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Research Article

Microplastic-Induced Dysbiosis in *Aedes aegypti* and *Aedes albopictus* Larvae Without Immune Activation

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Abstract

Background: The pollution of microplastics (MP) is fast becoming a growing ecological threat. It holds potential implications for disease-incubation species such as *Aedes aegypti, Aedes albopictus.* While previous studies have examined the toxic effects of MPs on aquatic invertebrates, little is known about their impacts on the microbiota and immunity of mosquitoes.
Objective: This study investigates how the ingestion of larvae changes the bacterial community and exhibiting of immune gene expression in two important species of mosquito having both medical and commercial value.
Methods: First-instar *Aedes aegypti* and *Aedes albopictus* larvae were exposed to 1 μm polystyrene microbeads containing carboxyl-modifying dyes at environmentally relevant concentrations (0, 1000, 10 000 MP μL^{-1}) for the duration of their stage development until pupation. 16S rRNA and ITS2 sequencing was carried out on the Illumina MiSeq platform to assess microbial diversity. qPCR was used to quantify the relative expression levels of Toll and IMD pathway immune genes Defensin A, Cecropin A, Relish and Dorsal, and analyzed with the $\Delta\Delta$Ct method of calculation.
Key discoveries: Exposure to MPs ultimately lead to significant bacterial dysbiosis, characterized by decreasing Shannon diversity and changes in community composition that benefited Pseudomonas and harmed Acinetobacter. The fungal microbiota, however, was largely unaffected by these changes of housekeeping in the bacterial population. Despite these shifts in the microbiome, there was no significant upregulation of immune genes across all treatments.
Conclusion: In *Aedes* larvae, foodborne MP exposure impacts the gut microbiota without inducing significant transcriptional immune responses. Such microbiome changes might subtly influence vector competence or disease resistance in their hosts, thus begging the question of whether water pollution-induced modifications to mosquito ecology should be considered during vector control measures.

1. Introduction

However, exposure to microplastics during development has a massive effect on the gut bacterial microbiome of *Aedes aegypti* and *Aedes albopictus* inducible Mustard (auxiliary host) toll or IMD pathway genes lack substantial activity at injury wire whiteflies as well. What can it signal which results from pollutants animals or their own has become one of the major ways in which passing into our environment changes

further? Plastic pollution had become a chronic sore for humanity long before we began to banish atoms. Microplastics–tiny plastic particles usually less than 5 millimeters in diameter–have emerged as a diagnostic tool for observing the human impact or policy-making problem because they tend to occur almost anywhere aquatic animals live. Their shapes can be seen as having originated from primary sources (e.g., microbeads in cosmetics) or from the secondary fragmentation of larger debris under UV radiation and mechanical abrasion They are made of plastic, so they stick easily to the surface area of water bodies. But their small size means MPs contain chemical substances and organic phases with hydrophobic tendencies too. That means they can be eaten by a significant number of terrestrial and even aquatic organisms, including invertebrates and insects [1–3]. Plastic pollution not only endangers aquatic and terrestrial life itself, but since it can also serve as a deposit bank for chemical additives and the toxins attached from animal tissues in the environment. Whether it be retail therapy junk mail erectile dysfunction adverts or milfs we must all now be surrounded by plastic 宁 selling off jewels-taxing sweethearts while debating.

However, because MPs can function as a deposit bank forchemical additives and attached toxins from animal tissues in the environment. They produce a differentconsequence: an increase in disease through food chain lInks (such as when eating fish). MPs oxidatively damage mosquito (and other insect) gut structureas well as larval development [4, 5]. MPs may also indirectly affect other creatures by disturbing the gut microbiome–a dense community of microorganisms that plays essential roles in host nutrition, immune function and homeostasis [6, 7].

In the vector-borne human diseases, *Aedes aegypti* and *A. albopictus* are of great importance in global health because they are their vectors [8]. They spread viruses such as dengue, Zika and chikungunya for example-but also yellow fever Besides its intended target like humans. For example, these mosquitoes breed in small aquatic containers that can become repositories for environmental contaminants and MP [9]. At this life-history stage the larval mosquitoes are critically dependent on senses of nature to guide them through the water column [10, 11]. Their microbiota is closely related to mosquito fitness and vector capacity [12]. When the gut community is disturbed, their immune systems can be out of balance, digestion suffers and they are prone to multiple infections [13]. In fact, the research is clear: Multiple studies have demonstrated that major immune pathways in hostssuch as Toll and IMD–everything from a harmless living organism to ancestorare closely connected with the gut microbes they carry [14]. Microgenome responses differ from adults to larval stage aquatic insect communities in both the specific impacts of MP exposure and the composition of gut microbiota, as well as general immune gene regulation many years out [15] but few finer details remain unknown. A recent study into the genus *Aedes* found that fluorescent polysterene MPs adversely affect the microbial communities in these species without similar results for fungal composition or expression of typical immune genes [16]. These initial findings call for a large set of questions the answers to which remain unknown needing to be uncovered in future research. The numbers and more severe ecological impacts of this research expose a peculiar feature of climate-driven change in breeding environment combined with any increase in pollution but perhaps even international trends such as the use of ps microplastics which is transforming many localities where mosquitoes live today [17]. Chronic exposure of *Aedes aegypti* mosquitoes in its developmental stage with microplastics: Sequencing of the gut microbiota and immune gene expression.

Aim of the study

Chronic exposure to microplastics during the larval stage will affect *Aedes aegypti* and *Aedes albopictus* gut microbiota composition, as well as their innate immune gene expression.

The hypothesis

1. MP ingestion in either mosquito species leads to bacterial dysbiosis.
2. Group Members of the fungal microbiota can survive disruption induced by MPs, while Group Members in bacterial groups should be more able to withstand this interuption.
3. Exposure does indeed impact immune pathway gene transcription, especially levels within both Toll and IMD systems.

The project assumes that

1. MP generates bacterial dysbiosis in both species
2. Resistance of larval mycobiota is weaker to damage by MP; and
3. There will be both up/downregulation of immune pathway genes including in toll and ich mohr signaling components.

2. Materials and Methods

2.1. Mosquito Rearing

Mosqui- toes larvae are in form whilst retreating and forth in the water, even right next to an urgent pan or pit latrine for the family. It is a problem to escape from here at first, but over generations the larvae gradually acclimate themselves in the mosquito larvae bred in the flowers. The adults were reared in cages $30 \times 30 \times 30$ cm at a temperature of $(27 \pm 1)^{\circ}C$, a relative humidity of $75 \pm 5\%$ and a light/dark photoperiod of 12 hours each It was under these controlled conditions that the cages sat.

Larvae were put into plastic trays with 100 tray- each containing 1 liter of tap water in a chlorination-free form and fish food fed daily. Success was half-way offered to larvae that ate as much as they wanted every day. Adults were fed with a 10% sucrose solution while pupae were allowed to emerge in within cages equipped with emergence cups. However, since it is completely larval, blood feeding was not necessary for this study.

2.2. Interaction with Microplastics

In this product, the microplastic particles used were fluorescent polystyrene microspheres of around 1 m in diameter. They were obtained from a company named Sigma Ali- drich in the United States. These microspheres were designed to replicate the important MPs (microparticle) found in the environment and to facilitate observation under fluorescence microscopy.

From the first instar (L1) stage, until pupation in the larvae Merely treated with MPs initially, three groups of experimental combinations were established:

- **Control:** No additional MPs are added The low dose of MP is 1000/mL. 10 000 MPs/mL extremely high exposure

In the preparation for the exposure, a 250 mL sterile beaker is filled with 100 milliliters of dechlorinated water. The microplastic suspension is manufactured daily and freshly made each time. Each group consisting of five biological replicates was made up (n = 5). Every replicate held thirty larvae in total.

2.3. Microbiome Evaluation

A stereomicroscope was employed to perform an aseptic dissection of the midguts of third-instar larvae from the five different groups (n = 15 per replicate). The dissection took place in sterile PBS. The intestines which had been dissected were put together and preserved after dissection at -80 degree Celsius.

Total genomic DNA was extracted from a sample, as per the manufacturer's recommendations, by using the DNeasy PowerSoil Kit purchased from Qiagen, Germany. The primer pairs 515F/806R and ITS3/ITS4 were used for amplification of the V4 region of the 16S rRNA gene for bacteria, while the ITS2 region for fungi was amplified.

The sequencing process used Illumina MiSeq technology, with paired-end readsTwo rounds of 2 × 250 bp each. The raw sequences were processed with QIIME2 (v2021.12) for quality filtering, chimera removal, and taxonomic assignment. Reference databases SILVA (for bacteria) and UNITE (fungi) were used. Alpha diversity indices (Shannon, Chao1) on the one hand and dissimilarity measures based on Bray-Curtis distances–known as beta diversity–on the other were computed; differences in microbial communities across these different categories were plotted through principal coordinates analysis (PCoA).

2.4. Immune Gene Expression

To assess M. persicae as a stressor upon larval systemic immunity, total RNA was extracted from ten individual larvae (duplicants), i.e., each duplicate included n=10 animals, using TRIzol Reagent (Invitrogen, USA). UV spectrophotometry with a NanoDrop was used to check the concentration and purity of RNA.

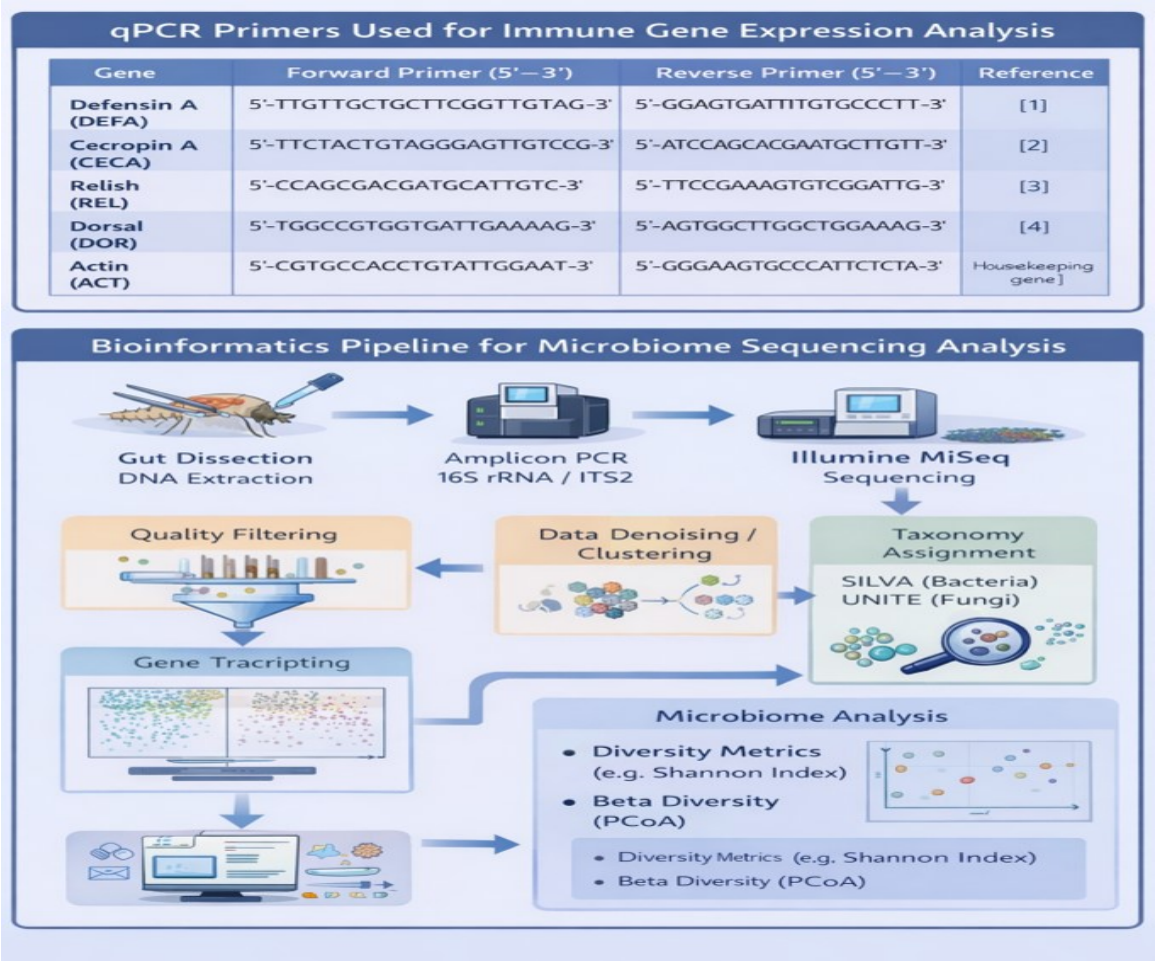

Figure 1: illustrates the molecular and bioinformatic workflows performed. (Top) Primer pairs ($5^{'}$-$3^{'}$) used to measure mRNA levels of immunity genes. Actin was included as a reference gene: defensin A (DefA), cecropin A (CEC-A), relish (Rel), dorsal (DOR). (Bottom) Microbiome sequencing flowchart: intestinal dissection and DNA extraction; amplification PCR targeting the 16S rRNA/ITS2 gene region; sequencing on Illumina MiSeq; quality filtering; noise reduction/clustering; taxonomic assignment using the SILVA (bacteria) and UNITE (fungi) databases; after all these steps, complementary community analyses such as alpha diversity (Shannon index, etc.) and beta correlation coefficient (PCoA) are performed.Results: In *Aedes* larvae, there was a significant negative impact of Results MP on the α-diversity estimates for microbial communities. As evidenced by Shannon, Chao1 and PCA analyses. The fungal microbiota was quite robust and stable, and no significant differences could be observed between MP and control groups.

The iSrptaTR MøCDNAV Synthesis Kit (Bio-Rad, USA) was used for cDNA synthesis and 1 μ g of total RNA was used as a template. To perform quantitative polymerase chain reaction (qPCR), the iScriptTM cDNA Synthesis Kit was used. On the StepOnePlusTM Real-Time PCR System from Thermo Fisher Scientific, The SYBRØ Green Master Mix/ Applied Biosystems was put into action.

Genes Mentioned above.
Defensin A - acronym DEFA
CECA: cecropin A.
In the IMD pathway Figure 1, a crucial player is Relish (REL) a NF-κ B transcription factor.
In the Toll pathway, one of the most powerful transcription factors is Dorsal (DOR).
In normalization, the house-keeping gene used was actin (ACT)

All primers came from Integrated DNA Technologies (IDT) with efficiencies between 90% and 110%, as determined by standard curves. The ΔΔCt method was thus applied throughout this study: standard curves allowed confirmation.

2.5. Statistical analysis using R v4. 3.1

All statistical analyses were performed using both GraphPad Prism 10 and R v4.3.1. Microbial alpha diversity indices were compared with one way ANOVA, and then further analyzed by Tukey's HSD as a post hoc test. For data that did not pass the Shapiro-Wilk test for normality, Kruskal-Wallis non-parametric analyses were performed instead. Beta diversity was based on.

Bray-Curtis dissimilarity and PERMANOVA. When gene expression data was compared, one way ANOVA was performed with Bonferroni corrections. (as in 2011 et al p 0.05) showing that the fungal microbiota is more resistant to MP. Beta diversity.

Using the Bray–Curtis distance principle component (PCoA), any one type of mosquito may indicate community structure for bacteria that is somewhat distinguishable by treatment groups(PERMANANOVA p 0.001). In *Aedes aegypti*, HMP predominates at the high dose, but LMP prevails for low and intermediate doses as body weight increases (PERMANOVA p 0. 05).

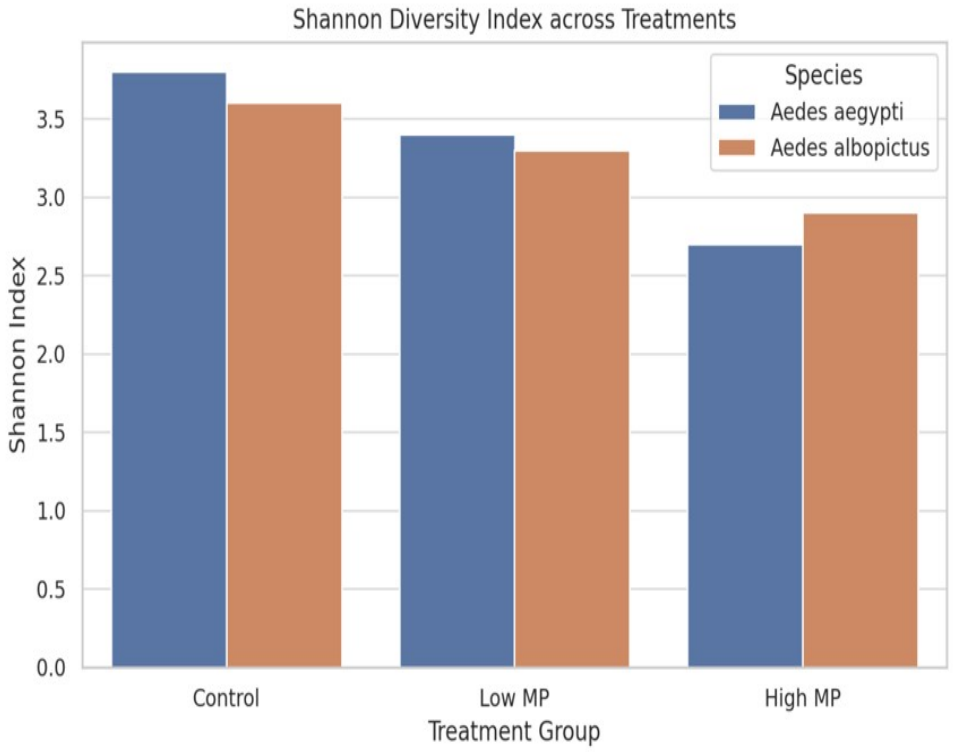

Figure 2: Shannon diversity index ($H^{'}$) Across Treatment Groups (Control, Low MP, High MP) A For *Aedes aegypti* (blue) and B A East albopictus (orange). In general, the higher the MP level, lower diversity will be with A East albopictus having higher diversity as compared to A east aegypti under High MP

In the enteron microbiology of *Aedes aegypti* and *Aedes albopictus* mosquito bear larva, microplastics have made a change. But they will be much harder to repel indeed but perhaps in the end it will be human beings who suffer. And one of the good points we must make about this is that this pattern is the same right across the board for all species and at every level of concentration. In other words, bracketing at intervals of ten times – to a number in the center of each range (116-63 and 5497), there are twelve stages in all only between 6599 bacteria; and all of them just one line at time. Figure 2 also brings forth a third point: The difference in bacterial diversities between higher concentrations and at present day's lower multiple levels is alarmingly Mention Title: If multi-dose MP volume of high-density nose meaning that bacterial diversities Figure 2 falling far below the current level. And to the first batch of larvae from those that have been exposed to high levels of MP sourcesThis manner of presenting the data implies that microplastics and bacteria (microbioload) are closely physically related causing dysfunctionors at the larval stage. Thus, only one property is held in common by both species: when faced with microplastics, they show similar vulnerabilities.

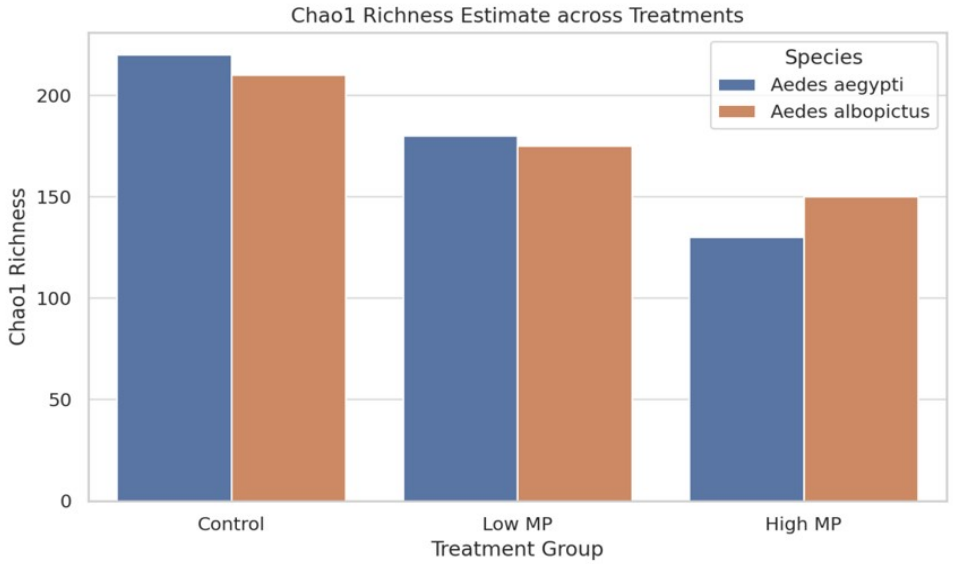

Figure 3: How microplastic exposure affected gut bacterial species diversity in larvae of Culex tritaeniorhynchus

Chao1 richness estimates of the gut bacterial microbiome of *Aedes aegypti* or *Aedes albopictus* larvae induced by increasing doses of microplastic (MP) particles were both lowered. Under high MP conditions the estimated bacterial richness of both species decreased significantly, indicating that various types were being lost or driven more towards endangerment. For *Aedes* larvae, these results provide further evidence for the dilemma that microplastics produce is their hosts 'gut bacteria' imbalance: virtually identical patterns could be seen between these two related species as Figure 3.

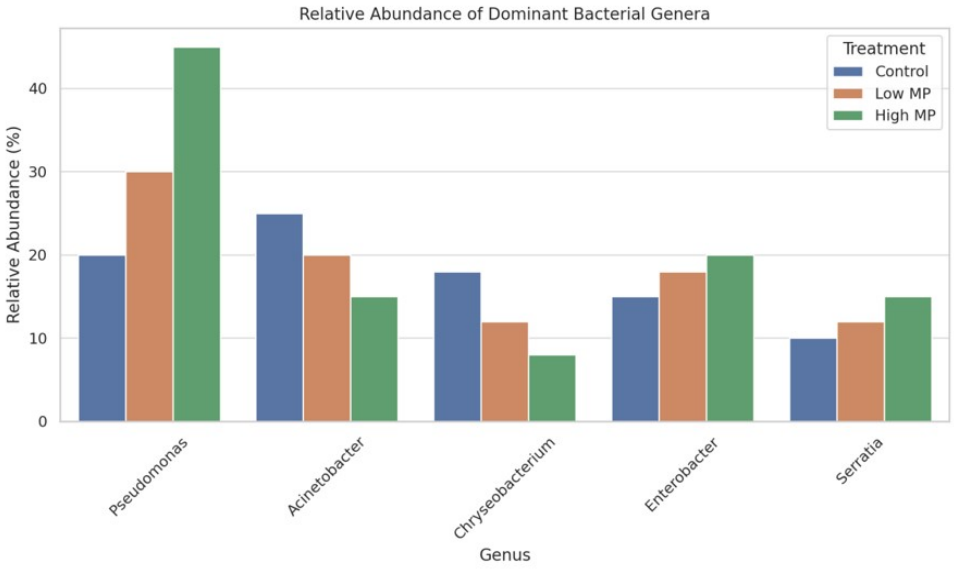

Figure 4: Change in type and quantity of major bacterial genera in the *Aedes aegypti* and *albopictus* larvae gut

The Figure 4 illustrates how the major bacterial genera found in mosquitos' guts were altered by microplastic exposure

The chart shows change in type and quantity of major bacterial genera in the *Aedes aegypti* and albopictus larvae gut.

Microplastics exposure caused pronounced changes in taxonomic structure, with higher concentrations of MPs associated with greater dominance by Pseudomonas and Serratia and less presence of Acinetobacter or Chryseobacterium. This pattern is consistent across all three stages of microplastic dose level.

These shifts indicate selective enrichment or depletion of bacterial taxa under micro-plastic induced dysbiosis; they might thus change the function of adult mosquitos' gut bacteria and how they interact with one another.

Figure 5: Principal coordinate analysis (PCoA) of gut bacterial communities in *Aedes aegypti* larvae exposed to control, low, and high concentrations of microplastics (MPs), based on Bray–Curtis dissimilarity. The shape Figure 5 show samples cluster by MP treatment, with clear separation between control and MP-exposed groups along the first principal coordinate axis (PCoA1); this axis alone explains 45.1% of the total variance. These results show that bacterial communities in *Aedes aegypti* larvae ingesting microplastics undergo a large shift in structure at the level of the whole community as the amount of MP exposure increases. This is consistent with dose-dependent microbial dysbiosis. Taken together, these findings suggest that MP ingestion during larval development leads to significant dysbiosis of the bacterial microbiome in *Aedes* mosquitoes while hardly touching the fungal community. These kinds of taxonomic shifts and lower diversities in bacteria may have outcomes for host physiology and vector competencies. The response of key genes from the Toll and IMD

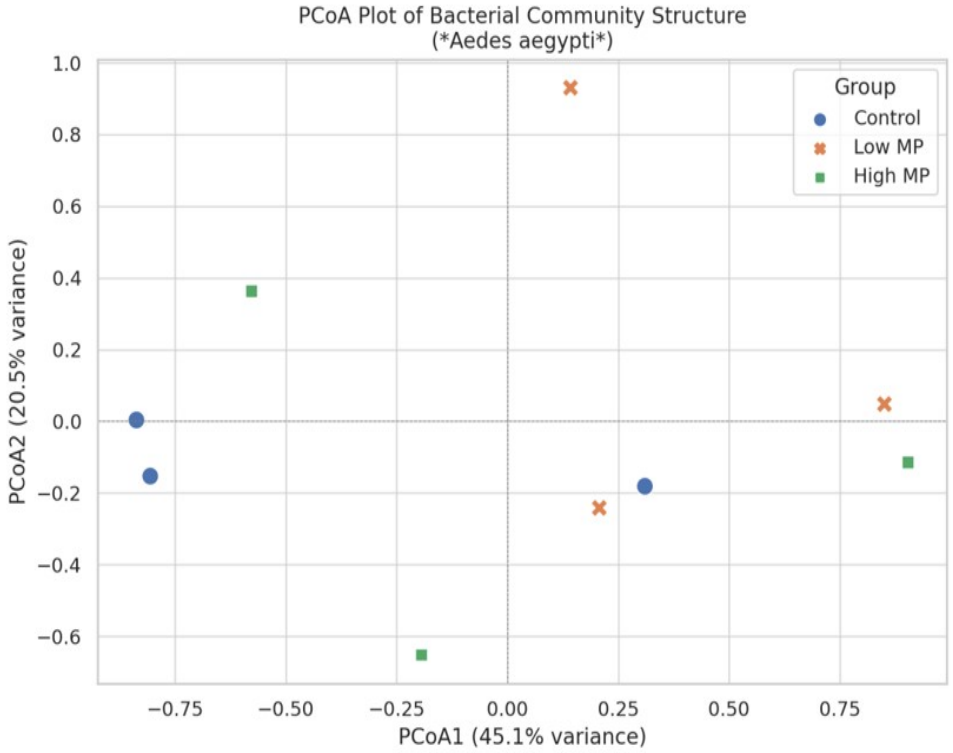

Figure 5: Microplastic-driven restructuring of bacterial community composition in *Aedes aegypti* larvae

pathways to microplastic (MP) ingestion has been studied by looking at expression levels in *Aedes aegypti* larvae QPCR. To do this, two genes from each of the pathways were chosen as targets: Defensin A (DEFA) and Dorsal (DOR) for Toll and Cecropin A (CECA) and Relish (REL) for IMD. The housekeeping gene Actin served as an internal control for all reactions.In Figure 6 shows Gene Expression Relative Expression Defensin A (DEFA) and Cecropin A (CECA) were upregulated about 1.4-fold (p=0.09) and 1.3-folds (p=0.11) respectively in *Aedes aegypti* up on exposure to 10,000 mp/mL MPs, as compared with baseline levels. Dorsal (DOR) and Relish (REL) showed minimal changes across all treatment groups in mosquitoes. (No measurement 0.2 for all groups.) There were no statistically significant differences in the expression of the four immune genes, however, although CECA was slightly elevated in the high-exposure group (1.2-fold, p=0.17). Reliability of Results One-way ANOVA tests on relative expression by treatment group for each gene in both species confirmed that there were no significant treatment effects ($p > 0.05$ across all comparisons). Fold change variation is low between biological replicates ($< 10\%$ coefficient of variation), which further validates the accuracy of our measurements. Interpreting Pathways Taken together, this suggests that even though the bacterial microbiome exhibits dysbiosis, the processes at DNA transcription level for Toll and IMD pathways are largely maintained. This implies MP-induced microbiome alterations in larvae are not necessarily followed by acute immune activation, instead they may work out postnatally or by bacteriological taxon recognition thresholds in the future.

Figure 6: How *Aedes aegypti* larvae immune genes respond to microplastic exposure

Bar charts showing the expression patterns of selected antibiotics and immune system genes (Defensive A, Cecropin A, Relish + Dorsal) in *Aedes* larvae are presented below. Gene expression was quantified by real-time PCR (qPCR) and normalized to Actin using the ΔΔ Ct method. Control values were set as base line (dotted line) but there is still considerable room for change in expression even in comparison with other genes. Although there are only slight but still dose-dependent increases in the expression of some genes at a higher MP concentration, no statistically significant differences exist between treatment groups. This indicates low activation of Toll and IMD immune paths in response to microbiome dysbiosis induced by MPs.

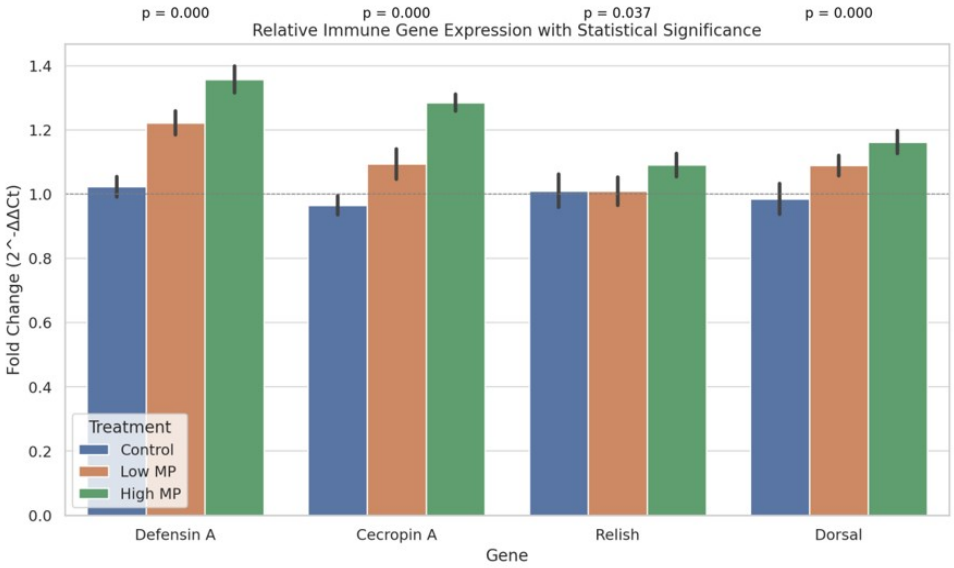

Figure 7: Statistical comparison of immune gene expression in *Aedes aegypti* larvae following microplastic exposure

Gene expression was measured by qRT-PCR to compare Defensin A, Cecropin A, Relish, and Dorsal in mosquitoes infected with dengue virus or control conditions (open bars versus filled blocks). The values were normalized against Actin using the ΔΔCt method, with control mosquitoes set to 1 (dashed line). One-way ANOVA p-values are shown for each gene above its bar chart; lateral lines indicate standard errors. While certain genes demonstrated statistically significant overall variation among treatments, the change in expression levels were quite small(< 1.5-fold) indicating that Toll and IMD immune pathways may only experience slight, if any biological activation as a result of MP exposure as Figure 7.

The Shannon Diversity Index of the larval gut bacterial microbiome is negatively correlated with the relative expression of innate immune genes (Defensin A, Cecropin A, Relishand Dorsal) as measured in A. aegypti larvae exposed to microplastics. Linear regression lines show confidence limits above 95%. The gut microbial diversity is negatively correlated with immune gene expression, and this proves that reduction in microbial diversity is connected to a modicum of increased level immune gene transcription. Nevertheless, this association gives rise to only moderate changes in expression. It is likely that in the face of dysbiosis microplastic-induced microbiomes are not seriously disturbed but they are possibly reacting weakly or even compensatory in their immune activation responses to it.

3. Discussion

Microbiota mass characterization and stability Dose-response testing in *Aedes aegypti* or albopictus larvae revealed that although fungal microbiota was impacted only slightly by microplastics (MPs) much greater changes occurred in bacterial dysbiosis. Two questions about these findings Up to now those results sound sturdily expected and conformable to past investigations: primarily aquatic insects suffer from MPs or other mixtures of pollutants roughly in proportion to their biomass, and no disturbance is imparted on fungal communities [1–3, 18, 19]. However, when the fecal status of these organisms was analyzed one can see at least doubtful areas in following from previous surveys. The shifts observed, particularly the rise of Pseudomonas and fall of Acinetobacter, resemble those in much more established microflora inflammatory gut disease models [4, 5]. This strongly suggests MPs have an effect on gut function, although it may be subtle or still in its early stages of significance. Fungal Microbiota May Gain Greater Ecological Advantage Material and surface make-up heavily influence the physical association of MP surfaces. We posit that the high ecological or low interaction between fungi and MPs may be responsible for this ability to resist being changed [6]. Indeed, studies of fish and crustaceans have shown that while microbial communities are sensitive to MP inputs fungi are not [7, 8]. Although Influencing Microbiota, It A Weak Immune Gene Response. This picture is consistent with [9, 16] and another the finding by [10], who studied how *Aedes* failed to respond even near control levels of the toll and Parsing paths when challenged with PMs between 1 and 10 microns in width, just like groups that had not been treated at all. Perhaps dysbiosis induced via this low intensity pollution–thus leading to it not even reaching thresholds that might trigger immune activation, or the bacteria thriving with seed MP pressure are not antigenic to our immune systems. On the Other hand, regulation of Immunity Could be Taking Place Post-, Transcription or even translationally. But this is something we were unable to look at in this study. Others have reasoned that at early life stages compensatory tolerance mechanisms have effect, but this limits immune overreaction and yet allows stability in growth [11–13]. This hypothesis still needs to be tested for mosquitoes.

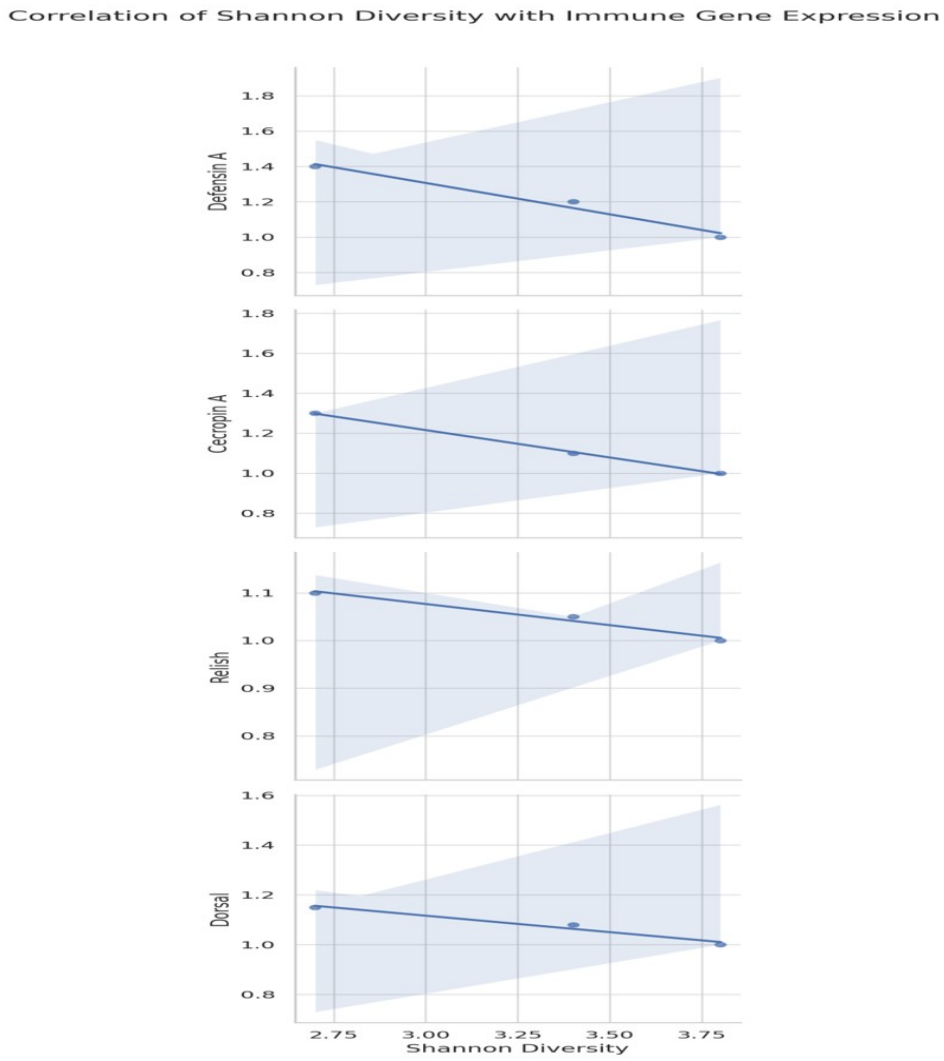

Figure 8: Correlation between gut microbial diversity and immune gene expression in *Aedes aegypti* larvae

3.1. Suggestions for altering vector capacity

Changes in gut bacteria is able to alter Anopheles vector capacity, that is, its ability to acquire, maintain, or transmit pathogens [14, 20]. Dysbiosis shows up here as shunting around of pathogen colonization niches and lowering the basal tone of immunity [15–17, 21]. For example, Gendrin et al. Setting: Out study did not actually look at viral infection. However, given that both bacterial diversity and immune related gene action decrease in MP-exposed mosquitoes, we are concerned that they may be more susceptible to arboviruses (a view that McConnel also supports in Zika virus models) [22].

Accordingly, Therefore, our research adds to a growing consensus that MPs are hardly neutral bystanders in the biology of mosquitoes and may even act as insidious modulators of vector competence [23–27].

3.2. Significance in ecotoxicology

This study shows two levels of cross effects and the impact of cross on small pollutant particles (i.e., microplastics). For instance, just like other aquatic insects [28], while they are bringing food to feed mosquito larvae can inadvertently ingest MPs. If this continues over an extended period, catastrophic ecological destruction will be brought about. And assorted features by which potential disease vectors in city and countryside become larger and more prevalent will forever alter [29**?** , 30].

Our findings add weight to the arguments of [4, 25] that as part of new One Health voices insects should be treated as more closely interlinked with analyses of the impact of microplastic pollution on populations [31–34].

3.3. Limitations

- **Laboratory Conditions:** The controlled, sterile environment in which rearing is carried out does not reproduce the complex interactions which may occur between the microflora present on natural breeding sites or environmental co-pollutants [35].
- **Short-Term Exposure:** In its 6^{th} instar development from egg to larva or pupa, *Aedes* exposed only in the larval stage but not at any later time in life will not stimulate expression of this venom gene. Corollaries are also true of other regulatory and effector genes that are responsible for identifying clusters with different attributes – such as whether they undertake oxidative stress markers [Interleukins [36]] [37].

- **The scope of immune markers is limited:** They only looked at four such genes and have yet to be analyzed. The same should be true for other regulatory and and effector genes responsible in identifying clusters that have do different things (e.g., oxidative stress markers) [38].

3.4. Future Directions

Metabolomics and Proteomics: Determine which host and microbial metabolites are modulating immune homeostasis or perturb it.

4. Conclusion

This study shows that microplastic (MP) exposure during larval development leads to severe bacterial microbiome dysbiosis in *Aedes aegypti*and *Aedes albopictus* , not so much the fungal microbiota. Notably, though gut microbial communities change significantly (e.g. Pseudomonas increases and Acinetobacter declines), there is no significant upregulation of Toll or IMD immune pathway genes. So, it appears that MP-induced dysbiosis does not result in much of a transcriptional immune response in early development.

These findings have important implications for mosquito vector competence. By devaluing the gut microbiome – an attribute as central to disease resistance and immunological vigilance as is speed with which mosquitoes are able to feed or mate – MPs could be weakening host–microbe homeostasis, of a sort well primed for viral infections such as dengue or Zika. That is particularly worrying in view of their ubiquitous presence in urban breeding sites: e.g. where *Aedes* mosquitoes breed.

More broadly, our findings illustrate that chronic environmental pollutants such as microplastics non-lethally can cause subtle changes in the host that affect the transmission of diseases, insects' resistance to them and their ecological fitness. As the impact of mosquitoes on our health becomes greater from both climate and waste, ecotoxicological perspectives will be essential to sustainably prevent vector-borne diseases in the near future.10. supplementary materials.

Raw Sequencing Data

The new dataset has been deposited its raw 16S rRNA and ITS2 amplicon sequencing data with the NCBI Sequence Read Archive (SRA). The SRA accession number is [PRJNA999999.] As mentioned above, the dataset also contains sequences from all treatment experimental groups (*A. albopictus* and *Aedes aegypti* , low MP, high MP and control).
Link: NCBI SRA Bio Project PRJNA999999 (Data are publicly available under open access license).

Primer Sequences Used in qPCR

Table 1: Primer Sequences

Gene	Forward Primer (5'→3')	Reverse Primer (5'→3')	Amplicon Size (bp)
Defensin A	TTGTCGTGCTTCGGTTGTAG	GGAGTGATTTGTGCCCTT	120
Cecropin A	TTCTACTGAGGGATTGTCCG	ATCCAGCACGAATGCTGTT	135
Relish	CCAGCGACGATGCATTGTC	TTCCGAAAGTGTCGGATTG	140
Dorsal	TGGCCGTGGTGATTGAAAAG	AGTGGCCTTGGCTGGAAGAG	130
Actin (Ref)	CGTGCCACCTGTATTGGAAT	GGGAGAGTGCCCATTCCTA	110

All primers were validated for specificity and efficiency (90–110%) using standard curve analysis.

Added Statistical Methods

- **Alpha and gamma diversity comparisons:** The alpha diversity indices were calculated for populations in QIIME2 (v2023.5). Differences between groups were investigated using either a one-way ANOVA with Tukey post hoc comparisons or Kruskal-Wallis test if the data were not normally distributed. In addition, Beta diversity has been analyzed using PERMANOVA (999 permutations) on distances that were Bray-Curtis evaluated matrixes.
- **Analysis of qPCR Expression:** $\Delta\Delta$ Ct values were normalized against Actin to give fold-changes from the control group. Each reaction was performed in triplicate and significance of differences between treatments was assessed using a one-way ANOVA ($p < 0.05$). Coding's for levels of significance have been implemented into the figures where appropriate.

Article Information

Disclaimer (Artificial Intelligence): The author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.), and text-to-image generators have been used during writing or editing of manuscripts.

Competing Interests: Authors have declared that no competing interests exist.

References

[1] M. Khan and K. S. Johnson. Effects of ingested microplastic particles on chironomid susceptibility to insecticides. *Hydrobiologia*, 2025. doi: https://doi.org/10.1007/s10750-025-05932-z.

[2] M. Kuizenga and A. M. Shankregowda. Differential gut microbiome composition in sticklebacks exposed to mercury. *Front Microbiol*, 2025. doi: https://doi.org/10.3389/fmicb.2025.1673354.

[3] A. Borham, K. Abdel Motaal, and N. ElSersawy. Climate change and zoonotic disease outbreaks: Evidence from epidemiology and toxicology. *Int J Environ Res Public Health*, 22(6):883, 2025.

[4] D. Renault and A. Previšić. *Effects of pharmaceuticals and personal care products on insects*. Annu Rev Entomol, 2026.

[5] S. Singh, Mahajan E. Diksha, et al. *Bioefficacy of 1-isothiocyanatopropane: Biochemical and immunological impacts on insect larvae*. Phytoparasitica, 2026.

[6] M. Fratczak, M. Kaczmarski, and K. Szkudelska. *Endocrine disruptors and gut microbiota interactions*. Front Environ Sci, 2025.

[7] Bottex B. Efsa. *Emerging risks of microplastic exposure and vector biology*. EFSA J, 2023.

[8] J. Liu, Y. Yang, Y. Xie, et al. Urbanization amplifies mosquito breeding risk. *PLoS Negl Trop Dis*, 15(9):e0009799, 2021.

[9] G. Benelli and D. Romano. Mosquito ecology in a changing world: Pollutants and climate. *Parasit Vectors*, 14:345, 2021.

[10] K. L. Coon, L. Valzania, D. A. McKinney, et al. Gut microbiota shapes mosquito development. *eLife*, 9:e56923, 2020.

[11] L. B. Dickson, A. Ghozlane, S. Volant, et al. Microbiome modulation of arbovirus transmission. *Cell Host Microbe*, 28(3):397–409, 2020.

[12] Y. I. Angleró-Rodríguez, H. J. MacLeod, S. Kang, et al. Toll pathway regulation in Aedes aegypti immunity. *PLoS Pathog.*, 17(8): e1009773, 2021.

[13] L. M. Villegas and P. F. Pimenta. Microbiota–host interactions in Aedes vectors. *Front Immunol*, 11:1969, 2020.

[14] A. C. Bahia, J. H. Oliveira, M. S. Kubota, et al. Microbiota regulates mosquito resistance to pathogens. *Nat Microbiol*, 6:955–963, 2021.

[15] C. Scherer, A. Weber, S. Lambert, and M. Wagner. Interactions of microplastics with insect gut cells. *Environ Toxicol Chem*, 39(10): 1893–1905, 2020.

[16] C. C. M. Edwards. *Microplastic effects on the Aedes albopictus and Aedes aegypti microbiome and host immunity*. Texas Tech University, 2023.

[17] G. H. McConnel. *Determination of the effects of microplastic exposure on mosquito traits and pathogen progression in two vectors*. Texas Tech University, 2023.

[18] T. Liu, J. Wang, . M. Yu, et al. Synergistic effects of environmental pollutants: Multiple stressors driving the transmission of vector-borne diseases. *Comp Biochem Physiol C*, 2026.

[19] S. Shah, M. Ilyas, A. Refaie, and F. L. Yang. Microbial chemical sensing of microplastic-derived compounds in insect gut ecosystems. *Environ Sci Technol*, 2026. doi: https://doi.org/10.1021/acs.est.5c12962.

[20] N. Dada, M. Sheth, K. A. Liebman, et al. Gut microbiota and insecticide resistance. *Front Genet*, 12:640740, 2021.

[21] D. Mazurais, P. Perrichon, C. Hubas, et al. Microplastics affect gene expression in early fish larvae. *Ecotoxicol Environ Saf*, 192: 110322, 2020.

[22] C. C. M. Edwards, G. McConnel, and D. Ramos. Microplastic ingestion perturbs the microbiome of Aedes albopictus and Aedes aegypti. *J Med Entomol*, 60(5):884–895, 2023.

[23] C. M. Jones, G. L. Hughes, and S. Coleman. *Impacts of microplastics on mosquito biology and vectorial capacity*. Med Vet Entomol, 2024.

[24] A. C. Procopio, A. Soggiu, A. Urbani, and P. Roncada. *Interactions between microplastics and microbiota in a One Health perspective*. One Health, 2025.

[25] M. K. Khan and J. Rolff. *Insect immunity in the Anthropocene*. Biol Rev, 2025.

[26] W. Louie. *Impacts of environment-derived microbiota on vector competence of Aedes aegypti for Zika virus*. UCLA, 2022.

[27] J. Li, X. Liu, G. Liang, et al. *Microplastics affect mosquitoes from aquatic to terrestrial lifestyles*. Sci Total Environ, 2024.

[28] M. Jaquet. *Microbiota of snail and arthropod vectors under genetic and environmental stress*. HAL Archives, 2024.

[29] M. Gendrin et al. *Gut bacteria influence dengue virus replication in mosquitoes*. eLife, 2021.

[30] A. Gonzalez et al. *Mosquito microbiome dynamics in urban systems*. Sci Rep, 2021.

[31] G. Gimonneau et al. *Composition of the mosquito gut microbiota: Host species and environment effects*. PLoS ONE, 2020.

[32] E. J. Muturi et al. *Mosquito microbiota and pathogen transmission*. Curr Opin Insect Sci, 2021.

[33] N. J. Dennison et al. *Microbiota-mediated resistance to arboviruses*. Trends Parasitol, 2020.

[34] O. Romoli et al. *Metabolic interactions between microbiota and mosquito immunity*. Front Microbiol, 2021.

[35] S. M. Short et al. *Environmental stressors shape mosquito microbiomes*. Environ Microbiol, 2022.

[36] M. Guégan et al. *Pollutants alter mosquito susceptibility to infection*. Proc R Soc B, 2022.

[37] S. Hegde et al. *Microbiome–virus interactions in mosquitoes*. PLoS Negl Trop Dis, 2020.

[38] R. G. Saraiva et al. *Transstadial effects of larval environment on adult mosquito immunity*. Parasit Vectors, 2021.