Impact of sublethal pyrethroid exposure on resistant *Anopheles gambiae* mosquitoes’ fitness [version 1; peer review: 2 approved with reservations]

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**Abstract**

**Background:** There is increasing evidence of insecticide resistance spreading among wild mosquito populations, which is widely believed to compromise vector control once it reaches a threshold that enables mosquitoes to survive exposure to long lasting treated bed-net (LLIN) or indoor residual spraying (IRS). However, very little is known about the long-term impact of insecticide resistance on malaria transmission, which makes the consequence of insecticide resistance spreading difficult to predict.

**Methods:** To gain more clarity, we have assessed four life-history traits of a resistant *Anopheles gambiae* laboratory strain that was repeatedly exposed to a LLIN and compared with individuals issued from the same strain but exposed to an untreated bed-net.

**Results:** The non-parametric Kruskal-Wallis test did not show any significant impact of gonotrophic cycle on the five traits. However, the Kolmogorov-Smirnov non-parametric test revealed a significant (i) drop in blood feeding mean rates (D = 0.800; P< 0.0001), (ii) increase in 24-hours post-exposure (D = 0.600; P< 0.001) and (iii) end of gonotrophic cycle mortality (D = 0.611; P <0.006), and (iv) drop in egg laying rate (D = 0.730, P< 0.0001) when mosquitoes were exposed. Surprisingly, there was rather an upward trend in the number of L3 larvae/female mosquito for the exposed group comparing to the unexposed one, although the difference was not significant (D = 0.417, P> 0.05).

**Conclusion:** Our study shows that in a context of widespread of resistance to insecticides, current pyrethroid-based vector control tools can still confer protection against malaria.
Keywords
Key words: Malaria, Vector, Insecticide, Resistance, Fitness

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Introduction

Malaria is a parasitic disease that is transmitted to humans by female mosquitoes of the genus Anopheles. It is considered as a major obstacle to development in Sub-Saharan African countries. Large and sustained funding in the two last decades has enabled widespread geographical prevention of malaria disease. The most widely utilized strategies are the mass distribution of long-lasting insecticidal nets (LLINs), indoor residual spraying (IRS) and artemisinin-based combination therapy (ACT). According to Bhatt et al., global malaria incidence has been reduced by 40% between 2000 and 2015. The authors estimate LLINs to be accountable for 68% of this reduction and ACT and IRS for 19% and 13%, respectively. For the period of 2016 to 2030, new goals and a new benchmark to reduce and eliminate malaria have been established taking into account the current situation. The core methods of malaria control remain unchanged and rely on insecticides-based vector control. To date, only few classes of insecticides are approved by the World health organization (WHO) to be used in public health with only one, the class of pyrethroids, being recommended for LLINs. Unfortunately, insecticide resistance is now widespread among malaria vector populations especially in sub-Saharan African regions. Even more worrisome are reports from countries such as Côte d’Ivoire where resistance to all four classes of insecticides have been recorded within the same mosquito populations. While much information has been gained on the biological and genetic mechanisms behind insecticide resistance, its long term impact on vector control methods is poorly known and remains difficult to predict. As an evolutionary process, insecticide resistance has an associated fitness cost that can be reduced, for instance, through phenotypic plasticity. This associated cost has previously been assessed in regards to age, multiple exposures and delayed mortality. The current study aims to investigate over the course of the mosquito’s lifespan whether individuals that have survived insecticide exposure have a reduced fitness and reproductive success. This was assessed through the measurement after exposure to a sub-lethal dose of a public health insecticide of various life-history traits including blood-feeding rate, 24-hour post-exposure mortality rate, oviposition rate, mortality rate at the end of each gonotrophic cycle and the reproductive success. By assessing this, we hoped to gain a better understanding of insecticide resistance implication for malaria vector control.

Methods

Mosquito strain

All experiments were conducted on a strain of An. gambiae isolated from an An. gambiae s.l. strain, originating from the locality of Tiassalé in Côte d’Ivoire and bred without selection at the insectary of the Centre Suisse de Recherches Scientifiques en Côte d’Ivoire (CSRS) since 2010. According to WHO criteria, Tiassale mosquito population has been described as resistant to at least three of the four classes of insecticides. Both the Kdr and Ace1R point mutations, respectively responsible of resistance to pyrethroids and carbamates and organophosphates have been identified as a resistance mechanism in the Tiassalé strain, as well as metabolic resistance. The strain was a mixed of about 80% An. coluzzii and 20% An. gambiae. In order to isolate the An. gambiae from the original Tiassalé strain, pupae were isolated individually in plastic cups. After emergence, couples composed of a female and a male were formed and left for observation for five days to allow them to mate. A number was assigned to each couple. Following the oviposition, the Mosquitoes parent couple was removed, killed in the freezer at -20°C and processed for molecular analysis through the Sine PCR for species identification. Only larvae whose both parents were An. gambiae were kept and nurtured to adulthood. More than 200 couples were used to build up the new colony in order to reduce the effect of inbreeding. A subsample randomly selected in the F2 progeny was used to confirm the species status. The breeding conditions were standard insectary conditions of 27 ± 2 °C, 70 ± 5 % relative humidity (RH) and a 12 hours light:12 hours dark photoperiods. Eggs were placed in plastic trays of 15x30x5cm and filled with 800mL of dechlorinated tap water. Hatched larvae were separated into groups of about 200 larvae/tray in order to limit size variation. They were fed on mashed Friskies dry cat food. Starting day ten after hatching, pupae were collected for two days and transferred into 25x25x25cm holding cages for emergence. All emerged adults, before and after experimentation, were maintained under standard insectary conditions of 27 ± 2°C, 70 ± 5 % RH. They were provided with a 10% glucose solution daily. Experiments started with the F3 progeny.

Experimental design and processing

Prior the experimentation, a sub-lethal exposure time to deltamethrin treated bed-net that procured 20% mortality was determined. The experimental design used has been drafted in order to mimic exposure to insecticides in natural settings where mosquitoes are coming in contact to the chemical on bed-net when they are trying to reach a person sleeping underneath for blood meal. Thus, a treated bed-net has been used. To increase accuracy, the bed-net was furnished by the National Control Program for Malaria (NCPM) of Côte d’Ivoire and was issued from the pool of Dawa 2.0 LLINs distributed to the population. It is coated with 80mg/m² of deltamethrin. Net
Quality was assessed with a WHO standardized cone-test with the susceptible An. gambiae Kisumu strain maintained at the CSRS laboratory in Abidjan.

During the course of the experiment, four cohorts of 30 to 100 females aged four to five days depending on mosquito availability were separated into two same-sized groups. Each group was either exposed to a LLIN or to an untreated bed-net for 10 minutes, corresponding to the sub-lethal exposure time. For the exposure, mosquitoes were released into a 15X15X15cm cage that was covered with either a LLIN or an untreated net. The cage frames were wrapped with either treated fabric for the treated group or untreated fabric for the untreated group. After exposure, all individuals were transferred into a new holding cage according to their respective groups for 30 minutes recovery period whereafter, they were given the opportunity to have a blood meal for 30 minutes. All mosquitoes were allowed to rest and digest for two days before being transferred into individual 125 ml plastic cups. At this point, 24-hour mortality-post exposure and the blood feeding rates were recorded for each group. Each cup was lined at the bottom with damp cotton wool covered with a filter paper to stimulate oviposition. Each blood fed female was allowed to lay eggs for three days or until over 50% of the control group has laid. The egg laying rate or oviposition rate was defined as the number of females having laid at least one egg out of the total number of females that have taken a blood meal for each group. A second mortality was recorded at this point, corresponding to the mortality after a gonotrophic cycle. Afterwards, all females, fed and not fed, were transferred back into their initial cages and the experiment was repeated. Each cohort was exposed four times. Intervals between exposures were adapted to the times needed for egg laying.

Laid eggs were collected and pooled accordingly as exposed or non-exposed group and reared under standard insectary conditions. When larvae from each group grown to stage three (L3), they were counted and recorded. The reproductive success was estimated as the mean number of L3 counted per female and per batch that had laid eggs.

Data analysis

From the exposure to the end of gonotrophic cycle, four life-history traits were measured including the blood-feeding rate, the mortality rate 24-hour post-exposure, the mortality rate at the end of gonotrophic cycle, the egg laying rate and the reproductive success defined here as the mean number of L3 counted per female and per batch that had laid eggs.

Results

A total of 477 females An. gambiae from the TIAS strain split into two groups were either exposed or not to a LLIN and allowed to complete four gonotrophic cycles. Both groups of mosquitoes were well balanced. Details on sample sizes, cohort composition over the different gonotrophic cycles are given in Table 1 as supplementary information. The unexposed mosquitoes consisted of four cohorts (or replicates) of 50, 100, 28 and 60 mosquitoes for a total of 238 versus four cohorts of 50, 100, 29 and 60 mosquitos for the exposed group for a total of 239 mosquitoes (Table 1, Data availability).

For both the unexposed and exposed groups, none of the five traits studied (including the blood-feeding rate, the mortality rate 24-hour post-exposure, the mortality rate at the end of gonotrophic cycle, the egg laying rate and the mean number of L3 counted per female and on per batch that had laid eggs) appear to be significantly affected by the gonotrophic cycle within each group as their variation was not significant from gonotrophic cycles 1 to 4. However, these parameters were negatively affected once the mosquitoes were exposed to the treated net. Their variation was significant when the deltamethrin-exposed group was compared to unexposed group.

Regarding the blood-feeding, its rate was relatively stable between the different gonotrophic cycles in the unexposed group, and varied between 66.8% and 82.7%, the difference was not significant (P=0.909; Figure 1a). In the exposed group, this parameter varied between 32.2% and 51.3% within the gonotrophic cycles. As in the unexposed group, the difference however was not significant (P=0.351; Figure 1a). But when compared the unexposed to the exposed group, a significant drop in blood feeding mean rates was observed (D = 0.800; P<0.0001; Figure 1b).

With regards to the mortality 24 hours post-exposure, we did not observe any significant variation between the gonotrophic cycles either in the unexposed group (5.9 to 11.9%; P= 0.847; Figure 2a) or in the exposed group (11.1 to 38.9%; P= 0.071; Figure 2a) despite that the trend was upward in the exposed group (Figure 2a). However, when the two groups are compared together, the increase in mean mortality rate in the exposed group is significant (D = 0.600; P< 0.001; Figure 2b).

Concerning the mortality at the end of the gonotrophic cycle or delayed mortality, we noted a non-significant variation in the unexposed group between the gonotrophic cycles (19.2 to 35%; P= 0.307; Figure 3a) as well as in the exposed group (31.7 to 74.5%; P= 0.197; Figure 3a), despite that it was increasing over the gonotrophic cycles in the latter. However, when the two groups were compared together, the increase in the mortality at the end of gonotrophic cycle was significant (D = 0.611; P< 0.006; Figure 3b).

Like the blood-feeding rate or the mortality, the oviposition rate did not vary significantly between the gonotrophic cycles in the unexposed (33.3 to 21.4%; P= 0.841) nor in exposed group (15.0 to 9.6%; P=0.667), although there was a slight downward trend in both groups (Figure 4a). As for the other
above traits, the egg laying rate was significantly affected by exposure to the treated net (D = 0.730, P< 0.0001, Figure 4b).

As all for the four traits listed above, we did not find any significant variation in the reproductive success between the different gonotrophic cycles in both the unexposed group (P= 0.472) and the exposed group (P= 0.778; Figure 5a). Contrary to what might have been expected in view of the results obtained on the other traits, the reproductive success did not decrease following exposure. Although it was not significantly different between the two groups (D = 0.417, P> 0.05, Figure 5b), there was rather an upward trend in the exposed group comparing to the unexposed one.

**Discussion**

Resistance to insecticide has a certain fitness cost on the short and long-term that can be observed on a phenotypic level. However, the extent to which this can maintain the sustainability of the species requires further investigation. Here, we assessed four energy-cost related life-history traits in resistant mosquitoes after a sublethal exposure to insecticide treated net and their epidemiological importance.
The first examined trait being the blood-feeding rate. Females that were exposed to deltamethrin fed less than females that were not exposed. Our findings were consistent with Glunt et al.\textsuperscript{28} and contrary to what was observed by Tchakounte et al.\textsuperscript{27} who showed that exposure of mosquitoes to PermaNet 2.0 deltamethrin-treated bednet presented no effect on their blood feeding ability. From an epidemiological point of view, our findings can be translated into a reduction in transmission as the drop in the rate of mosquito bites also means less risk of infection with plasmodium. In areas of low- to moderate-coverage with impregnated mosquito nets, it is obvious that the irritant effect of pyrethroids repels mosquitoes from treated households to untreated households. However, according to our study, we can imagine that the prior contact of these mosquitoes with insecticides in treated dwellings should favor a limitation of bites of individuals in untreated dwellings\textsuperscript{26}. Such beneficial effect should lead in a decrease of plasmodium-infected mosquito bites. This is probable one of the reasons which explains the beneficial effect of indoor residual sprayings (IRS) in areas of insecticide resistance in mosquito populations, where there is no physical barrier between the sleeper and the mosquito as is the case with mosquito bednets. Indeed, our study suggests that in such areas, despite that IRS can fail to kill mosquitoes because of their resistance to insecticides, simple prolonged
contacts can be enough to cause a drop in host seeking behavior\textsuperscript{26}, and therefore a decrease in malaria transmission. Possible explanation is that insecticide exposure can have an effect on chemo-sensitivity, hence, exposed female may be less likely to detect physical and chemical cues from their prey. The success of blood feeding is doubly important as it affects not only pathogen transmission but also reproduction and thus population density and dynamic. In our study, in addition to the low rate in the bloodfeeding of exposed mosquitoes, we also noted among this group a low rate of egg laying compared to non-exposed mosquito group. This effect can be explained through a resources based trade-off between fecundity and survival\textsuperscript{28} as the over-production of detoxifying enzymes triggered upon exposure to insecticide is probably associated with energetic cost, which impacts the resource available for egg laying. Such a regulation would indicates a high adaptive cost related to insecticide resistance\textsuperscript{29}, which have been already demonstrated in previous studies\textsuperscript{30–34}. The low rate of egg laying would logically be translated epidemiologically into a decrease in vector density and a thus decrease in transmission.

Reduction of vectors longevity is the target of current insecticide-based malaria control methods and insecticide resistance management strategies\textsuperscript{33,35–37}. In our setting, the longevity was expressed by the mortality both at 24 hours post exposure, and at the end of the gonotrophic cycle. These two mortalities were affected by exposure to LLIN at each gonotrophic cycle contrary to observation made in Burjina Faso where following single and multiple exposures to a PermaNet 2.0 LLIN only one of the four mosquito populations tested showed evidence of delayed mortality\textsuperscript{38}. In our study, it increases by twice between the first and fourth gonotrophic cycles in unexposed mosquitoes, while it tripled over the same interval in the exposed group. By the end of gonotrophic cycle 4, mosquitoes were aged 25 to 28 days. At this point, 85% of the control population was eliminated and 97% of the treated group. Such an overall mortality rate approximates what can be observed in the field where only about 20% of the female live up to day 20\textsuperscript{39}. Those 13% difference in population survival are important for malaria transmission as only old female transmit the parasite. Indeed, under favorable conditions in tropical regions, the principal malaria \textit{plasmodium falciparum} parasite complete its development cycle in 10–12 days in mosquitoes. Thus, from our results, repetitive exposure to a sub-lethal dose of deltamethrin through LLIN can still be efficient in controlling transmission in presence of pyrethroids resistance. This may partly explain why LLINs efficacy does not seem to have been curtailed despite the widespread of pyrethroids resistance in African malaria vectors\textsuperscript{11,40}.

However, when looking at the reproductive success, although the number of offspring per female was not significantly different between the two groups, we observed high numbers of L3 larvae in the exposed group comparing to the unexposed group. Maybe this is a result of a resource-based trade-off for species perpetuation as final investment. Nevertheless if the same observation is made in natural settings, it can be considered as a counterbalance to the decrease in the vector population due to combination of high mortality, low blood intake rate and low egg laying rate following mosquitoes exposure to treated surfaces. Although this counterbalance is not enough to compensate for the losses caused by exposure to insecticides, it can nevertheless explain why despite the large-scale deployment of vector control strategies and/or insecticide resistance management, there is no eradication of mosquito populations despite a significant drop in their densities. This justifies the spread of resistance phenomena and the maintenance of malaria transmission despite decades of insecticide-based
vector control activities. In this study, no evidence of differed impacts of insecticide exposure on the offspring viability was observed at larval stage. These effects were not further analyzed as we consider the variance acting on those parameters to be too high to draw conclusions.

While mortality/longevity remains one of the most sensitive parameter in vector control and thus malaria transmission, several other mosquito life traits that are negatively affected by exposure to insecticide for a sublethal time can reduce vector transmission potential to a certain extent, and when combined together, the overall impact on can be even much higher, although we have not estimate the magnitude. This study reinforces the hypothesis that in the current context of resistance to insecticides, especially to pyrethroids, the existing toolbox for vector control can still confer protection against malaria. However, these findings should be considered with cautious as observations can vary the intensity of insecticide resistance, especially that the rapid and widely spread of insecticide resistance which threatens the insecticide-based vector control programs is problematic and highlights the urgent need to develop new strategies to mitigate these phenomena and preserve the efficacy of control interventions.

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**Data availability**

Zenodo. Impact of sublethal pyrethroid exposure on resistant Anopheles gambiae mosquitoes’ fitness. DOI: http://doi.org/10.5281/zenodo.5105928

Data are available under the terms of the Creative Commons Zero “No rights reserved” data waiver (CC BY 4.0 Public domain dedication).

**Authors’ contributions**

BKF contributed to laboratory experiments and revised the manuscript. AK carried out the laboratory study and drafted the manuscript. GSC, AKFP and ZGM helped with the laboratory experiments. KGB revised the manuscript. CSM conceived and initiated the study, ran data analysis and improved the manuscript. All authors have seen and approved that last draft.

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OVERVIEW

Overall an excellent, well conducted and important paper but does need some work on the finer points.

MAJOR COMMENTS

1. The following sentence is vitally important to assessing the validity of the study design and statistics applied to the data: “Laid eggs were collected and pooled accordingly as exposed or non-exposed group and reared under standard insectary conditions.” If eggs were pooled within group separately for each of the four cohorts, then that’s fine. However, if groups from the four different cohorts were pooled then that means there would have been no replication. I presume the former design is what the authors implemented and, if so, they need to make that completely unambiguous in their explanation. If not, the study design would be un-replicated and therefore invalid.

2. In the abstract and main text centered around figure 5, it is normally considered redundant and even overly defensive to say much about a non-significant result unless there is a good reason to suspect there is a real effect that has not been conclusively demonstrated by the study, specifically a very large effect size with a P value at least approaching significance (P ≤0.10) and/or some indication that the study was underpowered to evaluate that particular effect. I’d therefore like to see an exact P value for the following statement, and equivalent text in the main narrative around figure 5, rather than a crude simple statement of being above or below P=0.05: “Surprisingly, there was rather an upward trend in the number of L3 larvae/female mosquito for the exposed group comparing to the unexposed one, although the difference was not significant (D = 0.417, P> 0.05).” If there is no convincing evidence to suggest this statistically insignificant difference may be real and biologically significant, I would delete from the abstract and edit the main text accordingly. Unless a strong case can be made, I think the second last paragraph is excessively speculative and can be deleted entirely or at least reduced to a single sentence.
3. In fact, this deficit applies throughout: In the Data Analysis subsection, I would like to see exact P values for all tests, rather than simply setting an a priori cut off of 5%, so that I can critically assess the risks of underpowering and spurious significance.

4. Surely for many of these parameters, survival analysis statistics are more appropriate? I'm thinking of Kaplan-Meyer analysis, Cox regression, etc.

MINOR COMMENTS
1. Second sentence of introduction: Malaria is not prevented geographically. I think the authors mean “geographically widespread”, but the second word makes the first redundant, so I'd just delete “geographically”.

2. Last sentence of the introduction: “implications” plural.

3. Methods section, mosquito strain subsection: Replace “mixed” with “mixture” in “The strain was a mixed of about 80% An. coluzzii and 20% An. gambiae.”

4. Same section: While PCR is a widely understood acronym, “Sine PCR” is jargon and non-obvious even to most experts. Please use enough text to give the reader some idea of how this PCR test differs from the regular test of Scott et al.

5. Opening sentence of the Experimental design and processing section: Insert “to” and delete “the”: “Prior to experimentation....”

6. Following sentence: “designed” not “drafted”.

7. Following sentence at the bottom right-hand corner of page 3: I suggest “To ensure representativeness of relevant real world processes...” instead of “To maximize accuracy...”

8. Opening sentence at the start of the following paragraph, top left corner of page 4 needs some extra punctuation: “During the course of the experiment, four cohorts of 30 to 100 females aged four to five days, depending on mosquito availability, were separated into two same-sized groups.”

9. Page 3, opening sentence of the last paragraph: This sentence does not make sense as written, needs to be made far more explicit, and I suspect the authors mean something different when they use the word “procured”.

10. Opening sentence of data analysis subsection: Insert and extra “the” into “From the exposure to the end of the gonotrophic cycle...”.

11. Results section, opening sentence: “female” singular.

12. There is no link to table 1 in the supplementary online material.

13. I could not understand the following sentence: “For both the unexposed and exposed groups, none of the five traits studied (including the blood-feeding rate, the mortality rate 24-hour post-exposure, the mortality rate at the end of gonotrophic cycle, the egg laying rate and the mean number of L3 counted per female and per batch that had laid eggs)”
appear to be significantly affected by the gonotrophic cycle within each group as their variation was not significant from gonotrophic cycles 1 to 4. I suspect the authors need to be clearer about what they mean by "gonotrophic cycle": do they mean the length of the gonotrophic cycle or do they mean gonotrophic age? I suspect the latter, but good to be clear.

14. Last sentence at the end of the second paragraph of the results section: I would rephrase a little more explicitly as: “As explained in the following paragraphs, the distributions of all these parameters differed when the deltamethrin-exposed group was compared to unexposed group.” Also, please remember that the word “significant” is not required and considered redundant because one would not normally state a difference exists in a solid scientific journal unless it was statistically significant.

15. Opening sentence third paragraph of the results section: “across” not “between”.

16. Bottom left corner of page six: Capitalize and italicize “Plasmodium”.

17. Bottom right hand corner of page six: Insert “the fact” into “...despite the fact that IRS can...”

18. End of first paragraph on page 7: “thus a” instead of “a thus”.

19. Bottom left hand corner of page 7: “Burkina Faso” not “Burjina Faso”.

20. Following sentence: Capitalize first word: “In our study”.

21. Second paragraph, page 7: The word “eliminated” has a very important and specific meaning in epidemiology and is inappropriate here. Replace “were eliminated” with “had died”.

22. End of the same paragraph: some grammatical errors, so correct to “...despite widespread pyrethroid resistance among African malaria vector populations”.

23. Last paragraph: Opening sentence is too long, so break into two sentences and be clearer in each.

24. Same final paragraph: “caution” not “cautious”.

25. Final sentence also far too long and wanders around quite a bit, leaving me unclear about what the authors are trying to say. Please break down into smaller, clearer sentences, that explain the perspectives of the authors in smaller, more precise steps.

Is the work clearly and accurately presented and does it cite the current literature? Yes

Is the study design appropriate and is the work technically sound? Yes

Are sufficient details of methods and analysis provided to allow replication by others?
Yes

**If applicable, is the statistical analysis and its interpretation appropriate?**
Partly

**Are all the source data underlying the results available to ensure full reproducibility?**
Yes

**Are the conclusions drawn adequately supported by the results?**
Partly

**Competing Interests:** No competing interests were disclosed.

**Reviewer Expertise:** Malaria vector biology and control, as well as malaria transmission dynamics and epidemiology,

I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

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**Author Response 01 Oct 2021**

**BEHI KOUADIO FODJO,** Centre Suisse de Recherches Scientifiques en Côte d’Ivoire, Abidjan, Cote d’Ivoire

**MAJOR COMMENTS**

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  **For each group of mosquitoes, eggs were pooled separately. Each exposure was repeated four times. Eggs were not pooled.**

- In the abstract and main text centered around figure 5, it is normally considered redundant and even overly defensive to say much about a non-significant result unless there is a good reason to suspect there is a real effect that has not been conclusively demonstrated by the study, specifically a very large effect size with a P value at least approaching significance (P \leq 0.10) and/or some indication that the study was underpowered to evaluate that particular effect. I’d therefore like to see an exact P value for the following statement, and equivalent text in the main narrative around figure 5, rather than a crude simple statement of being above or below P=0.05:
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- In fact, this deficit applies throughout: In the Data Analysis subsection, I would like to see exact P values for all tests, rather than simply setting an a priori cut off of 5%, so that I can critically assess the risks of underpowering and spurious significance. Done

- Surely for many of these parameters, survival analysis statistics are more appropriate? I’m thinking of Kaplan-Meyer analysis, Cox regression, etc. Done

You’re right, we could use survival analysis statistics, but we didn’t just assess mosquito survival (Mortality/longevity) after exposure to deltamethrin treated bed-net. We also assessed other parameters and some of which were not necessarily related to the survival of mosquitoes exposed, such as productivity.

**MINOR COMMENTS**

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○ Last sentence at the end of the second paragraph of the results section: I would rephrase a little more explicitly as: “As explained in the following paragraphs, the distributions of all these parameters differed when the deltamethrin-exposed group was compared to unexposed group.” Also, please remember that the word “significant” is not required and considered redundant because one would not normally state a difference exists in a solid scientific journal unless it was statistically significant. Done

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○ End of the same paragraph: some grammatical errors, so correct to “…despite widespread pyrethroid resistance among African malaria vector populations”. Done
Competing Interests: No competing interests were disclosed.
The authors say they used a resistant strain whereas in the material and method section they say the strain is maintained without selection in laboratory since 2010. Does it mean that the strain is still resistant? Have they checked whether the strain has become susceptible. Moreover if they have checked for the susceptibility to different insecticide they can add this information in the material and method section.

Page 7 sentence “These two mortalities were affected by exposure to LLIN at each gonotrophic cycle contrary to observation made in Burjina Faso……” “Burjina Faso.” Change to “Burkina Faso”

Page 7 “Indeed, under favorable conditions in tropical regions, the principal malaria plasmodium falciparum parasite... « Please start with a capital letter Plasmodium falciparum

Page 7 “Although this counterbalance is not enough to compensate for the losses caused by exposure to insecticides, it can nevertheless explain why despite the large-scale deployment of vector control strategies and/or insecticide resistance management, there is no eradication of mosquito populations despite a significant drop in their densities. This justifies the spread of resistance phenomena and the maintenance of malaria transmission despite decades of insecticide-based vector control activities.”

The result of the study cannot explain or justify everything because the influence of outdoor biting mosquitoes and other factors such as the diversity of species taking part to malaria transmission or usage of control measures were not considered so the authors need to revise this section accordingly and talk of other factors which could affect vector control and which were not investigated in the present study.

Is the work clearly and accurately presented and does it cite the current literature?  
Yes

Is the study design appropriate and is the work technically sound?  
Yes

Are sufficient details of methods and analysis provided to allow replication by others?  
Yes

If applicable, is the statistical analysis and its interpretation appropriate?  
Yes

Are all the source data underlying the results available to ensure full reproducibility?  
Yes

Are the conclusions drawn adequately supported by the results?  
Partly

*Competing Interests:* No competing interests were disclosed.

*Reviewer Expertise:* Vector biology, and vector control
I confirm that I have read this submission and believe that I have an appropriate level of expertise to confirm that it is of an acceptable scientific standard, however I have significant reservations, as outlined above.

Author Response 01 Oct 2021

BEHI KOUADIO FODJO, Centre Suisse de Recherches Scientifiques en Côte d'Ivoire, Abidjan, Cote d'Ivoire

Material and method section:
Page 3 “To increase accuracy, the bed-net was furnished by the National Control Program for Malaria (NCPM) of Côte d’Ivoire...” should be written National Malaria Control Program. 
Done

Results:
It should have been interesting to also report the duration of the gonotrophic cycle for each group
Paragraph 3 “With regards to the mortality 24 hours post-exposure, we did not observed any significant variation between the gonotrophic cycles either in the unexposed group (5.9 to 11.9%; P= 0.847; Figure 2a)...” It should be written “..... significant variation of the mortality after 24 hours between the gonotrophic cycle ......” Done

Discussion
Page 5: “Here, we assessed four energy-cost related life-history traits in resistant mosquitoes after a sublethal exposure to insecticide treated net and their epidemiological importance.”

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**Competing Interests:** No competing interests were disclosed.