes sensoriels permettent notamment aux femelles de percevoir différents stimuli environnementaux susceptibles de les attirer ou au contraire de les repousser (Wooding et al. 2020). Elles seraient ainsi capables de discerner la composition physico-chimique de l'eau entre différents gîtes, et de sélectionner ceux abritant les conditions qui stimulent davantage la ponte et/ou le développement de leur descendance (Hery et al. 2021a). La présence de larves conspécifiques (c'est-à-dire de la même espèce) ou de certains microorganismes semble également jouer un rôle dans l'attractivité du gîte pour les femelles gravides (Shragai et al. 2019). En d'autres termes, les propriétés biotiques et abiotiques de l'eau sont des facteurs importants à prendre en compte dans la biologie de cette espèce et sa dynamique d'invasion. En milieu urbain, les gîtes artificiels dans lesquels se développent les larves sont directement exposés à différentes sources de pollution (trafic routier, chauffage urbain, industrie, agriculture, résidus pharmaceutiques...) et peuvent contenir des polluants susceptibles de modifier ces propriétés, ce qui pourrait avoir des conséquences sur les comportements de pré-oviposition et d'oviposition des femelles ainsi que sur le développement des larves.

Dans cette étude, nous avons réalisé une analyse de la composition physico-chimique et microbienne de l'eau prélevée dans des réservoirs d'eau stagnante au sein de 23 jardins collectifs de la métropole de Lyon. Contrairement aux études menées jusque-là, l'originalité de notre étude tenait au fait d'avoir élargi cette analyse à (i) la détection plus globale et sans *a priori* des molécules organiques présentes dans l'eau (analyse non ciblée) tout en permettant un niveau de sensibilité compatible avec des concentrations de polluants présents à l'état de traces et (ii) la prise en compte des microorganismes eucaryotes, relativement peu étudiés en comparaison des communautés procaryotes. De plus, la plupart des études pour caractériser

l'eau des gîtes larvaires s'intéressait à l'une ou l'autre des composantes (biotiques ou abiotiques) indépendamment sans considérer leurs influences réciproques.

4.1 Impact de la composition biotique et abiotique de l'eau sur son état de colonisation

A l'exception de deux composés (CH_4 , N_2O) et des communautés fongiques, nos analyses n'ont pas révélé de différence marquée dans la composition physico-chimique et microbienne de l'eau entre les gîtes colonisés ou non par le moustique. Un plus grand nombre de jardins et de gîtes aurait peut-être permis de mettre en évidence des différences. Cependant, de par la complexité des analyses et la réalité du terrain, prélever des échantillons dans plus de jardins s'est avéré difficile à mettre en place. En effet, pour obtenir les 23 jardins de l'étude, nous nous sommes finalement rendus dans 31 jardins, et il n'a pas été possible de prélever dans huit d'entre eux soit parce que nous nous sommes heurtés à un refus soit parce que tous les réservoirs du jardin étaient colonisés, soit parce que le moustique n'était pas présent dans le jardin. Au commencement de cette étude, nous nous étions fixés un nombre raisonnable d'analyses et de traitements de données à réaliser dans le temps imparti et pour cela deux stratégies d'échantillonnages avaient été envisagées. La stratégie 1 était de maximiser le nombre de jardins en collectant dans chacun d'eux un gîte colonisé et un gîte non colonisé tandis que la stratégie 2 priorisait la collecte d'un plus grand nombre de gîtes mais dans un nombre plus limité de jardins. S'agissant d'un travail exploratoire, nous avons opté pour la première stratégie qui selon nous avait l'avantage de diversifier les lieux de prélèvements et ainsi de maximiser les chances d'observer des différences dues aux pratiques (voir chapitre IV-1) ou au contexte de pollution. Pour limiter les biais d'analyse, la majorité des réservoirs d'eau échantillonnés était du même type, à savoir des récupérateurs d'eau de pluie. La matière du réservoir pouvant influencer les propriétés biotiques et abiotiques du gîte (Hery et al. 2021b), il serait intéressant de poursuivre ce travail en diversifiant le type de réservoirs étudiés et leur localisation dans les jardins. En dehors des récupérateurs d'eaux de pluie retrouvés dans les jardins collectifs, la mosaïque urbaine renferme toute une multitude d'autres habitats aquatiques propices au développement du moustique. C'est le cas notamment des avaloirs, des coffrets techniques ou encore des terrasses sur plots. Il serait intéressant de réaliser un inventaire de ces gîtes à grande échelle (type, prévalence, état de colonisation...) puis d'évaluer leurs propriétés biotiques et abiotiques afin de déterminer les conditions les plus propices à la colonisation par le moustique.

Nos analyses ont néanmoins permis de montrer une plus forte concentration de méthane (CH_4) et de protoxyde d'azote (N_2O) dans les eaux non colonisées. Les activités humaines et notamment l'agriculture intensive sont à l'origine de l'émission de ces gaz (Hinshaw and Dahlgren 2013; Borges et al. 2018). A l'exception de l'oxygène dissous, les gaz dissous sont encore très peu étudiés dans les gîtes larvaires de moustiques. Contrairement à nos résultats, une étude montre que la présence de méthane dans l'eau des gîtes de *Cx. pipiens* favorise l'oviposition (Bentley and Day 1989). A notre connaissance, cette étude est la première à mesurer l'ensemble des gaz présents dans l'eau des gîtes larvaires du moustique tigre. Ces deux gaz étant retrouvés préférentiellement associées à de plus fortes concentrations dans les gîtes non colonisés, il serait intéressant de tester en condition contrôlée de laboratoire leurs effets sur le comportement de pré-oviposition des femelles et le développement des larves. En ce qui concerne les communautés microbiennes, les micro-eucaryotes identifiés à partir du séquençage ITS présentent des variations significatives selon que l'eau soit colonisée ou non. En effet, on observe une plus grande diversité dans les eaux colonisées, ce phénomène pouvant être attribué, d'une part, à leur influence sur l'attraction des femelles pour le site de ponte, et d'autre part, à l'introduction de certaines espèces fongiques lors de la ponte (Malassigné et al. 2020).

4.2 Présence de polluants dans les gîtes larvaires en milieux urbains

Des études antérieures *in situ* semblent montrer que les larves d'*Ae. albopictus* sont capables de se développer dans des eaux avec des indices de pollution élevés et contenant des métaux lourds (Dom et al. 2016; Dalpadado et al. 2022). En plus de l'analyse chimique de l'eau, chaque jardin a été caractérisé selon sa proximité à différentes sources de pollution (industrielles, atmosphériques et agricoles) nous permettant ainsi de définir leur répartition selon ces trois critères. Les discussions informelles avec les jardiniers nous laissent penser que les différents conteneurs échantillonnés (majoritairement des collecteurs d'eau de pluie) étaient composés d'eau de pluie ou d'un mélange d'eau de pluie et d'eau du robinet. L'eau de pluie peut contenir certains métaux toxiques comme le cuivre, le zinc ou encore le cadmium mais également des produits chimiques de synthèse comme des produits agrochimiques et des hydrocarbures aromatiques polycycliques (HAP) (Müller et al. 2020). D'autres contaminants émergents, comme les fluoroalkyles, sont aussi retrouvés (Müller et al. 2020). En ce qui concerne l'eau du robinet, les études font état de la présence de polluants n'étant pas éliminés dans les stations d'épuration. Des travaux montrent, par exemple, la présence de phtalates, de

bisphénol ou encore de perturbateurs endocriniens (Esteban et al. 2014; Domínguez-Morueco et al. 2014; Russo et al. 2022).

En dehors des paramètres physico-chimiques (température, la teneur en oxygène, le pH et la conductivité), cités plus haut, classiquement identifiés pour caractériser la productivité d'un gîte larvaire, nous avons également cherché à déterminer leur composition en micropolluants via une analyse non ciblée permettant la détection globale et sans *a priori* de molécules organiques. Contrairement aux analyses ciblées, où seuls les ions spécifiques des molécules visées sont analysés, cette technique permet de rechercher une gamme étendue de molécules présentes dans un échantillon d'eau (Kind and Fiehn 2007). Dans les échantillons prélevés dans les jardins collectifs, nous avons détecté 1382 molécules organiques dont seulement 23 ont pu être identifiées. Pour connaître l'origine et le rôle potentiel sur le moustique tigre de ces molécules organiques, il serait intéressant de déterminer une partie des 1359 molécules non identifiées. Cela pourrait se faire *a posteriori* en utilisant par exemple « l'analyse suspectée » qui consiste à comparer les données acquises (rapport masse/charge, profil isotopique, temps de rétention, fragmentation) à des bases de données, permettant l'identification de certains contaminants à un seuil acceptable sans la présence d'un étalon analytique (Schymanski et al. 2014). Parmi les molécules identifiées, nous avons retrouvé plusieurs composés caractéristiques de milieux aquatiques pollués, tels que des résidus pharmaceutiques (anti-inflammatoires, hormones, antioxydants) ou encore des fertilisants et des plastiques (dérivés de phtalates). Les micro et nanoplastiques sont aujourd'hui omniprésents dans les milieux aquatiques (Danopoulos et al. 2020; Campanale et al. 2022). Des études récentes ont indiqué que ces composés étaient détectés en forte concentration dans les réservoirs d'eau en zones fortement urbanisées (Carr et al. 2016). Une étude montre que les microplastiques, ici le polystyrène, sont accumulés dans l'intestin des larves et diminuent le taux d'émergence des moustiques *Ae. albopictus* et *Ae. aegypti* (Edwards et al. 2023). Par ailleurs, chez *Ae. aegypti* les nanoplastiques ingérés au stade larvaire s'accumulent chez la nymphe jusqu'à l'adulte mais également chez leur descendance. Dans ce cas-là, ces composés n'affectent pas la survie des moustiques mais retardent leur développement. Ils augmentent également l'activité de piqûre des femelles avec un risque de transmission de ces composés aux êtres humains (Gopinath et al. 2022). Par ailleurs, Muturi et al. (2016) ont montré que l'exposition d'*Ae. albopictus* à des fertilisants tels que le sulfate d'ammonium et le chlorure de potassium réduisait le temps de développement et le taux de survie des larves (Muturi et al. 2016). L'ingestion par les moustiques adultes de solution sucrée supplémentée en engrais de type NPK avait un impact significatif sur leur

fécondité (Darriet 2018a). Bara et al. (2014) ont également montré que l'ingestion d'atrazine, l'un des herbicides les plus fréquemment retrouvés dans les eaux de surface, par les larves d'*Ae. albopictus* augmentait le taux d'émergence et la taille des ailes des adultes (Bara et al. 2014). En dehors de ces composés dont l'effet a été étudié chez le moustique tigre, d'autres polluants ont été analysés chez différentes espèces de moustiques. Il a ainsi été montré que l'exposition du moustique *Anopheles* aux métaux lourds, généralement induite par la pollution industrielle, diminuait non seulement sa fécondité et sa longévité mais pouvait également augmenter sa tolérance aux insecticides et sa susceptibilité à l'infection par *Plasmodium falciparum*, l'agent responsable du paludisme (Jeanrenaud et al. 2020; Maya-Maldonado et al. 2021). Chez *Ae. aegypti*, une étude a révélé deux conséquences transgénérationnelles de l'exposition à l'ibuprofène, un anti-inflammatoire largement utilisé. D'une part, il a été montré une accélération du cycle de vie de la descendance et d'autre part une augmentation de la tolérance à la toxine Bti (Prud'homme et al. 2017).

Pour obtenir des informations sur la composition de l'eau des gîtes, nous avons initialement prévu de déterminer avec précision l'origine de l'eau et d'évaluer la durée pendant laquelle elle avait stagné. Pour cela, nous avons mesuré les rapports isotopiques de l'eau. Cependant, l'interprétation des résultats fut compliquée pour plusieurs raisons. Premièrement, ces valeurs dépendent de l'origine de l'eau et des variations climatiques (De Wet et al. 2020). Or dans notre cas, l'origine de l'eau n'était pas toujours connue et pouvait contenir des mélanges d'eau de ville et d'eau de pluie. Deuxièmement, ces valeurs sont également impactées par l'évaporation et donc les variations météorologiques (Cappa 2003). Des tests supplémentaires seraient nécessaires afin de déterminer le temps nécessaire à la colonisation de l'eau par le moustique tigre. Ceux-ci pourraient être réalisés en créant des gîtes artificiels sur le terrain, en utilisant de l'eau dont l'origine est connue. Cette approche permettrait de mesurer l'évolution du rapport isotopique de l'eau au fil du temps et d'estimer *in situ* depuis combien de temps elle stagne.

4.3 Interaction complexe entre le moustique tigre, les communautés microbiennes et les perturbateurs anthropiques

Les écosystèmes aquatiques sont des environnements complexes où les interactions entre les contaminants d'origine anthropique, les microorganismes et les moustiques sont interconnectées (Sigeo 2005). En effet, les communautés microbiennes de l'eau sont capables

de réduire la présence de perturbateurs anthropiques comme les hydrocarbures (Mandal et al. 2021). Les microorganismes peuvent également être impactés par les contaminants anthropiques (Gao et al. 2019; Pinto et al. 2022). Par exemple, Gao et al. (2019) montrent que la présence de micropolluants organiques module les communautés bactériennes dans les eaux de surface (Gao et al. 2019). Plusieurs d'entre eux, tels que les antibiotiques, les microplastiques ou encore les pesticides peuvent modifier la composition microbienne dans l'eau et moduler le microbiote des moustiques (Qing et al. 2020; Juma et al. 2020; Yang et al. 2022; Edwards et al. 2023). Par exemple, l'ingestion de micro-plastiques par les larves d'*Ae. albopictus* au premier stade engendre une diminution de la diversité des communautés fongiques et bactériennes associées aux larves. Les microorganismes qui ont contribué à ces différences appartiennent au genre *Elizabethkingia*, pour les bactéries et *Aspergillus*, pour les champignons (Edwards et al. 2023).

Lors de cette étude nous avons également testé l'influence d'expositions environnementales sur les communautés microbiennes de l'eau des gîtes larvaires. Cette analyse s'avérait pertinente pour notre étude à plus long terme visant à déterminer les propriétés du gîte jouant un rôle attractif pour les femelles gravides. Pour cela, nous avons inclus dans cette analyse l'impact combiné de la présence de molécules organiques et de la composition physico-chimique de l'eau ainsi que l'exposition à des pollutions (via la proximité du jardin à différentes sources de pollution). Nos résultats ont révélé un impact différentiel de ces expositions en fonction de la présence/absence du moustique. Dans les gîtes colonisés, la pollution industrielle et la composition ionique étaient positivement corrélées avec les UTOs appartenant à *Actinomycetospora* et *Rozellomycota* tandis que la composition en molécules organiques impactait positivement une UTO de la famille des Rhizobiaceae et un champignon phytopathogène *Ascochyta phaca*. Le phylum fongique *Rozellomycota* est également apparu corrélé avec certaines molécules organiques dans les gîtes non colonisés de même que la microalgue *Neochloris aquatica*. Les *Actinomycetospora*, déjà identifiés dans les gîtes de moustiques *Aedes* (Zouache et al. 2022), sont également conservés dans le microbiote des larves. Il a été montré que *Neochloris aquatica* présentait non seulement des effets larvicides mais pouvait également impacter les paramètres physico-chimiques de l'eau (Gil et al. 2021; Ummalyma et al. 2023). Bien que le rôle des *Rozellomycota* ne soit pas clairement défini, ces bactéries pourraient participer à la décomposition de la matière organique (Grossart et al. 2016). Il est important de préciser ici que les réseaux identifiés témoignent de corrélations observées *in situ* entre certains paramètres mais ne signifie pas forcément un lien de causalité. Il serait

intéressant de poursuivre les investigations en étudiant en conditions contrôlées de laboratoire le rôle de ces microorganismes dans la biologie du moustique, et leur réponse à une exposition à des polluants ou des paramètres physico-chimiques particuliers. Ces nouvelles expériences nous permettraient de déterminer si ces combinaisons pourraient représenter un facteur de risque pour la prolifération du moustique.

Pour finir, avec notre objectif à plus long terme de créer des pièges visant la capture spécifique du moustique, nous avons isolé des communautés microbiennes à partir de l'eau des gîtes colonisés. Cette approche nous a ainsi permis de créer une souchothèque de près de 580 bactéries et 230 levures dont la taxonomie a été réalisée par séquençage Sanger et a permis d'identifier des souches spécifiques des gîtes colonisés et non colonisés, y compris certains membres des groupes taxonomiques cités plus haut. Des travaux sont actuellement en cours afin d'évaluer leur pouvoir attractif ou répulsif vis-à-vis du comportement de pré-oviposition des femelles. Des résultats préliminaires tendraient à montrer que la bactérie *Serratia oryzae*, isolée de l'eau colonisée, stimulerait la ponte des femelles *Ae. albopictus*.

5. Mobilisation sociale et recherche scientifique : un levier pour ralentir la progression du moustique tigre en milieu urbain ?

A l'heure actuelle, bien que certains prototypes de vaccins soient en cours d'élaboration, la vaccination et la prophylaxie contre la plupart des maladies transmises par le moustique tigre sont limitées (Schwameis et al. 2016). Par conséquent, la lutte antivectorielle s'impose comme le seul moyen pour contrôler l'apparition de ces maladies. L'élimination totale des moustiques n'est pas envisageable, l'objectif étant de contrôler leur densité afin de diminuer le risque de transmission et d'épidémies. Une lutte antivectorielle intégrée, impliquant les chercheurs, les collectivités territoriales, les établissements publics comme l'Entente interdépartementale pour la démoustication (EID), les particuliers et certains acteurs économiques, apparaît aujourd'hui comme la seule approche capable d'obtenir un tel résultat. Le développement de nouvelles méthodes doit répondre à plusieurs critères : être efficaces, avoir peu ou pas d'impact sur l'environnement, être durables et enfin être acceptées par la population (Naillet 2020). Dans cette étude, l'un des objectifs initiaux était précisément de mettre en place des pièges en utilisant des microorganismes et de tester leur efficacité sur le terrain.

L'usage d'insecticides est réservé aux seuls traitements localisés autour de cas humains malades confirmés, en complément d'une destruction des gîtes larvaires dans le voisinage des personnes infectées. Ces interventions sont réalisées par l'EID. Depuis le mois de mai 2023, six communes du Rhône ont été concernées. Ce type d'intervention fait débat et fait naître un mouvement de désobéissance civile. Par exemple, en 2019 l'association de protection de l'environnement, « Arthropologia », a lancé un mouvement pour protester et empêcher l'utilisation de traitements insecticides de la famille des pyréthrinoïdes. De plus, l'augmentation croissante des niveaux de résistances aux insecticides pyréthrinoïdes remet en question leur utilité (ANSES 2021a). Il a d'ailleurs été proposé un lien entre l'exposition des moustiques à certains polluants (dont HAP et herbicides) et l'augmentation de leur tolérance aux insecticides chimiques (Suwanchaichinda and Brattsten 2002; Poupardin et al. 2008; Riaz et al. 2009; David et al. 2010; Poupardin et al. 2012). Il serait intéressant d'évaluer si les polluants retrouvés dans l'eau de gîtes colonisés impactent les mécanismes de résistance métabolique aux insecticides chez *Ae. albopictus*.

La lutte biologique est une alternative viable à l'utilisation d'insecticides et semble bien acceptée par la société civile (Lambert 2010). Ces méthodes, respectueuses de l'environnement, reposent sur l'utilisation d'organismes vivants ou de leurs produits dérivés pour lutter contre d'autres organismes jugés nuisibles (Van Driesche and Bellows 2012). Parmi elles, les insecticides biologiques tels que les spores de Bti (*Bacillus thuringiensis* var. *israelensis*) ont fait leur preuve pour lutter contre les moustiques. Cette bactérie produit, sous la forme de nanocristaux, quatre toxines spécifiques des larves de moustiques. Dans notre enquête, seuls 20% des répondants déclaraient connaître la lutte biologique et seulement neuf d'entre eux étaient capables d'expliquer ce que cela signifiait. Selon notre enquête, il y a clairement une demande de mieux connaître ce moyen de lutte, puisqu'une majorité (63%) des adhérents aux jardins collectifs ont répondu qu'ils souhaitaient être davantage informés sur les méthodes alternatives à l'utilisation d'insecticides chimiques. Une étude indique que la plupart des adhérents des jardins manifeste une sensibilité aux enjeux environnementaux (Den Hartigh 2012). Par conséquent, cette approche de lutte plus écologique pourrait être favorablement accueillie au sein de ces jardins. Classé dans la catégorie des méthodes biologiques, l'emploi de microorganismes semble également une méthode prometteuse pour limiter la prolifération des moustiques (Huang et al. 2017). Il a par exemple été montré que les bactéries du genre *Streptomyces* produisaient des composés actifs capables de tuer les œufs, les larves ou encore les nymphes de moustiques (Amelia-Yap et al. 2022). Le développement de pièges attractifs

basés sur l'utilisation de microorganismes est également à l'essai (Hapairai et al. 2021; Mosquera et al. 2023). En effet, il a été mis en évidence que les microorganismes produisaient des composés organiques volatiles (COV) capables d'attirer les moustiques (Mosquera et al. 2023). De nombreuses études visent à caractériser le microbiote de la peau humaine et à identifier les composés d'origine microbienne responsables des odeurs qui attirent les moustiques (Takken and Verhulst 2017; Michalet et al. 2019; Lucas-Barbosa et al. 2021). Les microorganismes colonisant les gîtes larvaires jouent également un rôle dans le comportement de pré-oviposition des femelles (Girard et al. 2021). Il a ainsi été montré que certains COVs émis par des bactéries du genre *Klebsiella* ou des levures du genre *Saccharomyces* pouvaient modifier le comportement de ponte des femelles *d'Aedes* en les attirant vers les sites où ils sont présents. Cependant, l'utilisation de microorganismes ou de leurs dérivés dans les pièges pourrait, dans certains cas, être mal acceptée par la population et faire l'objet d'une perception plus négative. En effet, pour de nombreuses personnes, le terme « microorganisme » est souvent synonyme de « pathogène » (Lambert 2010). Un travail de sensibilisation vis-à-vis de ces méthodes s'avère essentiel pour changer les mentalités (Warner 2012).

Quelle que soit la méthode de lutte privilégiée, celle-ci doit impérativement être associée à la lutte mécanique pour optimiser ses chances de succès (Shaukat et al. 2019). La lutte mécanique comprend non seulement l'élimination ou la protection de potentiels habitats larvaires, mais peut également viser les moustiques au stade adulte avec la pose de pièges. De plus, il est crucial de prendre en compte les avancées scientifiques dans la caractérisation des sites de reproduction des moustiques (type, couleur, composition) compte tenu de leur rôle dans la colonisation par le moustique (Hoel et al. 2011; Oliva et al. 2014; Pavlovich and Rockett 2018; Borel et al. 2021; Hery et al. 2021b).

V. Conclusion et perspectives

L'ensemble de ces travaux de thèse contribue à réaffirmer l'importance de prendre en compte plusieurs facteurs pour lutter efficacement contre l'expansion du moustique tigre en milieu urbain. Cette recherche a offert une perspective globale sur l'impact des comportements humains en parallèle des activités anthropiques dans un même contexte d'étude, à savoir les jardins collectifs. Ces jardins sont des initiatives de plus en plus fréquentes mais qui, parallèlement à leurs nombreux apports pour la ville et ses habitants, favorisent la prolifération du moustique en milieu urbain. Il est donc très important de caractériser avec précision les facteurs favorisant son expansion et contribuer à la recherche de solutions pour réduire sa vitesse de propagation. Notre étude montre que le moustique tigre est souvent considéré davantage comme une nuisance que comme vecteur de maladies. Il est alors crucial de mieux informer sur le risque futur afin de sensibiliser à l'émergence de ce problème de santé publique. Nos résultats mettent en avant que la gestion de l'eau semble être un paramètre clé dans les facteurs favorisant la prolifération du moustique dans les jardins collectifs. Renforcer l'information et la sensibilisation des adhérents sur les enjeux sanitaires futurs liés à la présence de ces réservoirs d'eau dans les jardins et sur les bonnes pratiques d'utilisation sont un élément clé dans la lutte contre le moustique. Nos résultats, à l'instar d'autres travaux, mettent également en avant un impact potentiel de la pollution sur la biologie de cet insecte en milieu urbain. Agir contre la pollution en ville est déjà reconnu comme un enjeu de santé publique majeur. Acquérir de nouvelles données sur les conséquences de l'exposition du moustique à ces différentes sources de pollution urbaine et en particulier sur sa capacité à transmettre des pathogènes serait un argument supplémentaire pour encourager les initiatives afin de réduire la pollution en ville.

Ce travail ouvre plusieurs perspectives de recherche à court, moyen et long terme. Les résultats préliminaires obtenus dans le chapitre IV-2 sur les tests d'attractivité de microorganismes semblent être une piste intéressante et méritent d'être poursuivis en testant un plus grand nombre de souches ainsi que des mélanges. Une fois leur effet confirmé en laboratoire et l'autorisation sous le règlement UE N°528/2012 associé aux usages biocides obtenue, les pièges pourront alors être développés et testés sur le terrain en mobilisant, sur la base du volontariat, les adhérents aux jardins volontaires. Selon l'ANSES, les pièges à moustiques déjà existants ciblant les femelles gravides démontrent une très bonne acceptabilité des populations de par leur maintenance peu contraignante, leur simplicité d'utilisation et leur coût abordable (ANSES 2021b).

Ce travail exploratoire est également une étape préliminaire à une étude plus globale, qui pourrait être élargie à d'autres écosystèmes urbains (Vezzani 2007; Dalpadado et al. 2022). Au-delà de la caractérisation des jardins selon leur contexte de pollution, d'autres paramètres sont susceptibles d'influer sur la colonisation de l'eau par le moustique et/ou le développement des larves. Par exemple, la pollution lumineuse ou les îlots de chaleur urbain, revêt une grande importance dans ces milieux et semble avoir un impact significatif sur les comportements des moustiques, leur capacité à transmettre des maladies, ainsi que sur les communautés microbiennes (Medeiros-Sousa et al. 2017; Cheloni and Slaveykova 2018; Kernbach et al. 2019; De Jesús Crespo and Rogers 2021; Coetzee et al. 2022; Wolkoff et al. 2023). Explorer davantage leurs incidences sur la colonisation de l'eau par le moustique tigre et son développement en milieu urbain serait une perspective intéressante pour poursuivre ces travaux (Ulpiani 2021).

Ce travail de thèse interdisciplinaire avait pour objectif de mettre en évidence certains facteurs contribuant à l'expansion du moustique tigre dans les jardins collectifs de la métropole de Lyon et pouvant conduire à plus long terme à la préconisation de nouvelles méthodes adaptées pour aller vers une diminution de sa propagation en milieu urbain. Réels lieux d'échanges, ces jardins offrent un terrain favorable aux actions d'information et de sensibilisation aux bonnes pratiques de gestion de cette espèce. De nouveaux programmes de lutte pourraient être développés, impliquant à la fois des experts en démoustication et des membres de la population locale. Par exemple, dans le Var, des initiatives de ce type ont été entreprises au sein de plusieurs communes et ont contribué à mettre en avant l'engagement de la population. Ce modèle semble être une solution efficace pour la transmission des connaissances, ainsi que pour la mise en œuvre de mesures de lutte mécanique à la fois dans les espaces publics et privés (Le Tyrant 2020). Plusieurs articles de quotidiens témoignent du mécontentement de la population générale vis-à-vis de l'implication des pouvoirs publics dans la gestion de cette espèce^{1,2,3,4}. Aujourd'hui, il semble alors essentiel que tous les acteurs ayant des leviers s'associent pour faire face à ce nouveau défi de santé publique.

¹ Face aux moustiques tigres, des villes de métropole demandent une action de l'État (bfmtv.com) (29/08/2023)

² "Le moustique tigre est une affaire politique", un député réclame en urgence des mesures (francetvinfo.fr) (25/07/2023)

³ Métropole de Lyon: la politique des élus écologistes contre le moustique tigre ne convainc pas les habitants (bfmtv.com) (26/08/2023)

⁴ À Lyon, les élus écologistes se défendent d'être responsables de l'invasion de moustiques-tigres - Le Parisien (26/08/2023)

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Annexe 1 : Affiches de sensibilisation au moustique tigre visant les jardins collectifs mis en place par la ville de Lyon en partenariat avec l'association Le passe jardin.

J

JARDINS URBAINS, ENVIRONNEMENT & SANTÉ

MOUSTIQUES TIGRES ET JARDINS



Comment se protéger et protéger son entourage ?

ENJEUX SANITAIRES ET ENVIRONNEMENTAUX

La prolifération des moustiques, notamment du moustique « tigre » (*Aedes albopictus*) qui est le vecteur de deux maladies virales, la dengue et le chikungunya, est désormais un réel problème de santé et de salubrité publique.

Le Rhône est concerné par le plan de Lutte Nationale contre la dengue et le chikungunya.

Ces virus sont transmis par des moustiques femelles du genre *Aedes* qui sont identifiables grâce à la présence de rayures noires et blanches sur

leur abdomen et leur petite taille. La femelle s'infecte quand elle pique une personne malade. Elle devient alors vectrice et peut transmettre le virus lors d'une nouvelle piqûre. Le moustique « tigre » est fortement affilié à l'homme et il vit au plus près de nous. Il se déplace peu. C'est donc à chacun de nous d'être vigilants pour détruire les larves. La ponte s'effectue sur des « supports » secs, proches de l'eau, indispensables pour le développement des œufs et des larves.



Albopictus en train de piquer : CDC / James Gathany
Aedes Albopictus et pièce de 1 centime : JB Ferré / EID Méditerranée

RECOMMANDATIONS

SOYONS SECS AVEC LES MOUSTIQUES ! DES GESTES SIMPLES - STOCKER L'EAU DANS DES CONDITIONS D'HYGIÈNE adaptées

EVITER LA CRÉATION DE GÎTES LARVAIRES : Le moyen le plus efficace pour freiner la reproduction des moustiques est d'empêcher la ponte et la croissance des larves. Cela ne peut être réalisé qu'en supprimant les gîtes larvaires où elles se développent.

- Rendre les bidons de récupération d'eau de pluie inaccessibles aux moustiques (les couvrir d'une moustiquaire ou d'un tissu fin, maille de 1mm maximum).
- Ranger à l'abri de la pluie tous les stockages pouvant contenir de l'eau : pneus, bâches plastique, les soucoupes des pots de fleurs ou les remplir de sable humide, « Dans la soucoupe, je mets du sable non traité, ainsi lorsque j'arrose ma plante, le surplus d'eau reste dans le sable, ce dernier reste humide et je fais des économies. Sans eau, les moustiques ne pourront pas pondre leurs œufs ».
- Arroser les jardinières de manière contrôlée afin d'avoir un terreau juste humide.



Grille anti-moustiques de maille de 1mm au maximum

POUR ÉLIMINER LES LIEUX DE REPOS DES MOUSTIQUES :

- Débroussailler et tailler les herbes hautes et les haies, élaguer les arbres, ramasser les fruits tombés et les déchets végétaux.
- Prévoir une pente suffisante pour que l'eau ne stagne pas dans les gouttières, veiller à la bonne évacuation des eaux de pluie.
- Entretien votre jardin.

POUR SE PROTÉGER : Il est recommandé avant d'utiliser un répulsif cutané, de demander conseil au pharmacien ou à votre médecin.

En partenariat avec :



BON A SAVOIR

Comment le moustique « tigre » se développe-t-il ?

Il se développe en quatre étapes : œuf, larve, nymphe et adulte. Le développement des larves s'étend de 7 à 20 jours, en fonction de la température. Plus il fait chaud, plus le développement des œufs est rapide.

Chaque femelle de moustique « tigre » pond environ 200 œufs. Au contact de l'eau, ils donnent des larves. C'est à ce stade qu'il faut agir...



Seules les femelles piquent, pendant la journée (contrairement aux autres espèces de moustiques présentes en zone urbaine qui piquent plutôt le soir et la nuit) avec une recrudescence d'activité le matin et en fin de journée.



Source EID MEDITERRANÉE

Arrêté préfectoral n° 2014/044-022 relatif aux modalités de mise en œuvre du plan national anti-dissémination du chikungunya et de la dengue dans le département du Rhône

- Loi n° 64-1246 du 16 décembre 1964 relative à la lutte contre les moustiques modifiée par la loi n° 2004-809 du 13 août 2004 relative aux libertés et responsabilités et responsabilités locales
- Règlement Sanitaire Départemental articles 12, 23-3, 30 et 121
- Arrêté préfectoral n° 2014-118-0015 relatif aux modalités de mise en œuvre du plan national anti-dissémination du chikungunya et de la dengue dans le Département du Rhône.
- Arrêté préfectoral n° 2015/077-0009 autorisant les agents chargés de la lutte contre les moustiques à pénétrer dans les propriétés publiques et privées pour procéder aux opérations relatives à la lutte contre les moustiques



inspiré de l'ARS (Agence Régionale de Santé)

LES CONTACTS UTILES

POUR L'IDENTIFICATION ET LE TRAITEMENT CONTRE LE MOUSTIQUE TIGRE :

Entente Interdépartementale Rhône-Alpes pour la Démoustication
04 79 54 21 58

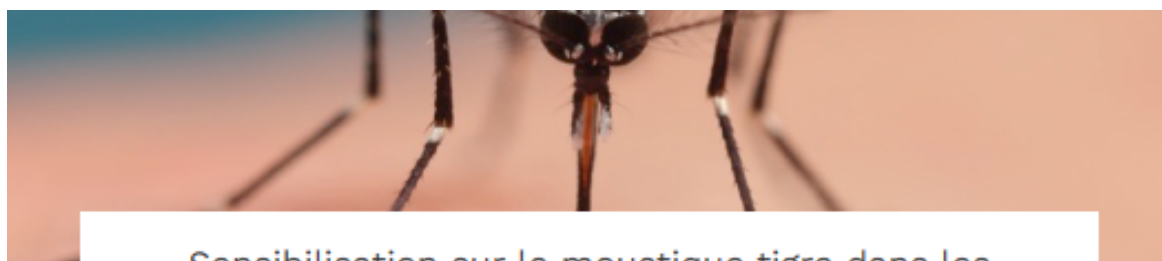
POUR SIGNALEMENT :

Ville De Lyon, Direction de l'Ecologie Urbaine
Service Hygiène Urbaine
ecg-urb@mairie-lyon.fr et 04 72 83 14 00

INFOS PLAQUETTE ARS :

[Moustique tigre, nuisances et maladies](#)

Annexe 2 : Extrait de la page internet de la ville de Strasbourg présentant des ateliers de sensibilisation au moustique tigre destinés aux membres des jardins collectifs (www.strasbourgcapousse.eu).



Sensibilisation sur le moustique tigre dans les jardins collectifs de l'Eurométropole de Strasbourg

10 JUL. 2022



● Le moustique tigre *Aedes albopictus*

Reconnaissable à ses rayures noires et blanches sur les pattes et l'abdomen, ce moustique de très petite taille (5 à 7-mm) vit en milieu urbain, à proximité des hommes et se déplace peu. À la différence du moustique commun, il pique tout au long de la journée. Les larves se développent dans les eaux stagnantes. Ce moustique peut transmettre certaines maladies comme la dengue, le chikungunya ou le zika, s'il pique une personne malade revenant de zones tropicales où ces maladies sont présentes.

● Les jardins collectifs, lieux propices au moustique tigre !

En plus d'être des milieux de vie favorables aux moustiques tigres adultes, ceux-ci offrent une multitude de sites de ponte potentiels : fûts de récupération d'eau de pluie, seaux, arrosoirs, coupelles...etc.

● Comment lutter contre sa prolifération ?

- Éliminer tous les endroits où l'eau peut stagner, même en petite quantité
- Couvrir tout ce qui contient de l'eau avec une moustiquaire, du tissu ou un couvercle hermétique
- Vérifier le bon écoulement des eaux

● Pour répondre à toutes vos questions sur ce moustique nuisible et recueillir des conseils pour lutter contre sa prolifération, des spécialistes du Syndicat mixte de Lutte contre les Moustiques dans le Bas-Rhin (SLM67) organisent plusieurs rencontres et ateliers pour tout public :

Des ateliers de sensibilisation en petits groupes, durée 2 à 3 heures pour en apprendre davantage sur ce moustique : sa biologie, son comportement, outils d'observation et de prévention, exposition,

Des stands nomades : situés aux entrées de certains jardins collectifs, sous la forme d'un point d'observation, documentation, conseils,

À la rencontre des usagers de jardins familiaux : des agents du SLM67 viendront à votre rencontre dans vos jardins (familiaux, partagés ou même résidentiels) pour vous informer et sensibiliser sur le moustique tigre.

Les dates de ces différentes actions seront indiquées sur les tableaux d'affichage des jardins concernés, des modifications ou annulations pourront toutefois survenir afin de s'adapter au mieux aux conditions météorologiques.

À télécharger : affiche sur le moustique tigre ci-dessous

Plus d'informations sur www.slm67.fr/moustique-tigre et sur la page de strasbourg.eu «accueil → pratique → logement → santé et cadre de vie → moustique tigre, soyons tous attentifs »

Annexe 3 : Version papier de l'enquête CAP mise en place vis à vis des adhérents aux jardins collectifs de la métropole de Lyon.



Enquête sur les jardins collectifs de la métropole de Lyon

La présence de nombreux espaces végétalisés en zones urbaines est source de biodiversité et de bien-être pour les habitants, mais peut également contribuer à la prolifération de certaines espèces nuisibles.

Dans le cadre de ma première année de thèse en biologie à l'université Lyon 1, je réalise une enquête sur les pratiques dans les jardins collectifs en milieu urbain et les nuisances dues à certaines espèces qui colonisent ces jardins. La métropole de Lyon, comptant à ce jour plus de 200 jardins collectifs, constitue mon terrain d'étude.

A terme, le but de cette étude est de trouver de nouveaux moyens de lutte contre les nuisibles qui soient plus durables, mieux ciblés et respectueux de l'environnement.

Au préalable, j'ai mis en place une enquête auprès des adhérents des jardins collectifs de la Métropole, afin de connaître la fréquentation de ces jardins, les pratiques de culture ainsi que les éventuelles pratiques mises en place pour lutter contre les nuisibles.

Le questionnaire dure 15min, je vous remercie par avance de votre participation.

Enquête conçue par Pénélope Duval, doctorante à l'UMR d'Ecologie Microbienne et l'UMR Environnement Ville et Société, financée par l'Ecole Urbaine de Lyon.

Conformément à la réglementation en vigueur en matière de protection des données à caractère personnel, vos réponses resteront strictement confidentielles et anonymes. Je m'engage à ne les utiliser que dans le cadre de ma recherche et à ne les diffuser à aucune personne, structure ou entreprise extérieure. Les informations recueillies dans ce formulaire sont enregistrées dans un fichier informatisé et sécurisé sur les serveurs de la TGIR Huma-num et au LEM (UMR 5557). Elles sont destinées à mon travail de doctorat. Vous disposez d'un droit d'accès, de rectification, de portabilité des informations qui vous concernent, et de limitation du traitement de vos données.

Pour faire valoir vos droits ou signaler des erreurs : penelope.duval@universite-lyon.fr

Fréquentation et pratiques au sein d'un jardin collectif

Quand avez-vous adhéré ou participé à des activités dans un jardin collectif pour la première fois ?

- Moins d'1 an
 Entre 6 ans et 10 ans
 Entre 1 et 5 ans
 Plus de 10 ans

**Si vous participez à plusieurs jardins collectifs en même temps, vous répondrez en prenant en compte le jardin que vous fréquentez le plus.*

Depuis quand avez-vous adhéré au jardin collectif dans lequel vous êtes actuellement (indiquez le nombre de mois ou années) ?

Quel est le nom du jardin que vous fréquentez actuellement ?

Dans quelle commune ou arrondissement est localisé ce jardin ?

Qui est le gestionnaire de ce jardin collectif ?

- Un bailleur social (par exemple GrandLyon Habitat)
 Le jardin est autogéré
 La mairie
 Ne sais pas
 Une association
 Autre

Autre, précisez

Quel est votre rôle au sein de ce jardin collectif ? (plusieurs réponses possibles)

- Initiateur du projet
 Participant/Jardinier
 Aménageur
 Partenaire financier
 animateur
 Maraîcher professionnel
 Ne souhaite pas répondre
 Autre

Autre(s), précisez

Au sein de ce jardin, quelles activités pratiquez-vous ? (plusieurs réponses possibles)

- Plantation de fruits et/ou légumes
 Plantation de plantes et/ou fleurs d'ornement
 Participation à des formations
 Participation à des ateliers pédagogiques
 Fabrication de mobilier de jardin
 Autre

Autre(s), précisez

Quelles ont été vos motivations pour intégrer un jardin collectif ? (plusieurs réponses possibles)

- Pour faire pousser soi-même des fruits et légumes
 Pour le plaisir de jardiner
 Pour pratiquer des activités physiques
 Pour créer des liens sociaux
 Pour pratiquer des activités en plein air
 Pour transmettre des pratiques de jardinage aux plus jeunes
 Autre

Autre(s), précisez

A quelle(s) saison(s) fréquentez-vous le plus le jardin collectif ? (plusieurs réponses possibles)

- Automne
 Hiver
 Printemps
 Été
 Pas de différence de fréquentation selon les saisons

A quel(s) moment(s) de la journée fréquentez-vous le plus le jardin collectif ? (plusieurs réponses possibles)

- Matin
 Entre midi et deux
 Après-midi
 Soirée
 Pas de différence de fréquentation

Pendant la ou les saisons où vous fréquentez le jardin collectif, vous y allez :

- Tous les jours
 Plusieurs fois par semaine
 Une fois par semaine
 Une fois toutes les deux semaines
 Une fois par mois
 Autre

Autre, précisez

Combien de temps restez-vous en moyenne dans le jardin collectif ?

- Moins de 30 min
 Entre 30 min et 1h
 Entre 1h et 2h
 Plus de 2h

Le type de parcelle que vous cultivez est :

	Oui	Non	Ne sait pas
Une parcelle individuelle	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>
Une parcelle collective	<input type="radio"/>	<input type="radio"/>	<input type="radio"/>

Si vous avez une ou des parcelles individuelles, combien en avez-vous ?

- 1
 2
 3
 Plus de 3

Connaissez-vous la superficie de votre ou vos parcelle(s) individuelle(s) ?

- Oui Non

Si oui, quelle est sa superficie (en mètres carrés) ?

Votre parcelle est-elle cultivée en intégralité ?

- Oui Ne sait pas
 Non

Si non, comment est aménagé le reste de votre parcelle ?

Parmi les items suivants, votre parcelle contient : (plusieurs réponses possibles)

- Aucun mobilier de jardin Jardinière
 Cabane Composteur
 Serre Récupérateur d'eau de pluie
 Pot de fleur Autre

Autre(s), précisez

Connaissez-vous la superficie du jardin que vous fréquentez ?

- Oui Non

Si oui, quelle est sa superficie (en mètres carrés) ?

Parmi les équipements collectifs, le jardin que vous fréquentez contient : (plusieurs réponses possibles)

- Aucun mobilier collectif Panneau informatif
 Toilettes sèches Barbecue
 Cabane collective Récupérateur d'eau de pluie
 Serre Autre
 Composteur

Autre(s), précisez

Parmi les propositions suivantes, qu'utilisez-vous comme pratique agricole : (plusieurs réponses possibles)

- Agriculture conventionnelle Permaculture
 Agriculture biologique Ne sais pas
 Agriculture hors sol Autre

Autre(s), précisez

Dans le jardin que vous fréquentez, utilisez-vous : (plusieurs réponses possibles)

- Engrais chimiques (engrais azotés, engrais phosphatés, etc.) Insecticides
 Fertilisants organiques (fumier, compost, etc.) Autre

Autre(s), précisez

Si vous utilisez des engrais ou des insecticides, connaissez-vous le nom ?

- Oui Non

Si oui, précisez le nom

Pensez-vous que la pollution de l'air de la ville influence la pollution au sein de votre jardin ?

- Pas du tout Beaucoup
 Un peu Sans avis

Avez-vous déjà fait faire des analyses de sols sur votre parcelle ou dans le jardin collectif ?

- Oui Non

Si oui, quel type d'analyse avez-vous effectué ?

Si vous avez effectué des analyses de sol, seriez-vous prêt à nous transmettre les résultats afin de faciliter notre travail de terrain ?

- Oui Ne souhaite pas répondre
 Non

Pour arroser vos plantes, utilisez-vous : (plusieurs réponses possibles)

- Je n'arrose pas L'eau de pluie (récupérateur d'eau)
 L'eau de ville Autre
 Forage

Autre(s), précisez

Par quel(s) moyen(s) arrosez-vous vos plantes : (plusieurs réponses possibles)

- Arrosoir Goutte à goutte
 Jet d'eau Autre

Autre(s), précisez

Nuisances

Dans les jardins, de nombreuses espèces végétales ou animales peuvent avoir des effets néfastes sur vos cultures ou sur votre bien-être. Dans cette partie, nous allons considérer seulement les espèces nuisibles animales.

Dans le jardin collectif que vous fréquentez, avez-vous rencontré une ou plusieurs espèce(s) nuisible(s) (provoquant des effets néfastes sur vos cultures ou sur votre bien-être) parmi les suivantes : (plusieurs réponses possibles)

- | | |
|-------------------------------------|-------------------------------------|
| <input type="checkbox"/> Pucerons | <input type="checkbox"/> Moustiques |
| <input type="checkbox"/> Escargots | <input type="checkbox"/> Rongeurs |
| <input type="checkbox"/> Limaces | <input type="checkbox"/> Tiques |
| <input type="checkbox"/> Oiseaux | <input type="checkbox"/> Autre |
| <input type="checkbox"/> Doryphores | |

Autre(s), précisez

Trouvez-vous que la présence de moustiques au cours des deux dernières années :

- | | |
|--------------------------------------|----------------------------------|
| <input type="radio"/> A augmenté | <input type="radio"/> A diminué |
| <input type="radio"/> N'a pas changé | <input type="radio"/> Pas d'avis |

Pendant quelle(s) période(s) de l'année les moustiques vous semblent présents ? (plusieurs réponses possibles)

- | | |
|--|--|
| <input type="checkbox"/> Janvier-Avril | <input type="checkbox"/> Septembre-Octobre |
| <input type="checkbox"/> Mai-Juin | <input type="checkbox"/> Novembre-Décembre |
| <input type="checkbox"/> Juillet-Août | <input type="checkbox"/> Pas d'avis |

Au cours de vos activités dans votre jardin collectif, les moustiques vous ont gêné :

- | | |
|---|--------------------------------|
| <input type="radio"/> A chaque fois | <input type="radio"/> Rarement |
| <input type="radio"/> Souvent | <input type="radio"/> Jamais |
| <input type="radio"/> Environ la moitié du temps passé dans les jardins | |

Si les moustiques vous ont gênés, est-ce du : (plusieurs réponses possibles)

- | | |
|--|---|
| <input type="checkbox"/> Aux moustiques qui me tournent autour | <input type="checkbox"/> A ma réaction aux piqûres (gonflement, démangeaison, etc.) |
| <input type="checkbox"/> A la piqûre en elle-même | <input type="checkbox"/> Autre |
| <input type="checkbox"/> Au nombre très important de piqûres | |

Autre(s), précisez

La présence des moustiques vous préoccupe-t-elle ?

- | | |
|-----------------------------------|--------------------------------|
| <input type="radio"/> Pas du tout | <input type="radio"/> Beaucoup |
| <input type="radio"/> Un peu | |

Si oui, pourquoi la présence des moustiques vous préoccupe-t-elle ?

Avez-vous modifié certaines de vos pratiques au sein du jardin collectif à cause de la présence de moustiques ?

- | | |
|---------------------------------|------------------------------|
| <input type="radio"/> Aucune | <input type="radio"/> Toutes |
| <input type="radio"/> Certaines | |

Si vous avez répondu "aucune" à la question précédente vous pouvez passer directement à la question : "Au sein du jardin collectif, avez-vous agi ou limité certaines pratiques pour éviter la prolifération des moustiques ?"

Comment avez-vous modifié vos pratiques au sein du jardin collectif ?

Avez-vous modifié la période où vous vous rendez dans le jardin collectif ?

- Oui
 Pas d'avis
 Non

Est-ce un changement au niveau de vos horaires ?

- Oui
 Non

Si oui, précisez

Est-ce un changement au niveau de la période/saison ?

- Oui
 Non

Si oui, précisez

Est-ce un changement au niveau d'autre chose ? (Précisez)

Au sein du jardin collectif, avez-vous agi ou limité certaines pratiques pour éviter la prolifération des moustiques ?

- Jamais
 Assez souvent
 Rarement
 Très souvent
 Occasionnellement

Si vous avez agi contre les moustiques, pouvez-vous expliquer la façon dont vous luttez contre ?

Si vous avez agi contre les moustiques, à quelle fréquence agissez-vous pour éviter la prolifération des moustiques dans le jardin ?

Si vous avez agi contre les moustiques, est-ce individuellement ou collectivement ?

- Individuellement
 En groupe
 Avec une autre personne
 Autre

Autre, précisez

Si vous avez agi contre les moustiques, comment et avec qui avez-vous agi ?

Pour lutter contre les moustiques, vous avez déjà utilisé au cours de l'année passée dans le(s) jardin(s) collectif(s) : (plusieurs réponses possibles)

- Répulsifs
 Vêtements couvrants
 Insecticides chimiques
 Lutte biologique (par exemple des prédateurs, hormones, etc)
 Elimination de réservoirs d'eau stagnante
 Autre

Autre(s), précisez

Le moustique tigre

Avez-vous déjà entendu parler du moustique tigre ?

- Oui
 Ne sais pas
 Non

Si oui, par quel(s) moyen(s) avez-vous été informé ? (plusieurs réponses possibles)

- Par les médias (journal, radio, télévision, site d'actualités, etc.)
 Par des campagnes de sensibilisation (ARS, EID, etc.)
 Par une campagne de communication (le Grand Lyon, la ville de Lyon, votre commune, etc.)
 Autre
 Par l'organisme gestionnaire de votre ou vos jardin(s) collectif(s) (par exemple une association)

Autre(s), précisez

Avez-vous déjà recherché par vous-même des informations concernant le moustique tigre ?

- Oui, souvent
 Non, jamais
 Oui, c'est déjà arrivé

Si oui, vous avez effectué des recherches par : (plusieurs réponses possibles)

- Internet
 Via les services de la Métropole
 Livre
 Autre
 Via les services de votre commune

Autre(s), précisez

D'après vous, le moustique tigre est : (plusieurs réponses possibles)

- Un nuisible (via les piqûres)
 Une espèce utile dans la chaîne alimentaire
 Une espèce de moustique qui peut transmettre des agents infectieux responsables de maladies chez l'homme et les animaux
 Une espèce invasive
 Une espèce de moustique comme les autres
 Autre
 Très présent sur le territoire français (métropole)

Autre(s), précisez

Pensez-vous reconnaître le moustique tigre parmi les autres espèces de moustiques ?

- Oui
 Ne sait pas
 Non

Si oui, selon quel(s) critère(s) : (plusieurs réponses possibles)

- La taille
 Les heures d'observation
 Les rayures sur les pattes
 Les ailes
 Le trait dorsal
 Autre

Autre(s), précisez

Selon vous, le moustique tigre se développe dans : (plusieurs réponses possibles)

- Grand plan d'eau (lacs, marais, etc.)
 Eaux vives (rivières, ruisseaux, etc.)
 Végétation
 Ne sait pas
 Moyen et petit réservoir d'eau stagnante (arrosoir, coupelle, récupérateur d'eau de pluie, etc.)
 Autre
 L'air

Autre(s), précisez

Selon vous, les moustiques se nourrissent de : (plusieurs réponses possibles)

- | | |
|--|---|
| <input type="checkbox"/> Eau | <input type="checkbox"/> Jus sucré de fruits mûrs |
| <input type="checkbox"/> Sang humain | <input type="checkbox"/> D'autres insectes |
| <input type="checkbox"/> Sang prélevé sur d'autres animaux | <input type="checkbox"/> Autre |
| <input type="checkbox"/> Nectar de plante | |

Autre(s), précisez

Aimeriez-vous être mieux informé quant à cette espèce de moustique ?

- | | |
|---------------------------|-----------------------------------|
| <input type="radio"/> Oui | <input type="radio"/> Ne sait pas |
| <input type="radio"/> Non | |

Si oui, comment aimeriez-vous être informé ? (plusieurs réponses possibles)

- | | |
|--|---|
| <input type="checkbox"/> Presse locale | <input type="checkbox"/> Via internet (mail, réseaux sociaux) |
| <input type="checkbox"/> Presse nationale | <input type="checkbox"/> La métropole ou la commune |
| <input type="checkbox"/> Radio | <input type="checkbox"/> Gestionnaire du jardin collectif |
| <input type="checkbox"/> Directement au sein des jardins | <input type="checkbox"/> Autre |

Autre(s), précisez

Lutte biologique

Comment évaluez-vous les moyens de lutte utilisés actuellement contre les moustiques dans le jardin fréquenté ?

- | | |
|--|---|
| <input type="radio"/> Efficace | <input type="radio"/> A ma connaissance, il n'y a pas de moyen de lutte dans le jardin que je fréquente |
| <input type="radio"/> Partiellement efficace | <input type="radio"/> Sans avis |
| <input type="radio"/> Inefficace | <input type="radio"/> Autre |

Autre, précisez

Parmi les méthodes de lutte contre les moustiques, avez-vous déjà entendu parler de la lutte biologique ?

- | | |
|---------------------------|-----------------------------------|
| <input type="radio"/> Oui | <input type="radio"/> Ne sait pas |
| <input type="radio"/> Non | |

Si oui, pouvez-vous, en quelques mots, expliquer en quoi elle consiste ?

Sur une échelle de 1 (je ne souhaite pas être informé) à 5 (je souhaite être mieux informé) aimeriez-vous être mieux informé sur les méthodes alternatives à l'utilisation d'insecticides chimiques ?

Au sein de votre jardin collectif, seriez-vous prêt à devenir acteur pour lutter contre la propagation du moustique tigre ?

- Oui
 Pas d'avis
 Non

Si oui, quel degré aimeriez-vous vous investir ?

- Un peu
 Énormément
 Beaucoup

Si vous le souhaitez, vous pouvez laisser vos coordonnées ci-dessous afin de recevoir les résultats de l'enquête et/ou participer à la suite de mon étude sur le terrain

Données descriptives

Dans quelle commune/arrondissement habitez-vous ?

Sexe

- Un homme
 Autre
 Une femme

Âge

- < 18 ans
 50-65 ans
 18-25 ans
 65-75 ans
 25-35 ans
 > 75 ans
 35-50 ans

Statut

- Célibataire
 Couple avec enfant(s)
 Couple sans enfant
 Autre

Autre, précisez

Vie professionnelle

- En activité
 Sans activité
 En formation
 Retraité
 En étude
 Ne souhaite pas répondre
 En recherche d'emploi
 Autre

Autre, précisez

Vous êtes :

- En activité à temps plein
 Autre
- En activité à temps partiel

Autre, précisez

Quelle est ou était votre profession (avant votre départ à la retraite) ?

- Agriculteur(e)
 Cadre
- Employé(e)
 Travailleur (travailleuse) indépendant(e)
- Ouvrier(e)
 Autre

Autre, précisez

Quel est votre niveau d'étude ?

- Niveau inférieur au bac
 Niveau supérieur au bac
- Niveau du bac
 Autre

Autre, précisez

Pour finir, avez-vous des remarques ou suggestions générales (y compris sur le questionnaire) ?

Merci pour le temps que vous avez consacré à cette enquête !

