TARGET MALARIA

A Vector Control Research Alliance

**Évaluation indépendante du risque écologique lié au lâcher à petite échelle d'une souche mâle stérile *Anopheles coluzzii***

## Contexte

En septembre 2015, l'Organisation du Commonwealth pour la recherche scientifique et industrielle (CSIRO) a publié une étude sur les risques écologiques liés aux études de laboratoire en milieu confiné d'une souche d'*Anopheles gambiae* mâle stérile génétiquement modifiée[1]. CSIRO est l'Agence fédérale australienne pour la recherche scientifique et est l'un des plus grands organismes mondiaux pour la recherche scientifique, et aussi l'un des plus diversifiés[2]. L'expertise multidisciplinaire de CSIRO se décline dans tout un éventail de disciplines scientifiques, elle a notamment une équipe spécialiste en évaluation quantitative de risque écologique qui a fait ses preuves dans ce domaine[3]. L'étude de risque réalisée en 2015 par CSIRO avait été commandée par la FNIH (Fondation des Instituts Nationaux de la Santé aux États-Unis) afin d'obtenir une évaluation externe des risques liés aux activités proposées avec la souche de moustiques mâles stériles en milieu confiné. Les conclusions de l'étude de risque de 2015 confirmaient celles de Target Malaria, telles que présentées dans les dossiers de soumissions réglementaires du projet, et la souche de moustiques mâles stériles a été importée en novembre 2016 dans l'insectarium de l'Institut de Recherche en Sciences de la Santé à Bobo-Dioulasso, au Burkina Faso. Depuis l'importation initiale, la souche de moustiques mâles stériles a fait l'objet de rétrocroisements continus avec une souche locale de moustiques du Burkina Faso ; des analyses poussées ont permis d'établir ses caractéristiques et de générer des données supplémentaires afin de mieux éclairer les considérations de sécurité et de performance.

## L'étude de CSIRO concernant les risques du lâcher à petite échelle

La prochaine phase de recherche proposée pour la souche de moustiques mâles stériles consiste en un lâcher à petite échelle, au Burkina Faso, qui permettra de générer plus de données de terrain sur le comportement et le cycle biologique de la souche dans l'environnement naturel. Ceci contribuera au développement d'un outil de lutte antivectorielle contre le paludisme lors de phases ultérieures de la recherche. CSIRO a donc été chargé par la FNIH de procéder à une étude de risque en rapport avec le lâcher à petite échelle [de moustiques mâles stériles], en partant du rapport 2015 et en prenant en compte les données générées lors d'expérimentations réalisées en milieu confiné à l'insectarium du Burkina Faso depuis novembre 2016. La méthodologie utilisée dans l'étude de risque de CSIRO était fondée sur les principes d'évaluation quantitative du risque écologique, évaluation qui passe par l'utilisation structurée d'outils décisionnels probabilistes afin de retracer les trajectoires de cause à effet et de quantifier les probabilités de résultats spécifiques. Bien que le projet de lâcher en question ne fasse pas intervenir des moustiques modifiés porteurs de la technologie du « gene drive », la méthodologie d'étude de risque de CSIRO se conforme aux recommandations récentes de l'Académie nationale américaine des sciences, de l'ingénierie et de la médecine (NASEM) pour les applications de gene drive[4]. Elle offre donc une

[1] https://targetmalaria.org/wp-content/uploads/pdf/target-malaria-risk-assessment-sterile-males-plus-executive-summary.pdf
[2] https://www.csiro.au/en/Research
[3] http://people.csiro.au/H/K/Keith-Hayes
[4] http://nas-sites.org/gene-drives/

excellente occasion d'intégrer des outils d'évaluation quantitative du risque à un stade précoce de la recherche. L'étude de risque examine les données et éléments fournis par Target Malaria et intègre un processus consultatif structuré faisant intervenir des experts indépendants. Le rapport de l'étude de risque écologique, finalisé en mai 2018, est un exemple exhaustif d'une étude quantitative de risque, mise à disposition dans le domaine public, concernant un lâcher à petite échelle délibéré et contrôlé de moustiques génétiquement modifiés (mais non porteur de la technologie du 'gene drive').

L'étude de risque concernant le lâcher de la souche de moustiques mâles stériles de CSIRO est fondée sur des paramètres issus des préoccupations des communautés locales ; celles-ci ont trait aux perceptions des risques pour l'environnement et la santé humaine et ont été rassemblées lors d'activités d'engagement des communautés sur les sites d'étude. Les sept paramètres spécifiques définis dans le rapport d'étude de risque peuvent être regroupés en quatre domaines concernant le potentiel de :

- Transmission accrue de la maladie,
- Survie des moustiques génétiquement modifiés relâchés ou de leur descendance au-delà des délais anticipés,
- Persistance et propagation de la construction génétique dans la population cible et au sein d'espèces étroitement apparentées,
- Résistance accrue aux insecticides des moustiques femelles génétiquement modifiées relâchées, par rapport à la population sauvage.

Globalement, CSIRO a conclu que les résultats de l'étude de risque n'indiquaient pas la nécessité de mesures supplémentaires de gestion des risques, hormis les activités de surveillance déjà planifiées par le projet. Il importe de noter que les calculs réalisés dans l'étude de risque ne tenaient pas compte de l'élimination des moustiques femelles sauvages, qui se poursuit par le biais des activités routinières du programme de surveillance de Target Malaria. Le nombre de moustiques femelles capturées dans la population locale sur le site du lâcher, dans le cadre des captures de surveillance, dépasse largement le nombre potentiel de moustiques femelles qui seraient susceptibles d'être introduit par inadvertance lors du lâcher. Il s'agit d'une considération pertinente pour l'estimation des risques, notamment celui de transmission de la maladie qui dépendrait du nombre de moustiques femelles présents, mais cette considération ne rentrait pas dans le périmètre des calculs réalisés par CSIRO pour cette étude de risque.

CSIRO HEALTH AND BIOSECURITY
www.csiro.au

# Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases: Controlled field release for Sterile Male Construct
# Risk assessment final report

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# Contents


**ACKNOWLEDGEMENTS** . . . . . 1

**EXECUTIVE SUMMARY** . . . . . 2

**1 INTRODUCTION** . . . . . 7

1.1 Project background and objectives . . . . . 8

1.2 Report structure . . . . . 9

1.3 Contextual information . . . . . 10

1.3.1 Entomological surveys and controlled field release sites . . . . . 10

1.3.2 Strain establishment, maintenance and release . . . . . 14

1.4 Risk assessment scope . . . . . 17

**2 COMMUNITY CONCERNS AND RISK ENDPOINTS** . . . . . 18

2.1 Community concerns and values . . . . . 18

2.1.1 Human-health concerns . . . . . 19

2.1.2 Environmental concerns . . . . . 20

2.2 Risk assessment endpoints . . . . . 21

2.3 Expert opinion, risk assessment and Bayesian inference . . . . . 22

**3 CAPACITY TO VECTOR BLOOD-BORNE PATHOGENS** . . . . . 27

3.1 Known blood-borne pathogens . . . . . 28

3.1.1 Informative priors . . . . . 28

3.1.2 Scope and parameter definitions . . . . . 29

3.1.3 Populations and lineages . . . . . 30

3.1.4 Definition of the relative risk assessment endpoint . . . . . 30

3.1.5 Human feeding rate . . . . . 31

3.1.6 Extrinsic incubation period . . . . . 31

3.1.7 Transmission efficiency . . . . . 33

3.1.8 Daily probability of mortality . . . . . 40

3.1.9 Relative risk results . . . . . 44

3.2 Novel blood-borne pathogen . . . . . 46

**4 TRANSGENE SPREAD AND PERSISTENCE** . . . . . 51

4.1 Spread and persistence pathways . . . . . 52

4.2 Female release pathways . . . . . 53

4.2.1 Incidental release of Ac(DSM)2 females . . . . . 53

4.2.2 Hybridisation with sexually compatible species . . . . . 53

4.2.3 Insecticide resistance . . . . . 56

4.3 Male release pathways . . . . . 59

4.3.1 Spread and survival of sterile males . . . . . 59

4.3.2 Probability of construct failure . . . . . 61

4.3.3 Probability of hybridisation with compatible species . . . . . 65

4.3.4 Bayesian update of FT50 and FT51 . . . . . 65

**5 NON-TARGET EFFECTS** . . . . . 68
5.1 Persistence and adverse outcomes pathways . . . . . 69

**6 DISCUSSION** . . . . . 71
6.1 Quantitative risk estimates . . . . . 72
6.2 Lessons and future analysis . . . . . 74

**REFERENCES** . . . . . 77

**Appendix A TECHNICAL DETAILS** . . . . . 82
A.1 Vectorial capacity: known blood-borne pathogens . . . . . 82
A.1.1 Predictive prior distributions . . . . . 82
A.1.2 Predictive posterior distribution and model evidence . . . . . 86
A.1.3 Predictive posterior distribution for daily probability of mortality . . . 87
A.2 Female survival . . . . . 90
A.3 Insecticide resistance . . . . . 93
A.4 Spread and survival of sterile males . . . . . 99
A.4.1 Latent process model . . . . . 99
A.4.2 Chemotaxis parameters . . . . . 100
A.4.3 Mortality rate . . . . . 103
A.4.4 Catchability parameters . . . . . 103
A.4.5 Bayesian inference . . . . . 103
A.4.6 Evaluating the PDE model . . . . . 106
A.4.7 Sampling algorithm . . . . . 107
A.4.8 Posterior distributions and model evidence . . . . . 107
A.4.9 Posterior and correlation . . . . . 110
A.4.10 Model validation . . . . . 114
A.5 Probability of construct failure . . . . . 117

**Appendix B Pre-elicitation document** . . . . . 120

**Appendix C Background material** . . . . . 134

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# EXECUTIVE SUMMARY

In September 2015, the Commonwealth Science and Industrial Research Organisation (CSIRO) assessed the human health and environmental risks associated with a hypothetical escape of transgenic Dominant Sterile Male *Anopheles gambiae* mosquitoes, designated Ag(DSM)2, from insectaries in Western Africa. Target Malaria used the risk assessment to support an application to import and rear these mosquitoes at the Institut de Recherche en Sciences de la Santé insectary in Bobo-Dialousso, Burkina Faso.

In November 2016, Target Malaria imported under permit Ag(DSM)2 eggs into a contained facility within the insectary. The insectary backcrosses Ag(DSM)2 females with male *An. coluzzii* mosquitoes born to gravid females originally sourced from a village in the Kou valley. The backcrossing replaces the genetic background of the original Ag(DSM)2 eggs with that of the wild-type mosquitoes, designated here as Ac(WT). The trangenic mosquitoes are subsequently designated Ac(DSM)2 to reflect the introgression of the Dominant Sterile Male construct into the *An. coluzzii* genetic background.

Target Malaria propose to conduct a controlled field release of between 2,000 and 10,000 male Ac(DSM)2 mosquitoes marked with fluorescent dust, and an equivalent number of males Ac(WT) mosquitoes as comparators, in Bana village in the Kou Valley, approximately 25 km west of the insectary. The primary purposes of the field release are to: (i) generate data on the daily survival rate of released Ac(DSM)2 males and to assess their movement from a defined release point; and (ii) strengthen local capacity in the handling, release and recapture of laboratory reared mosquitoes including through the establishment and validation of standard operating procedures and internal systems for the oversight of regulatory compliance.

Data generated from the field release will further inform an understanding of how outcomes from indoor contained use experiments can be extrapolated to a field entomology context. Additionally, although Ac(DSM)2 does not incorporate a gene drive mechanism, data on the population dynamics and dispersal behaviour of male Ac(DSM)2 collected from a field release will serve to further demonstrate and develop the methodology and mathematical models needed to support an environmental risk assessment to standards recommended for gene drive applications (National Academies of Sciences, Engineering and Medicine, 2016; Australian Academy of Sciences, 2017).

This report documents the results of a risk assessment conducted by the CSIRO to estimate the ecological and human-health risks associated with the proposed field release. The report is not a complete evaluation of all potential risks. Some potential risks, such as the risks to social endpoints identified in Burkina Faso's legislation, are not addressed in this analysis.

At the beginning of the assessment, CSIRO and Target Malaria agreed to base the risk calculations on a controlled release of 5,000 male Ac(DSM)2 mosquitoes at the beginning of the wet season (July). The field release requires regulatory approval and is contingent on the insectary generating a sufficiently large population of male Ac(DSM)2 mosquitoes. The actual release date, and number of mosquitoes released, may therefore change, but the number released will not exceed 10,000. Target Malaria have also stipulated that their female separation protocols will limit the incidental release of female Ac(DSM)2 mosquitoes to no more than 5 for every 1,000 male Ac(DSM)2 mosquitoes released. The risk

assessment assumes that this condition will be met.

In November 2016, the CSIRO asked Target Malaria's stakeholder engagement team to collate the local community's concerns about the field release to help identify risk assessment endpoints. The community subsequently identified four human health concerns and five environmental concerns that were deemed to have plausible Adverse Outcome Pathways. These concerns are represented in seven risk assessment endpoints that form the basis of this risk assessment:

1. The probability that the Dominant Sterile Male construct will increase the vectorial capacity of female Ac(DSM)2 mosquitoes for three pathogens - *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis - relative to Ac(WT) mosquitoes, and thereby increase the probability of these diseases being transmitted following the controlled field release.
2. The probability that incidentally released female Ac(DSM)2 mosquitoes will vector a novel blood-borne pathogen.
3. The survival of incidentally released female Ac(DSM)2 mosquitoes, and their transgenic offspring, expressed as the probability distribution of the time taken (measured in days after the release) for the expected number of Ac(DSM)2 females to drop below one.
4. The survival of released male Ac(DSM)2 mosquitoes again expressed as the probability distribution of the time taken for the expected number of Ac(DSM)2 males to drop below one.
5. The probability that the Dominant Sterile Male construct will spread and persist in local populations of wild-type *An. coluzzii*.
6. The probability that the Dominant Sterile Male construct will spread and persist in local populations of wild-type *An. gambiae* or *An. arabiensis*.
7. The probability that the Dominant Sterile Male construct will make female Ac(DSM)2 mosquitoes less susceptible (more resistant) then female Ac(WT) mosquitoes to commonly used insecticides in the region for which insecticide resistance has been reported in Anopheles mosquitoes.

The first two endpoints examine the potential for female Ac(DSM)2 mosquitoes to transmit *P. falciparum* or other pathogens, and are directly linked to human safety. The third endpoint examines how many female Ac(DSM)2 mosquitoes are in the environment, and how long they are likely to persist, assuming no fitness disadvantages other than that caused by male sterility. The potential for female Ac(DSM)2 mosquitoes to transit pathogens depends on how many there are in the environment. The potential for impacts on non-target organisms also depends on how long they persist in the environment. The third endpoint is therefore relevant to the potential for human health and ecological impacts.

The fourth endpoint examines how long male Ac(DSM)2 mosquitoes are likely to persist in the environment, assuming all male mosquitoes are sterile. The potential for impacts on local populations of *An. coluzzii*, and any subsequent knock-on effects, depends on how long Ac(DSM)2 male mosquitoes persist in the environment. The fourth endpoint is therefore relevant to the potential for human health and ecological impacts.

The fifth and sixth endpoints examine how likely it is that the transgene would spread in the species complex into which it is being released. This is not considered harmful per se, but it would be unexpected, and would trigger additional assessment as to whether it might lead to environmental harm. These two endpoints are therefore conservative.

The last endpoint examines the probability that female Ac(DSM)2 mosquitoes are more resistant to the insecticides that are commonly used in the controlled field release region. Enhanced resistance to insecticide could confer a strong fitness advantage and could therefore increase the probability that female Ac(DSM)2 mosquitoes will survive in the environment. The last endpoint is therefore relevant to the potential for human health and ecological impacts.

In accordance with recent recommendations by the National Academies of Science, Engineering and Medicine, and the Australian Academy of Sciences (National Academies of Sciences, Engineering and Medicine, 2016; Australian Academy of Sciences, 2017), the risk calculations in this analysis are quantitative and based on informative priors elicited from domain experts, updated wherever possible with data from field or laboratory observations. The risk assessment also discusses the possibility of non-target effects attributable to the field release.

The results of the risk assessment for each of the seven endpoints are summarised in Table 6.1. The initial vectorial capacity analysis calculates the relative risk of pathogen transmission by female Ac(DSM)2 after 29 backcrosses, compared to female Ac(WT) mosquitoes in the field (that is without any laboratory habituation) assuming an equal vector to human host ratio for the duration of time that female Ac(DSM)2 mosquitoes are in the environment. Under these circumstances the probability of increased transmission of *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis, attributable to the effect of the transgene, is predicted to be 0.29, 0.33 and 0.13 respectively. This means there is a 71%, 67% and 87% chance respectively that Ac(DSM)2 females will have a lower potential to transmit these pathogens than wild type females.

Depending on the time of the controlled field release, the population of wild *An. coluzzii* female mosquitoes in Bana village is anticipated to be about 400 to 2,000 times larger than the population of Ac(DSM)2 females (allowing for the transgenic offspring of the initial incidentally released population). If this difference between the two vector populations is accounted for by assuming that the female Ac(DSM)2 population is 1,000 times smaller than the wild female population, then the probability of an increase in the transmission of *P. falciparum*, o'nyong'nyong virus or lymphatic filariasis, during the time that female Ac(DSM)2 mosquitoes are in the environment, is predicted to be 0.03, 0.05 and 0.03 respectively.

Accounting for the relatively small numbers of Ac(DSM)2 females that may be incidentally released also reduces the probability that the Ac(DSM)2 mosquitoes will vector a novel blood borne pathogen. A fault tree analysis conducted by Hayes et al. (2015) concluded that the median probability of this event, conditioning on a release of 5,000 female Ag(DSM)2 mosquitoes, would be lower than $5.2 \times 10^{-7}$. Assuming that the elicitations conducted for Ag(DSM)2 are transferable to Ac(DSM)2, and accounting for the smaller number of females released reduces this median probability to a value of $1.3 \times 10^{-8}$, under the most conservative calculation method.

The analysis completed here predicts that male and female Ac(DSM)2 mosquitoes released

during the controlled field trial will die out over the course of the succeeding wet and dry seasons. The field release is not therefore predicted, and is not intended, to have any noticeable effects on local populations of predominately *An. coluzzii* mosquitoes.

A simple model of female population dynamics, with an initial population of 25 Ac(DSM)2 females, predicts that the expected number of female Ac(DSM)2 mosquitoes (allowing for offspring from the initial incidentally released population) will drop below one individual about 65 days after the release. The 90% central credible interval of this prediction is between 6 and 152 days. A detailed spatio-temporal model of male dispersal and survival, with an initial population of 5,000 Ac(DSM)2 males, predicts that it will take 10 days for the expected number of Ac(DSM)2 male mosquitoes to drop below one individual, assuming all 5,000 mosquitoes are sterile. The 90% central credible interval of this prediction is between 6 and 20 days. This predicted survival is too short, and the associated population sizes too small, to cause any noticeable effect on non-target organisms or ecosystem processes.

Hayes et al. (2015) used fault tree analysis to calculate the probability that the Dominant Sterile Male construct will spread in local populations of *An. gambiae* in a year following an accidental release of 5,000 Ag(DSM)2 females and 5,000 Ag(DSM)2 males. This risk assessment amends these calculations by updating (in a Bayesian sense) the prior probability that the construct will fail to sterilise individual mosquitoes. The change in target species from *An. gambiae* to *An. coluzzii*, however, requires that we assume that all other events in the fault tree analysis, except the probability of construct failure, are transferable between the two species. This assumption is reasonable given the high levels of dependency between the two species in the original analysis.

This assessment predicts that the probability of the construct spreading in local populations of *An. coluzzii* in a year following a controlled release of 5,000 Ac(DSM)2 males has a median value of $8.9 \times 10^{-4}$ (under the more conservative fault tree calculation strategy). The equivalent probability of the construct spreading in local populations of *An. gambiae* or *An. arabiensis* has a median value of 0.002 (again under the more conservative calculation strategy). This latter value, however, does not account for the low proportion of hybrids observed in Target Malaria's field samples, and is therefore likely to be an overestimate.

Simulation studies indicate that there is a 30% chance that male Ac(DSM)2 mosquitoes will be present in the vicinity of Bana if they are not observed in three sequential days of observation, with Mark Release Recapture equivalent survey effort, implemented ten days after the controlled field release. This is because capture efficiencies are typically less than a few percent, hence the absence of target (in this case Ac(DSM)2) mosquitoes in a sample does not provide a great deal of confidence that the target population is in fact absent from the survey site. This probability is a slight underestimate because these studies were unable to include the offspring of incidentally released female Ac(DSM)2 mosquitoes, approximately one-quarter of which will be male Ac(DSM)2.

The simulation studies also indicate that the absence of male Ac(DSM)2 mosquitoes in samples collected several months after the field release provide better assurance that Ac(DSM)2 mosquitoes are indeed absent. This is because the simulation model predicts that the male Ac(DSM)2 mosquito population will decline with time. Conversely, if samples collected three months after the field release return true positives for the presence of the transgene, then this would signal that the risk assessment predictions may be incorrect. The analysis would draw the same conclusions if the small number of Ac(DSM)2 males

born to female Ac(DSM)2 mosquitoes were included.

This risk assessment predicts that the probability of horizontal gene transfer of the Dominant Sterile Male construct causing potential impacts on the fertility of any specific Eukaryote (including humans) following the controlled field release will be lower than the probability of horizontal gene transfer to any eukaryote calculated by Hayes et al. (2015), and will therefore be less than $1.2 \times 10^{-10}$.

An analysis of the results of insecticide susceptibility experiments, conducted to World Health Organisation standards, indicates that female Ac(DSM)2 mosquitoes are either no less susceptible (Fenitrothion and Bendiocarb, because mortality is 100% and almost 100% respectively), somewhat more susceptible (Alpha-cypermethrin and Lambda-cyhalothrin) or much more susceptible (Permethrin, Deltamethrin and Dichlorodiphenyltrichloroethane) than female Ac(WT) mosquitoes. These results indicate that there is no evidence that female Ac(DSM)2 mosquitoes are more resistant to these insecticides than their wild-type counterparts.

Seven endpoints were evaluated in this risk assessment based on plausible Adverse Outcome Pathways (AOPs) for both potential human health and environmental risks derived from local community concerns. Overall only two endpoints - the potential for an increase in the transmission of *P. falciparum*, o'nyong'nyong virus or lymphatic filariasis, and for the potential spread of the Dominant Sterile Male construct in sexually compatible species - are identified as having median risks higher than $1 \times 10^{-6}$. The assessment in these two areas assumes that 25 female Ac(DSM)2 mosquitoes are incidentally released as an artefact of the manual sorting process. This, however, is considered to be a maximum threshold, and Target Malaria are seeking to release fewer females. If in fact the number of incidentally released female Ac(DSM)2 is lower than 25 then this would reduce the risk estimates. If no female Ac(DSM)2 are incidentally released then the transmission risks would be conditional on the failure of the Dominant Sterile Male construct and would be several orders of magnitude lower than the values reported here.

The risk assessment accounts for the very small contribution that the incidental releases would make to the overall female population (by assuming their vector to host ratios will be 1000 times smaller) and reveals that the potential transmission risks occur for a relatively short period of time, and are likely to be highly localised. The modelling does not account for Ac(DSM)2 mosquitoes that are removed by survey methods used at the proposed site, and does not account for additional male Ac(DSM)2 mosquitoes that are born to incidentally released females. Neither of these mechanisms however, will make a significant difference to the risk calculations because recapture rates are anticipated to be very low and very few Ac(DSM)2 males are expected to be born at the proposed release site. The possibility that the risk models and calculations are incorrect warrants surveys for Ac(DSM)2 mosquitoes after the proposed field release. Target Malaria are planning to conduct these surveys in the months following the proposed field release. Otherwise the risk results do not indicate the need for additional risk management measures. It should also be noted that the removal of wild female mosquitoes through surveillance and monitoring undertaken by Target Malaria, or any ongoing vector control activities, could reasonably be expected to incrementally reduce the risk of disease transmission as a consequence of reducing the vector population in the proposed release area, however the potential impacts of these activities are outside the scope of this risk assessment.

# 1 INTRODUCTION

**KEY POINTS**

1. In September 2015, CSIRO assessed the human health and environmental risks associated with a hypothetical escape of transgenic Dominant Sterile Male *Anopheles gambiae* mosquitoes, designated Ag(DSM)2, from insectaries in Western Africa.
2. Target Malaria used the risk assessment to support an application to import and rear Ag(DSM)2 mosquitoes at the Institut de Recherche en Sciences de la Santé insectary in Bobo-Dialousso, Burkina Faso.
3. Target Malaria imported Ag(DSM)2 eggs under permit into a contained facility within the insectary in November 2016. The insectary has since successfully maintained a contained transgenic colony by continually backcrossing transgenic females with a synchronously maintained wild type colony established with gravid female *An. coluzzii* mosquitoes.
4. The continual backcrossing replaces the genetic background of the original Ag(DSM)2 eggs with that of the wild-type mosquitoes. The transgenic mosquitoes are subsequently designated Ac(DSM)2 to reflect the introgression of the Dominant Sterile Male construct into the *An. coluzzii* genetic background.
5. Upon receipt of regulatory approval, Target Malaria propose to release between 2,000 and 10,000 male Ac(DSM)2 mosquitoes, and an equivalent number of Ac(WT) mosquitoes as comparators, in Bana village.
6. Target Malaria and Institut de Recherche en Sciences de la Santé have conducted (almost continuous) monthly entomological surveys in Bana, Souroukoudingan, and Pala since July 2012, and have completed five Mark Release Recapture experiments in Bana village.
7. In Bana, female *Anopheles gambiae* s.l. account for 77.8%, of all samples with male *Anopheles gambiae* s.l. accounting for 21.4%. Their numbers peak in September/October towards the end of the wet season (May to October) and are lowest in December/January toward the middle of the dry season (November to April). Molecular analysis reveals that 90.5% of the *An. gambiae* s.l. mosquitoes captured in Bana are *An. coluzzii*. Very low (0.4% of sub-samples) rates of hybridisation occur between the species in the *An. gambiae* complex.
8. The results of an analysis of the first four Mark Release Recapture experiments suggest that the male mosquito population in Bana village may reach 100,000 - 500,000 in the wet season, and decline by an order of magnitude to 10,000 - 50,000 in the dry.
9. Target Malaria have stipulated that their sex separation protocols will limit the incidental release of Ac(DSM)2 females to no more than 5 for every 1,000 deliberately released Ac(DSM)2 males. All subsequent analysis assumes that this condition will be met.

## 1.1 Project background and objectives

In February 2014, the CSIRO was engaged by the Foundation for the National Institutes of Health (FNIH) to conduct an independent assessment of the risks associated with a hypothetical escape from African insectaries of *Anopheles gambiae* mosquitoes genetically modified to be male-sterile (and to express two fluorescent marker genes) by the $\beta$2-Ppo2 construct (Klein et al., 2012; Windbichler et al., 2008).

The initial CSIRO risk assessment (Hayes et al., 2015, available at http://targetmalaria.org/resources/), submitted to FNIH in September 2015, quantifies the risk of five assessment endpoints using fault tree analysis and carefully structured elicitation of experts' beliefs, expressed as subjective probability statements. The assessment was used by Target Malaria to support a biosafety application for the importation and rearing of $\beta$2-Ppo2 transgenic mosquitoes, hereafter referred to as the Ag(DSM)2 strain[1], into a contained facility in Burkina Faso.

Regulatory approval was subsequently granted and Ag(DSM)2 eggs were imported into Burkina Faso under permit in November 2016[2]. These male-sterile mosquitoes are the first stage in the development pathway of a new genetic-control technology intended to provide a durable and cost-effective tool for reducing the burden of malaria in the Africa by reducing transmission of the malaria parasite. Currently there are no plans to use these mosquitoes as a Sterile Insect Technique. Their primary purpose is to provide a mechanism to study the effect of transferring the transgene into local (African) genetic backgrounds, and to allow Target Malaria to conduct a controlled field release of trangenic mosquitoes.

Following completion of the initial risk assessment, FNIH approached CSIRO to provide an independent assessment of the risks associated with the next stage of the development pathway for this new technology, namely a small scale, reproductively-contained, field release of male Ac(DSM)2 strain[3] mosquitoes in Burkina Faso. The objective of this project is to estimate the ecological and human-health risks associated with this controlled field release.

The primary purposes of the field release are to: (i) generate data on the daily survival rate of released Ac(DSM)2 males and to assess their movement from a defined release point; and (ii) strengthen local capacity in the handling, release and recapture of laboratory reared mosquitoes including through the establishment and validation of standard operating procedures and internal systems for the oversight of regulatory compliance.

Data generated from the field release will further inform an understanding of how outcomes from indoor contained use experiments can be extrapolated to a field entomology context. Additionally, although Ac(DSM)2 does not incorporate a gene drive mechanism, data on the population dynamics and dispersal behaviour of male Ac(DSM)2 collected from a field release will serve to further demonstrate and develop the methodology and mathematical models needed to support an environmental risk assessment to standards recommended for gene drive applications (National Academies of Sciences, Engineering and Medicine,

[1] *An. gambiae* Dominant Sterile Male phenotype

[2] Since then, the facility has operated successfully with no accidental escape of Ac(DSM)2 mosquitoes (*pers comm* J. Mumford, Target Malaria, 2nd November 2017)

[3] This nomenclature reflects the introgression of the Dominant Sterile Male construct into the wild-type genetic background by back-crossing female Ag(DSM)2 mosquitoes with locally collected wild-type *An. coluzzii* males.

2016; Australian Academy of Sciences, 2017).

This risk assessment is designed to support a regulatory application for the controlled field release. The risk assessment endpoints reflect the results of the hazard analysis conducted by Hayes et al. (2015), and community-identified concerns associated with the persistence of the genetic construct in the environment, pathogen transmission, horizontal gene transfer and effects on non-target organisms. The scope of the risk assessment is restricted to the risks associated with the use of Ac(DSM)2 mosquitoes in the field trial. The scope extends to activities within the insectary that maintains the Ac(DSM)2 strain only to the extent that these activities are relevant to the probability or consequences (losses) of these risks.

## 1.2 Report structure

This report is divided into six sections. The remainder of this first section provides contextual information on the location of the controlled field release and the lineage of the mosquito strain that will be released. This section also highlights some of the results of previous field studies conducted by Target Malaria at the release site that are pertinent to the risk assessment.

Section 2 documents the results of the consultation process conducted by Target Malaria's stakeholder engagement team, at CSIRO's request, to identify any concerns that the local community may have about the controlled field release. This section describes the concerns that are addressed by the risk assessment, and those that are not, provides a rationale for this choice and concludes with a summary of the risk assessment endpoints.

Target Malaria does not plan to release female Ac(DSM)2 mosquitoes during the field trial. It acknowledges, however, that the sex separation protocols that it will implement prior to the field release may not be 100% effective. This leaves open the possibility that a small number of female Ac(DSM)2 mosquitoes will be incidentally released with the males. Section 3 of this analysis therefore considers disease transmission by female Ac(DSM)2 mosquitoes, including the possibility that they may transmit novel blood-borne pathogens.

Many of the local communities' concerns are contingent on the spread and persistence of the sterile male construct in the environment. Section 4 addresses the mechanisms by which male and female Ac(DSM)2 mosquitoes could survive following the controlled field release. This section describes the model of dispersal and survival developed for male Ac(DSM)2 mosquitoes, and the separate model developed for the survival of female Ac(DSM)2 mosquitoes that might be incidentally released during the field trial. It also addresses the probability that the construct will spread through local populations of wild type mosquitoes, and the probability that Ac(DSM)2 female mosquitoes have enhanced resistance to insecticide treatments.

Section 5 draws on the results of the previous section to assess the extent to which impacts on non-target organisms may or may not be anticipated following the field release. The report concludes in Section 6 with a discussion of the results. The models, analysis methods and risk calculations associated with each part of the risk assessment are described in detail in Appendix A.

## 1.3 Contextual information

### 1.3.1 Entomological surveys and controlled field release sites

The Ac(DSM)2 mosquitoes are housed in a secure insectary run by the Institut de Recherche en Sciences de la Santé (IRSS) in Bobo Dioulasso, 356 kms south west of Ouagadougou, the capital of Burkina Faso (Figure 1.1). Subject to regulatory approval, the controlled field release is proposed to take place in Bana village in the Kou valley, approximately 25 km west of the insectary. This village is one of three villages – Bana, Souroukoudingan and Pala – that have been entomologically characterised by Target Malaria and the IRSS since 2012 (Target Malaria, unpublished data). Souroukoudingan village has also been identified as a possible alternative site for the field release.

Bana village comprises 315 houses grouped into 65 extended-family compounds (known as "concessions"). Houses typically consist of one or two rooms, constructed with beaten earth walls and corrugated tin roofs. At the time of a local census in October 2014, 380 people were estimated to live in the village (Epopa et al., 2017). About one kilometer to the west of the village is a second settlement, Bana market, consisting of an additional 68 concessions. The two settlements are separated by an intermittent river. The next nearest villages to Bana are Dindresso, some 5 km to the east, and Souroukoudingan, 5.8 km to the west (Target Malaria, unpublished data).

Target Malaria and IRSS have conducted almost uninterrupted monthly entomological surveys at four sites in Burkina Faso since July 2012. The entomological surveys use four capture methods: (i) Pyrethroid Spray Catches (PSC) inside houses; (ii) sweep netting of swarms; (iii) Human Landing Catches (HLC); and, (iv) larval habitat exploration. Mosquitoes are identified morphologically to genus, and all *An. gambiae* s.l. mosquitoes preserved for subsequent molecular analysis.

The entomological surveys reveal that in Bana (village and market) female *Anopheles gambiae* s.l. account for $77.8\%$, of all samples with male *Anopheles gambiae* s.l. accounting for $21.4\%$. Three other species – *An. rufupes*, *An. funestus* and *An. flavicosta* – have also been captured in Bana but together they account for only $0.5\%$ of all samples collected to date. *Anopheles gambiae* s.l. numbers peak in September/October towards the end of the wet season (May to October) and are lowest in December/January toward the middle of the dry season (November to April) (Figure 1.2).

Molecular analysis of a sub-sample (usually between 30 to 50 individuals) of captured *An. gambiae* s.l. mosquitoes is performed according to the method described by Fanello et al. (2002). The results indicate that the vast majority $(90.5\%)$ of male and female mosquitoes captured in Bana are *An. coluzzii* (formerly *An. gambiae* M form). *An. gambiae* s.s. mosquitoes account for $8.8\%$ of the sub-samples, while *An. arabiensis* accounts for only $0.3\%$. Hybridisation between the three species occurs at low rates $(0.4\%)$. Hybrids have only been detected in December, March, May and June in Bana (Figure 1.3) when mosquito numbers are at their lowest, possibly because alternative mates are hard to find at this time (Target Malaria, unpublished data).

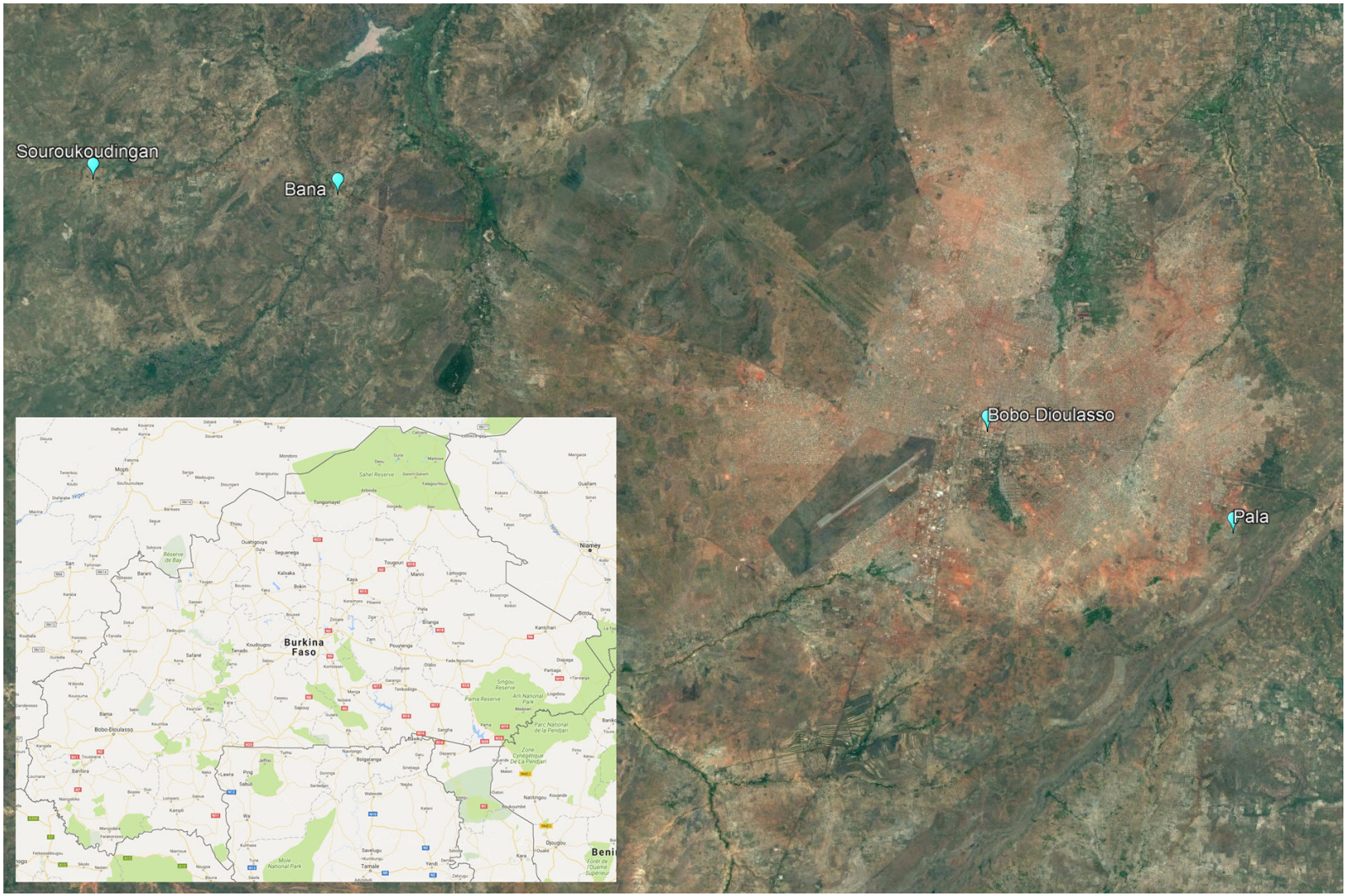


**Figure 1.1:** Geographical context of the risk assessment. The IRRS insectary is located in Bobo-Dioulasso, 356 kms to the south west of Ouagadougou, the capital of Burkina Faso. The location of the proposed controlled field release is Bana village, about 25 kms west of the insectary. A second possible site is Souroukoudingan, which is further west. Monthly entomological surveys are conducted in Bana, Souroukoudingan and Pala which is approximtely 10 kms to the east of the insectary.

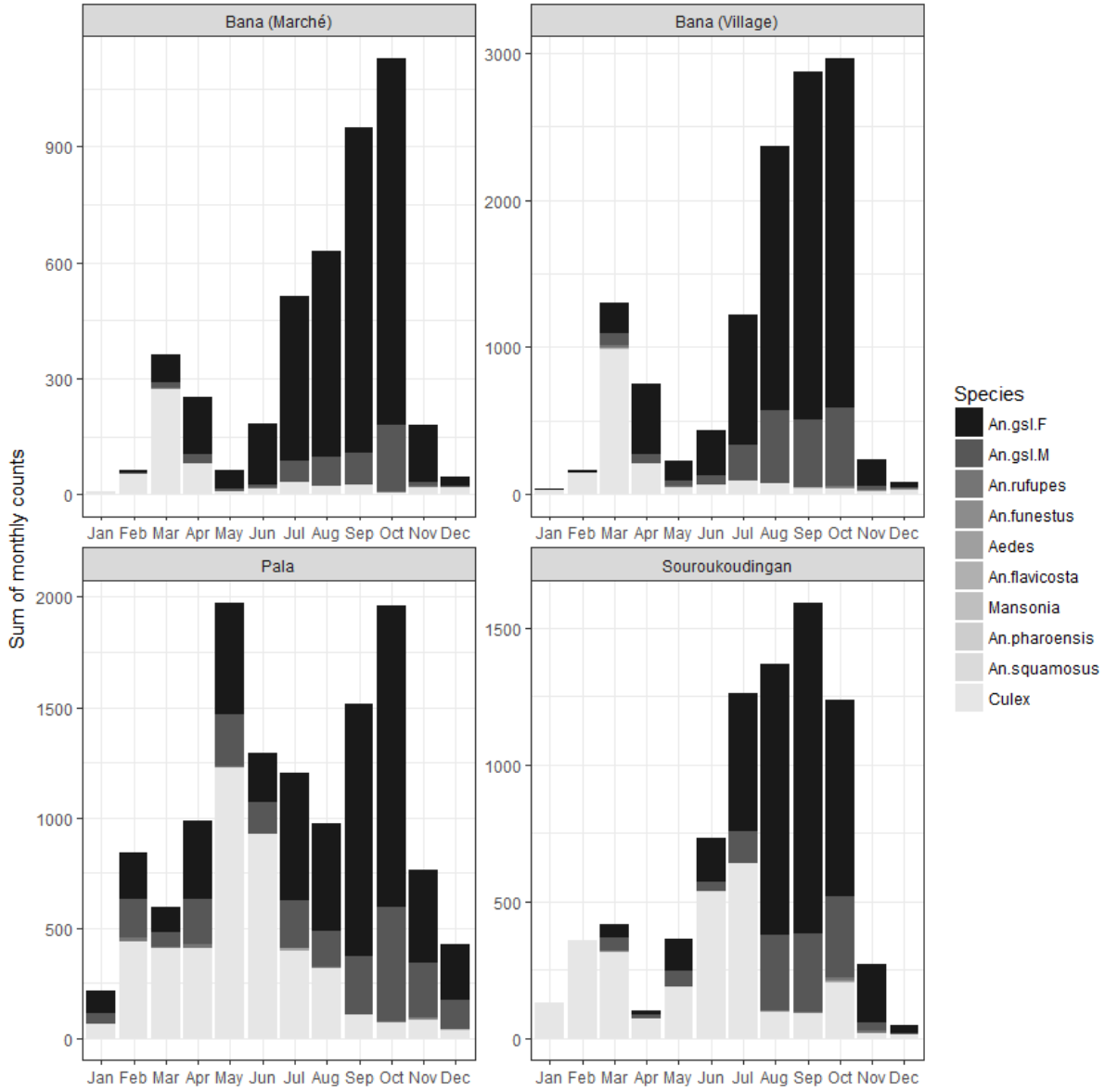


**Figure 1.2:** Summary of entomological surveys conducted by Target Malaria and IRSS between July 2012 and February 2015 in Bana Marché, Bana Village, Pala and Souroukoudingan (Data provided courtesy of Target Malaria). The monthly counts of morphologically identified mosquitoes captured during the entomological surveys show that the population of the dominant species – *An. gambiae* s.l. (An.gsl) – increases from May to October (the wet season) and then decreases from November to February. In Bana Village and Bana Marché, mosquitoes from the Culex genus are relatively more abundant towards the end of the dry season (February, March and April) but otherwise these mosquitoes, and all other species of Anopheline mosquitoes, are comparatively rare.

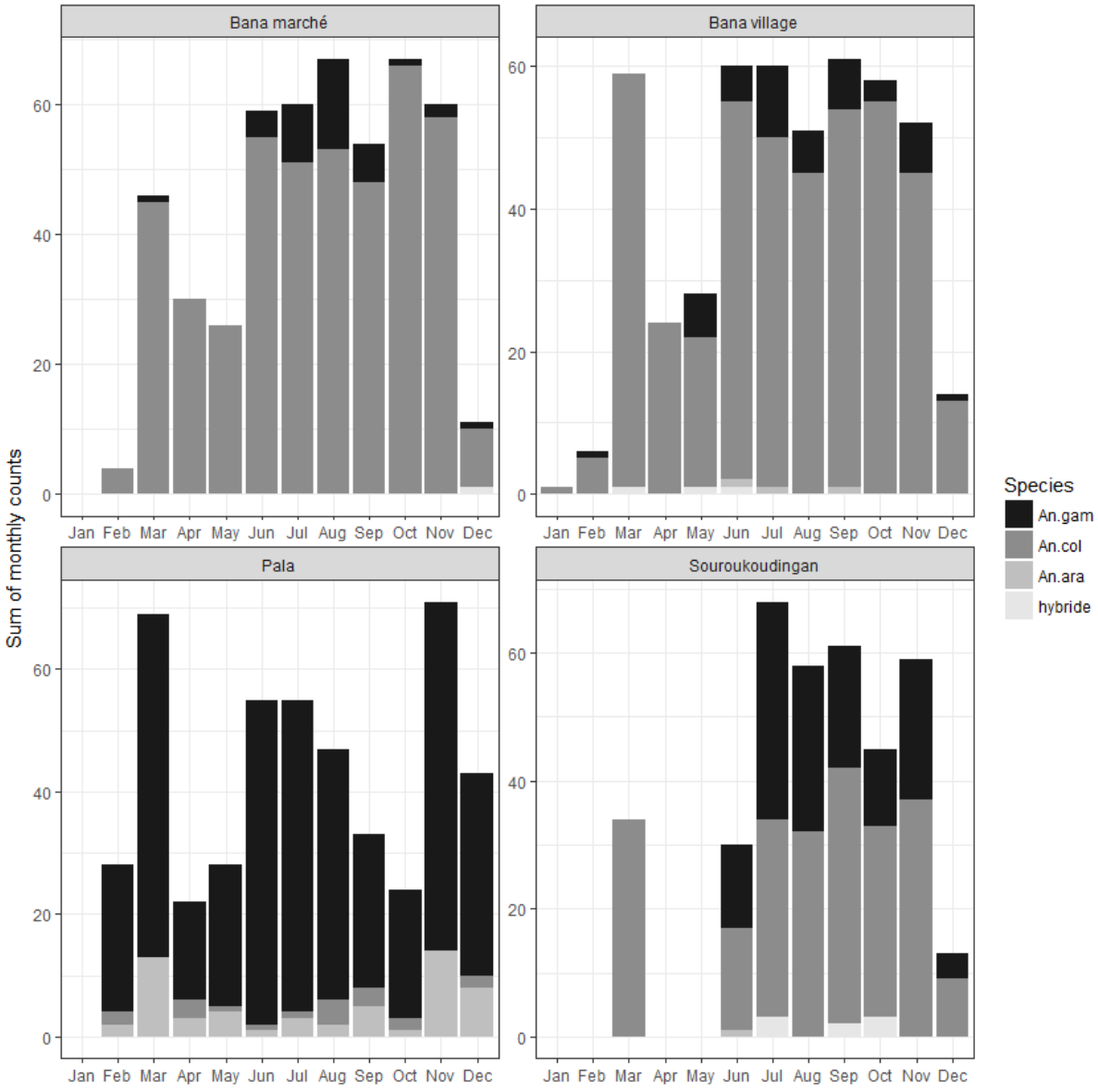


**Figure 1.3:** Summary of the molecularly identified mosquitoes subsampled from the entomological survey samples (Data provided courtesy of Target Malaria). Molecular identification confirms that the vast majority of mosquitoes identified morphologically as *An. gambiae* s.l. (An.gam) are *An. coluzzii* (An.col). Molecular methods are also able to identify the occurrence of hybridisation between *An. arabiensis* (An.ara), *An. gambiae* s.s. and *An. coluzzii*. In Bana village and and Bana Marché hybrids are rare and appear to occur only in December, March, May and June when populations of Anopheline mosquitoes are relatively low.

### 1.3.2 Strain establishment, maintenance and release

The Ac(DSM)2 strain is maintained by constant back-crossing of hemizygous transgenic females, with wild type *An. coluzzii* males, sourced from a synchronously maintained wild type colony (Figure 1.4). The wild-type colony was started in the IRSS insectary in October 2014 by collecting over 100 gravid *An. coluzzii* females from a village in the Kou valley, western Burkina Faso (Target Malaria, unpublished data).

The genetic identity of the females that laid eggs used to establish the colony was confirmed as *An. coluzzii* by PCR amplification of single nucleotide polymorphisms within the internally transcribed spacers ITS1 and ITS2 (Wilkins et al., 2006). The colony's identity was tested again by genetically identifying 5 randomly selected larvae hatched from the founding egg family, and its purity through time is confirmed by genetically identifying 25 males and 25 females randomly selected from the wild type *An. coluzzii* colony every fifth generation (Target Malaria, unpublished data).

The Ac(DSM)2 colony was started in November 2016 by importing Ag(DSM)2 eggs from Perugia, Italy, into approved facilities within the IRSS insectary. Ag(DSM)2 female pupae from these founding eggs were subsequently mixed with Ac(WT) male pupae to allow the emerging adults to breed. The resulting backcross between transgenic females and wild type males creates a mixed population containing approximately half hemizygous transgenic and half non-transgenic individuals. Transgenic individuals can be visually identified because they express DsRed (red florescence) in the nerve tissues, which is evident in the larvae's optic lobe. The hemizygous transgenic males also express eGFP florescence in their testes which is visible in the male larvae's abdomen.

The Ac(DSM)2 colony is maintained in a two-step procedure that manually separates DsRed positive L3 or L4 larvae, and then manually identifies females from amongst this group by visual inspection of the terminalia of individual Ac(DSM)2 pupae (Target Malaria, unpublished data). Ac(DSM)2 female pupae are then mixed with wild type male pupae separated from the wild type colony in a similar manner (Figure 1.5). This process of constant backcrossing ensures that the Ac(DSM)2 strain is maintained but also quickly replaces the original Ag(DSM)2 genetic background with the local wild type genetic background.

Following at least three backcrosses, Target Malaria performed a series of experiments to confirm the specificity and sensitivity of the fluorescent markers, and to compare key characteristics (insecticide resistance, sexual sterility, mating competitiveness and life history traits) of male and female Ac(DSM)2 mosquitoes against their wild-type comparators.

Target Malaria initially planned to conduct the controlled field release in July 2018 by which time the wild type colony will have been raised under contained laboratory conditions for 62 generations and backcrossed into the Ac(DSM)2 line 29 times. The field release, however, is contingent on the insectary generating a sufficiently large population of male Ac(DSM)2 mosquitoes and receipt of regulatory approval. The actual release date may therefore change.

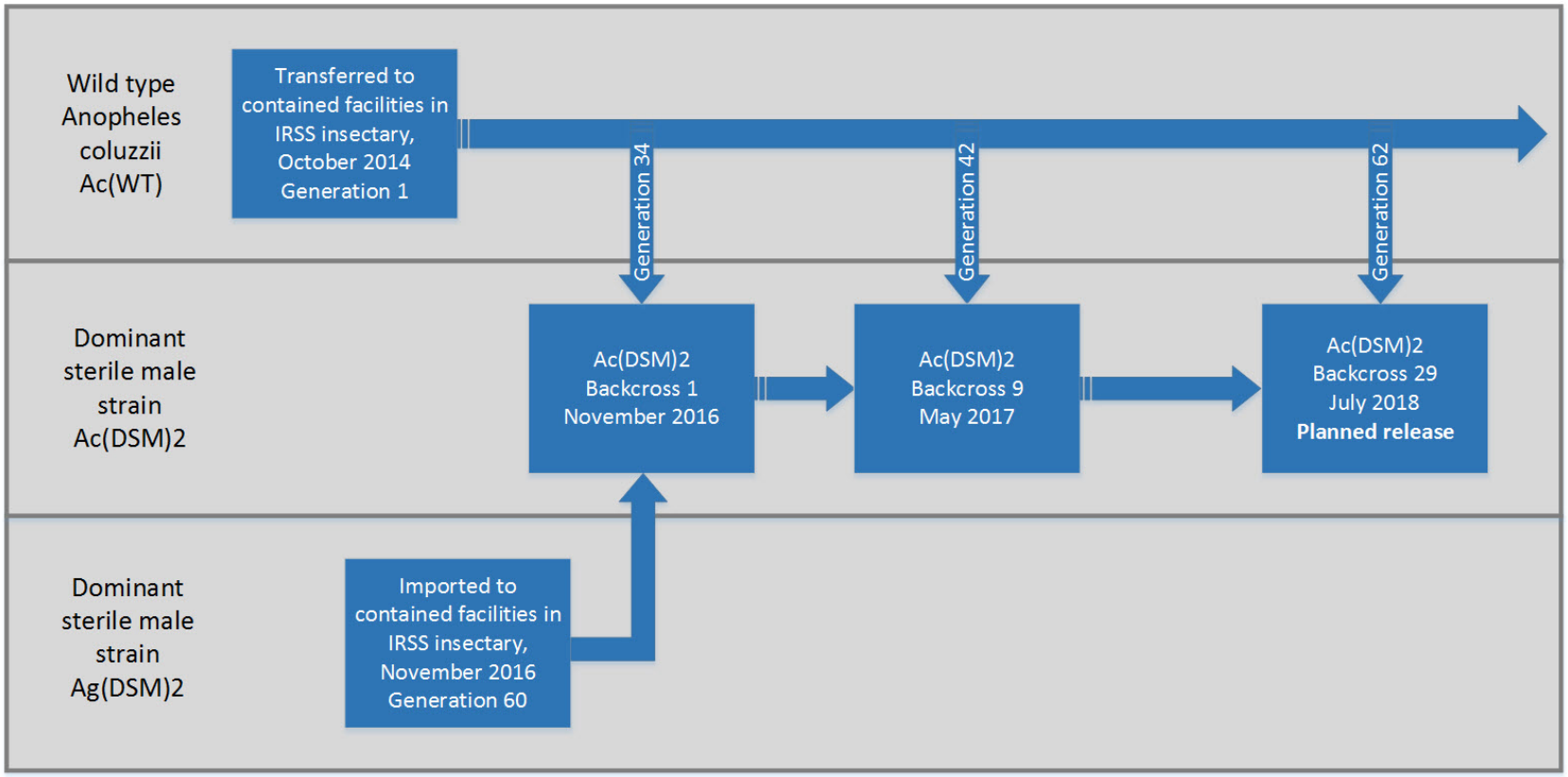


**Figure 1.4:** Schematic representation of the timeline from importation to controlled field release. Eggs of the Ag(DSM)2 strain mosquitoes were imported under permit into contained facilities in the IRSS insectary in November 2016. Adult females raised from these eggs were subsequently crossed with wild type *An. coluzzii* males taken from a colony established in the insectary in October 2014 in order to create the Ac(DSM)2 strain. This strain is maintained by continual back-crossing of hemizygous Ac(DSM)2 females, with Ac(WT) males. By July 2018 (the initial planned release date) the wild type colony will have been raised under laboratory conditions for 62 generations, and backcrossed into the genetic strain 29 times.

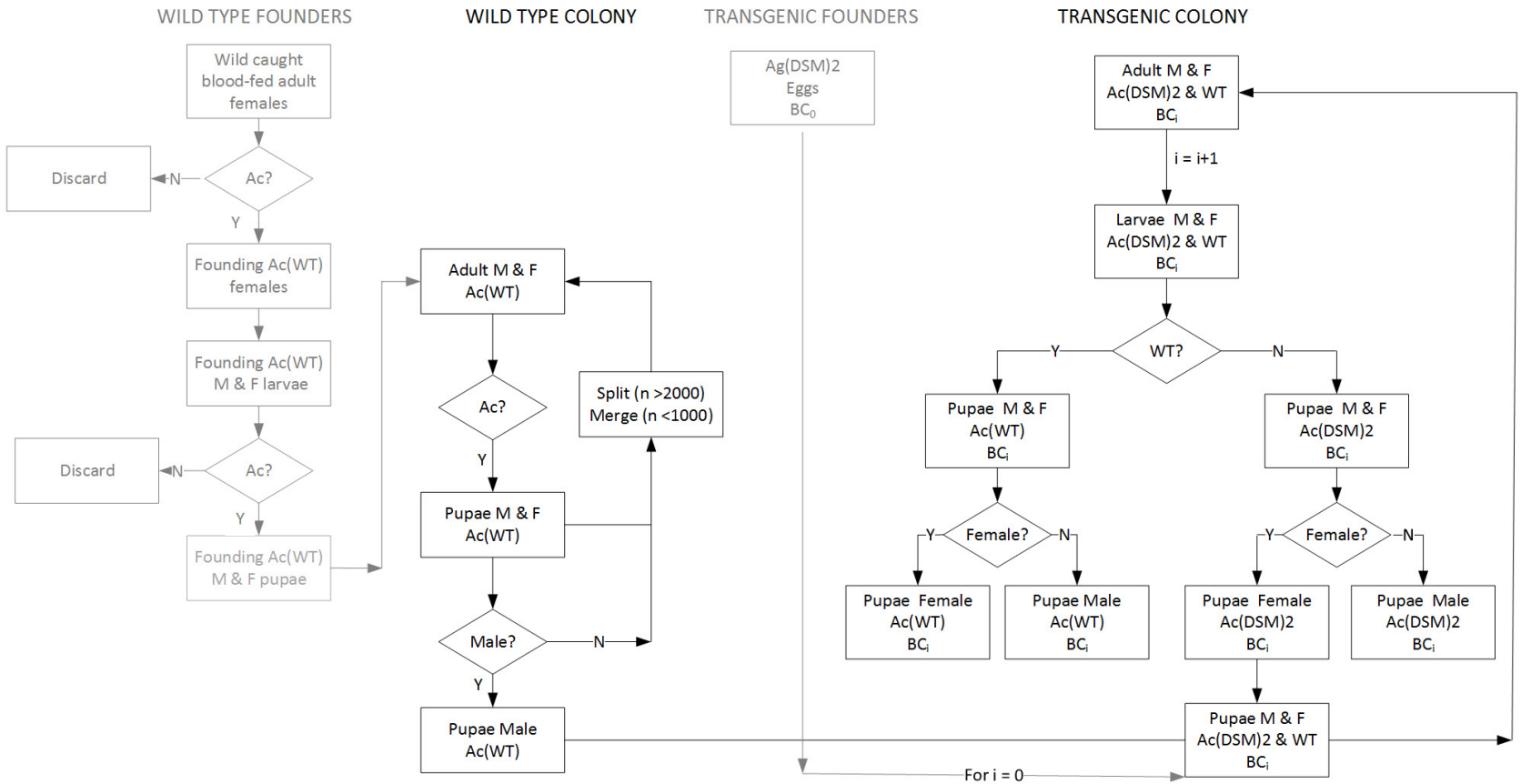


**Figure 1.5:** Flow chart showing how the Ac(DSM)2 strain is maintained in the insectary. Ac(DSM)2 males are sterile so the Ac(DSM)2 line can only be maintained by continuously backcrossing hemizygous Ac(DSM)2 females with Ac(WT) males, sourced from the wild type colony. Larvae from these backcrosses are manually separated into transgenic (DsRed positive) and non-transgenic (DsRed negative) strains. Transgenic individuals are then separated into male and female by visual inspection of the terminalia of individual pupae, and female Ac(DSM)2 pupae mixed with wild type male pupae to continue the backcross cycle.

The field release will involve releasing male Ac(DSM)2 mosquitoes and male Ac(WT) comparators. Both groups will be dusted with fluorescent powder as per the methods described in Epopa et al. (2017). The field release will occur sometime between the beginning of the wet season and the middle of the dry season. At this stage it is unclear how many mosquitoes will be released because this depends on the insectary's ability to scale-up production of mosquitoes to the requisite number. The minimum useful release size, however, is considered to be 2,000 Ac(DSM)2 and 2,000 Ac(WT) mosquitoes. The maximum release size has been set at 10,000 Ac(DSM)2 and 10,000 Ac(WT) mosquitoes. For the purposes of this analysis we assume that 5,000 Ac(DSM)2 male mosquitoes will be released.

Target Malaria will implement sex separation protocols and testing procedures on the mosquito batch earmarked for release, and as a result have stipulated that they will release no more than 5 Ac(DSM)2 females for every 1,000 Ac(DSM)2 males released. All subsequent analysis assumes that this condition will be met.

## 1.4 Risk assessment scope

The risk assessment endpoints (Section 2) have been determined by local community concerns, and the scope of the assessment is restricted to the deliberate controlled field release of male Ac(DSM)2 mosquitoes, and the incidental field release of female Ac(DSM)2 mosquitoes. The risk assessment does not address the effect of the subsequent Mark Release Recapture experiment, or the monthly entomological surveys conducted by Target Malaria after the controlled field release, on the risk assessment predictions. The risk assessment assumes that any vector control activities in the proposed release location, such as the use of insecticide treated bed nets, will continue to be implemented after the controlled field release but does not consider the effect of these activities on the risk predictions.

# 2 COMMUNITY CONCERNS AND RISK ENDPOINTS

**KEY POINTS**

1. In November 2016, CSIRO asked Target Malaria's stakeholder engagement team to collate the local community's concerns about the field release to help identify risk assessment endpoints.
2. The community identified four human health concerns and five environmental concerns with plausible Adverse Outcome Pathways (AOPs). These concerns are reflected in seven risk assessment endpoints.
3. The first two endpoints address the relative difference between the pathogen transmission capacity of female Ac(WT) and female Ac(DSM)2 mosquitoes (for *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis) and the possibility that female Ac(DSM)2 mosquitoes may vector a novel blood-borne pathogen.
4. The third endpoint is the time taken for the expected number of Ac(DSM)2 females (including their offspring) to fall below one individual.
5. The fourth endpoint is the time taken for the expected number of Ac(DSM)2 males (assuming complete sterility) to fall below one individual.
6. The fifth and sixth endpoints are the probability that the construct will spread in local populations of *An. coluzzii* and other sexually compatible Anopheline species in a year following the controlled field release.
7. The last endpoint is the probability that the female Ac(DSM)2 strain experiences lower rates of mortality than the female Ac(WT) strain when exposed to commonly used insecticides under standardised experimental conditions.
8. The assessment predicts that the probability of horizontal gene transfer (HGT) of the Dominant Sterile Male causing potential impacts on human fertility following the field release will be lower than the probability of HGT to any eukaryote calculated by Hayes et al. (2015), and therefore remains less than $1.2 \times 10^{-10}$.
9. The risk assessment considers the plausibility of effects on non-target organisms following the field release in light of the predicted survival of Ac(DSM)2 male and female mosquitoes.
10. The risk calculations in this assessment are quantitative and all assumptions are clearly stated. The assessment adopts a transparent and coherent approach to uncertainty, and uses informative priors elicited from domain experts, updated wherever possible with data from empirical observations.
11. In some instances the informative prior and currently available data are insufficiently aligned to allow the update to occur. In these instances the assessment of risk is solely based on the informative prior.

## 2.1 Community concerns and values

Target Malaria maintains an active stakeholder engagement strategy in Bana that provides for information exchange, complaint management, and community involvement in the

project's progress and field activities. In November 2016, the CSIRO asked Target Malaria's stakeholder engagement team to collate a list of any concerns that the community had about the field release in order to help identify appropriate endpoints for the risk assessment. A local team working in consultation with in-country Target Malaria partners subsequently surveyed stakeholders in Bana (and Mali) in December and recorded a number of health- and environment-related concerns.

### 2.1.1 Human-health concerns

The human-health concerns expressed by the community can be divided into two categories: (i) those in which an Adverse Outcome Pathway (AOP) is plausible (irrespective of its probability); and (ii) those in which the AOP is implausible. The community identified four human health concerns with plausible AOPs:

- **Will the Ac(DSM)2 mosquitoes have a better capacity to transmit pathogens?** The CSIRO risk assessment addresses this concern by assessing the relative capacity of female Ac(DSM)2 mosquitoes to transmit three known mosquito-vectored pathogens; *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis (Section 3.1). The assessment also addresses the related possibility that female Ac(DSM)2 mosquitoes may vector novel blood-borne pathogens (such as Hepatitis and Ebola).
- **Could there be a potential impact on human fertility following a bite from a female mosquito from the sterile male strain?** The 15 base pair I-PpoI target site is present in the large subunit rRNA gene of all eukaryotes but in humans the target site does not reside on a sex chromosome. This AOP is plausible but it requires horizontal transfer of the genetic construct from the mosquito into a human germ cell, and possibly mutation of the construct to target gene sequences that influence human fertility. Hayes et al. (2015) concluded that the median probability of horizontal gene transfer of the genetic construct to any non-target eukaryote in a year following the accidental release of 10,000 Ag(DSM)2 mosquitoes was $1.2 \times 10^{-10}$. This probability will be further reduced for the field trial because the number of mosquitoes released will likely be less, and because of the additional steps necessary for impacts on human fertility to occur.
- **Could other disease emerge from the decrease of mosquitoes?** The AOP for this concern presumes that the field release will cause wild mosquito populations to diminish, as occurs for example in traditional Sterile Insect Technique (SIT) releases. The field release, however, will not be repeated and will only introduce a relatively small number of sterile mosquitoes into the wild population. The AOP is nonetheless plausible. Section 4 therefore addresses the survival of Ac(DSM)2 male and female mosquitoes following the controlled field release.
- **Will the Ac(DSM)2 mosquitoes impact other aspects of human health?**. This concern is ambiguous. In addition to the potential impacts discussed previously it is plausible that the release could create stress or anxiety in the community. This risk will be managed by Target Malaria's Stakeholder Engagement team by monitoring complaints regarding the release during (for example) the collection of consent forms, and is not addressed further in this assessment.

In addition to these concerns, community members in Bana also identified a number of issues that whilst legitimate have less plausible AOPs. The first of these relate to the poten-

tial for toxicity or allergic reaction to the I-PpoI protein. Stakeholders asked if humans could get sick from eating animals bitten by female mosquitoes from the sterile male strain. The I-PpoI protein, however, has no toxic or allergic properties and is not present in mosquito saliva or carcasses (Target Malaria, unpublished data). There does not therefore appear to be a plausible mechanism for toxicity to humans.

Secondly, community members expressed concern around the possibility of mental disorder as a result of a bite from a female mosquito of the sterile male strain. Whilst it is possible that the field release could cause anxiety and stress within some parts of the local community (see above) we could not envisage a plausible AOP for this concern at this time. Stakeholders also raised concerns about the impact of the gene editing technique used for sterile male on human health. The risk assessment maintains its focus on the human health issues discussed previously because this concern appears to be addressed by them.

### 2.1.2 Environmental concerns

In addition to the human-health issues, the survey of stakeholder concerns also identified five environmental issues with plausible AOPs:

- **What is the risk of sterility not being complete and the males being able to reproduce and therefore persist?** Section 4 of this report calculates the posterior probability of incomplete sterility. This posterior probability is subsequently used to update the fault tree analysis developed by Hayes et al. (2015) to calculate the probability that the construct will spread in local populations of *An. coluzzii* or *An. gambiae*.
- **How can the non-persistence of a flying animal be monitored?** Target Malaria plan to monitor the released cohort of Ac(DSM)2 mosquitoes through an intensive Mark Release Recapture experiment designed to confirm the expected mortality and disappearance rate of that cohort. They also plan to monitor for the possibility of subsequent generations in the months after the release with a monitoring effort equivalent to that used in the monthly entomological surveys.
- **Do the Ac(DSM)2 mosquitoes have a lower susceptibility to insecticides?** Section 4 of this report calculates the posterior probability that Ac(DSM)2 strain has a lower mortality rate (higher resistance) than the Ac(WT) strain when exposed to a number of commonly used insecticides, using non-informative priors and experimental data.
- **What is the risk of sterility affecting all mosquito species?** This concern has two plausible AOP's. The first is through vertical transmission of the I-PpoI gene via hybridisation between Ac(DSM)2 mosquitoes and other sexually compatible Anopheline species. This issue is addressed in Section 4. The second is via horizontal gene transfer of the Dominant Sterile Male construct to sexually incompatible species of mosquitoes.
- **Will the field release affect non-target species?** Section 4 of the report examines the predicted survival of Ac(DSM)2 male and female mosquitoes. Section 5 of this report uses this analysis to comment on the possibility of non-target effects following the controlled field release.

Community members also identified three further issues. The first is related to the concern about impacts on non-target species, and was expressed in terms of the risk of an "unbalance in nature" because of the reduction of *An. coluzzii* following the field release. This

concern appears to be related to effects on non-target species and this is addressed in Section 5.

The second issue centered around the potential difference between the imported strain and local mosquitoes, and was expressed in terms of any “new issues” that may result because of this difference. The imported strain will be backcrossed with local wild type mosquitoes at least 29 times prior to release (Figure 1.4). We therefore anticipate that the main differences between the field release mosquitoes and their wild type equivalents will be the presence of the Dominant Sterile Male construct and the effects of habituation to laboratory conditions. Wherever possible these issues are explicitly addressed in the risk assessment.

Finally community members expressed concerns about the “stability of the modification”. This concern could be related to several possibilities including: (i) the construct fails to sterilize male mosquitoes; (ii) the construct is mobile and can be excised from its target locus in the genome; (iii) the construct mutates between generations; or (iv) it is not possible to phenotypically identify Ac(DSM)2 mosquitoes because the DsRed marker fails. The first possibility is addressed in Section 4. The second and third possibilities can contribute to horizontal gene transfer and the fourth relates to the possibility of false negative identification in the laboratory, in the Mark Release Recapture experiment following the field release or in any subsequent post-release monitoring. Target Malaria have investigated the second and third possibility and found no evidence for mobilisation of the construct in 178 independent tests and no evidence for failure of the DsRed construct in 215 individual tests (Target Malaria, unpublished data). Furthermore, after the field release and Mark Release Recapture experiment is completed, the presence of Ac(DSM)2 mosquitoes will be detected by molecular and phenotypic methods.

## 2.2 Risk assessment endpoints

The measurement endpoints for this risk assessment are informed by the plausible AOPs identified by the community, and the hazard analysis and endpoints identified by Hayes et al. (2015). The measurement endpoints for this risk assessment are:

1. Probability of an increase in the vectorial capacity of Ac(DSM)2 mosquitoes relative to Ac(WT) for *P. falciparum*, o’nyong’nyong virus and lymphatic filariasis.
2. Probability that the Ac(DSM)2 mosquitoes will vector a novel blood-borne pathogen in a year following the field release.
3. The time taken for the expected number of Ac(DSM)2 females (including their offspring) to fall below one following the incidental release of a small (less than 25) founding population of female Ac(DSM)2 during the controlled field release.
4. The time taken for the expected number of Ac(DSM)2 males (assuming complete sterility) to fall below one following the intentional release of 5,000 male Ac(DSM)2 mosquitoes during the controlled field release.
5. The probability that the Dominant Sterile Male construct will spread in local populations of wild type *An. coluzzii* in a year following the field release.
6. The probability that the Dominant Sterile Male construct will spread in local populations of wild type *An. gambiae* s.s, or *An. arabiensis* in a year following the field release.

7. The probability that the female Ac(DSM)2 strain experiences lower rates of mortality than the female Ac(WT) strain when exposed to commonly used insecticides under standardised experimental conditions.

Following this analysis, the risk assessment considers the possibility of non-target effects, including the possibility that other diseases will increase due to suppression of local populations of *An. coluzzii*, in light of the predicted survival of the female and male Ac(DSM)2 mosquitoes released during the field trial, and the predicted probability that the construct will spread through wild type mosquito populations.

Hayes et al. (2015) predicted that the probability of horizontal gene transfer of the Dominant Sterile Male construct to any eukaryote, following the accidental release of 10,000 Ag(DSM)2 mosquitoes, would have a median probability of $1.2 \times 10^{-10}$. This assessment predicts that the probability of impacts on human fertility following the controlled field release will be lower than this.

## 2.3 Expert opinion, risk assessment and Bayesian inference

The objective of the proposed controlled field release of male Ac(DSM)2 mosquitoes is to develop local capacity in the maintenance, handling and re-capture of Ac(DSM)2 mosquitoes, as a pre-cursor to the development and application of a self-sustaining genetic control methodology for malaria and possibly other mosquito vectored diseases. Importantly, the field release, related field surveys and laboratory experiments, also provide an opportunity to develop and apply probabilistic risk assessment methodologies for novel genetic control techniques within a staged-release protocol as advocated by the World Health Organisation (World Health Organisation, 2009, 2014) and the National Academies of Science Engineering and Medicine (National Academies of Sciences, Engineering and Medicine, 2016).

A key challenge to probabilistic risk assessment for a novel technology is the lack of empirical information on its safety and reliability. Classical actuarial approaches to probabilistic risk assessment are not possible because the technology's operational history is limited or its potential adverse outcomes occur at a very low frequency (Rasmussen, 1981). At least initially, the risk assessment must rely on expert opinion, and in these circumstances probability theory can be used to coherently express experts' degrees of belief in uncertain outcomes (Kaplan and Garrick, 1981). Furthermore, Bayes theory describes the correct way to incorporate data into expert-based risk predictions and update them as operating experience develops (Apostolakis, 1981, 1990).

Bayes theory enables assessors to make and update probabilistic risk predictions for novel technologies in a manner entirely consistent with the scientific process of prediction, observation and (in)validation. Challenges and choices surface, however, when this method is implemented within the staged-release protocols advocated for novel genetic control technology. Bayesian probabilistic risk assessment updates expert judgements, elicited as prior probability distributions, with data generated by experimental observations. The experimental observations and expert elicitation, however, must be independent and carefully aligned for this process to work.

The prior opinion available to this risk assessment includes the elicitation performed previously in the first assessment and the elicitations performed during the course of this assessment. In all cases the elicitations focus on the most directly relevant outcomes – that

is outcomes that occur in the field following a deliberate, accidental or incidental release of Ac(DSM)2 mosquitoes. The data available to this risk assessment consists of a mixture of field experiments with wild type strain *An. coluzzii* and laboratory experiments with Ac(DSM)2 and Ag(DSM)2 strain mosquitoes. The two sources of information are independent for all the risk assessment endpoints but their degree of alignment varies across the endpoints (Table 2.1).

Misalignment between the priors and data occurs because: (i) the prior and data do not refer to the same species for a given endpoint; or (ii) laboratory outcomes are thought to be very sensitive to experimental protocols and likely not representative of outcomes that would have occurred in the field. Vectorial capacity parameters, including mortality, are particularly vulnerable to this problem (Aguilar et al., 2005; Brady et al., 2013), and dependence between mortality rate and age (Clements and Paterson, 1981) is a further complication. In this risk assessment we have responded to these issues in the following way:

- For the transmission efficiency from human to vector (vectorial capacity endpoint) we do not update the informative prior with evidence from the laboratory observations on the grounds that the laboratory information is not sufficiently representative of field conditions.
- For female mortality (vectorial capacity endpoint) we update the informative prior for Ac(WT) with data from field observations collected in the first Mark Release Recapture experiment.
- For male mortality and dispersal distance (survival of Ac(DSM)2 males) we assume that Ag(WT) and Ac(WT) are equivalent and update the informative priors for Ag(WT) with data from field observations of Ac(WT) collected in the Mark Release Recapture experiments.
- When updating priors for endpoints with multiple models (multiple informative priors) we assume that the prior probabilities of each model are equal, and use Bayesian model averaging (Kass and Raftery, 1995; Wasserman, 2000), with weights given by the model evidence $w_j = Pr\left(Model_j|Data\right)$, in order to calculate a mixture posterior distribution.
- For male mortality and dispersal we assume that the model weights calculated for male Ac(WT) apply also to male Ac(DSM)2 and perform simulations for the dispersal and survival of male Ac(DSM)2 using a weighted average of informative priors.
- For the probability that the construct fails to sterilise male Ac(DSM)2 mosquitoes (spread of the construct through local populations) we assume that the laboratory outcomes are representative of field outcomes and update the informative prior with data from laboratory observations.

**Table 2.1:** Summary of alignment issues between the prior and evidence (empirical observations) for some of the endpoints addressed in this risk assessment

| EP Ref. | Parameter | Prior | Evidence | Alignment |
|---|---|---|---|---|
| 1 | Transmission efficiency from humans to vector (*P. falciparum*) | Informative prior for field conditions that accounts for level of backcross and laboratory habituation | Standard Membrane Feeding Assay with Ag(DSM)2 and laboratory strain (G3) mosquitoes | Expert commentary suggest laboratory protocols have strong influence on results. Difficult to align for Ac(DSM)2 and Ac(WT) under field conditions |
| 1 | Transmission efficiency from humans to vector (ONNV) | Informative prior for field conditions that accounts for level of backcross and laboratory habituation | Transmission efficiency experiments with Ag(DSM)2 and G3 | Expert commentary suggest laboratory protocols have strong influence on results. Difficult to align for Ac(DSM)2 and Ac(WT) under field conditions |
| 1,3 | Female mortality | Informative prior for Ag(WT), Ag(DSM)2, Ac(WT) and Ac(DSM)2 in field, latter accounting for level of backcross and laboratory habituation | Ac(WT) under field conditions from MRR 1. Ac(DSM)2 with and without construct under laboratory conditions | Good alignment for Ac(WT) in field. Poor alignment for Ac(DSM)2 under field conditions; difficult to replicate field conditions in laboratory |
| 4 | Male mortality | Informative prior for Ag(WT) and Ag(DSM)2 in field | Ac(WT) under field conditions from MRR 1 to 5 | Only aligned for field conditions assuming Ac(WT) and Ag(WT) are equivalent |
| 4 | Male dispersal | Informative prior for Ag(WT) and Ag(DSM)2 in field | Ac(WT) under field conditions from MRR 1 to 5 | Only aligned for field conditions assuming Ac(WT) and Ag(WT) are equivalent |
| 5,6 | Pr(Males sterile) | Informative prior for Ag(DSM)2, Ac(DSM)2 and Aa(DSM)2 in field | Ag(DSM)2 and Ac(DSM)2 in laboratory | Only aligned for field conditions assuming performance in laboratory and field are equivalent |
| 7 | Insecticide resistance | Weakly informative priors | Ac(WT) and Ac(DSM)2 under standard laboratory tests | Aligned for laboratory conditions |

Another important facet of this risk assessment is the use of Bayesian model averaging during the calculation of the posterior distribution. Bayesian model averaging assigns weights to experts' informative priors according to how well their predictions agree with observed data. In the results that follow these weights are calculated during the Bayesian update of the expert's informative priors for the dispersal and daily probability of mortality for male wild type mosquitoes, the daily probability of female wild type mosquitoes and the probability that the construct will fail sterilise male mosquitoes when introgressed into *An. coluzzii* and *An. gambiae*. All of these prior distribution were collected in the analysis conducted by Hayes et al. (2015).

The calculations and expert weights for the wild type female mortality rate are described in detail in Section A.2. For the male dispersal and survival modelling the calculations and expert weights are described in Section A.4.8. For the probability of construct failure the calculations and weights are described in Section A.5.

The risk assessment subsequently applies the Bayesian model average weights to each of the expert's informative priors about the dispersal and mortality of Ac(DSM)2 mosquitoes in order to simulate the behaviour of male Ac(DSM)2 upon release. This approach assumes that experts who make predictions about wild type mosquitoes that are closer to the truth also make better predictions about Ac(DSM)2 and Ag(DSM)2 strains. The analysis also assumes that in all other respects (chemotaxis and catchability) male Ac(DSM)2 mosquitoes behave in an identical fashion to male Ac(WT) mosquitoes.

# 3 CAPACITY TO VECTOR BLOOD-BORNE PATHOGENS

**KEY POINTS**

1. The first endpoint focusses on the prevalent malaria parasite *Plasmodium falciparum*, the potentially emergent o'nyong'yong virus (ONNV) and the neglected tropical disease lymphatic filariasis (*Wuchereria bancrofti*).
2. Independent domain experts provided probabilistic assessments of parameters that govern the pathogen transmission potential of Ac(DSM)2 and local Ac(WT) mosquitoes in the Sudanian zone of Burkina Faso and the neighbouring Sudano-Guinean zone.
3. The Ac(DSM)2 strain was assessed when positive (hemizygous) for the DSM construct, and Ac(DSM)2 females were assessed for different numbers of backcrosses with Ac(WT) males. Experts also considered the effects of genetic founder effects, drift, and selection that might arise from the establishment and maintenance of the Ac(DSM)2 and Ac(WT) strains in the laboratory.
4. An initial analysis calculates the relative risk of pathogen transmission by Ac(DSM)2 at backcross 29 compared to Ac(WT) at laboratory generation F = 0, assuming equal vector to human host ratio. Under these circumstances the predictive priors indicate that the probability of increased transmission of *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis, attributable to the effect of the transgene, is 0.28, 0.3 and 0.14 respectively. This means that across the three pathogens there is about a 70% chance that Ac(DSM)2 at backcross 29 will have a lower transmission capacity than Ac(WT) at F = 0.
5. Evidence of adult female Ac(WT) mortality rate in Bana Village from the first Mark Release Recapture experiment enables a Bayesian update of the daily probability of mortality, and hence the transmission capacity calculations. The predictive posterior probability of increase transmission of *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis is 0.29, 0.33 and 0.13 respectively.
6. If the female Ac(WT) population is 1000 times larger than the population of female Ac(DSM)2, the predictive prior probability of increased transmission attributable to the incidental release of Ac(DSM)2 females drops to 0.04, 0.05 and 0.04 for *P. falciparum*, o'nyong'nyong and lymphatic filariasis respectively. The predictive posterior probability of increased pathogen transmission, during the time that female Ac(DSM)2 mosquitoes are in the environment, drops to 0.03, 0.05 and 0.03 respectively.
7. Amending the results of the fault tree analysis conducted by Hayes et al. (2015) to reflect the probability of contacting an infected individual given an incidental release of 25 female Ac(DSM)2 mosquitoes, reduces the median probability of transmitting a novel blood-borne pathogen from $5.2 \times 10^{-7}$ to $1.3 \times 10^{-8}$ under the Aggregate First Then Convolute (AFTC) calculation method.
8. A conservative assessment that allows for the possibility that laboratory habituated Ac(DSM)2 mosquitoes may survive longer in the field than G3 mosquitoes slightly increases the median probability from $1.3 \times 10^{-8}$ to $1.5 \times 10^{-8}$ under the AFTC strategy.

## 3.1 Known blood-borne pathogens

Anopheline mosquitoes are known to vector a number of blood-borne pathogens. During the initial project scoping Target Malaria requested CSIRO to focus on the prevalent malaria parasite *Plasmodium falciparum*, the potentially emergent o'nyong'yong virus (ONNV) and the neglected tropical disease lymphatic filariasis (*Wuchereria bancrofti*) because:

- *Plasmodium falciparum*: *Anopheles coluzzii* is one of the primary vectors of malaria in Africa (Sinka et al., 2012). Burkina Faso and Mali both had more than 50% parasite rate for *Plasmodium falciparum* in 2010 (Noor et al., 2014).
- O'nyong'nyong virus (ONNV): as an alphavirus, ONNV is unique in its ability to be vectored to high epidemic levels by *Anopheles* mosquitoes (Powers, 2014). ONNV is a potentially re-emergent disease with major epidemics having occurred in East Africa in 1959 and 1996 (Corbet et al., 1961; Williams et al., 1965; Lanciotti et al., 1998). Evidence of ONNV transmission occurrence has been found in countries that neighbour Mali and Burkina Faso such as Ghana, Côte d'Ivoire and Senegal (Woodruff et al., 1978; Posey et al., 2005).
- Lymphatic filariasis: this neglected tropical disease, which is vectored by Anopheline mosquitoes, occurs in both Burkina Faso and Mali (Lindsay and Thomas, 2000; Gyapong et al., 2002).

Anopheline mosquitoes are also known to vector other viruses, namely West Nile virus, Rift Valley Fever virus and Tataguine virus, which Target Malaria determined to be out of scope. West Nile Virus is maintained in nature in a cycle between birds and mosquitoes but infections are primarily due *Culex species* (Rossi et al., 2010).

Rift Valley Fever (RVF) is most commonly observed in domestic animals but epizootic outbreaks can lead to infections in humans (Flick and Bouloy, 2005). RVF is most prevalent in eastern and southern Africa. It is relatively rare in Burkina Faso and Mali (https://www.cdc.gov/vhf/rvf/index.html) but is considered to have 66% - 99% chance of occurring in Mali, and a 10%-66% chance of occurring in Burkina Faso, during the 2017 wet season (Food and Agricultural Organisation of the United Nations, 2017).

Tataguine virus (TV) causes symptoms that are similar to malaria but much milder, and is likely under-reported because patients may not report to medical authorities (Fagbami and Tomori, 1981). TV has been isolated from *An. gambiae* in Burkina Faso (Tomori, 2001) but its prevalence is unknown (possibly due to its mild symptoms).

### 3.1.1 Informative priors

This analysis addresses the parameters associated with the well-known basic reproduction number ($R_0$) and the closely related vectorial capacity (Table 3.1). These parameters underlie some of the most commonly used analyses and modelling approaches applied to vector-borne pathogen transmission. The list of parameters addressed by each expert in the elicitation session were determined by the individual's domain knowledge, expertise and experience. Experts were asked to address only those parameters that they believed were within their domain of expertise, and to omit questions which they did not feel comfortable responding to.

Each elicitation was carefully structured to document each expert's domain experience, ex-

**Table 3.1:** Parameters subject to expert elicitation.

| Parameter | Definition | Varies by pathogen? |
|---|---|---|
| $a$ | Number of bites on humans per day per mosquito | No |
| $q$ | Daily probability of mortality of female mosquitoes | Yes* |
| $b$ | Transmission efficiency from female mosquitoes to humans | Yes |
| $c$ | Transmission efficiency from humans to female mosquitoes | Yes |
| $\tau$ | Duration of extrinsic incubation period in days | Yes |

*Mortality rate may vary for female mosquitoes infected with lymphatic filariasis.

pertise and commentary, supported wherever possible with reference to established theory and scientific literature. The elicitation method deliberately allows for uncertainty that may arise from knowledge gaps or variability in a target parameter. This information is used to develop a probabilistic (Bayesian) model (see Section A.1.1 for technical detail). Where relevant, independent data is available, the model can coherently assimilate it and update the expert's contributed assessments.

Prior to the start of the elicitation each expert was required to sign two copies of an ethics document, and given the opportunity to review the project's objectives, together with a description of the Dominant Sterile Male construct and details of the planned field release.[4] The elicitation session began with a presentation on probabilistic risk assessment that included examples of common heuristic biases and example problems to enable the expert to practice and become comfortable with the elicitation procedure.

### 3.1.2 Scope and parameter definitions

The parameters addressed in the elicitation are defined in Table 3.1. The human feeding rate ($a$) and daily probability of mortality ($q$) are assumed independent of disease pathogen. The daily mortality rate may vary for female mosquitoes infected with lymphatic filariasis, and is related to other traditional indices of mosquito mortality such as the daily probability of survival $p$ or the daily mortality rate $\mu$:

$$p = 1 - q$$
$$\mu = -\log(1 - q).$$

The extrinsic incubation period ($\tau$), transmission efficiency from vectors to humans ($b$) and transmission efficiency from humans to vectors ($c$) may also vary with pathogen.

All parameters were assessed with respect to a pre-determined spatial and temporal scope. The spatial scope was defined by Target Malaria to be the Sudanian zone of Burkina Faso and the neighbouring Sudano-Guinean area. Insecticide treated nets were assumed to

[4]This information was provided to each expert two weeks in advance of the elicitation in the form of a pre-elicitation document (Appendix B) and background information document compiled by Target Malaria. The experts were asked to reviewed these materials carefully and were allowed to refer to them throughout the elicitation.

have been deployed at each release site. The elicitation focused on how vectors were predicted to behave in the field environment outside of controlled laboratory conditions.

The parameters in Table 3.1 were defined as annual averages for non-aestivating mosquitoes. Aestivating mosquitoes were excluded from the scope of the elicitation. The precise date of the controlled field release is uncertain, and although the release was initially planned to occur at the beginning of the wet season in Burkina Faso, released mosquitoes may in theory survive and reproduce into and beyond the following dry season.

### 3.1.3 Populations and lineages

The laboratory population of Ac(DSM)2 was originally sourced from the laboratory G3 strain (Figure 1.4). The genetic composition of the original G3 strain appears to be a mixture of *An. gambiae* and *An. coluzzii*. Prior to backcrossing with Ac(WT), the genetically engineered lineage is referred to as Ag(DSM)2.

The Ac(DSM)2 lineage began by crossing male Ac(WT) with female Ag(DSM)2 (Figure 1.5). For the Ac(DSM)2 strain experts were asked to consider scenarios that vary the number of backcrosses with the Ac(WT) lineage. For the Ac(WT) strain the genetic composition of the local wild type population within the laboratory production facility may diverge from the local field wild-type population with each generation through loss of genetic diversity and adaptation to the laboratory environment (Aguilar et al., 2005) . Experts were therefore asked how values for the target parameter may depend on the number of generations that Ac(WT) was maintained in the laboratory.

### 3.1.4 Definition of the relative risk assessment endpoint

The risk assessment endpoint for known blood-borne pathogens is defined as the probability of an increase in the pathogen transmission potential of Ac(DSM)2 mosquitoes relative to Ac(WT) for *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis.

We assess the potential for pathogen transmission using the basic reproduction number, which for the Ross-Macdonald model[5] of malaria transmission is defined as

$$R_0 = \frac{a^2 bcme^{-\mu\tau}}{r\mu} = \frac{a^2 bcmp^{\tau}}{-r\log p},$$

where $r$ is the recovery rate of humans from infectiousness and $m$ is the vector to human host ratio (see Smith and McKenzie, 2004, for review). If $R_0 < 1$ then the disease free equilibrium of the Ross-Macdonald model is stable such that the disease cannot remain established in the long term if introduced into a susceptible population.

In this analysis we define a relative risk index $\mathcal{R}$ as the ratio of the basic reproduction number of the female Ac(DSM)2 mosquitoes that will be released – that is the Ac(DSM)2 mosquitoes at backcross 29 ($F = 62$) over the local wild population – that is Ac(WT) at $F = 0$ (i.e. without any laboratory habituation):

$$\mathcal{R} = \frac{R_0^{GE}}{R_0^{WT}} = \frac{\mu_{WT} a_{GE}^2 b_{GE} c_{GE} e^{-\mu_{GE}\tau_{GE}}}{\mu_{GE} a_{WT}^2 b_{WT} c_{WT} e^{-\mu_{WT}\tau_{WT}}}, \tag{3.1}$$

[5]The closely related vectorial capacity omits the human related parameters – that is the human recovery rate $r$ and the host to vector transmission efficiency $c$

where we use the notation $GE$ to indicate Ac(DSM)2 at backcross 29 ($F = 62$), and $WT$ to indicate Ac(WT) at $F = 0$. Note also that the human recovery rate is assumed to be unchanged and therefore cancels out of Equation (3.1).

If $\mathcal{R} < 1$ then there is decreased relative risk attributable to the Ac(DSM)2 strain. Conversely, if $\mathcal{R} > 1$ then there is increased risk of pathogen transmission associated with the Ac(DSM)2 strain during the time that it is present in the environment. Comparisons could also be made using other choices of backcross, number of generations in the laboratory or strains but here we focus on the endpoint that compares the local wild population to Target Malaria's initial Ac(DSM)2 release plan.

### 3.1.5 Human feeding rate

Five external experts predicted the human feeding rate for female Ac(DSM)2 and Ac(WT) mosquitoes at each of the elicitation's design points (Section A.1.1). The statistical models fitted to their prior beliefs for the comparison relevant to the relative risk endpoint defined above are summarised in Figure 3.1. These results indicate that four out of the five experts believe that wild type mosquitoes bite humans about once every two to three days. One of the experts indicated that the wild type human feeding rate could be higher because *An. coluzzii* are aggressive but cautious and hence may bite the same human multiple times in order to complete a meal.

The predictive priors for Ac(DSM)2 reflect three of the experts' beliefs that the Ac(DSM)2 strain mosquitoes will feed on humans at the same rate as the wild type. Two experts, however, suggested that the human feeding rate will be slightly lower due to the domestication effects of the laboratory. For example, laboratory reared mosquitoes may be less likely to search for a blood meal because they don't need to in the laboratory.

### 3.1.6 Extrinsic incubation period

The predictive priors for the duration of the extrinsic incubation period for Ac(WT) at $F = 0$ and Ac(DSM)2 at backcross 29 for *P. falciparum*, o'nyong'nyong and lymphatic filariasis are shown in Figures 3.2, 3.3 and 3.4 respectively.

Four experts responded to the elicitation for *P. falciparum*. The experts held similar views, indicating that the extrinsic incubation period is highly dependent on the ambient temperature and for wild-type mosquitoes in the release site would likely range from about 9 to 18 days. Overall the experts did not believe that this parameter would change dramatically in the Ac(DSM)2 strain, with one expert suggesting that the Ac(DSM)2 strain may not provide as good a host for the parasite as the wild type, hence the extrinsic incubation period may be a day or two longer.

Only one expert was able to respond to the elicitation for this parameter for o'nyong'nyong. The model of this belief shows the extrinsic incubation period for wild type mosquitoes in the range of about 4 to 27 days, with a median value of 11 days at the time of planned release. The expert indicated that the range could be somewhat narrower for the Ac(DSM)2 strain and the model suggests a similar median value of 11 days.

Three experts responded to the elicitation for lymphatic filariasis, and again noted that the extrinsic incubation period in wild type mosquitoes varies with temperature. In this case, however, the external experts held almost identical beliefs. The model fitted to their elicitation shows the extrinsic incubation period for wild types at the release site falling between

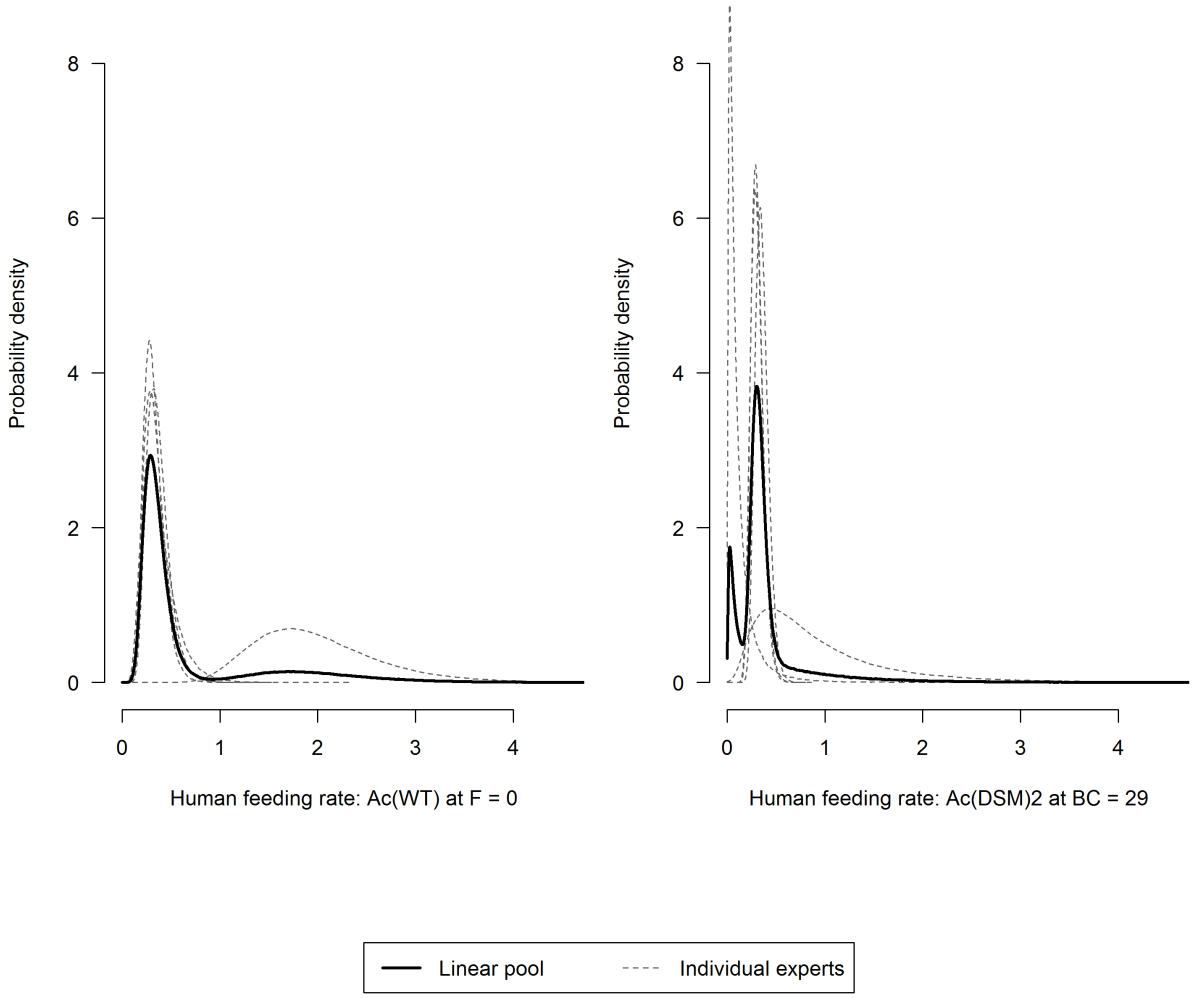


**Figure 3.1:** Predicted prior distributions for human feeding rate The individual priors (dashed lines) indicate that four out of five experts believe that wild *An. coluzzii* will bite about once every 2 to 3 days. One expert believes it could be higher because the mosquitoes may need to make multiple bites to complete a blood meal. The experts' opinions are more variable for the Ac(DSM)2 strain but 3 out of the 5 experts do not believe that the human feeding rate will change, whilst 2 believe it will be lower than wild type due to domestication effects caused by laboratory rearing. The linear pool (solid line) is a weighted average of the experts' opinions. In the absence of data each expert is a given an equal weight.

about 9 and 18 days, with a median value of 13 days at the time of the planned release. All of the experts elicitations indicated similar responses for the Ac(DSM)2 strain, with the fitted model showing the same median value as the wild type.

### 3.1.7 Transmission efficiency

The predictive priors for the transmission efficiencies – vector to human host and human host to vector – for *P. falciparum*, o'nyong'nyong and lymphatic filariasis are shown in Figures 3.5, 3.6 and 3.7 respectively. Both transmission efficiency parameters $b$ and $c$ in Equation (3.1) lie on the range (0,1). It is clear from these figures that the level of agreement between the experts about the value of these parameters is generally lower than that associated with the vectorial capacity parameters described previously.

For *P. falciparum* in wild type mosquitoes, the low level of agreement between the experts means that the fitted linear pool predictive prior for the vector to host, and human host to vector, transmission efficiency could almost be described by a uniform distribution on the parameter's range – that is the value could lie anywhere on the range (0,1) with almost equal probability. For the Ac(DSM)2 strain, the linear pool of the model fitted to the experts' beliefs suggests that transmission efficiencies are a little less variable, and values less than 0.5 somewhat more probable.

This level of uncertainty is consistent with the experts' commentary that *P. falciparum* host to vector transmission efficiency is dependent on the dynamics of infection within the human host population, where infectiousness could exhibit substantial heterogeneity and temporal change. The experts also noted that laboratory data generated by membrane feeding assays will vary depending on the details of the laboratory protocols, for example, the specific levels of gametocytes present in the infected blood.

Only one expert was able to respond for o'nyong'nyong virus. The statistical model fitted to this expert's elicited beliefs suggests that transmission efficiencies from vector to host and host to vector are generally quite high, with a median value of 0.8 and 0.6 respectively in Ac(WT) and Ac(DSM)2 strain mosquitoes. In this context, the expert commentary emphasises that the host to vector transmission efficiency is dependent on the strain of o'nyong'nyong virus and the level of viremia. The literature also suggests that laboratory observations of transmission, as evidenced by presence of virus in the salivary glands, might be sensitive to the choice of experimental protocol.

For lymphatic filariasis, the model fitted to the experts' response to the elicitation for vector to human host transmission efficiency indicates that two of the three experts who responded believe that the efficiency will be well below $0.1$ for both the Ac(WT) and Ac(DSM)2 strains. One expert suggested that efficiency could be higher but the commentary that accompanied this elicitation (and succeeding out-of-session follow-up) does not provide a clear reason why this might be the case. In the absence of empirical data, the equally weighted linear pool places twice as much emphasis on the probability that vector to host transmission efficiency will be less than about 0.04.

The fitted model of human host to vector transmission efficiency for lymphatic filariasis also indicates that the efficiency will be less than $0.1$ but in this instance the level of agreement between the three experts is high, and the linear pool predictive prior places almost no probability mass on transmission efficiency's greater than $0.1$ for either Ac(WT) or Ac(DSM)2 strain mosquitoes.

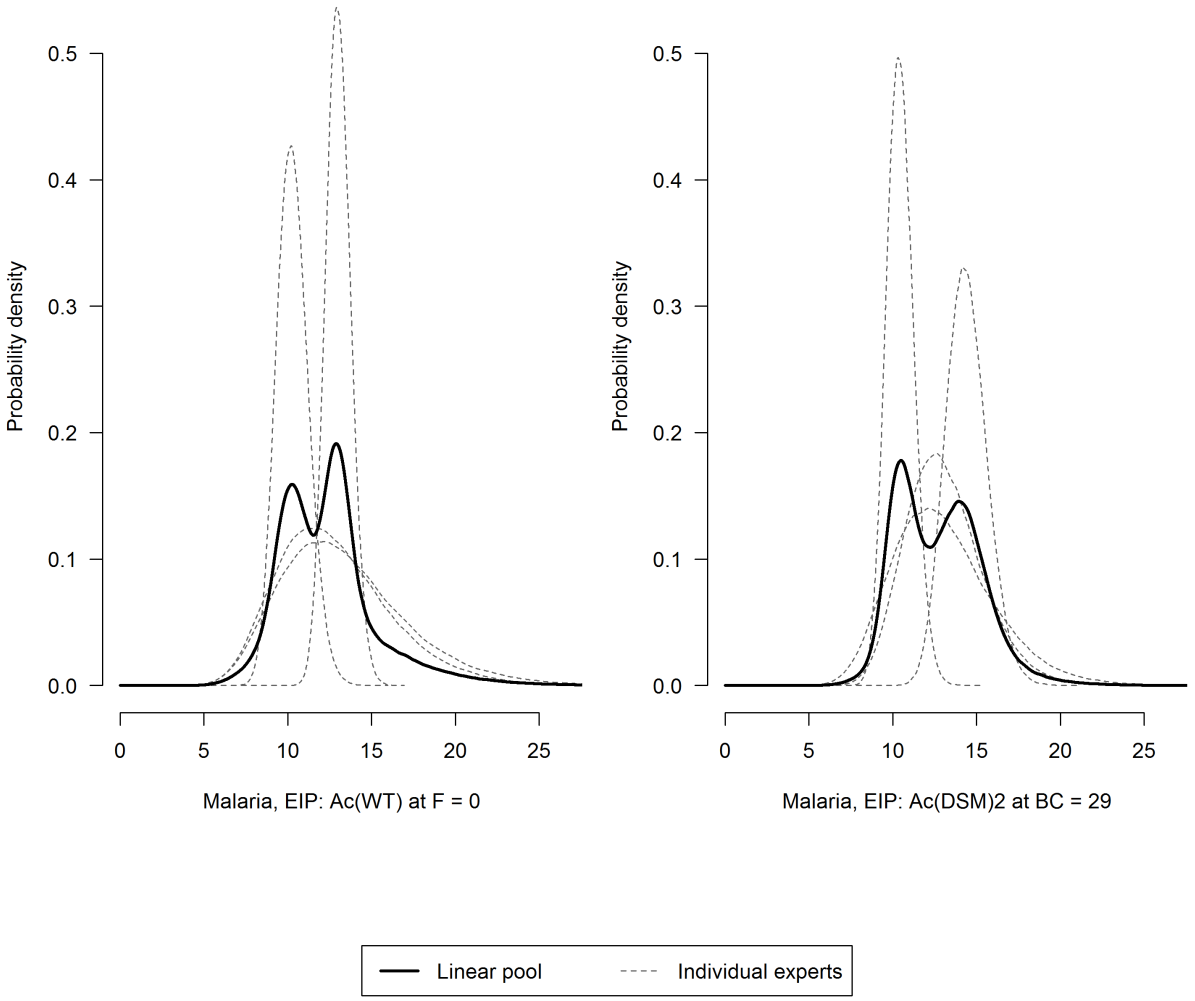


**Figure 3.2:** Predictive prior distributions for *P. falciparum* extrinsic incubation period (EIP) in days. The individual priors (dashed lines) indicate that the four experts who responded to this parameter held similar views. The statistical model fitted to their beliefs indicates that the extrinsic incubation period for Ac(WT) and Ac(DSM)2 will be similar, and likely vary between 9 and 18 days depending on the ambient temperature. The linear pool (solid line) is a weighted average of the experts' opinions. In the absence of data each expert is a given an equal weight.

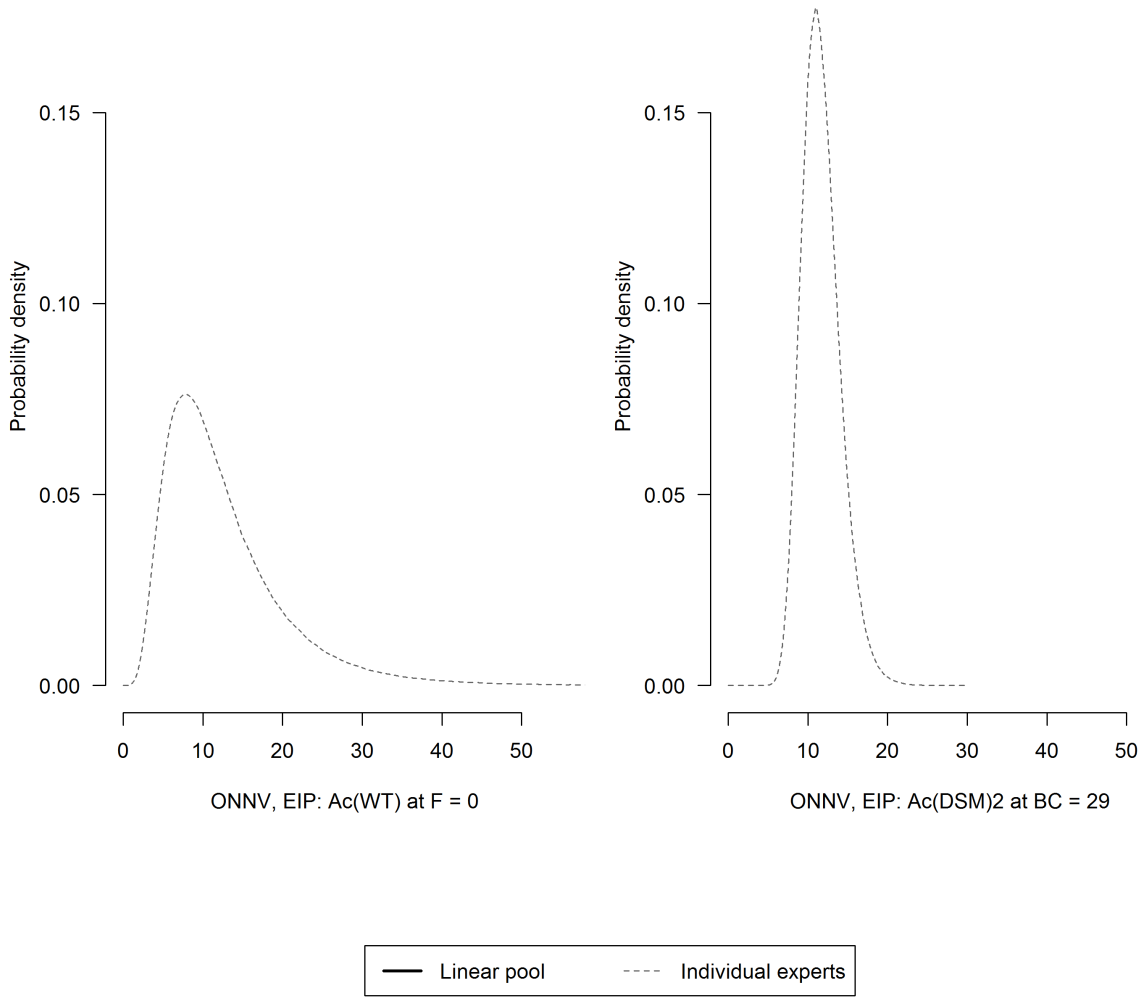


**Figure 3.3:** Predictive prior distribution for o'nyong'nyong extrinsic incubation period (EIP) in days. The statistical model fitted to this experts' beliefs (dashed line) indicates that the extrinsic incubation period in wild type mosquitoes will lie in the range of 4 to 27 days with a median value of 11 days at the time of the planned release. The model for the Ac(DSM)2 strain reflects the experts' belief that the overall range in the Ac(DSM)2 strain will be narrower but with a similar median value of 11 days. The linear pool, which is identical to the individual prior, is not shown here because only one expert was able to respond to this parameter.

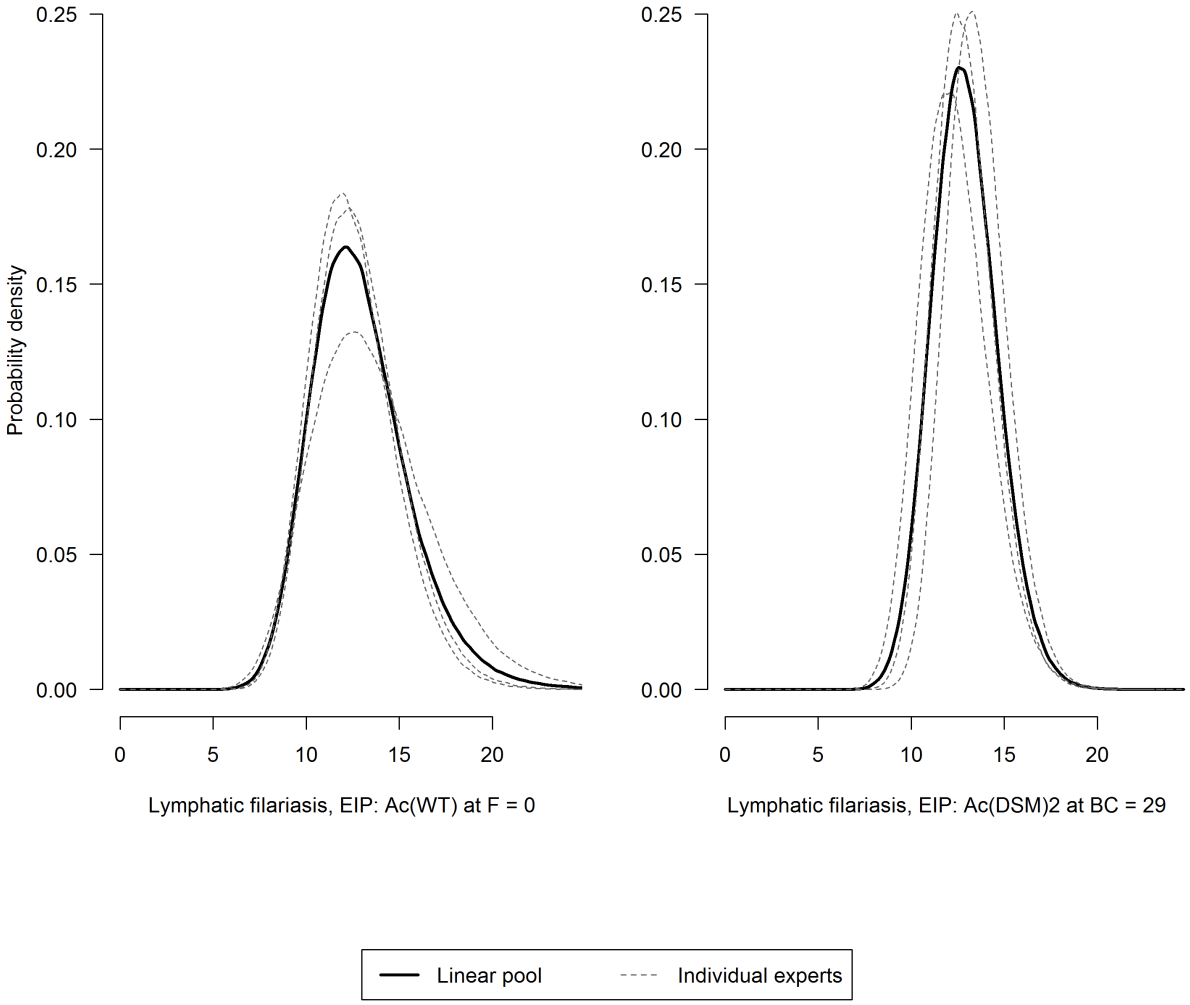


**Figure 3.4:** Predictive prior distributions for lymphatic filariasis extrinsic incubation period (EIP) in days. These individual priors (dashed lines) indicate that the three external experts who responded to this parameter held almost identical views. In wild type mosquitoes the model fitted to the experts' beliefs suggests that the extrinsic incubation period will lie somewhere between 9 and 18 days with a median value of about 13 days at the time of the planned release. For the Ac(DSM)2 strain, the model reflects the experts' beliefs by indicating that the range will be slightly narrower, but with a similiar median value of 13 days. The linear pool (solid line) is a weighted average of the experts' opinions. In the absence of data each expert is a given an equal weight.

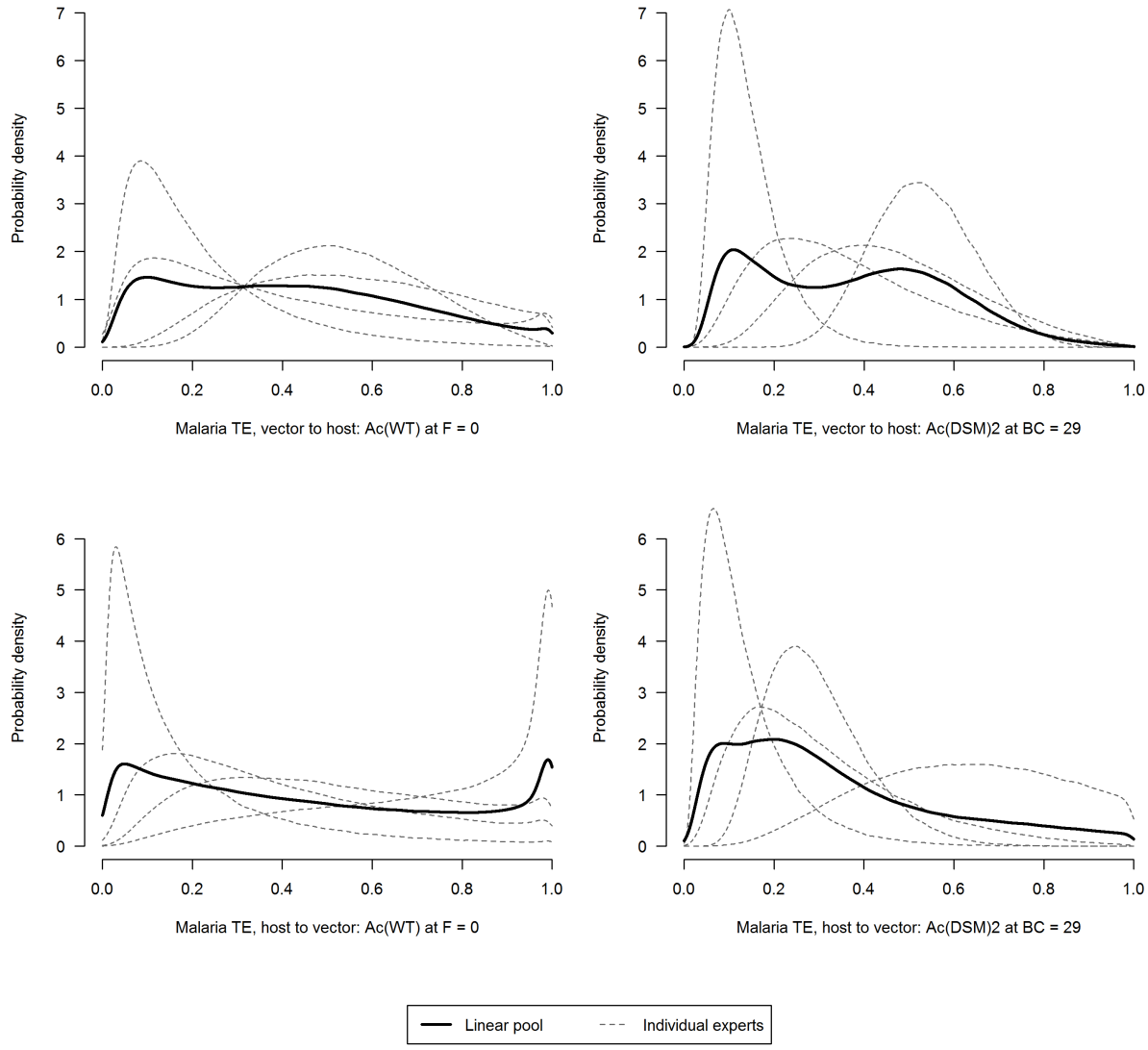


**Figure 3.5:** Predictive prior distributions for *P. falciparum* vector to host transmission efficiency (top) and host to vector transmission efficiency (bottom).The individual priors (dashed lines) fitted to the experts' response to the elicitation reflect a low level of agreement between the experts for the transmission efficiencies (vector to host and host to vector) for wild type strains. This low level of agreement is consistent with the expert commentary that *P. falciparum* transmission efficiency is highly variable from place to place and between the seasons. The level of agreement for the Ac(DSM)2 strain mosquitoes is slightly better and the fitted model suggests that transmission efficiencies may be slightly lower, but the overall level of uncertainty remains relatively high. The linear pool (solid line) is a weighted average of the experts' opinions. In the absence of data each expert is a given an equal weight.

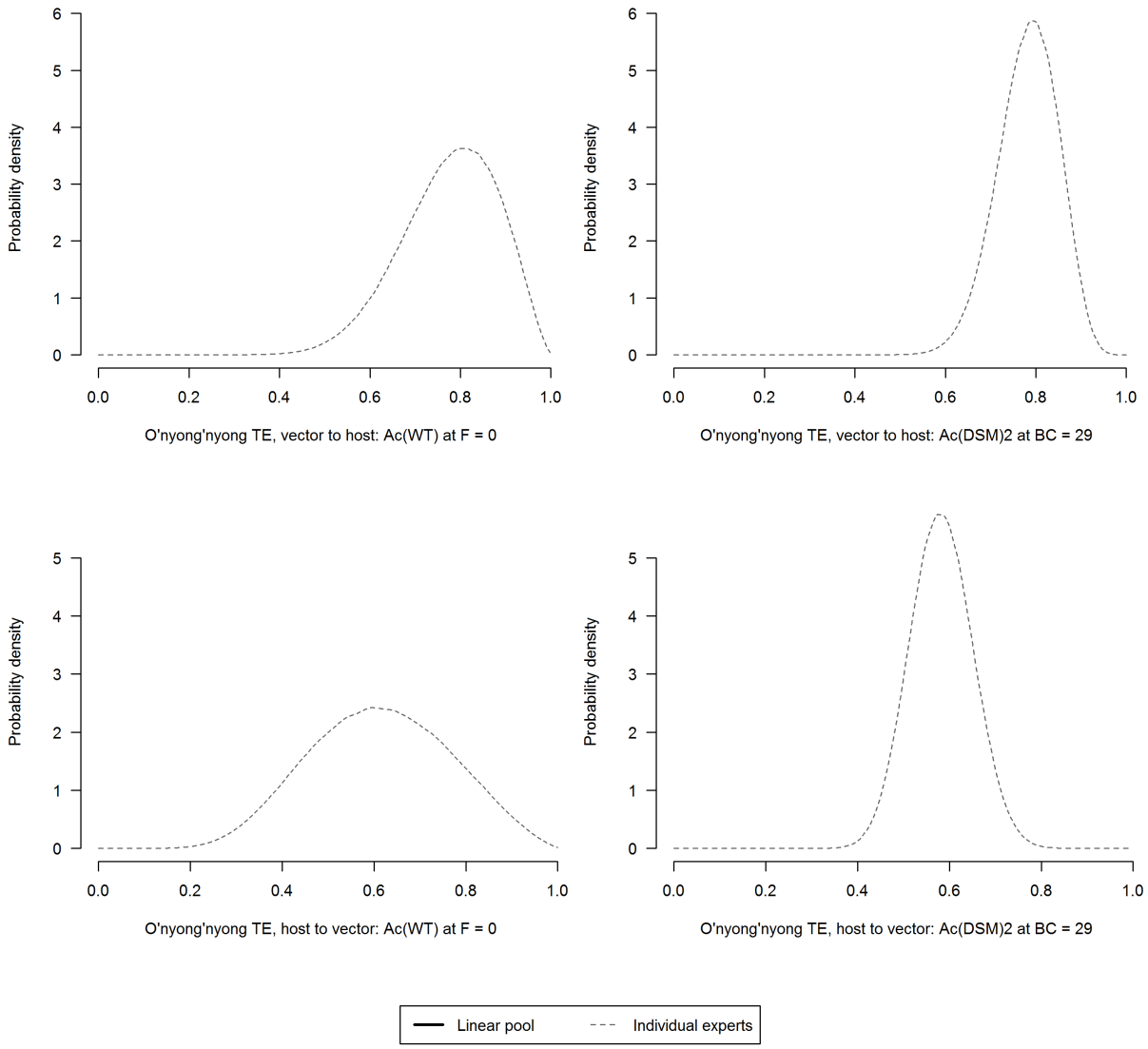


**Figure 3.6:** Predictive prior distributions for o'nyong'nyong virus vector to host transmission efficiency (top) and host to vector transmission efficiency (bottom). Only one expert was able to respond to the elicitation for o'nyong'nyong virus, and the model fitted to their response suggests that vector to host transmission efficiency will be relatively high (median value of 0.8 ) for both Ac(WT) and Ac(DSM)2 strains. Similarly, the model fitted to the expert's host to vector response indicates moderately high (median value of 0.6 ) efficiency for both strains. The linear pool is not shown here because it is identical to the individual prior.

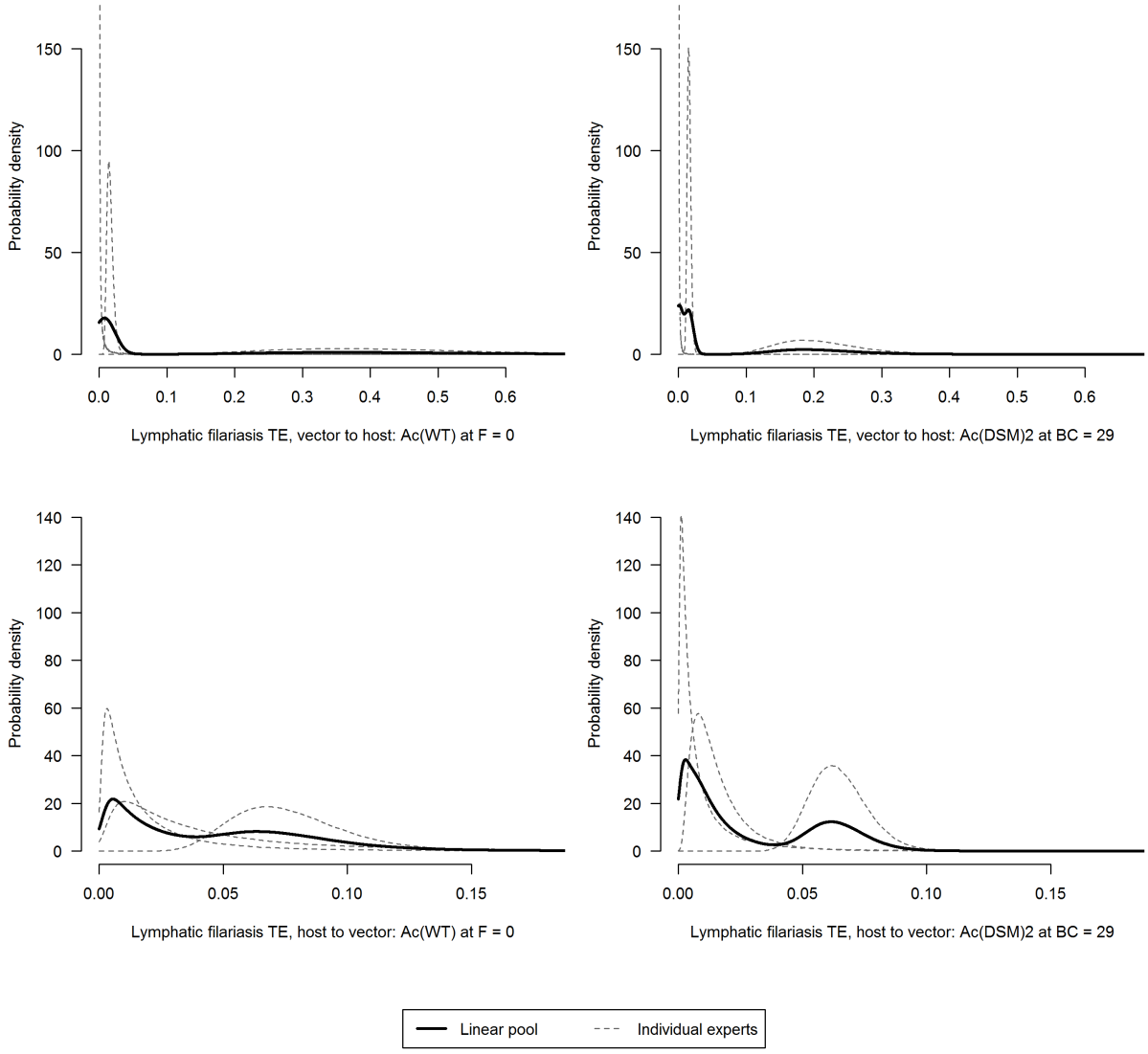


**Figure 3.7:** Predictive prior distributions for lymphatic filariasis vector to host transmission efficiency (top) and host to vector transmission efficiency (bottom). The individual priors (dashed lines) show that two of the three experts who responded to the lymphatic filariasis transmission efficiency elicitation believe that the vector to host efficiency is very less than 0.1 and this is reflected in statistical model fitted to their beliefs. The remaining expert, however, indicated that it could be as high as 0.4 and 0.3 in the Ac(WT) and Ac(DSM)2 strains respectively. For the host to vector transmission efficiency the experts' beliefs were more closely aligned and the fitted model suggests that this will almost certainly be less than 0.1 for both Ac(WT) and Ac(DSM)2 strains. The linear pool (solid line) is a weighted average of the experts' opinions. In the absence of data each expert is a given an equal weight.

### 3.1.8 Daily probability of mortality

The predictive priors for the daily probability of survival[6] for Ac(WT) ($F = 0$) and Ac(DSM)2 (backcross 29) are shown in Figure 3.8. Figure 3.9 shows the predictive priors for only those experts who responded to the elicitation for lymphatic filariasis. These elicitations are separated because mortality rate may vary substantially for female mosquitoes infected with lymphatic filariasis, whereas malaria and o'nyong'nyong infection were believed to not substantially affect the mosquitoes' mortality rate.

The predictive priors for mosquitoes that are uninfected with lymphatic filariasis reflect the fact that most experts (four of the five that responded to the elicitations) believe that daily survival probability of wild strain mosquitoes would likely fall in the range of $0.9$ to $0.95$, whereas one expert suggested it is more likely to be around $0.8$.

One of the experts expressed the belief that the probability of daily survival of the Ac(DSM)2 strain could be higher than the wild type strain because insectary procedures, such as manual handling of mosquitoes, may select for strong individuals and this might lead to more robust laboratory individuals at release. Their predictions were uncertain, however, and also allows for the possibility that environmental conditions could still favour Ac(WT).

One of the experts also noted that the stress experienced by Ac(DSM)2 mosquitoes during transportation to the field release site may increase their mortality rate once released into the field. The wide range of views expressed during the elicitation is reflected in the substantial uncertainty in the predictive prior distributions for daily probability of survival of the Ac(DSM)2 strain (right hand side of Figure 3.8).

The predictive prior probability of survival for mosquitoes infected with lymphatic filariasis conditions only on those experts that contributed a mortality estimate specifically for mosquitoes infected by lymphatic filariasis (Figure 3.9). Both experts who responded to this pathogen agreed that infection increases the daily probability of mortality but their opinion about the magnitude of increase are quite different. Both also agreed that the mortality of Ac(DSM)2 strain mosquitoes infected with lymphatic filariasis will be substantially higher than the wild type strain.

Evidence of the daily mortality of female Ac(WT) mosquitoes at $F = 0$ is available from the empirical data collected during the first mark release recapture experiment, conducted as part of a larger study that focused primarily on male mosquitoes (Epopa et al., 2017). This empirical data enables the risk assessment to calculate the model evidence (Section A.1.2) which in turn allows the assessment to calculate the Bayesian model average predictive posterior distribution of the probability of daily mortality for female mosquitoes (Section A.1.3). The model evidence weights the contribution of each expert's prior to the average predictive posterior according to how well their prior predicted the data.

Figure 3.10 shows the predictive prior posterior distributions for the daily probability of survival for Ac(WT) (F = 0) and Ac(DSM)2 (backcross 29) with and without lymphatic filariasis infection. The analysis of the Mark Release Recapture data conducted here suggests that the daily probability of survival for wild strain mosquitoes[7] that are not infected with lymphatic filariasis is lower than that predicted by all of the experts, but quite close to the values

[6] Note: the daily probability of survival $p$ is defined as one minus the daily probability of mortality $q$

[7] Mosquitoes in the first Mark Release Recapture experiment were collected as larvae from the field, raised to adults in the laboratory and then dusted before release

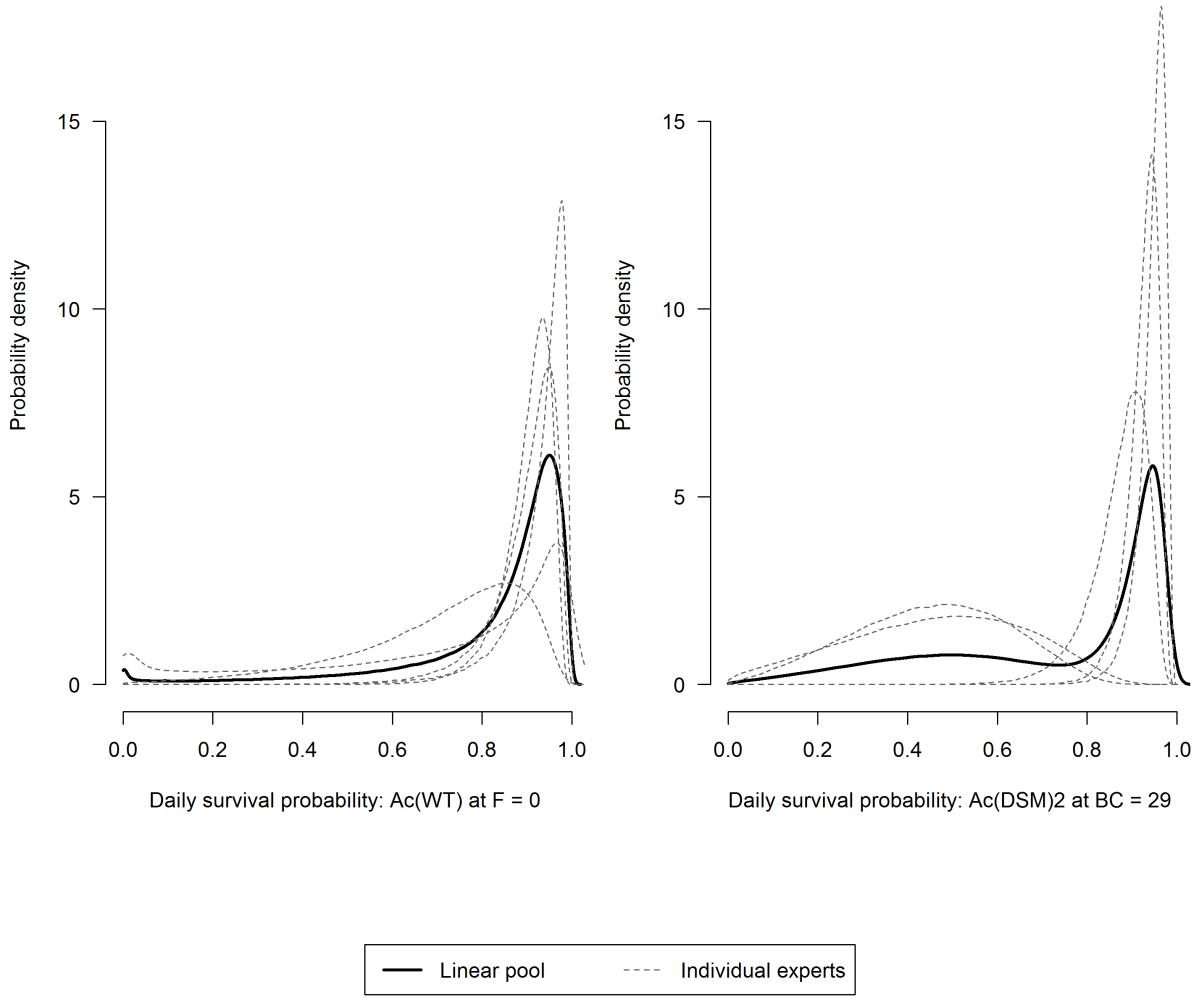


**Figure 3.8:** Predictive prior distributions of the probability of daily survival for mosquitoes that are not infected with lymphatic filariasis. The models fitted to the experts' individual responses (dashed lines) for wild strain mosquitoes reflect the fact that four of the five experts who responded were fairly confident that the daily survival probability would be greater than 0.8. The remaining expert indicated that it was likely to be about 0.8. Three of the five experts believed that female Ac(DSM)2 mosquitoes would experience higher mortality (lower survival) than Ac(WT) mosquitoes once released into the field. One, however, indicated that survival may be higher because handling of mosquitoes in the laboratory could select for strong individuals. The linear pool (solid line) is an equally weighted average of the experts' opinions.

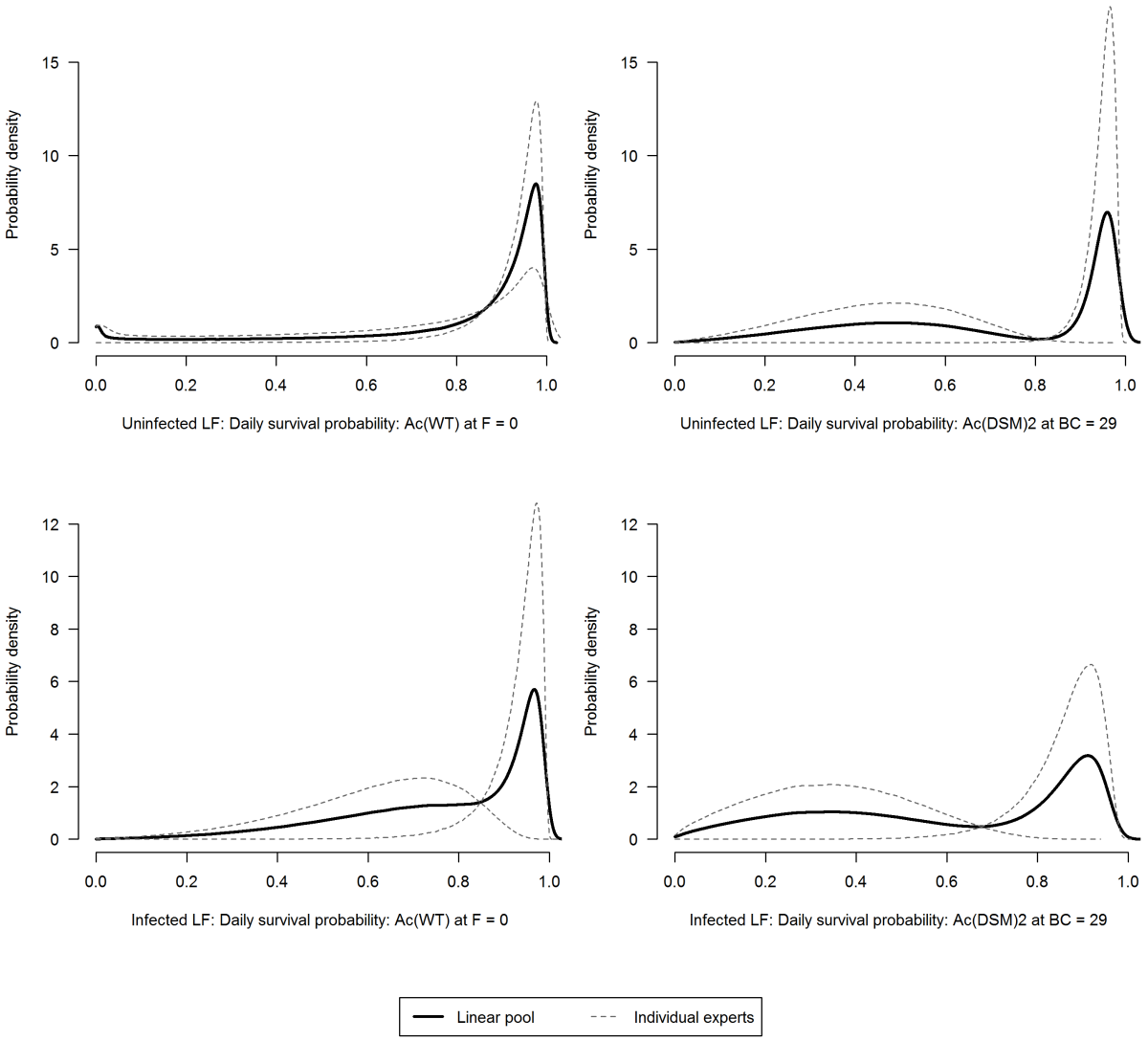


**Figure 3.9:** Predictive prior distributions of the probability of daily survival for mosquitoes uninfected with lymphatic filariasis (LF) (top) and those infected with lymphatic filariasis (bottom). The individual priors (dashed lines) for mosquitoes infected with lymphatic filariasis are singled out here because infected mosquitoes infected may experience higher rates of mortality. The individual expert's predictive prior distributions for uninfected mosquitoes are the same as those shown in Figure 3.8 but the linear pool is different because it incorporates only those experts who responded to lymphatic filariasis. The predictive priors for wild type mosquitoes infected with LF reflect a difference of opinion between the two experts who responded. One believed that the infection has quite a dramatic effect on survival, whilst the other believes the effect is smaller. Both experts believed that if the female Ac(DSM)2 mosquitoes are infected with lymphatic filariasis in the field then their daily probability of survival will be lower than the wild types. The linear pool (solid line) is an equally weighted average of the experts' opinions.

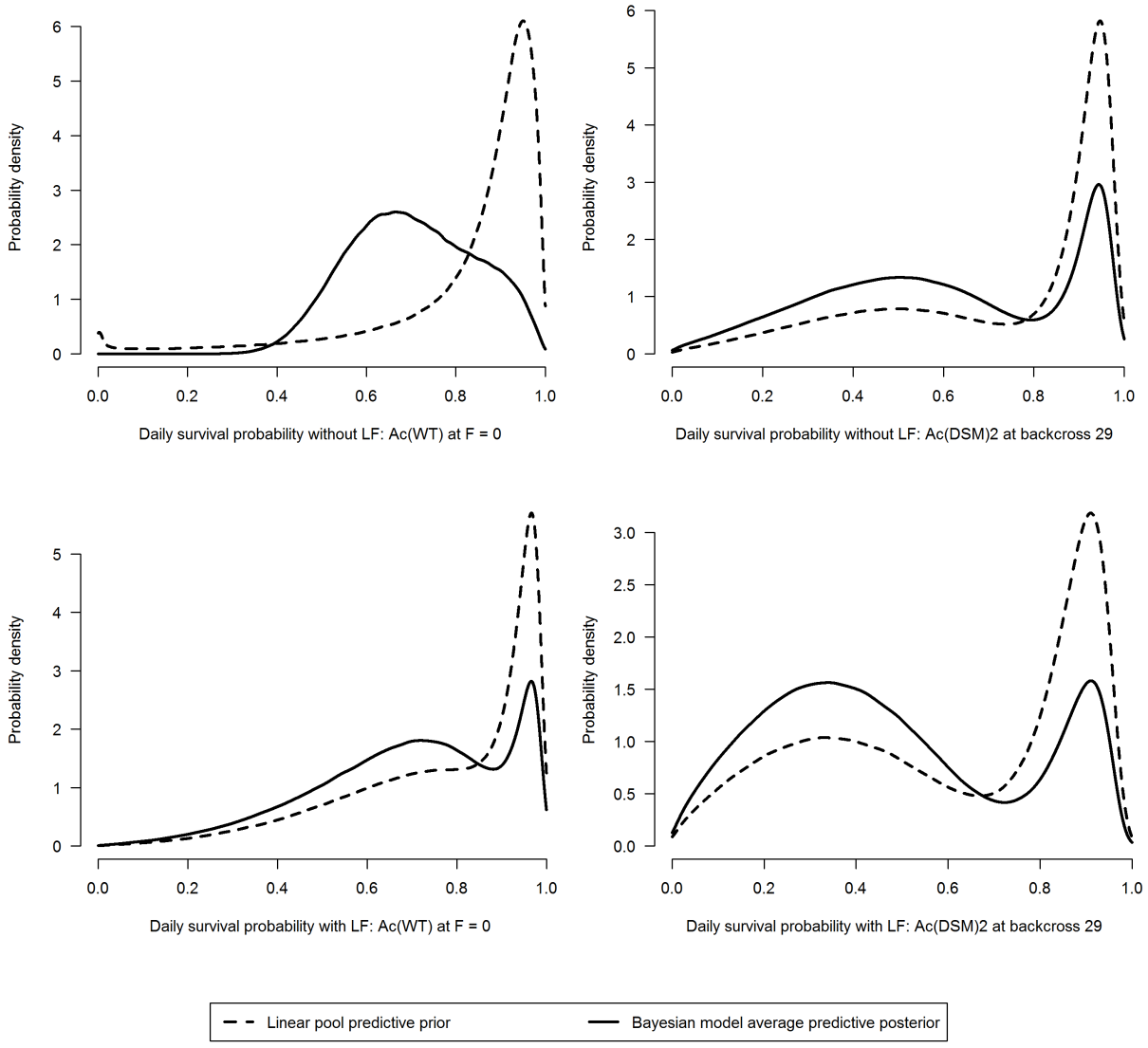


**Figure 3.10:** Predictive prior and posterior probability of daily survival for Ac(WT) at $F = 0$ and Ac(DSM)2 at backcross 29 without (top) and with (bottom) lymphatic filariasis infection. Data and empirical estimates of daily survival rates of dusted, wild type, female mosquitoes enables the risk assessment to calculate a posterior distribution of daily survival probability for the wild type strain. The predictive posterior distribution of daily survival shown here (solid line) is a Bayesian model average of the expert's individual posterior distributions. The Bayesian model average approach gives more credence to (places a higher weight on) expert's priors that are closer to actual outcomes, and here we assume that experts who make better predictions about Ac(WT) mosquitoes will also make better predictions about Ac(DSM)2 mosquitoes (Appendix A.1). As a consequence the predictive posterior distributions of daily survival probability for female Ac(WT) and female Ac(DSM)2 mosquitoes, with and without lymphatic filariasis infection, suggest that survival rates will be lower than those predicted by the linear pool prior (dashed line). In the case of female Ac(WT) mosquitoes without lymphatic filariasis infection, the median daily survival probability drops from 0.9 to 0.7 .

predicted by the expert who *a priori* believed it to be about 0.8. The predictive posterior distribution therefore shows a reduction to a median value of $0.7$ from a median value of $0.9$ under the linear pool prior.

The predictive posterior distribution for the daily probability of survival of female Ac(DSM)2 mosquitoes that are not infected with lymphatic filariasis also places a higher probability on lower values of survival than the linear pool because the expert who predicted lower values of survival for the female Ac(WT) mosquitoes has been given a higher weight in the posterior calculation of the Ac(DSM)2 strain – i.e. the risk assessment has applied the model evidence derived from the analysis of wild type data to the Ac(DSM)2 strain calculations on the assumption that experts who make predictions about female Ac(WT) mosquitoes that are closer to the truth will also make better predictions about female Ac(DSM)2 mosquitoes (see Appendix A.1).

Similarly, the predictive posterior distribution for the daily probability of female Ac(DSM)2 mosquitoes that are infected with lymphatic filariasis (bottom row of Figure 3.10) places more emphasis on lower values of daily survival probability than the linear pool because of the change in weight allocated to the experts' informative priors.

### 3.1.9 Relative risk results

Figure 3.11 shows the predictive prior and posterior distributions of the base 10 logarithmic ratio of the basic reproduction number for Ac(DSM)2 at backcross 29 versus Ac(WT) at $F = 0$ for the three known blood borne pathogens – *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis. The assessment uses the base 10 logarithm of the ratio to help present the results: the base 10 logarithm of 1 is 0 so positive values of the ratio on the base 10 logarithmic scale mean that the basic reproduction number of the female Ac(DSM)2 mosquitoes is higher than that of the female Ac(WT) mosquitoes, and conversely, negative values mean that the basic reproduction number of the female Ac(WT) mosquitoes is higher than that of the female Ac(DSM)2 mosquitoes.

The risk of Ac(DSM)2 at backcross 29 having a higher capacity to transmit pathogens than Ac(WT) at F = 0 is given by the area under the probability density function to the right of the dashed vertical line – i.e. the area where the ratio of the reproduction number on the base 10 logarithmic scale is positive. If this area is smaller than the area to the left of the dashed vertical line, as is most clearly seen with lymphatic filariasis, then female Ac(DSM)2 mosquitoes are predicted to have a lower transmission capacity than female Ac(WT) mosquitoes. If the areas on either side are roughly similar then so are the odds of pathogen transmission.

The results of this assessment, assuming that the vector to human host ratio $m$ is the same for both Ac(WT) and Ac(DSM)2, are as follows:

- ***P. falciparum***: the prior probability of an increased risk of pathogen transmission is $P(\mathcal{R} > 1) = 0.28$ and the posterior probability is $P(\mathcal{R} > 1|y) = 0.29$.
- **O'nyong'nyong**: the prior probability of an increased risk of pathogen transmission is $P(\mathcal{R} > 1) = 0.3$ and the posterior probability is $P(\mathcal{R} > 1|y) = 0.33$.
- **Lymphatic filariasis**: the prior probability of increased risk of pathogen transmission is $P(\mathcal{R} > 1) = 0.14$ and the posterior probability is $P(\mathcal{R} > 1|y) = 0.13$.

Note that the change between the predictive prior and posterior probabilities reported here

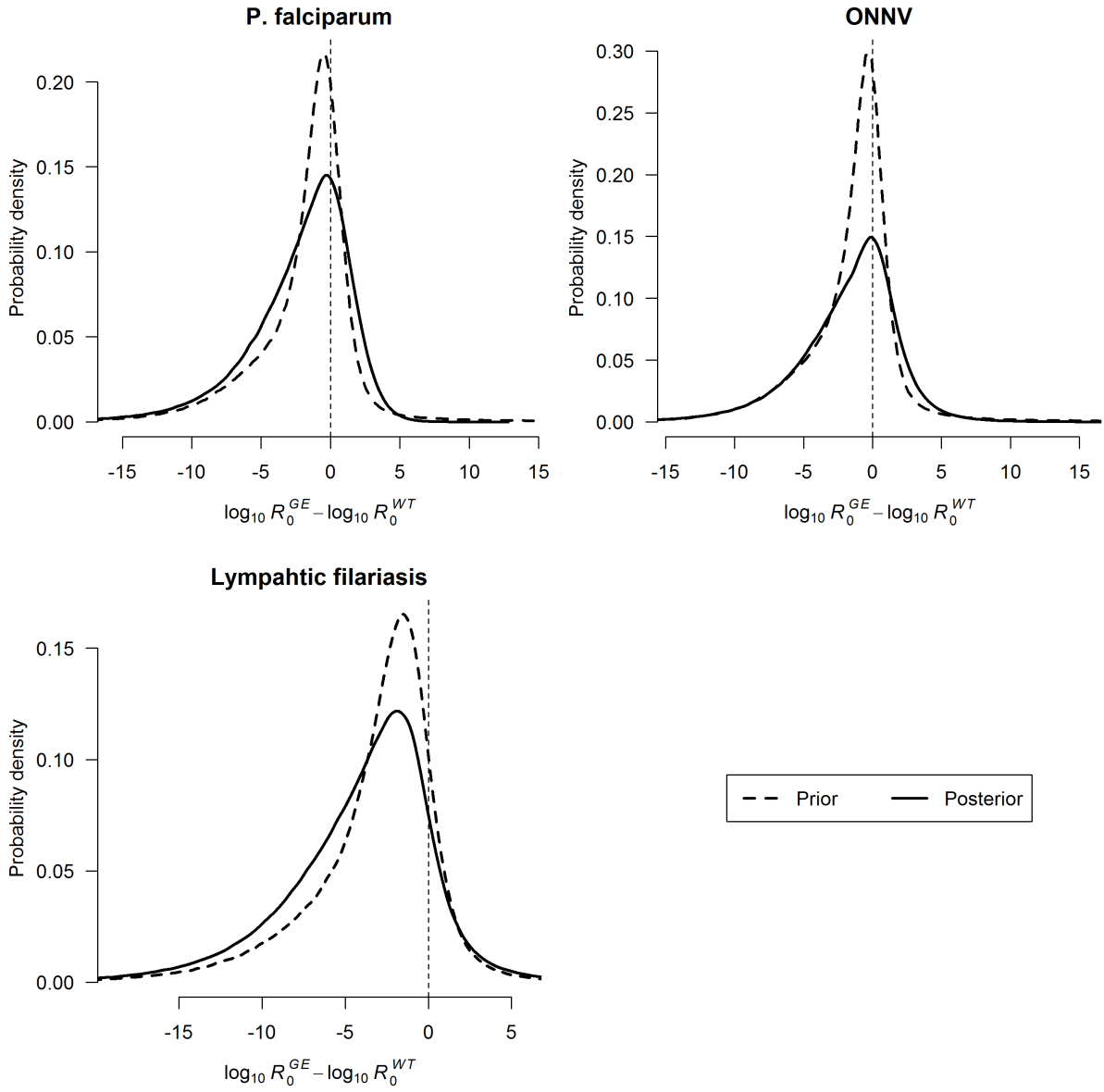


**Figure 3.11:** Prior and posterior probability distributions for the base 10 logarithm of the ratio of $R_0$ for Ac(DSM)2 at backcross 29 versus Ac(WT) at $F = 0$ for *P. falciparum*, (top left), ONNV (top right) and lymphatic filariasis (bottom left). The area under the probability density curves to the right of the dashed vertical line gives the realtive risk of Ac(DSM)2 at backcross 29 having a higher capacity to transmit pathogens than Ac(WT) at F = 0, assuming equal host to vector ratio (parameter $m$ in the basic reproduction number). The area under the probability density curves to the left of the vertical dashed line gives the relative risk of Ac(WT) having a higher vectorial capacity than Ac(DSM)2. These results indicate that the relative risk of female Ac(WT) mosquitoes having a higher pathogen transmission capacity than the female Ac(DSM)2 mosquitoes is about 0.70 for *P. falciparum* and o'nyong'nyong, and about 0.9 for lymphatic filariasis.

reflects the posterior update of the daily probability of mortality of the Ac(WT) strain, and the change in weight given to the experts when calculating the Bayesian model average of the posterior probability of daily mortality for the Ac(WT) and Ac(DSM)2 strains.

The Ross-Macdonald model forms the theory that guides modern models of vector-borne disease transmission (Reiner et al., 2013). The model is widely applied despite a number of important assumptions (Smith et al., 2012), including the assumption that the human host and vector populations remain static. The initial analysis presented here assumes that the vector to human host ratio $m$ is the same for both Ac(WT) and Ac(DSM)2 and so this parameter cancels out in the quantity $\mathcal{R}$ (along with the recovery rate from infectiousness in the human host population, $r$). The index $\mathcal{R}$ thereby shows the relative risk posed by an equivalent number of mosquitoes for each strain.

The quantity $\mathcal{R}$ can also be used to assess the consequences of letting the vector to human host ratio for the two mosquito strains – that is $m_{GE}/m_{WT}$ in Equation (3.1) – vary. Epopa et al. (2017) suggest that the population of male Ac(WT) in Bana village varies from between 100,000 - 500,000 in the wet season to between 10,000 - 50,000 during the dry season. The results of the entomological surveys also suggest that female Ac(DSM)2 are more numerous than males (Figure 1.2). If we conservatively assume, however, that males and females occur in equal numbers in Bana village, then the number of incidentally released Ac(DSM)2 females (stipulated to be less than 25) will be in the order 400 - 2,0000 times smaller then the local wild population depending on the time of the controlled field release[8].

Accounting for the larger Ac(WT) population will decrease the risk of transmission attributable to female Ac(DSM)2 mosquitoes. For example, if the female Ac(WT) population is 1000 times greater than the female Ac(DSM)2 population then the relative ratio of $R_0$ for the two populations is divided by 1000 because $m_{GE}/m_{WT} = 0.001$. Under this situation the predictive prior probability of increased pathogen transmission attributable to the incidental release of Ac(DSM)2 females is 0.04, 0.05 and 0.04 for *P. falciparum*, o'nyong'nyong virus and lymphatic filariasis respectively, whilst the predictive posterior probability is 0.03, 0.05 and 0.03 respectively. Note that the three orders of magnitude change in the vector-host ratio does not lead to a three-order magnitude reduction in risk of disease transmission because the quantity $P(\mathcal{R} > 1)$ is a non-linear function.

## 3.2 Novel blood-borne pathogen

Hayes et al. (2015) define a novel blood-borne pathogen to be any blood-based pathogen that has not previously been documented to be vectored by *An. gambiae* and in this context includes diseases such as Ebola and Hepatitis (Section 2). We apply this same definition here to *An. coluzzii*.

Hayes et al. (2015) used Fault Tree Analysis to estimate the probability that a G3 strain mosquito would transmit a novel pathogen in a year following an accidental escape of 10,000 mosquitoes from an insectary. The analysis subsequently examined if and how Ac(DSM)2 strain mosquitoes would differ from the G3 strain, and concluded that on the basis of a linear pool of expert opinion the risk would be the same or lower than that for G3.

[8]Here we have allowed for the possibility that the Ac(DSM)2 female population may increase if incidentally released at the beginning of the wet season (see Section 4.2) but have not accounted for the Ac(WT) or Ac(DSM)2 female mosquitoes that are removed from the population by any sampling conducted after the controlled field release

The fault tree focused on two primary pathways: (i) biological transmission where the pathogen is delivered in a malaria-like fashion via the saliva of the mosquito; and (ii) mechanical transmission where the pathogen is delivered via adhesion to the proboscis or via simple transport of contaminated blood between infected and uninfected individuals. We again note that whilst mechanical transmission of blood-borne pathogens has been previously documented in hematiphagous flies (see for example Vickerman, 1973; Hoch et al., 1985; Desquesnes and Dia, 2003) and mosquitoes (see for example Chamberlain and Sudia, 1961; Motha et al., 1984) we are unaware of any documented cases involving *An. coluzzii*.

In this analysis we assume that the experts' commentary and analysis regarding the effect of the construct in the G3 genetic background is transferable to the effect of the construct in the *An. coluzzii* genetic background. We also assume that the experts' responses for the G3 analysis are transferable to the Ac(WT) strain reared under laboratory conditions for 62 generations, with the exception of the probability that female mosquito will contact an infected human or vertebrate (fault tree event FT2000), and the probability that the mosquito survives the pathogen's incubation period (fault tree event FT2021).

In light of the previous analysis of female mortality, we allow for the possibility that experts may have allocated a higher probability to FT2021 by conservatively setting the probability of this event to 1 for all experts. The analysis also amends FT2000 to condition on the incidental release of 25 female mosquitoes rather than the accidental release of 5000 females mosquitoes that the original elicitation was conditioned on. To do this we assume a similar prevalence of novel blood-borne pathogens in the host populations and adjust the original elicitations for FT2000 ($p$) to be a probability of contact per mosquito ($p^*$)

$$\begin{aligned}(1-p) &= (1-p^*)^{5000} \\ p^* &= 1-(1-p)^{\frac{1}{5000}}\end{aligned} \tag{3.2}$$

so that the probability of at least one mosquito contacting an infected individual in a year following an incidental release of 25 female mosquitoes is $1-(1-p^*)^{25}$. Figure 3.12 illustrates the effect of applying Equation (3.2) to the linear pool prior elicited by Hayes et al. (2015).

The results of the amended fault tree analysis, accounting for the change in the number of female mosquitoes released and the possibility of increased survival are shown in Figure 3.13 for the two calculation strategies Aggregate First Then Convolute (AFTC) and Convolute First Then Aggregate (CFTA)[9].

The reduction in the number of incidentally released females has an overwhelming influence on the overall probability of transmitting a novel blood-borne pathogen, reducing the median probability from $5.1\times10^{-7}$ to $7.6\times10^{-9}$ under the AFTC strategy, with a similar two order of magnitude reduction under the CFTA strategy.

A conservative assessment that allows for a higher probability of survival slightly increases this median probability to $1.3\times10^{-8}$ under the AFTC strategy and $7.2\times10^{-9}$ under the CFTA strategy. Assuming that the experts' response to the Ac(DSM)2 versus G3 assessment are transferable, the assessment predicts that the probability of a female Ac(DSM)2 mosquito

[9]See Hayes et al. (2015) for further details of the fault tree analysis methods and differences between these two computational strategies.

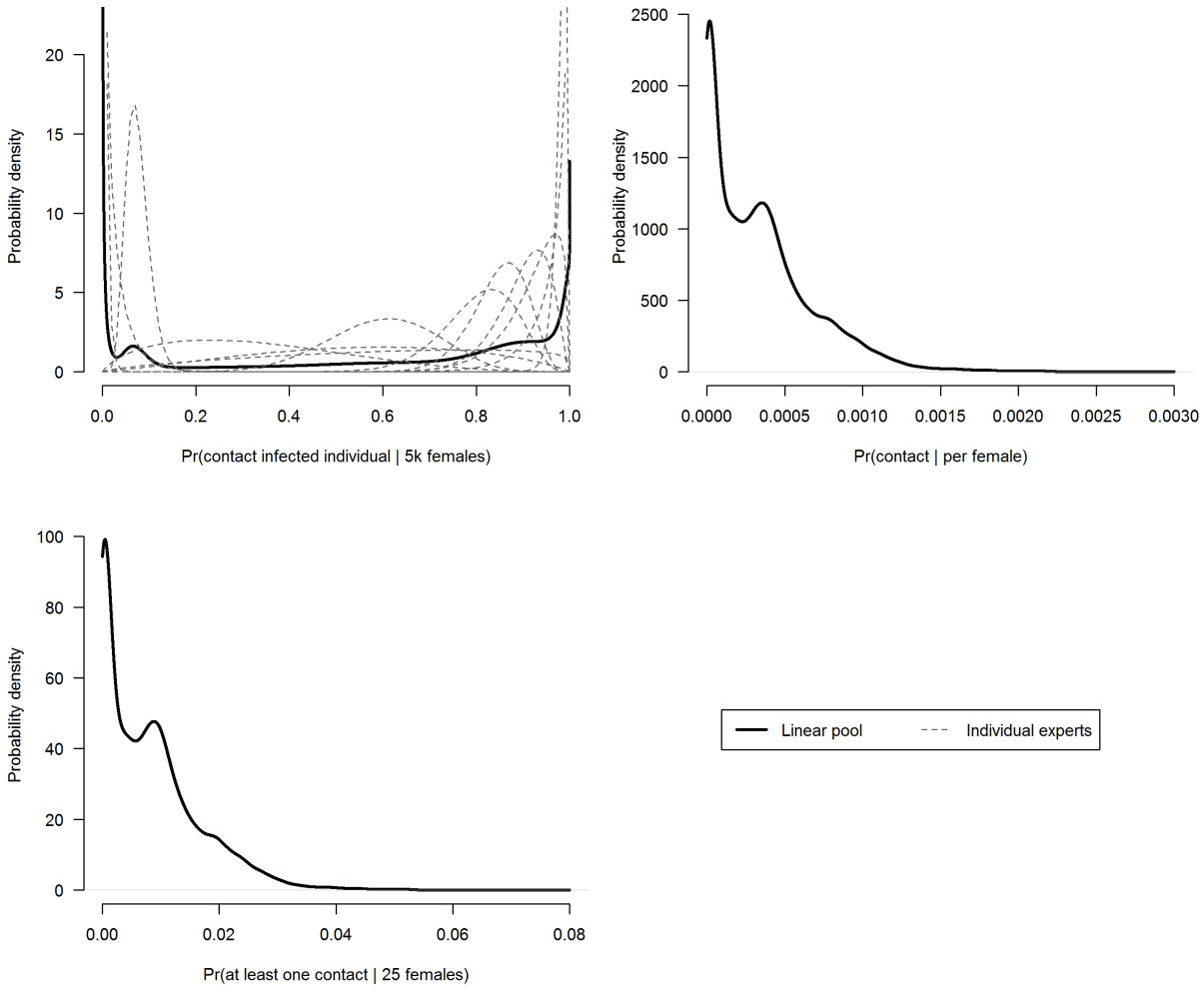


**Figure 3.12:** Informative prior probability that a female mosquito will contact a human or vertebrate infected with a novel blood-borne pathogen (initially elicited by Hayes et al., 2015) amended for n = 25 incidentally released females. The top left panel shows the original informative priors (and the linear pool) fitted to the experts' response to the probability of contacting an infected individual given an accidental escape of 5,000 female mosquitoes. The top right panel shows the probability of contact with an infected individual per female mosquito derived by applying Equation (3.2) to random samples drawn from the original linear pool prior. From this the probability of contact with at least one infected individual given 25 incidentally released female mosquitoes can be calculated (bottom left panel).

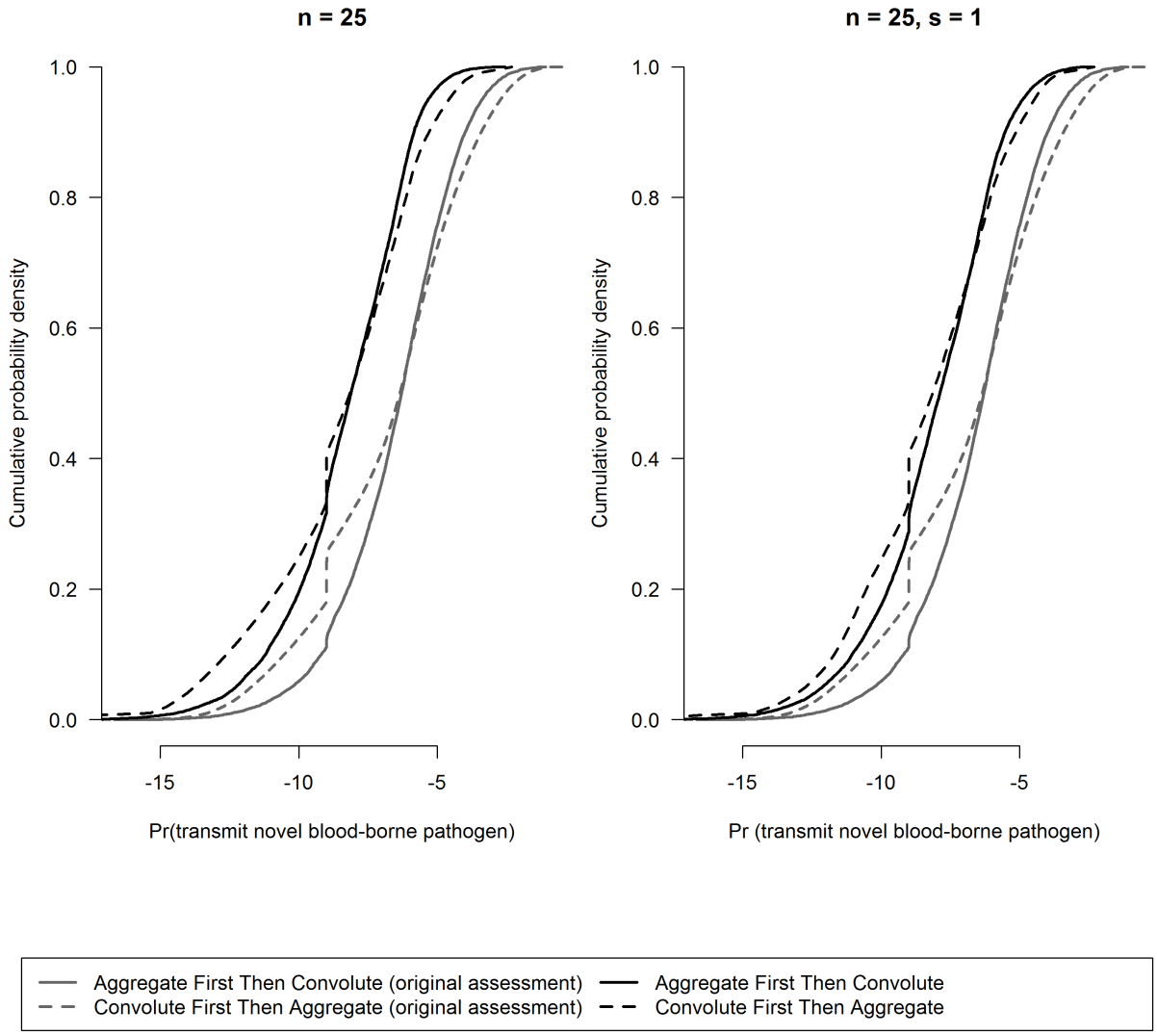


**Figure 3.13:** Updated fault tree analysis for the probability of vectoring a novel blood borne pathogen. The left hand panel shows the original results of the fault tree analysis (in grey) for two computation strategies – Aggregate First Then Convolute and Convolute First Then Aggregate. The updated analysis that accounts for a reduction in number of females released from 5,000 to 25 is shown in black. The right hand panel show the same information except this time the probability that the female mosquitoes will survive the pathogen's incubation period has been conservatively set to 1 to allow for the possibility of a higher daily probability of survival than was elicited by Hayes et al. (2015)..

transmitting a novel blood-borne pathogen in a year following the field trial will be the same or less than this.

# 4 TRANSGENE SPREAD AND PERSISTENCE

**KEY POINTS**

1. A simple model of female population dynamics, with an initial population of 25 Ac(DSM)2 females, predicts that the expected number of Ac(DSM)2 female mosquitoes (including offspring from the initial incidentally released population) will drop below one individual about 65 days after the release.
2. A detailed spatio-temporal model with an initial population size of 5,000 Ac(DSM)2 males predicts that the expected number of Ac(DSM)2 male mosquitoes (assuming males are not fertile) will drop below one individual 10 days after the release.
3. The median posterior probabilities of the catch efficiency of Pyrethroid Spray Catches, pots and swarm samples range from 0.5% to 3.5%. These values are not unusual and are typical of catch efficiency reported elsewhere, and they will influence the probability that post-release monitoring will detect Ac(DSM)2 mosquitoes.
4. Simulations based on the male dispersal and survival model indicate that there is a 30% chance that male Ac(DSM)2 mosquitoes will still be present in Bana if they are not observed in three sequential days of Mark Release Recapture equivalent survey effort implemented ten days after release.
5. The updated median probability of spread of the construct in local populations of *An. coluzzii* drops from a prior value of $1.4 \times 10^{-3}$ to a posterior value of $8.9 \times 10^{-4}$ under the Aggregate First Then Convolute calculation strategy, assuming all other elicitations in the fault trees for *An. gambiae* and *An. coluzzii* are transferable between the two species.
6. The updated median probability of spread of the construct in local populations of *An. gambiae* or *An. arabiensis* increases from a prior value of $1.7 \times 10^{-6}$ to a posterior value of $7.6 \times 10^{-6}$ under the Aggregate First Then Convolute calculation strategy. Under the Convolute First Then Aggregate strategy it increases from a prior value of $3.0 \times 10^{-5}$ to a posterior value of $2.1 \times 10^{-3}$. The increase is more marked because a change occurs in the number of experts responding to the updated events in the fault tree and this has a more pronounced effect on the overall results under this strategy.
7. The risk estimates for spread of the construct in local populations of *An. gambiae* or *An. arabiensis* are likely an overestimate because the analysis is unable to update the prior probability of hybridisation to reflect the low proportion of hybrids seen in the entomological survey samples taken from Bana Village.
8. Female Ac(DSM)2 are either no less susceptible (Fenitrothion and Bendiocarb, because mortality is 100% and almost 100% respectively) somewhat more (Alpha-cypermethrin and Lambda-cyhalothrin) or much more (Permethrin, Deltamethrin and Dichlorodiphenyltrichloroethane) susceptible than female Ac(WT) to insecticides.

## 4.1 Spread and persistence pathways

The survival and geographic dispersal of Ac(DSM)2 mosquitoes, or the persistence and spread of the construct into other non-target species, is a necessary event in many of the plausible Adverse Outcome Pathways identified in Section 2, and hence a key factor in the likelihood of adverse events associated with the field trial. The Dominant Sterile Male construct can persist and spread in the environment through four mechanisms:

- Female release pathways: Target Malaria has stipulated that no more than 5 Ac(DSM)2 females will be released for every 1,000 Ac(DSM)2 males. Female Ac(DSM)2 mosquitoes are hemizygous fertile. If these females mate with wild type *An. coluzzii* males half of their offspring will be transgenic, and approximately half of these individuals will be female and therefore capable of passing the Dominant Sterile Male construct on to their offspring. Ac(DSM)2 females may also mate with other sexually compatible Anopheline species in the field opening the possibility for introgression of the construct into a non-target species.
- Male release pathways: Male Ac(DSM)2 mosquitoes are hemizygous sterile. Following the initial dispersal of the deliberately released Ac(DSM)2 males, the Dominant Sterile Male construct can only persist and spread if it fails to sterilize transgenic male mosquitoes that might arise from three possible sources: (i) the deliberately released male Ac(DSM)2 population; (ii) Ac(DSM)2 males born to Ac(DSM)2 mothers in the field; or (iii) transgenic males born to transgenic mothers of another sexually compatible species.
- Enhanced resistance to insecticide: If insecticide resistance emerges it will exert a positive selection pressure, particularly on female mosquitoes in areas where insecticide treated bed nets are widely used. If female Ac(DSM)2 mosquitoes are more resistant to insecticides than female Ac(DSM)2 mosquitoes then this selection pressure would contribute to the persistence and spread of the Dominant Sterile male construct through female and male pathways.
- Horizontal gene transfer pathways: the genetic construct could persist and spread in populations of other eukaryotes and non-eukaryotes through horizontal gene transfer mechanisms.

Based on the analysis conducted by Hayes et al. (2015), this assessment predicts that the probability of horizontal gene transfer following the controlled field release will be less than $1.2 \times 10^{-10}$. This probability is too small to warrant further attention, hence this pathway is not addressed any further in this analysis.

The following subsections model the female and male release pathways to estimate the survival (and dispersal in the case of males) of the released populations of Ac(DSM)2 mosquitoes. The probability of hybridization with sexually compatible species, and the probability of increased insecticide resistance are addressed as part of the female pathways. The probability of introgression of the construct into local wild type populations, and local populations of sexually compatible species, is addressed under the male pathways by amending the fault tree analysis conducted by Hayes et al. (2015).

## 4.2 Female release pathways

### 4.2.1 Incidental release of Ac(DSM)2 females

If Ac(DSM)2 females are incidentally released during the field trial then vertical transfer of the genetic construct is most likely to occur through mating with Ac(WT) males in the field. The likelihood of this event decreases as the number of females released declines because the probability of the Ac(DSM)2 female population becoming extinct through demographic stochasticity increases as population size decreases (Pimm et al., 1988). The challenge in this context, however, is that stochastic spatio-temporal models of female population dynamics are complex and difficult to accurately parameterise with the relatively limited amount of information on female *An. coluzzii* dynamics.

In light of these constraints, the risk assessment developed a simple model of Ac(DSM)2 females population dynamics that does not include demographic stochasticity or related Allee effects. The model, described in detail in Section A.2, assumes a seasonally fluctuating birth rate, a constant mortality rate and no net annual population growth, and is parameterised using the observed time series of female *An. gambiae* s.l. abundance (Figure 4.1).

Figure 4.2 shows the summary statistics (median, 5th and 95th percentiles) of 5,000 model simulations of the expected number of Ac(DSM)2 females following the controlled field release. The model predicts that on average the population will steadily decline, although some simulations allow the population to grow through the wet season before collapsing with the onset of the dry season. Given the parameterisation and model assumptions, the median model prediction is that the expected number of Ac(DSM)2 female mosquitoes will drop below one individual about 65 days after the release.

### 4.2.2 Hybridisation with sexually compatible species

The incidental release of fertile Ac(DSM)2 females opens the possibility of vertical transmission of the genetic construct to other sexually compatible species within the *An. gambiae* complex. The probability of vertical transmission to species outside of this complex is considered to be virtually nil (*pers. comm.* Nora Besansky, 2nd March 2015) because:

- the closest relatives outside of the *An. gambiae* complex are species within the Pyretophorus series, only one of which, *An. christyi*, is known from Africa. *An. christyi* is a highland species occurring mainly between 4,700 and 8,000 feet in eastern and central Africa. It has not been recorded in any of the entomological surveys conducted by Target Malaria in Burkina Faso (Figure 1.2).
- a Target Malaria commissioned analysis of genetic introgression between the sequenced genomes of *An. gambiae* complex species and *An. christyi*, using the methods described in Fontaine et al. (2015), found no evidence of gene flow between *An. christyi* and any other member of the complex.

Within the *An. gambiae* complex hybridisation rates are typically low. F1 hybrids between the two most closely related species (*An. gambiae* s.s. and *An. coluzzii*) are only thought to occur in about 0.1% of samples (*pers. comm.* Nora Besansky, 2nd March 2015), an assertion supported by the results of the Bana entomological surveys that detected hybrids in only 0.4% of samples (Figure 1.3). The small predicted population of Ac(DSM)2 females, together with the low rates of hybridisation, suggests that hybridisation will not be a highly probable event in the spread and persistence pathways.

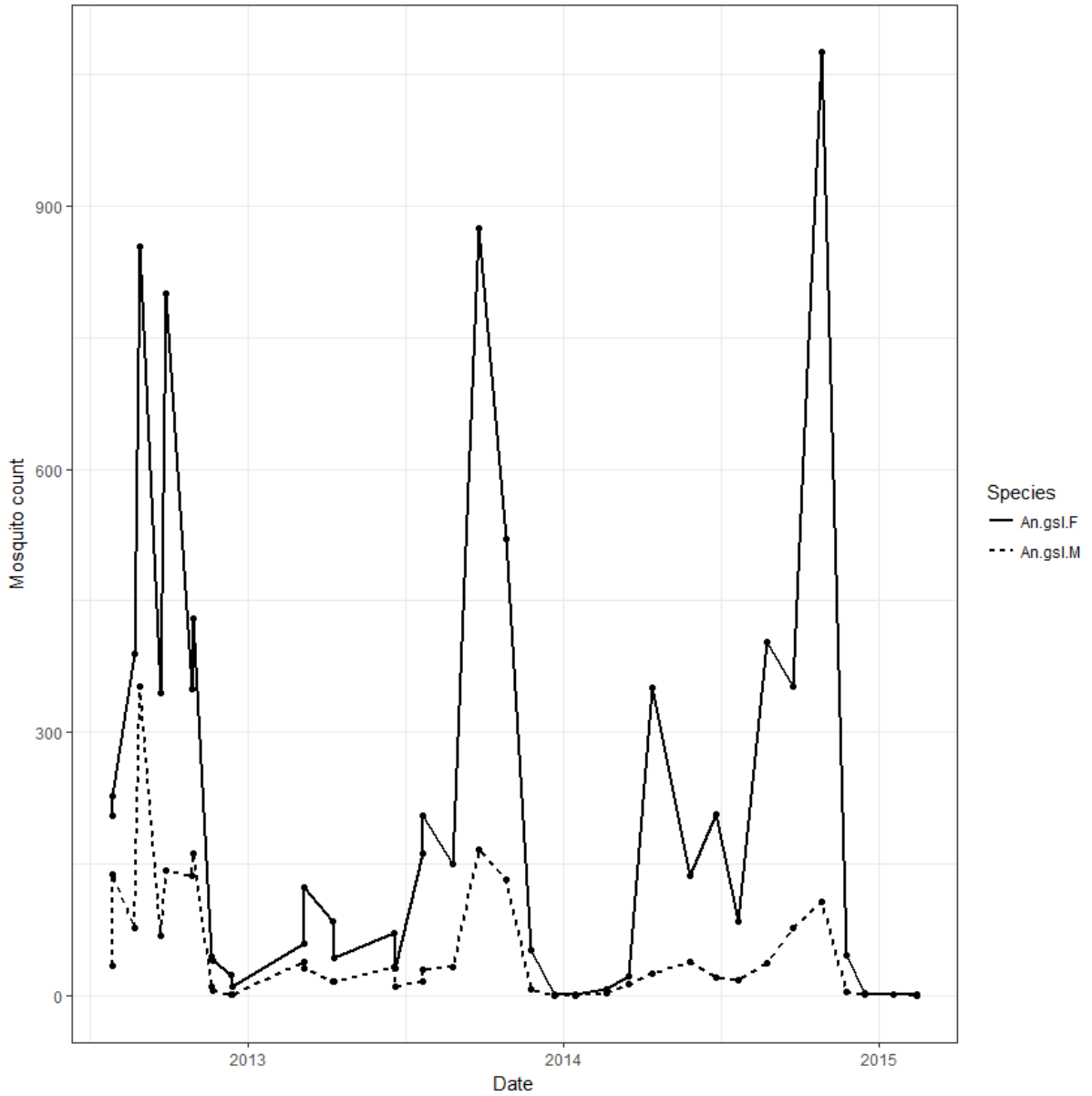


**Figure 4.1:** Time series of male and female *An. gambiae* s.l. abundance in Bana Village between July 2012 and February 2015 (Data provided courtesy of Target Malaria). The abundance of male (dashed line) and female (solid line) *An. gambiae* s.l. (An.gsl) mosquitoes exhibits strong seasonal fluctuations, increasing during the wet season and declining during the dry. These observations are used to parameterise a simple model of female Ac(DSM)2 population dynamics that matches this seasonal cycle in abundance by assuming birth rate varies seasonally whilst death rate remains constant.

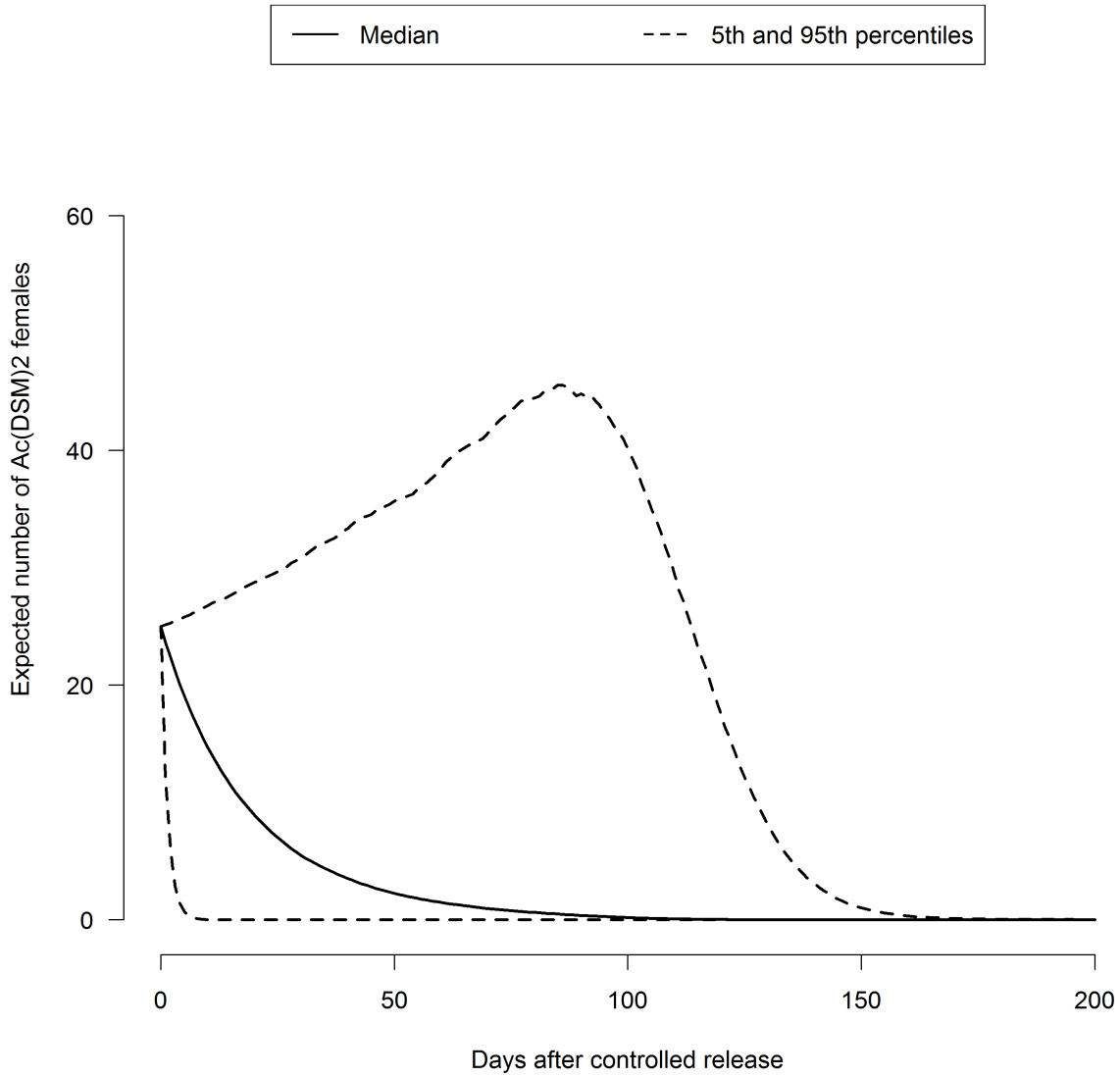


**Figure 4.2:** Simulations of Ac(DSM)2 female abundance following an incidental release of 25 Ac(DSM)2 females at the beginning of the wet season in Bana Village. This plot shows that 95% of the simulations of a temporal model of Ac(DSM)2 female survival predict that the expected number of females will drop below one individual 150 days after the planned field release. The median prediction is that the expected Ac(DSM)2 female population will drop below one individual after 65 days.

### 4.2.3 Insecticide resistance

In 2016 and 2017 Target Malaria conducted insecticide resistance tests on an *An. gambiae* Kisumu strain housed in the IRSS insectary's original bioassay laboratory, and on the Ac(WT) and Ac(DSM)2 strains housed in a separate confined laboratory. In each case susceptibility tests with 3-5 day old, non blood-fed adult females were carried out with seven active ingredients (Alpha-cypermethrin, Bendiocarb, Dichlorodiphenyltrichloroethane (DDT), Deltamethrin, Fenitrothion, Lambda-cyhalothrin and Permethrin) according to internationally recognised testing procedures (Target Malaria, unpublished data).

The insecticide susceptibility tests were performed in seven separate batches comprising four treatment replicates, each consisting of 20 to 25 mosquitoes, and two control replicates with an equivalent sample size. The number of alive and dead mosquitoes at the end of each test was recorded and converted into probability of death per replicate. An initial exploratory data analysis shows high levels of survival in the 14 control replicates and signs of resistance[10] to Alpha-cypermethrin, Dichlorodiphenyltrichloroethane, Deltamethrin, Lambda-cyhalothrin and Permethrin in Ac(WT) strains (Figure 4.3).

In this analysis we focus on the Ac(WT) and Ac(DSM)2 strains and seek to quantify the probability that the Ac(DSM)2 strain has a lower susceptibility (higher resistance) to any of the active ingredients. The analysis uses a Bayesian hierarchical model (described in detail in Section A.3), with weakly informative priors, to calculate the risk endpoint $\mathcal{R}$ – that is the posterior predictive difference in the probability of mortality of female Ac(DSM)2 and Ac(WT) mosquitoes under laboratory conditions:

$$\mathcal{R} = \left(p_i^{GE} - p_i^{WT}\right)$$

where $p_i^{GE}$ is the probability of mortality for the Ac(DSM)2 strain under insecticide treatment $i$, and $p_i^{WT}$ is the equivalent probability of mortality for the wild type strain. Positive values of the risk endpoint indicate that the Ac(DSM)2 strain has a higher susceptibility (higher probability of mortality) to the insecticide treatment than the wild type strain. Negative values indicate the reverse. The results of this analysis are summarised in Figure 4.4.

The analysis shows that the probability of Ac(DSM)2 mortality in the controls is slightly lower than Ac(WT) (median difference is $-0.0049$). For Bendiocarb and Fenitrothion the difference in the median probability of mortality between the two strains is $1.84 \times 10^{-4}$ and $8.43 \times 10^{-10}$ respectively because both of the these treatments are almost (in the case of Bendiocarb) and totally (in the case of Fenitrothion) 100% effective.

For the remaining insecticide treatments Ac(DSM)2 appears to be more susceptible than Ac(WT) with median differences of $0.19$, $0.24$ and $0.13$ for Permethrin, Deltamethrin and Dichlorodiphenyltrichloroethane respectively, and lower but still positive differences for Alpha-cypermethrin and Lambda-cyhalothrin. The evidence therefore suggests that female Ac(DSM)2 mosquitoes will be just as, or more, susceptible than their wild counterparts to these insecticide treatments.

[10] According to the World Health Organisation protocol mortality rates below 80% are considered to be indicative of insecticide resistance (World Health Organisation, 2016)

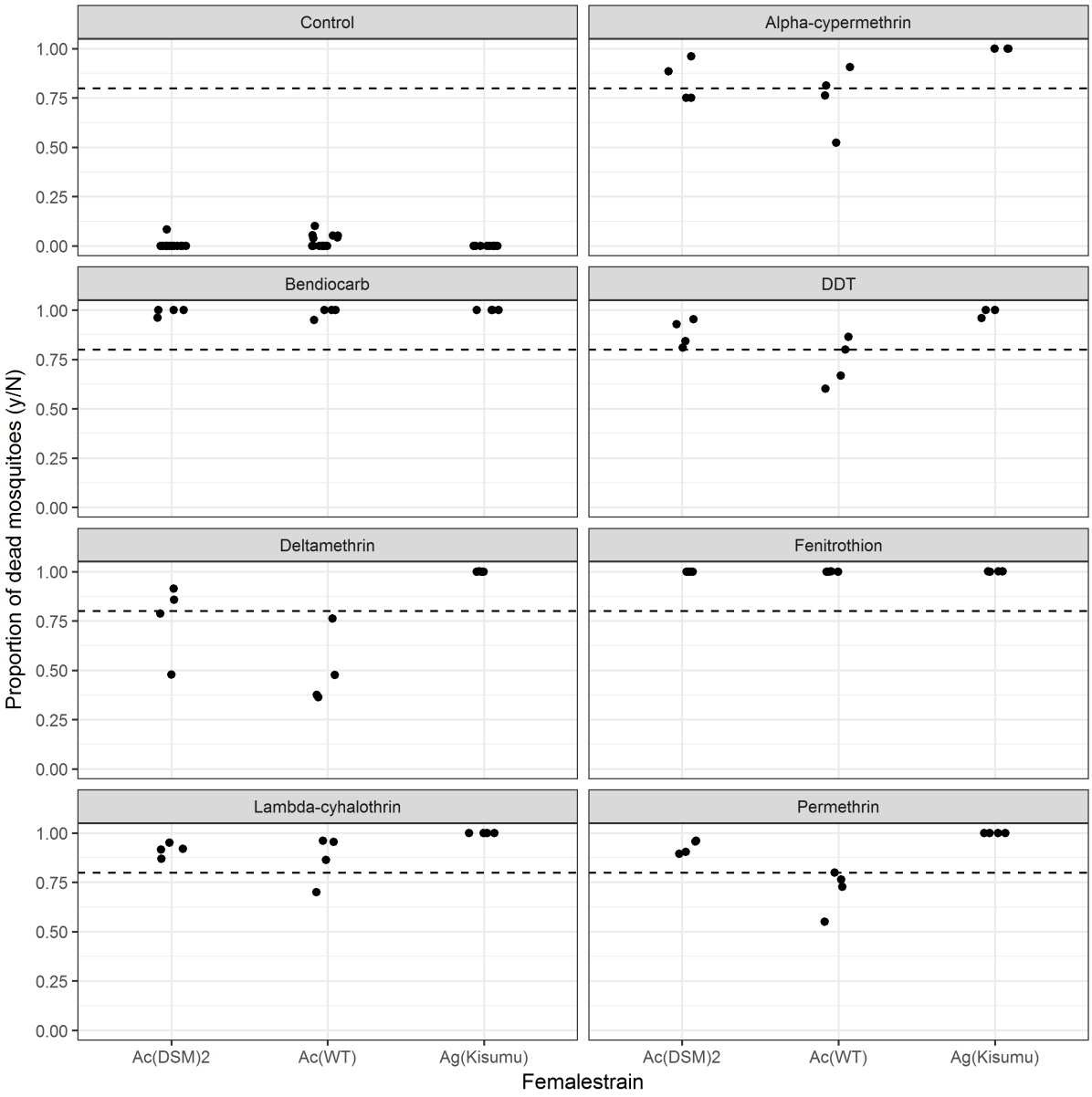


**Figure 4.3:** Results of insecticide resistance tests conducted to World Health Organisation standards in the IRSS insectary on adult female Ac(WT) and Ac(DSM)2, and on an An. gambiae strain housed in a separate laboratory (Data provided courtesy of Target Malaria). This plot shows the contrast between the proportion of dead mosquitoes in the experiment's control groups (top left panel) compared to the proportion of dead mosquitoes under each of the seven insecticide treatment groups. The levels of mortality in the wild type strain varies from 100% in the Fenitrothion treatment, to less than 50% in three of the four Deltamethrin replicates. Observations of mortality below the dashed horizontal line are indicative of insecticide resistance according to the World Health Organisation protocol.

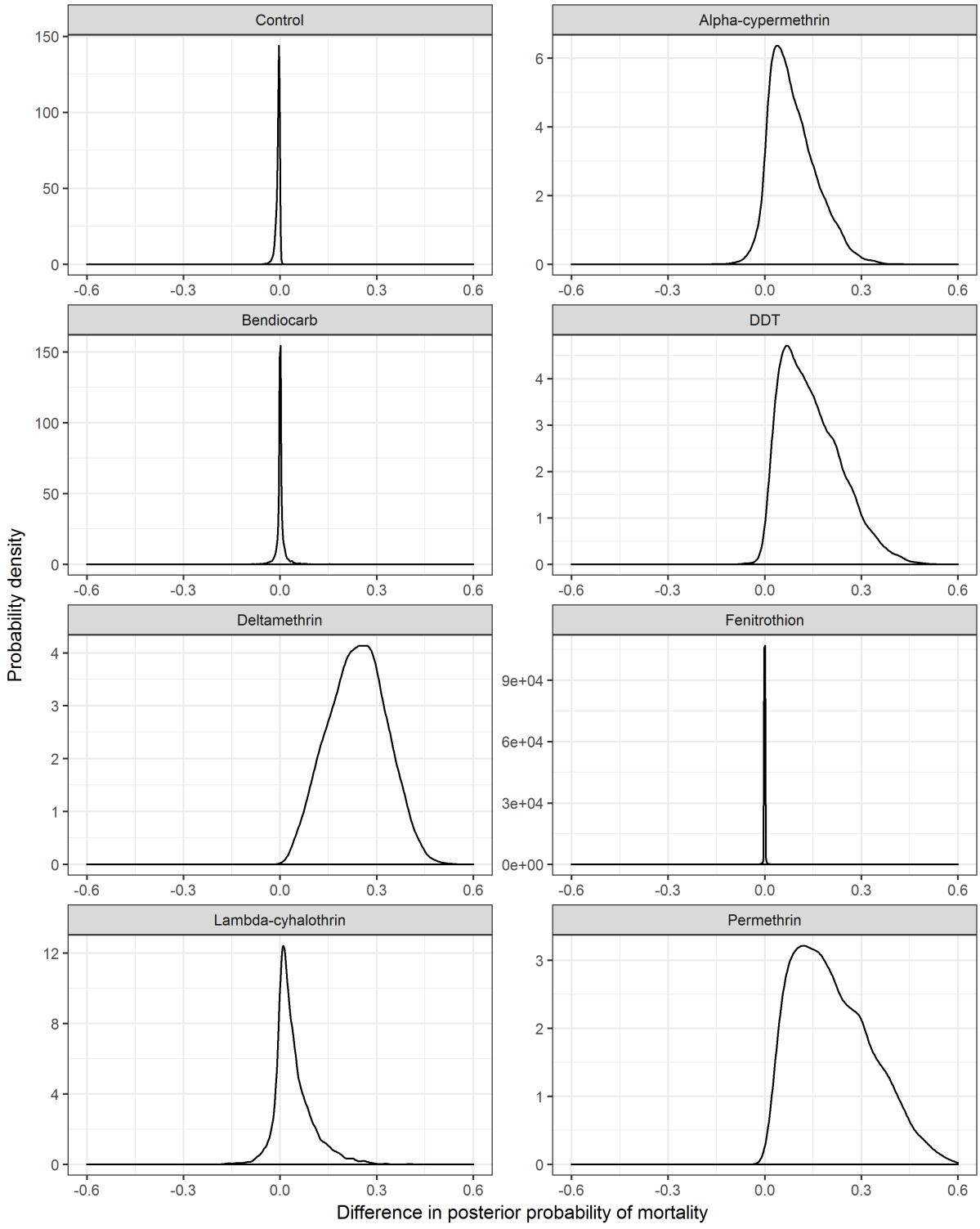


**Figure 4.4:** Posterior predictive difference in the probability of mortality between female Ac(DSM)2 and female Ac(WT) mosquitoes. If the posterior predictive difference in mortality is positive then this indicates that female Ac(DSM)2 mosquitoes are more susceptible (experiences higher levels of mortality) than female Ac(WT) mosquitoes to the insecticide. Negative values indicate the reverse. These results indicate that Ac(DSM)2 females are just as, or more, susceptible to insecticide than their Ac(WT) counterparts.

## 4.3 Male release pathways

### 4.3.1 Spread and survival of sterile males

Target Malaria have completed five Mark Release Recapture experiments in Bana village, and have published analysis of data from the first four (Epopa et al., 2017). The experiments used swarm sampling, Pyrethroid Spray Catches (PSC) and humidified clay pots to recapture mosquitoes dyed with one of three colours (Figure 4.5). The results of the analysis of the first four experiments suggest that the male mosquito population in Bana village may reach 100,000 - 500,000 in the wet season, and decline by an order of magnitude to 10,000 - 50,000 in the dry season. The analysis also indicates that recapture rates are low (0.3% to 1.7%), but well within the limits typically reported in the literature, and that male mosquito dispersal distance varies from about 40m to 550m (Epopa et al., 2017).

The availability of empirical data on wild type male dispersal and mortality, and catch rates, from these Mark Release Recapture experiments, together with the planned size of the controlled release of male Ac(DSM)2 mosquitoes, warrants a more thorough analysis of the dispersal and survival of the male Ac(DSM)2 population. The risk assessment therefore developed a hierarchical, Bayesian spatio-temporal model to predict the survival and geographical dispersal of male Ac(DSM)2 mosquitoes in Bana following the field release. The model also enables a simulation-based analysis of the probability that post-release monitoring will detect male Ac(DSM)2 mosquitoes. Here we provide a summary of the results of this analysis for Target Malaria's initial post-release monitoring plan.

The model is described in detail in Section A.4, but essentially consists of: (i) a partial differential equation that describes the unobserved (latent) process of male mosquito dispersal and mortality in time and space; and, (ii) a two-stage observation model that accounts for: a) the variation in the number of mosquitoes in the vicinity of a trap given the expected population size; and, b) the variation in mosquito counts given the number of mosquitoes in the vicinity of a trap and the probability of capture. The model is parameterised with informative priors (derived from the literature and Hayes et al. (2015)) and weakly informative priors. Posterior estimates of the model parameters are derived where possible using the Mark Release Recapture data.

From a risk assessment perspective the key issue following the release is the expected time to extinction. This event underlies many of the community concerns (Section 2) and is a key determinant in the probability of non-target effects (Section 5). For the purposes of the risk assessment we define extinction to have occurred when the expected number of mosquitoes, denoted $\lambda_t(\theta_d) = E\left[n(t_e)\right]$ falls below one individual. The expected number of mosquitoes is given by the spatio-temporal process model, which is dependent on the model parameters $\theta_d$.

It is important to note that the predicted number of mosquitoes in the vicinity of a trap is a modelled as a Poisson process with mean $\lambda_t(\theta_d)$ – that is the expected number of mosquitoes in the vicinity of the trap (Equation (A.18)). This Poisson process will still lead to simulated observations of mosquitoes in the observation model (Equation (A.17)), albeit in very low numbers, when the expected number of mosquitoes falls below 1. The definition of extinction used here is simple and easy to communicate but it is not meant to preclude alternative choices in other analyses.

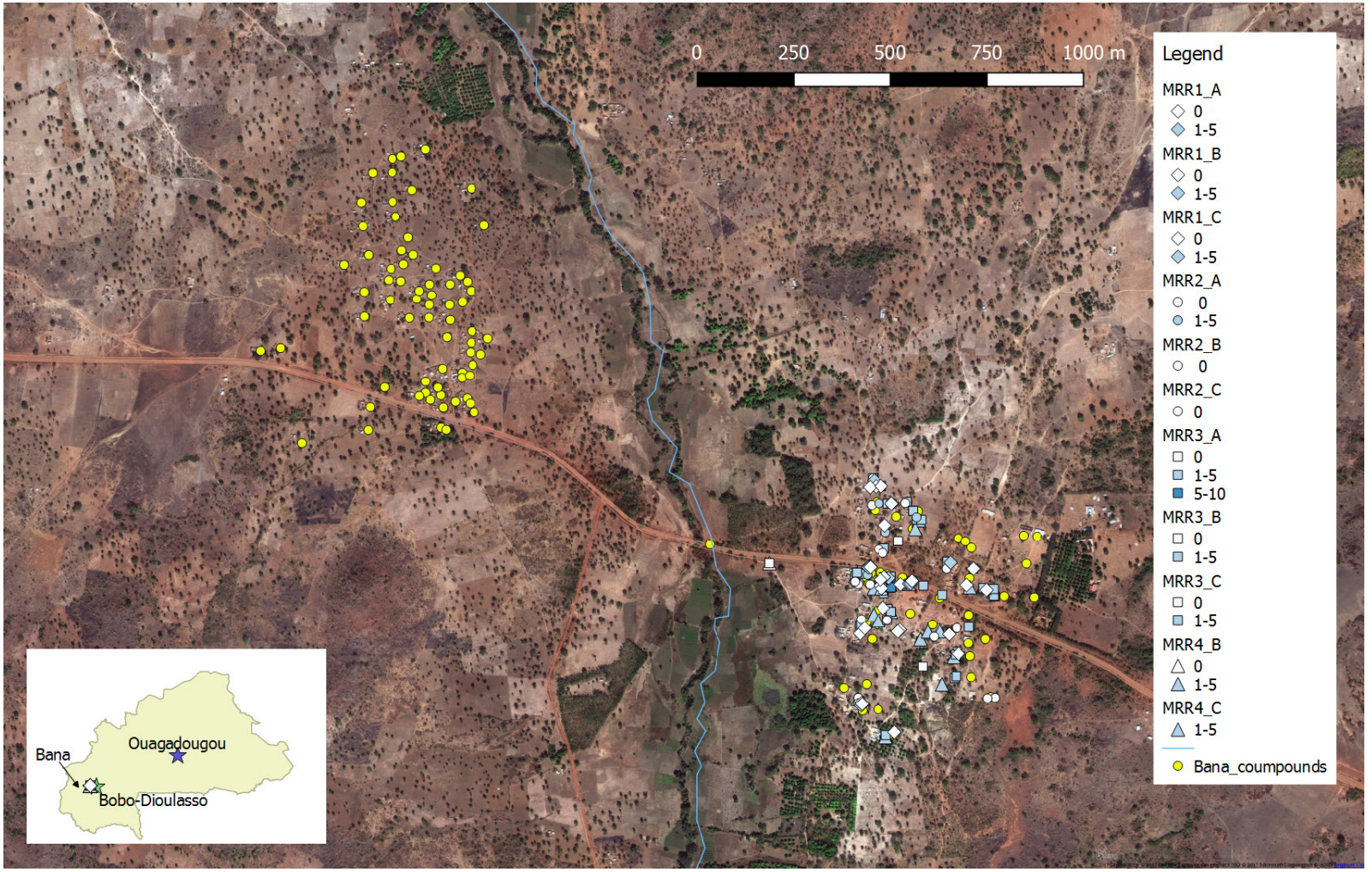


**Figure 4.5:** Summary of the results of the first four Mark Release Recapture experiments conducted in Bana Village with wild type strain *An. coluzzii*. In each experiment mosquitoes were dyed with one of three colours, labelled here A, B and C. The plot shows the number and location of dyed mosquitoes captured by all methods in the first four experiments.

Figure 4.6 captures the key risk-relevant result from the posterior simulations of male Ac(DSM)2 dispersal and survival. The results of the posterior simulations indicate that the 90% central credible interval for the time taken for the expected number of Ac(DSM)2 mosquitoes to fall below one individual, following a controlled field release of 5000 individuals, is $6$ to $20$ days, with a median value of $10$ days.

In this context it is important to note that the posterior estimates of mosquito trap efficiencies are consistent with the values reported in the literature (see for example Guerra et al., 2014). The posterior distributions for the Pyrethroid spray catches, pot and swarm catches suggest that catchabilities range from 0.5% to 3.5%. This will reduce the power of any planned post-release monitoring strategy, and thereby make it harder to confirm that the male Ac(DSM)2 population is actually extinct.

Simulations based on the male dispersal and survival model developed here, for example, indicate that there is a 30% chance that male Ac(DSM)2 mosquitoes will be still be present in the vicinity of Bana, even if they have not been observed after three sequential days of observation with equivalent survey effort[11] ten days after the initial release. If, however, three days of equivalent survey effort fails to detect male Ac(DSM)2 mosquitoes 30 days after the release, then the model predicts that there is $95\%$ chance that the probability of male Ac(DSM)2 mosquitoes still being present in the vicinity of Bana is less than $0.003$ because the model predicts with a high probability that the expected number of mosquitoes in the vicinity of Bana village will be less than one individual at this time.

### 4.3.2 Probability of construct failure

The results of the analysis described in Section 4.3.1 assume that the male mosquitoes are sterile. As a result there is no birth term in the reaction component of Equation (A.14) (Section A.4). Hayes et al. (2015) elicited informative priors for the probability that the Dominant Sterile Male construct will fail in the field (thereby allowing fertile Ac(DSM)2 males) as part of an analysis that the construct is able to persist and spread in the environment through vertical transmission to local populations of *An. gambiae*, *An. coluzzii* or *An. arabiensis*.

During the development and testing of the Dominant Sterile Male construct, Target Malaria conducted a number of sterility trials with Ag(DSM)2 to test the efficacy of the construct (Windbichler et al., 2008; Klein et al., 2012). These trials were continued after the initial risk assessment was completed in order to increase the sample size. Target Malaria have also conducted additional sterility trials with Ac(DSM)2 in the IRSS insectary (Target Malaria, unpublished data).

For the purposes of this analysis, CSIRO and Target Malaria collated the results of all of the sterility trials completed to date. These results are summarised in Figure 4.7 which shows the male experimental treatments – that is: (i) Ac(DSM)2 males mated with Ac(WT) females (top row); and, (ii) Ag(DSM)2 males mated with laboratory G3 strain females (bottom left panel)[12]. Also shown are the experimental controls – that is (i) Ac(WT) males mated with Ac(WT) females (middle row); and, (ii) G3 strain males mated with Ag(DSM)2 females and G3 strain females (bottom right panel).

[11] Equivalent to the effort expended in the first four Mark Release Recapture experiments

[12] The results for Ag(DSM)1 have been excluded from this analysis because the Dominant Sterile Male construct is different

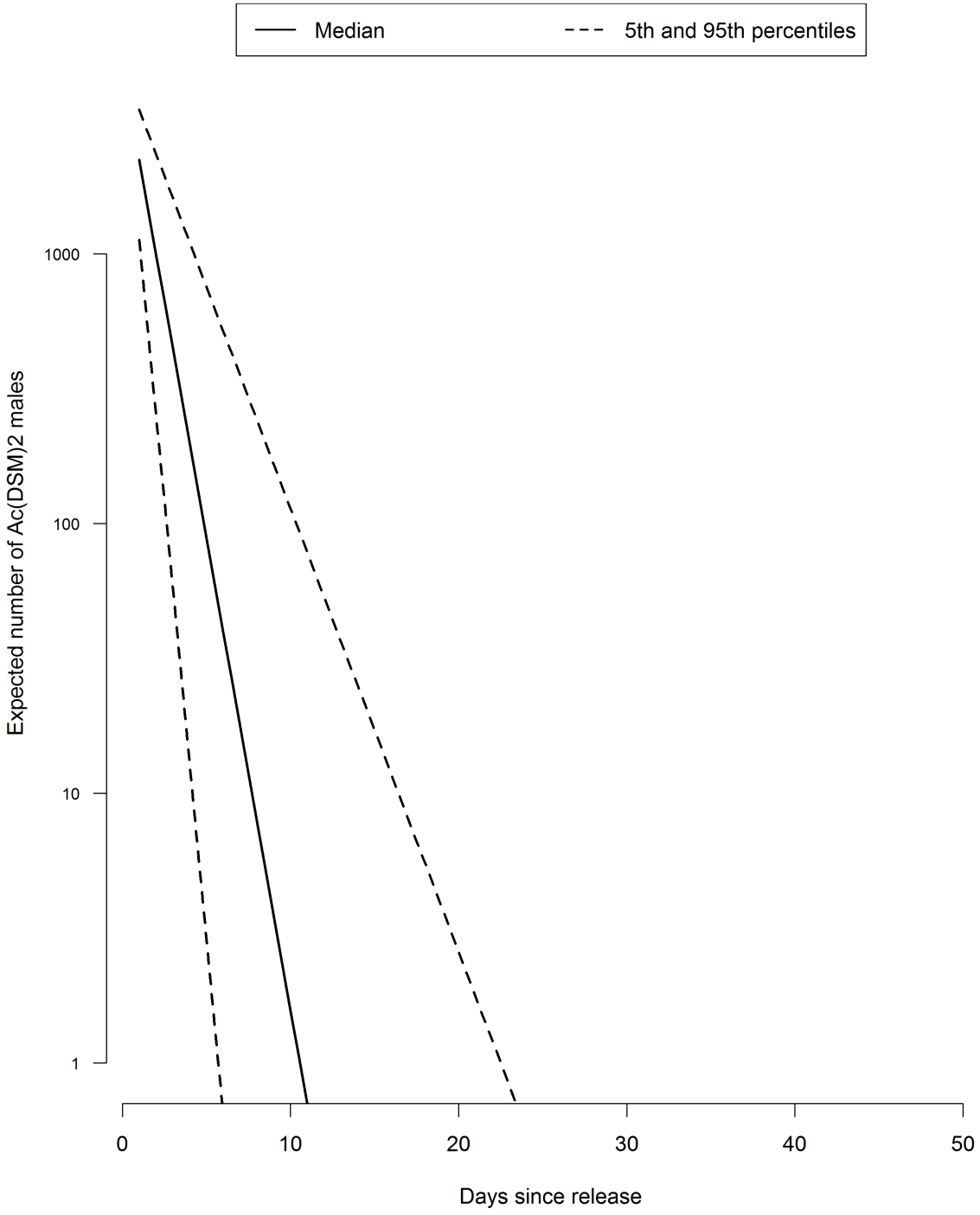


**Figure 4.6:** Simulations of Ac(DSM)2 male abundance following a controlled release of 5,000 male Ac(DSM)2 mosquitoes in Bana village. This plot shows that 95% of the simulations of a spatio-temporal model of Ac(DSM)2 male dispersal and survival predict that the expected number of males will drop below one about 20 days after the controlled field release.

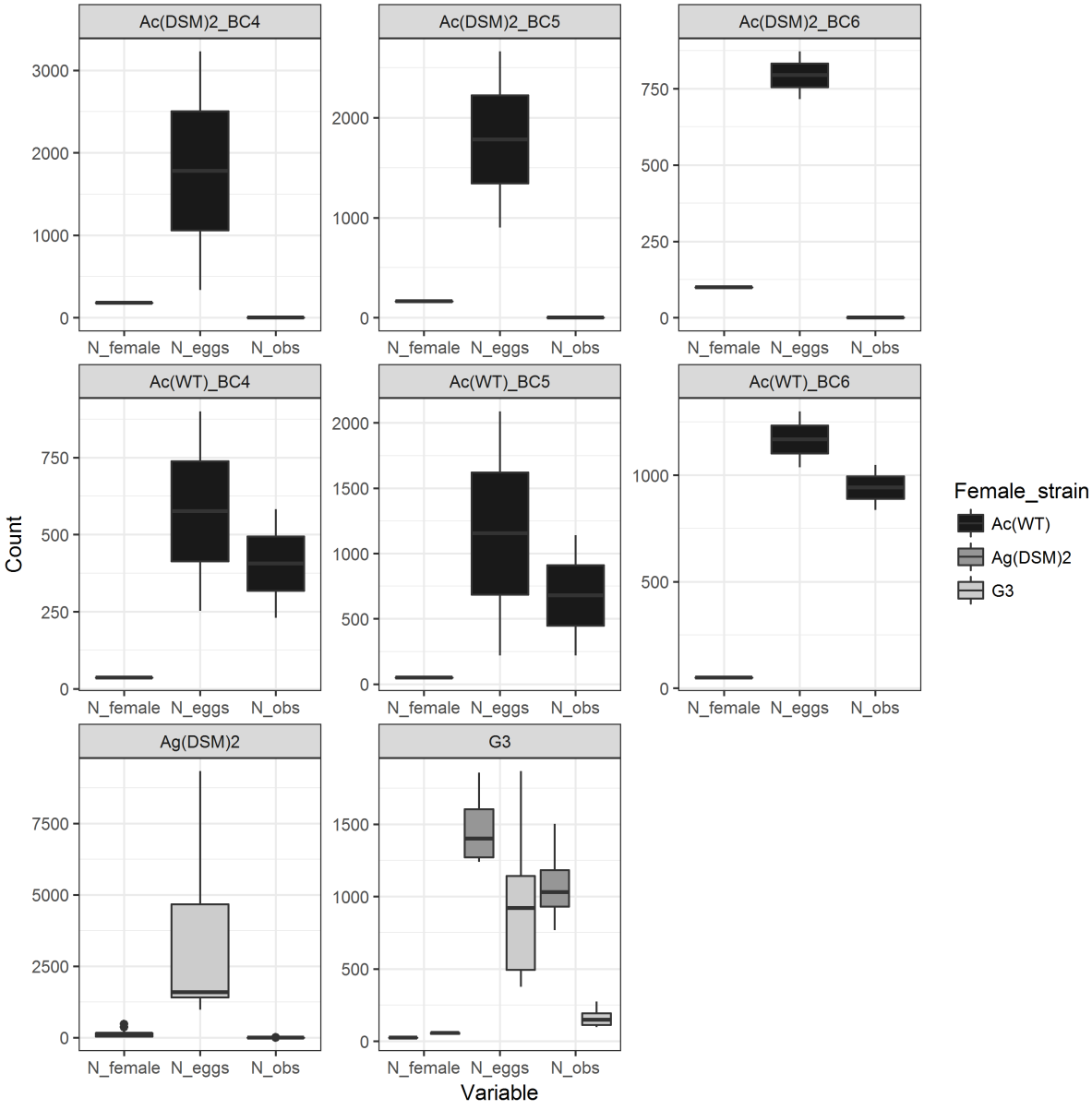


**Figure 4.7:** Summary of sterility experiments conducted with male and female Ag(DSM)2 and Ac(DSM)2 strain mosquitoes under laboratory conditions. All experiments involving male Ac(DSM)2 mosquitoes mated to female Ac(WT) mosquitoes (top row) have resulted in no observations of hatched eggs or larvae (N_obs = 0), whereas when male Ac(WT) mosquitoes are mated to female Ac(WT) mosquitoes hundreds to thousands of hatched eggs or larvae are observed (middle row). On three occassions matings between male Ag(DSM)2 mosquitoes and female G3 strain mosquitoes have led to 8 hatched eggs or larvae (bottom left panel) none of which subsequently survived to adulthood.

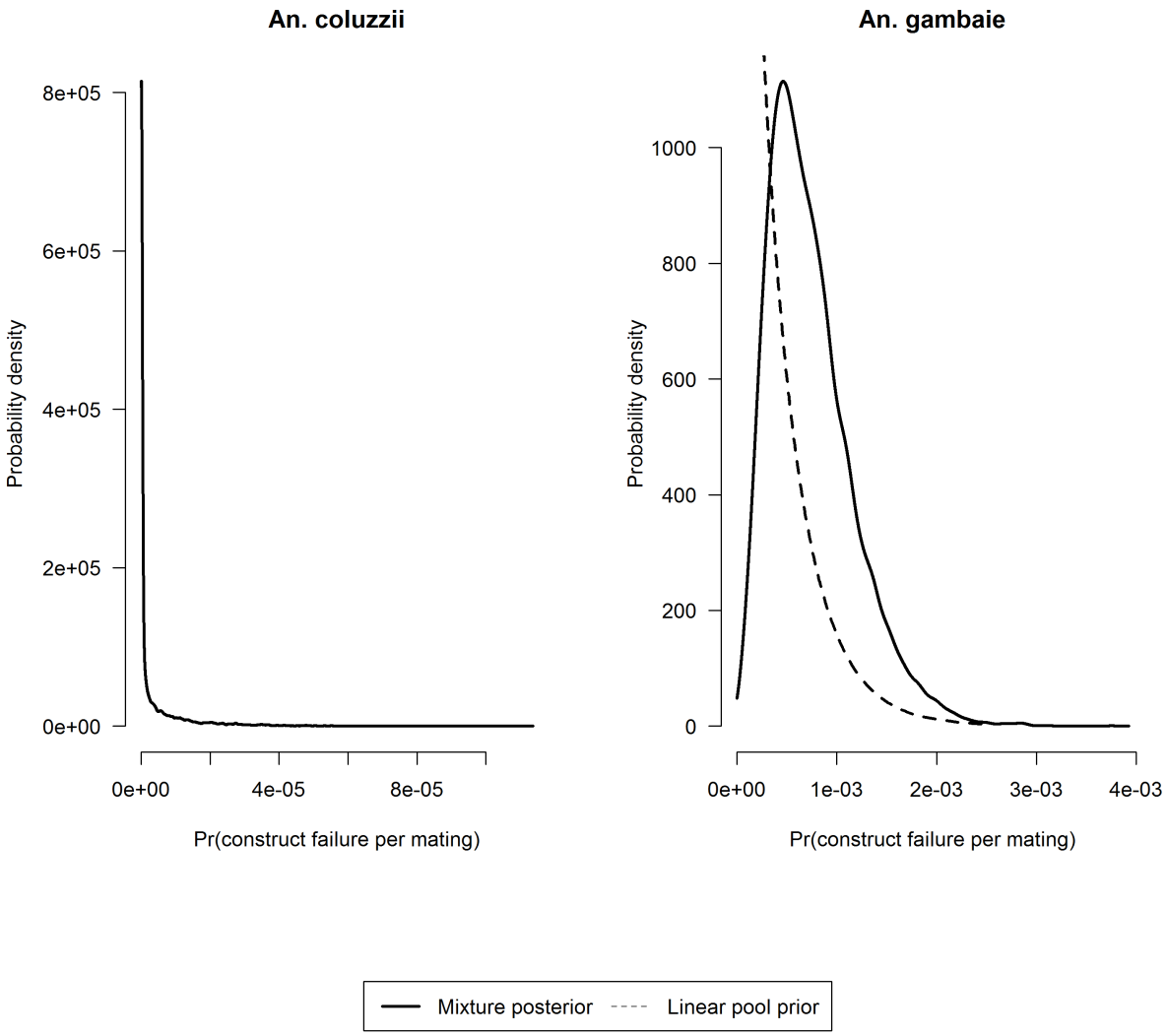


**Figure 4.8:** Linear pool prior and mixture posterior for the event that the construct fails to sterilise *An. coluzzii* (left) and An. gambiae (right). The linear pool priors shown here (dashed line) were collected by Hayes et al. (2015), and have been adjusted to a probability of failure per mating to allow for a Bayesian update using laboratory trials of the efficacy of the Dominant Sterile Male construct. The posterior prediction (solid line) is a Bayesian model average that weights the contribution of each expert's prior according to how well they predicted the data. The analysis shown here assumes that the construct's efficacy in the field will be the same as that observed laboratory conditions. Note that the linear pool prior for *An. coluzzii* is masked here by the mixture posterior.

The summary statistics from the sterility trials conducted to date with Ac(DSM)2 and Ag(DSM)2 mosquitoes are as follows: (i) control matings between wild type (or transgenic) females and non-transgenic control males (median number of matings = $50$) lead to large numbers of fertile eggs (median n = $1.14 \times 10^3$), and hence larvae or hatched eggs (median n = $272$); (ii) none of the matings between Ac(DSM)2 males and Ac(WT) females (total = $888$) have resulted in any observations of larvae or hatched eggs; and, (iii) matings between Ag(DSM)2 males and G3 strain females (total = $2086$) have led to a total of 8 larvae or hatched eggs from $y = 3$ observations of apparent construct failure. None of these larvae, however, survived to adulthood.

The availability of these experimental observations allows the risk assessment to calculate the Bayesian posterior probability of construct failure, which we subsequently use to update the fault tree analysis for the probability that the construct will spread into local populations originally completed by Hayes et al. (2015) using informative priors. The Bayesian inference is described in detail in Section A.5.

The results of the analysis for *An. coluzzii* are summarised in Figure 4.8. These results suggest that the median posterior probability of construct failure in one or more Ac(DSM)2 males, following the release of 5,000 males, will be $2.5 \times 10^{-4}$.

### 4.3.3 Probability of hybridisation with compatible species

The results of the entomological surveys point to very low rates of hybridisation between *An. coluzzii*, *An. gambiae* and *An. arabiensis* in Bana Village, and during the wet season (when mates are plentiful) hybrids are absent from the field samples (Figure 1.3). These results suggest that the prior probabilities elicited from experts by Hayes et al. (2015) may be too high. We do not believe, however, that it is possible to derive a posterior distribution for this event in the fault tree analysis because the elicitations do not condition on the time of release and the large fluctuations in the wild populations that this entails.

It is also difficult to adjust the hybridisation priors for the small number of incidentally released females in the same way that the other priors have been adjusted (Section 3.2) because the elicitations were conditioned on the release of male and female mosquitoes but they do not distinguish between that component of the prior attributable to the release of the Ag(DSM)2 females and that component attributable to the release of Ag(DSM)2 males.

### 4.3.4 Bayesian update of FT50 and FT51

Hayes et al. (2015) used fault tree analysis to examine the probability that the construct will spread in local populations of *An. gambiae* in a year following an accidental release of 5,000 Ag(DSM)2 females and 5,000 Ag(DSM)2 males (FT50). The same approach was used to calculate the probability that the construct will spread in local populations of *An. coluzzii* or *An. arabiensis* under the same conditions.

In this assessment we re-examine two events in the fault tree analysis completed by Hayes et al. (2015). The first is the event: "Given that genetically modified males carrying the construct are viable, what is the probability that the construct allows some fertile males (not all are sterilised)?" for the construct in *An. coluzzii*. The informative priors here can be replaced with the posterior estimates described above. The change in target species[13],

[13]The target species in the initial risk assessment was Ag(DSM)2. The target species in this risk assessment is Ac(DSM)2.

however, requires that we assume that all other events in the fault tree analysis, except the probability of construct failure, are transferable between *An. coluzzii* and *An. gambiae*. This assumption is reasonable given the high levels of dependency between the original analysis for the two species (see Appendix D of Hayes et al., 2015).

The second events is: "Given that genetically modified males carrying the construct are viable, what is the probability that the construct allows some fertile males (not all are sterilised)?" but this time for the construct in *An. gambiae*. The informative priors here can be replaced with the posterior estimates described above for *An. gambiae*, assuming again that all other events in the fault tree analysis, except this one, are transferable between the two species.

In the original analysis the event "Given that there are compatible species in the vicinity, what is the probability that Coluzzii hybrids will be formed, following a catastrophic release of all 10,000 mosquitoes?" are probably too high and therefore not transferable to *An. gambiae* hybrids, but as noted above it is not clear how to sensibly adjust the informative priors for this event.

The results of the analysis are summarised in Figure 4.9. The change in the median risk and the width of the credible intervals reflects the effect of the updated posterior for the probability of sterility. For *An. coluzzii* the median probability of construct failure (per mating) changes from a prior value of $4.51 \times 10^{-8}$ to a posterior value of $5 \times 10^{-8}$. As a consequence there is very little difference between the original and updated results of the fault tree analysis. For example, under the Aggregate First Then Convolute strategy the median probability drops slightly from $1.39 \times 10^{-3}$ to $8.87 \times 10^{-4}$. Note that the number of experts who responded to the events within the fault tree that have been updated has changed and this also has a small effect on the results.

For *An. gambiae* the change in the median probability of construct failure (per mating) increases from a prior value of $1.31 \times 10^{-6}$ to a posterior value of $6.55 \times 10^{-4}$. As a result of this the probability of the construct spreading in local populations of *An. gambiae* or *An. arabiensis* is predicted to increase from a median value of $1.82 \times 10^{-6}$ to a median value of $8.02 \times 10^{-6}$ under the Aggregate First Then Convolute strategy. The magnitude of this increase is not as large as the increase between the prior and posterior probability of construct failure because the change in probability is mediated by the other events within the fault tree.

For *An. gambiae* or *An. arabiensis*, the change in median probability reported in Hayes et al. (2015) and this one is larger under the Convolute First Then Aggregate strategy – the median increases from $3.12 \times 10^{-5}$ to $1.96 \times 10^{-3}$ – because the number of experts who answered the updated events, and their weights, has changed between the two risk assessments, and the effect of this change is more pronounced under this strategy.

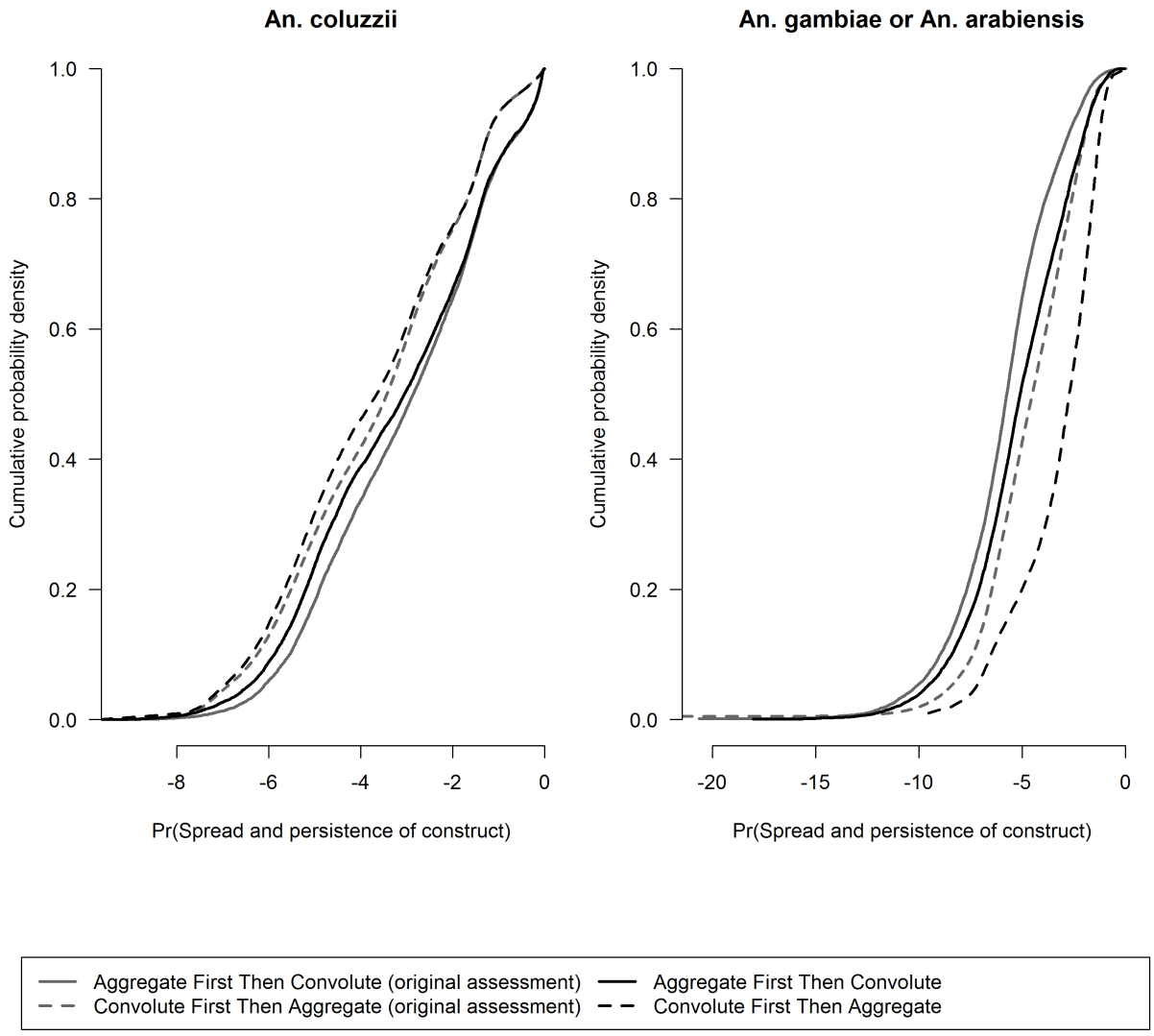


**Figure 4.9:** Updated fault tree analysis incorporating the posterior probability of construct failure in *An. coluzzii* and *An. gambiae*. These plots summarise the effect of updating the fault tree analysis conducted by Hayes et al. (2015) for the spread and persistence of the construct in local populations of *An. coluzzii* (left panel) and *An. gambiae* or *An. arabiensis* (right panel) for two types of fault tree calculation strategies (Aggregate First Then Convolute and Convolute First Then Aggregate). The updated analysis incorporates the results of laboratory trials on the efficacy of the Dominant Sterile Male construct. The analysis assumes that the efficacy of the construct in the field will be the same as that observed under laboratory conditions and that all events in the fault tree analysis (other than the probability of construct failure) are transferable between Ac(DSM)2 and Ag(DSM)2. The results for *An. gambiae* or *An. arabiensis* are conservative and do not reflect the low rate of hybridisation observed in field samples collected from Bana village.

# 5 NON-TARGET EFFECTS

**KEY POINTS**

1. There are a number of plausible Adverse Outcome Pathways for non-target effects following the controlled field release but all of these require the released mosquitoes to disperse and survive, or the Dominant Sterile Male construct to spread and persist, over spatial and temporal scales that are large enough for permanent or transient effects.
2. The spatio-temporal footprint of the planned field release is predicted to be very small and this mitigates the possibility of non-target effects.
3. This risk assessment predicts that there is a 95% chance that the expected number of Ac(DSM)2 males will drop below one individual 20 days after the controlled field release. This predicted survival is too short, and the associated population size too small, to cause any noticeable effect on non-target organisms or ecosystem processes.
4. If 25 Ac(DSM)2 females are incidentally released at the start of the wet season, the risk assessment predicts that there is a 95% chance that the expected number of Ac(DSM)2 females (including offspring of the initial incidentally released population) will drop below one individual 150 days after the controlled field release. The predicted decline in the population of Ac(DSM)2 females precludes the possibility of significant effects on non-target organisms or ecosystem processes.
5. If the construct's performance in laboratory tests are indicative of its performance in the field, then the median posterior probability that male Ac(DSM)2 mosquitoes will be fertile (per individual mating) is $4.51 \times 10^{-8}$ with a 90% central credible interval of $[2.76 \times 10^{-11}, 2.05 \times 10^{-5}]$. This equates to a median probability that all 5,000 Ac(DSM)2 males will be sterile and therefore unable to pass the construct on to sexually compatible species of 0.9998.

## 5.1 Persistence and adverse outcomes pathways

In the context of synthetic gene drives the National Academies of Sciences, Engineering and Medicine (2016) define non-target effects as, “a direct, unintended, short- or long-term consequence for one or more organisms other than the organism intended to be affected by an action or intervention”. There are a number of plausible Adverse Outcomes Pathways for non-target effects associated with the deliberate field release, many of which are reflected in the community’s concerns:

- Horizontal gene transfer: horizontal transfer of the Dominant Sterile Male construct to non-target species may have adverse effects by changing (for example) the behavioural characteristics or fitness of the non-target host.
- Vertical gene transfer: transmission and spread of the construct to non-target but sexually compatible species within the *An. gambiae* complex could impose a fitness effect on these species (although this may be a desirable side-effect).
- Knock-on effects of permanent suppression: if the construct remains functional whilst spreading through target or non-target species, and thereby causes a permanent reduction in their abundance, then this could have knock-on effects through the food-webs and ecosystem processes that these species form part of.
- Knock-on effects due to partial suppression: temporary suppression of target or non-target species could lead to changes in host-vector dynamics (such as the loss of herd immunity or changes in the prevalence of existing pathogens) and/or alter population dynamics by pushing species into alternative, possibly non-reversible, stable states.
- Evolutionary consequences: Long term persistence of the construct may have unintended consequences on evolutionary host-pathogen or predator-prey relationships.

A common thread that underlies all of these pathways is the persistence and spread of the transgene, through vertical or horizontal transmission, over spatial and temporal scales that are large enough for permanent or transient effects on non-target species. The spatial and temporal scales over which Ac(DSM)2 males and females are predicted to spread and persist, however, are relatively small, and need to be put in the context of current conventional mosquito-control practice, such as the use of insecticide treated bed nets.

The limited spatio-temporal footprint of the field release mitigates the possibility of non-target effects following the field release. In the first instance this analysis predicts that the probability of horizontal gene transfer of the Dominant Sterile Male construct to non-target eukaryotes and non-eukaryotes following the field release is too small to be a practical concern in this context.

The detailed model of Ac(DSM)2 male dispersal and survival developed for this risk assessment predicts that there is a 95% chance that the expected number of Ac(DSM)2 males will drop below one individual 20 days after the controlled field release (Section 4.3.1). Whilst it is still possible for small numbers of mosquitoes to be observed when the expected value of the population is less than one, this predicted survival is too short, and the associated population size too small, to cause any noticeable effect on non-target organisms or ecosystem processes. Importantly the uncertainty in the models predictions can be resolved with further analysis of Ac(DSM)2 male recapture rates following the field release.

A detailed (and necessarily complicated) analysis of female survival and spread is difficult

to justify given the small number of Ac(DSM)2 females that may be incidentally released. The simple analysis conducted here suggests that if 25 Ac(DSM)2 females are incidentally released at the start of the wet season, their expected number will decline to median estimate of less than one individual 65 days after the release (Section 4.2). The expected number of Ac(DSM)2 females falls below one individual about 150 days after the release with 95% certainty. Again these predictions preclude the possibility of significant effects on non-target organisms or ecosystem processes.

These predictions are contingent on the models and assumptions described in the previous sections of the report. An important consideration in this context is the predicted efficacy of the Dominant Sterile Male construct. The male dispersal and survival model, for example, assumes that all male Ac(DSM)2 mosquitoes are sterile and therefore sets the birth rate of Ac(DSM)2 males to zero. This assumption seems reasonable in light of the analysis and data collected to date.

If the construct's performance in laboratory tests are indicative of its performance in the field, then the median posterior probability that male Ac(DSM)2 mosquitoes will be fertile (per individual mating) is $4.51\times10^{-8}$ with a 90% central credible interval of $[2.76\times10^{-11}, 2.05\times 10^{-5}]$. This equates to a median probability of 0.9998 that all 5,000 Ac(DSM)2 males will be sterile. In other words, the risk assessment predicts there is about a 0.02% chance that there will be at least one fertile Ac(DSM)2 male among the 5,000 released.

# 6 DISCUSSION

**KEY POINTS**

1. There is about a 70% chance that female Ac(DSM)2 mosquitoes will have a lower capacity than female Ac(WT) moquitoes to transmit *P. falciparum* and o'nyong'nyong virus, and about a 90% chance for lymphatic filariasis, assuming equal vector to human host ratios.
2. Accounting for the likely difference in vector to host ratios at the time of the controlled field release reduces the probability that the incidental release of Ac(DSM)2 females will cause an increase in the transmission of these pathogens, during the time that Ac(DSM)2 female mosquitoes are present in the environment, to values in the range 0.03 to 0.05.
3. Accounting for the small number of females released reduces the median probability of them transmitting a novel blood-borne pathogen to a value of $1.3 \times 10^{-8}$
4. The median probability of released Ac(DSM)2 females and males becoming effectively extinct – that is the expected population size falling below one individual – is predicted to be about 65 days and 10 days respectively.
5. The probability that the construct will persist and spread in local populations of *An. coluzzii*, and in *An. gambiae* or *An. arabiensis* in a year following a release of 5,000 Ac(DSM)2 males is predicted to have a median value of $8.87 \times 10^{-4}$ and $1.96 \times 10^{-3}$ respectively (using the more conservative fault tree calculation strategies). The latter value, however, does not account for the low proportion of hybrids observed in Target Malaria's field samples, and is therefore likely to be an overestimate.
6. Simulation studies suggest that the probability of an extant population of male Ac(DSM)2 mosquitoes becomes less than 0.005 if male Ac(DSM)2 mosquitoes are not seen for three successive days in samples collected with Mark Release Recapture equivalent sampling effort at least thirty days after the release.
7. The posterior probability that the construct will fail (per mating) for male Ag(DSM)2 mosquitoes has a median value of $6.5 \times 10^{-4}$ and a 90% credible interval of $[2.0 \times 10^{-4}, 1.6 \times 10^{-3}]$.
8. Laboratory tests with female Ac(DSM)2 indicate that these mosquitoes have the same or higher susceptibility to insecticides that are commonly used in the release region for which insecticide resistance has been reported in Anopheles mosquitoes.
9. One of the lessons of this assessment is that within a staged-released strategy it is important to ensure that the risk assessment endpoints, elicitations and field/laboratory data align as closely as possible.

## 6.1 Quantitative risk estimates

The results of the risk assessment for each of the seven endpoints are summarised in Table 6.1. The first risk assessment endpoint addresses the possibility that the construct modifies the vectorial capacity of female Ac(DSM)2 mosquitoes for *P. falciparum*, o'nyong'yong and lymphatic filariasis. The endpoint refers directly to the first of the community's concerns, and is expressed here as a vectorial capacity index – that is the base 10 logarithm of the ratio of the basic reproduction number of female Ac(DSM)2 at backcross 29 over that of the local wild type females at laboratory generation F = 0, conservatively assuming in the first instance equal vector to human host ratios for the two strains. Negative values of this quantity indicate that the pathogen transmission potential of female Ac(DSM)2 mosquitoes is lower than that of female Ac(WT) mosquitoes, whereas positive values indicate increased transmission potential of Ac(DSM)2 mosquitoes.

The summary quantiles of the vectorial capacity index indicate that the bulk of the index's probability density (about 70%) lies below zero for all three pathogens, with about a 30% chance that the vectorial capacity of the Ac(DSM)2 strain is higher for *P. falciparum* and o'nyong'nyong, and about a 15% chance for lymphatic filariasis. These figures should be interpreted as the risk attributable to the Dominant Sterile Male construct, for equal vector to human host ratios, for the duration of time that Ac(DSM)2 female mosquitoes are present in the environment.

At the time of field release, however, the wild type population is likely to be at least three orders of magnitude larger than the population of incidentally released females, which reduces the risk attributable to the field release activities, but not by three orders of magnitude because the probability that the index is greater than zero is a non-linear function. For *P. falciparum*, for example, accounting for the difference in vector to human host ratio reduces the probability of an increase in *P. falciparum* transmission, during the time that Ac(DSM)2 females are present in the environment, to 0.03.

Accounting for the relatively small numbers of Ac(DSM)2 females that may be incidentally released also reduces the probability that the female Ac(DSM)2 mosquitoes will vector a novel blood borne pathogen. A fault tree analysis conducted by Hayes et al. (2015) concluded that the median probability of this event, conditioning on a release of 5,000 Ag(DSM)2 mosquitoes, would be lower than $5.2 \times 10^{-7}$. Assuming that the elicitations conducted for Ag(DSM)2 are transferable to Ac(DSM)2, and accounting for the smaller number of females released reduces this median probability to a value of $1.3 \times 10^{-8}$ under the most conservative calculation strategy.

Many of the communities concerns regarding the planned field release are contingent on the spread and survival of Ac(DSM)2 mosquitoes. The models for female survival, and male dispersal and survival, developed for this risk assessment predict that the released Ac(DSM)2 mosquitoes will have a small spatio-temporal footprint: the median probability of released females and males becoming effectively extinct is predicted to be about 65 days and 10 days respectively. These predictions, and the relatively small population sizes of Ac(DSM)2 mosquitoes, mitigate against the possibility of non-target effects attributable to the field release.

The male and female survival models assume *inter alia* that all Ac(DSM)2 males are sterile by: (i) not including a birth rate in the reaction terms of the male model; and, (ii) imposing

| Log10 ratio of vectorial capacity relative to Ac(WT) | | | |
|---|---|---|---|
| | **5%** | **50%** | **95%** |
| *P. falciparum* | $-9.5$ | $-1.49$ | 2.44 |
| ONNV | $-12.6$ | $-3.33$ | 1.79 |
| Lymphatic filariasis | $-8.75$ | $-1.18$ | 3.14 |
| Vector novel blood-based pathogen | | | |
| | **5%** | **50%** | **95%** |
| AFTC estimate | $7.54 \times 10^{-13}$ | $1.32 \times 10^{-8}$ | $1.29 \times 10^{-5}$ |
| CFTA estimate | $1.86 \times 10^{-13}$ | $7.17 \times 10^{-9}$ | $3.37 \times 10^{-5}$ |
| Survival of Ac(DSM)2 females given release of 25 female Ac(DSM)2 | | | |
| | **5%** | **50%** | **95%** |
| First day expected number of females $< 1$ | 6 | 71 | 152 |
| Survival of Ac(DSM)2 males given release of 5,000 male Ac(DSM)2 | | | |
| | **5%** | **50%** | **95%** |
| First day expected number of males $< 1$ | 6 | 10 | 20 |
| Spread and persistence of construct in *An. coluzzii* | | | |
| | **5%** | **50%** | **95%** |
| AFTC estimate | $3.34 \times 10^{-7}$ | $8.87 \times 10^{-4}$ | 0.64 |
| CFTA estimate | $9.88 \times 10^{-8}$ | $2.24 \times 10^{-4}$ | 0.17 |
| Spread and persistence of construct in *An. gambiae* or *An. arabiensis* | | | |
| | **5%** | **50%** | **95%** |
| AFTC estimate | $2.28 \times 10^{-10}$ | $8.02 \times 10^{-6}$ | 0.03 |
| CFTA estimate | $5.82 \times 10^{-8}$ | $1.96 \times 10^{-3}$ | 0.1 |
| Difference in the probability of mortality between Ac(DSM)2 and Ac(WT) | | | |
| | **5%** | **50%** | **95%** |
| Alpha-cypermethrin | $-0.0028$ | 0.076 | 0.23 |
| Bendiocarb | $-0.017$ | $1.84 \times 10^{-4}$ | 0.023 |
| DDT | 0.026 | 0.13 | 0.32 |
| Deltamethrin | 0.092 | 0.24 | 0.39 |
| Fenitrothion | $-8.27 \times 10^{-4}$ | $8.43 \times 10^{-10}$ | 0.0011 |
| Lambda-cyhalothrin | $-0.029$ | 0.027 | 0.16 |
| Permethrin | 0.048 | 0.19 | 0.43 |

**Table 6.1:** Risk estimates for seven endpoints following the deliberate release of 5,000 male Ac(DSM)2 mosquitoes, and the incidental release of 25 female Ac(DSM)2 mosquitoes, in Bana village in Burkina Faso at the beginning of the wet season. Risk calculations performed by fault tree analysis show results for two computational strategies (labelled AFTC and CFTA). Column headings 5%, 50% and 95% refer to the 5th, 50th and 95th quantiles.

a fitness cost of 0.5 on Ac(DSM)2 females in the female model. This assessment updates the probability of construct failure by collating the results of all the sterility trials conducted to date on Ag(DSM)2 and Ac(DSM)2 males. The Bayesian update takes a conservative stance by treating the number of matings (not the number of eggs) as the sample size in these trials, and also accounts for change in assessment unit between the prior elicitation conducted by Hayes et al. (2015) and the likelihood.

If the performance of the Dominant Sterile Male construct in the laboratory is the same as its performance in the field, then the results of this analysis suggest that there is a median probability of $4.51 \times 10^{-8}$ that the construct will fail to sterilise individual males. Updating the equivalent event in the fault tree analysis conducted by Hayes et al. (2015), and assuming that in all other respects the elicitations performed for Ag(DSM)2 are transferable to Ac(DSM)2, this analysis predicts that the probability that the construct will persist and spread in local populations of *An. coluzzii* in a year following a release of 5,000 Ac(DSM)2 males has a median value of $8.9e-04$.

The equivalent probability for spread and persistence in local populations of *An. gambiae* or *An. arabiensis* using the more conservative of the two fault tree calculation methods is $2e-03$. The later estimate, however, does not reflect the low probability of hybridisation in Bana Village indicated by the low proportion of hybrids (0.4%) in the entomological surveys, and is therefore likely to be an overestimate because the informative priors collected by Hayes et al. (2015) suggested that the median probability of hybrids being formed between *An. coluzzii* and *An. gambiae*, following the accidental release of 5,000 Ag(DSM)2 mosquitoes, was 0.76.

Simulation studies suggest that the absence of male Ac(DSM)2 mosquitoes in samples collected with a few days of Mark Release Recapture equivalent survey effort in the months after the field release, provides a better assurance that male Ac(DSM)2 mosquitoes are indeed absent, than observing the same result in the days that immediately follow the field release. This is because the male dispersal and survival model predicts that the population of Ac(DSM)2 mosquitoes will decrease with time, and because mosquito capture efficiencies are typically less than a few percent, hence the absence of target (in this case Ac(DSM)2) mosquitoes in a sample does not by itself provide a great deal of confidence that the target population is in fact absent from the survey site. Conversely, if samples collected in the three months following the field release return true positives for the presence of the transgene, then this would signal that the risk assessment predictions may be incorrect.

## 6.2 Lessons and future analysis

The analysis completed here predicts that male and female Ac(DSM)2 mosquitoes released during the field trial will die out over the course of the succeeding wet and dry seasons. The field release is not therefore predicted, and is not intended, to have any noticeable effects on local populations of predominately *An. coluzzii* mosquitoes.

The primary purposes of the field release are to: (i) generate data on the daily survival rate of released Ac(DSM)2 males and to assess their movement from a defined release point; and (ii) strengthen local capacity in the handling, release and recapture of laboratory reared mosquitoes including through the establishment and validation of standard operating procedures and internal systems for the oversight of regulatory compliance.

Data generated from the field release will further inform an understanding of how outcomes from indoor contained use experiments can be extrapolated to a field entomology context. Additionally, although Ac(DSM)2 does not incorporate a gene drive mechanism, data on the population dynamics and dispersal behaviour of male Ac(DSM)2 collected from a field release will serve to further demonstrate and develop the methodology and mathematical models needed to support an environmental risk assessment to standards recommended for gene drive applications (National Academies of Sciences, Engineering and Medicine, 2016; Australian Academy of Sciences, 2017).

This risk assessment places considerable emphasis on Bayesian methods. This is not the only paradigm for probabilistic risk assessment but it is well suited to scientific assessments for novel technologies. Elsewhere (see for example Hayes et al., 2013) we have also argued the merits of a staged-release strategy for genetic control strategies, consistent with World Health Organisation recommendations for transgenic mosquitoes (World Health Organisation, 2009, 2014).

One of the lessons of this assessment is that within a staged-released strategy it is important to ensure that the risk assessment endpoints, elicitations and field/laboratory data align as closely as possible. The relevance of the available data to the risk endpoints, and informative priors, can vary substantially.

This issue is highlighted in the vectorial capacity analysis (Section 3.1). The available evidence for vectorial capacity parameters varies from laboratory experiments on transmission efficiency that are possibly relevant to field conditions, to laboratory observations of longevity that are probably not representative of field outcomes to field-based observations that are unquestionably relevant.

Target Malaria's capacity to generate unquestionably relevant observations with future more persistent, dispersive genetic control products will likely be constrained. These constraints can be managed in part by judicious choice of endpoints and by further elaboration of expert elicitation to allow (for example) for a specific analysis of the expected responses under laboratory versus field conditions. The most relevant endpoints would typically be those that refer to outcomes in the field but laboratory endpoints and comparisons may form testable go/no-go benchmarks in their own right.

The Bayesian approach to probabilistic risk assessment provides a transparent and coherent process for amending expert beliefs in light of empirical observations. It is important, however, that the Bayesian updates are performed with data sources that are independent of the sources that an expert uses when forming their opinions and subjective risk estimates.

To facilitate this process future risk assessment and elicitations procedures should adhere to three principles:

- If data are relevant to a laboratory-based endpoint (for example insecticide resistance under internationally recognised testing procedures) or a field-based endpoint (for example pathogen transmission under field conditions), then the experts should not be allowed to examine it prior to or during an elicitation because it will be incorporated into the analysis via the likelihood.
- Wherever possible experts should be provided with finalised protocols that describe

the laboratory or field protocols that are used to collect data prior to, or during, an elicitation.

- If data are irrelevant to the endpoint, but somehow still "speak to" parameters associated with the endpoint (for example estimates of the basic reproduction number for other diseases) then experts may examine it prior to an elicitation and include the results in their probabilistic assessments.

Again these principles presume close communication between project teams and risk assessment teams to ensure that relevant information on, for example, field or experimental protocols can be made available to experts in pre-elicitation documentation.

# REFERENCES


Aguilar, R., Dong, Y., Warr, E., and Dimopoulos, G. (2005). *Anopheles* infection responses; laboratory models versus field malaria transmission systems. *Acta Tropica*, 95:285–291.

Apostolakis, G. (1981). *Bayesian Methods in Risk Assessment*, pages 415–465. Springer US, Boston, MA.

Apostolakis, G. (1990). The concept of probability in safety assessments of technological systems. *Science*, 250(4986):1359–1364.

Australian Academy of Sciences (2017). Synthetic Gene Drives in Australia: Implications of Emerging Technologies. Technical report, Australian Academy of Sciences, Canberra, Australia, 24 pp.

Bedrick, E. J., Christensen, R., and Johnson, W. (1996). A new perspective on priors for generalized linear models. *Journal of the American Statistical Association*, 91(436):1450–1460.

Brady, O. J., Johansson, M. A., Guerra, C. A., Bhatt, S., Golding, N., Pigott, D. M., Delatte, H., Grech, M. G., Leisnham, P. T., Maciel-de Freitas, R., Styer, L. M., Smith, D. L., Scott, T. W., Gething, P. W., and Hay, S. I. (2013). Modelling adult aedes aegypti and aedes albopictus survival at different temperatures in laboratory and field settings. *Parasites & Vectors*, 6(1):351.

Chamberlain, R. W. and Sudia, W. D. (1961). Mechanism of transmission of viruses by mosquitoes. *Annual Review of Entomology*, 6:371–390.

Chkrebtii, O., Campbell, D., Calderhead, B., and Girolami, M. (2016). Bayesian solution uncertainty quantification for differential equations. *Bayesian Analysis*, 11(4):1269–1273.

Christensen, R., Johnson, W., Branscum, A., and Hanson, T. E. (2011). *Bayesian Ideas and Data Analysis*. CRC Press, Boca Raton, USA.

Clements, A. N. and Paterson, G. D. (1981). The analysis of mortality and survival rates in wild populations of mosquitoes. *Journal of Applied Ecology*, 18:373–399.

Corbet, P. S., Williams, M., and Gillett, J. (1961). O'nyong-nyong fever: An epidemic virus disease in East Africa: IV. vector studies at epidemic sites. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 55(5):463–480.

Desquesnes, M. and Dia, M. L. (2003). Mechanical transmission of *Trypanosoma congolense* in cattle by the African tabanid *Atylotus agrestis*. *Experimental Pa*, 105:226–231.

Epopa, P. S., Millogo, A. A., Collins, C. M., North, A., Tripet, F., Benedict, M. Q., and Diabate, A. (2017). The use of sequential mark-release recapture experiments to estimate population size, survival and dispersal of male mosquitoes of the *Anopheles gambiae* complex in bana, a west african humid savannah village. *Parasites and Vectors*, 10:376.

Fagbami, A. H. and Tomori, O. (1981). Tataguine virus isolates from humans in nigeria, 1971-1975. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 75:788.

Fanello, C., Santolamazza, F., and Della Torre, A. (2002). Simultaneous identification of

species and molecular forms of the Anopheles gambiae complex by PCR-RFLP. *Medical and Veterinary Entomology*, pages 461–464.

Flick, R. and Bouloy, M. (2005). Rift valley fever virus. *Current Molecular Medicine*, 5:827–834.

Fontaine, M. C., Pease, J. B., Steele, A., Waterhouse, R. M., Neafsey, D. E., Sharakhov, I. V., Jiang, X., Hall, A. B., Catteruccia, F., Kakani, E., Mitchell, S. N., Wu, Y.-C., Smith, H. A., Love, R. R., Lawniczak, M. K., Slotman, M. A., Emrich, S. J., Hahn, M. W., and Besansky, N. J. (2015). Extensive introgression in a malaria vector species complex revealed by phylogenomics. *Science*, 347(6217).

Food and Agricultural Organisation of the United Nations (2017). Rift Valley Fever in Niger; Risk Assessment. Technical report, FAO Animal Health RIsk Analysis - Assessment, Issue No. 1, Rome, FAO.

Gelman, A., Carlin, J. B., Stern, H. S., Dunson, D. B., Vehtari, A., and Rubin, D. B. (2014). *Bayesian data analysis*, volume 2. CRC press Boca Raton, FL.

Gillies, M. T. and Wilkes, T. J. (1972). The range of attraction of animal baits and carbon dioxide for mosquitoes. Studies in a freshwater area of West Africa. *Bulletin of Entomological Research*, 61(03):389.

Guerra, C. A., Reiner, R. C., Perkins, T. A., Lindsay, S. W., Midega, J. T., Brady, O. J., Barker, C. M., Reisen, W. K., Harrington, L. C., Takken, W., et al. (2014). A global assembly of adult female mosquito mark-release-recapture data to inform the control of mosquito-borne pathogens. *Parasites & vectors*, 7(1):276.

Gyapong, J., Kyelem, D., Kleinschmidt, I., Agbo, K., Ahouandogbo, F., Gaba, J., Owusu-Banahene, G., Sanou, S., Sodahlon, Y., Biswas, G., et al. (2002). The use of spatial analysis in mapping the distribution of bancroftian filariasis in four West African countries. *Annals of Tropical Medicine & Parasitology*, 96(7):695–705.

Haario, H., Saksman, E., and Tamminen, J. (2001). An adaptive metropolis algorithm. *Bernoulli*, 7(2):223–242.

Hayes, K. R., Barry, S., Beebe, N., Dambacher, J. M., De Barro, P., Ferson, S., Ford, J., Foster, S., Gonçalves da Silva, A., Hosack, G. R., Peel, D., and Thresher, R. (2015). Risk Assessment for Controlling Mosquito Vectors with Engineered Nucleases, Part I: Sterile Male Construct Final Report. Technical report, CSIRO Biosecurity Flagship, Hobart, Australia, 148pp.

Hayes, K. R., Leung, B., Thresher, R. T., Dambacher, J. M., and Hosack, G. R. (2013). Meeting the challenge of quantitative risk assessment for genetic control techniques: a framework and some methods applied to the common carp (*Cyprinus carpio*) in australia. *Biological Invasions*.

Hoch, A. L., Gargan, T. P., and Bailey, C. L. (1985). Mechanical transmission of Rift Valley fever virus by hematophagous Diptera. *American Journal of Tropical Medicine and Hygiene*, 34:188–193.

Hosack, G. R., Hayes, K. R., and Barry, S. C. (2017). Prior elicitation for Bayesian ge-

neralised linear models with application to risk control option assessment. *Reliability Engineering & System Safety*, 167(Supplement C):351 – 361.

Kaplan, S. and Garrick, J. B. (1981). On the quantitative definition of risk. *Risk A*, 1(1):11–27.

Kass, R. E. and Raftery, A. E. (1995). Bayes factors. *Journal of the American Statistical Association*, 90:773–795.

Klein, T. A., Windbichler, N., Deredec, A., Burt, A., and Benedict, M. Q. (2012). Infertility resulting from transgenic I-PpoI male *Anopheles gambiae* in large cage trials. *Pathogens and Global Health*, 106:20–31.

Lanciotti, R. S., Ludwig, M. L., Rwaguma, E. B., Lutwama, J. J., Kram, T. M., Karabatsos, N., Cropp, B. C., and Miller, B. R. (1998). Emergence of epidemic O'nyong-nyong fever in Uganda after a 35-year absence: genetic characterization of the virus. *Virology*, 252(1):258–268.

Lindsay, S. and Thomas, C. (2000). Mapping and estimating the population at risk from lymphatic filariasis in Africa. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 94(1):37–45.

Liu, J. S. (2004). *Monte Carlo Strategies in Scientific Computing*. Springer-Verlag New York.

Martin, T. G., Wintle, B., Rhodes, J. R., Kuhnert, P. M., Field, S., Low-Choy, S. J., Tyre, A. J., and Possingham, H. P. (2005). Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. *Ecology Letters*, 8(11):1235–1246.

McGoff, K., Mukherjee, S., and Pillai, N. (2015). Statistical inference for dynamical systems: A review. *Statistics Surveys*, 9:209–252.

McIver, S. B. and McElligott, P. E. (1989). Effects of release rates on the range of attraction of carbon dioxide to some southwestern Ontario mosquito species. *Journal of the American Mosquito Control Association*, 5(1).

Motha, M. X. J., Egerton, J. R., and Sweeney, A. W. (1984). Some evidence of mechanical transmission of reticuloendotheliosis virus by mosquitoes. *Avian Diseases*, 28:858–867.

National Academies of Sciences, Engineering and Medicine (2016). Gene drives on the horizon: Advancing science, navigating uncertainty, and aligning research with public values. Technical report, The National Academies Press, Washington DC, USA.

Noor, A. M., Kinyoki, D. K., Mundia, C. W., Kabaria, C. W., Mutua, J. W., Alegana, V. A., Fall, I. S., and Snow, R. W. (2014). The changing risk of *Plasmodium falciparum* malaria infection in Africa: 2000–10: a spatial and temporal analysis of transmission intensity. *The Lancet*, 383(9930):1739–1747.

Pimm, S. L., Jones, H. L., and Diamond, J. (1988). On the risk of extinction. *The American Naturalist*, 132:757–785.

Posey, D. L., O'Rourke, T., Roehrig, J. T., Lanciotti, R. S., Weinberg, M., and Maloney, S. (2005). O'nyong-nyong fever in West Africa. *The American journal of tropical medicine and hygiene*, 73(1):32–32.

Powers, A. M. (2014). Infection patterns and emergence of o'nyong'nyong virus. In Singh, S. K., editor, *Viral Infections and Global Change*, pages 433–444. Wiley Online Library, first edition.

Rasmussen, N. C. (1981). The application of probabilistic risk assessment techniques to energy technologies. *Annual Reviews in Energy*, 6:123–138.

Reiner, R. C., Perkins, T. A., Barker, C. M., Niu, T., Chaves, L. F., Ellis, A. M., George, D. B., Le Menach, A., Pulliam, J. R. C., Bisanzio, D., Buckee, C., Chiyaka, C., Cummings, D. A. T., Garcia, A. J., Gatton, M. L., Gething, P. W., Hartley, D. M., Johnston, G., Klein, E. Y., Michael, E., Lindsay, S. W., Lloyd, A. L., Pigott, D. M., Reisen, W. K., Ruktanonchai, N., Singh, B. K., Tatem, A. J., Kitron, U., Hay, S. I., Scott, T. W., and Smith, D. L. (2013). A systematic review of mathematical models of mosquito-borne pathogen transmission: 1970–2010. *Journal of The Royal Society Interface*, 10(81).

Robert, C. P. (2007). *The Bayesian Choice: From Decision-Theoretic Foundations to Computational Implementation*. Springer Science & Business Media, 2nd edition.

Rossi, S. L., Ross, T. M., and Evans, J. D. (2010). West nile virus. *Clinics in laboratory medicine*, 30(1):47–65.

Ruggeri, F., Sawlan, Z., Scavino, M., and Tempone, R. (2017). A hierarchical bayesian setting for an inverse problem in linear parabolic pdes with noisy boundary conditions. *Bayesian Analysis*, 12(2):407–433.

Sinka, M. E., Bangs, M. J., Manguin, S., Rubio-Palis, Y., Chareonviriyaphap, T., Coetzee, M., Mbogo, C. M., Hemingway, J., Patil, A. P., Temperley, W. H., Gething, P. W., Kabaria, C. W., Burkot, T. R., Harbach, R. E., and Hay, S. I. (2012). A global map of dominant malaria vectors. *Parasites & vectors*, 5(1):69.

Smith, D. L., Battle, K. E., Hay, S. I., Barker, C. M., Scott, T. W., and McKenzie, F. E. (2012). Ross, macdonald, and a theory for the dynamics and control of mosquito-transmitted pathogens. *PLoS pathogens*, 8(4):e1002588.

Smith, D. L. and McKenzie, F. E. (2004). Statics and dynamics of malaria infection in *Anopheles* mosquitoes. *Malaria journal*, 3(1):13.

Soetaert, K. and Herman, P. M. J. (2008). *A Practical Guide to Ecological Modelling: Using R As a Simulation Platform*. Springer Publishing Company, Incorporated, 1st edition.

Soetaert, K. and Meysman, F. (2012). Reactive transport in aquatic ecosystems: Rapid model prototyping in the open source software r. *Environmental Modelling & Software*, 32:49–60.

Tomori, O. (2001). *Encyclopedia of Arthropod-transmitted Infections of Man and Domesticated Animals*, chapter Tataguine virus, pages 484–485. CABI publishing, Wallingford, Oxon, UK.

Vickerman, K. (1973). The mode of attachment of *Trypanosoma vivax* in the proboscis of the tsetse fly *Glossina fuscipes*: an ultrastructural study of the epimastigote stage of the trypanosome". *Journal of Protozoology*, 20:394–404.

Wasserman, L. (2000). Bayesian model selection and model averaging. *Journal of Mathematical Psychology*, 44:92–107.

Wikle, C. K. (2003). Hierarchical bayesian models for predicting the spread of ecological processes. *Ecology*, 84(6):1382–1394.

Wilkins, E. E., Howell, P. I., and Benedict, M. Q. (2006). IMP PCR primers detect single nucleotide polymorphisms for *Anopheles gambiae* species identification, Mopti and Savanna rDNA types, and resistance to dieldrin in *Anopheles arabiensis*. *Malaria Journal*, 5:125.

Williams, M., Woodall, J., Corbet, P. S., and Gillett, J. (1965). O'nyong-nyong fever: An epidemic virus disease in East Africa VIII. Virus isolations from *anopheles* mosquitoes. *Transactions of the Royal Society of Tropical Medicine and Hygiene*, 59(3):300–306.

Windbichler, N., Papathanos, P. A., and Crisanti, A. (2008). Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in *Anopheles gambiae*. *PLoS Genetics*, 4:e1000291.

Woodruff, A., Bowen, E., and Platt, G. (1978). Viral infections in travellers from tropical Africa. *Br Med J*, 1(6118):956–958.

World Health Organisation (2009). Progress and prospects for the use of genetically modified mosquitoes to inhibit disease transmission. Technical report, World Health Organisation, Geneva, Switzerland.

World Health Organisation (2014). Guidance framework for testing genetically modified mosquitoes. Technical report, Technical report, Special Programme for Research and Training in Tropical Diseases, World Health Organisation, Geneva, Switzerland.

World Health Organisation (2016). Test procedures for insecticide resistance monitoring in malaria vector mosquitoes. Technical report, World Health Organisation, Geneva, Switzerland.

Zhu, L., Qualls, W. A., Marshall, J. M., Arheart, K. L., DeAngelis, D. L., McManus, J. W., Traore, S. F., Doumbia, S., Schlein, Y., M'uller, G. C., and Beier, J. C. (2015). A spatial individual-based model predicting a great impact of copious sugar sources and resting sites on survival of *Anopheles gambiae* and malaria parasite transmission. *Malaria Journal*, 14(1):59.

# Appendix A TECHNICAL DETAILS

## A.1 Vectorial capacity: known blood-borne pathogens

### A.1.1 Predictive prior distributions

The structure of statistical model used to generate the predictive prior distributions is set in advance to allow for interactions between predictive terms. The model is initially parameterised by expert opinion followed by independent empirical data as available. Table A.1 summaries the predictors that are inputs into the model.

**Table A.1:** Model predictors.

| Covariate | Description |
|---|---|
| $u_F$ | number of Ac(WT) generation (in situ) |
| $u_B$ | number of backcrosses between Ac(WT) and Ac(DSM)2 |
| $u_{GE}$ | binary indicator for genetically engineered strain (GE) (Ag(DSM)2, Ac(DSM)2) |
| $u_{WT}$ | binary indicator for Ac(WT) |

For a given target parameter, the elicitations occur at pre-specified design points. A design point can be thought of as a scenario that is defined by the choice of strain or lineage, for example, G3 or Ag(DSM)2. In some cases, it may include a numeric covariate for number of generations in the laboratory for Ac(WT) and the number of backcrosses for Ag(DSM)2.

Each expert's assessment of a target parameter at a given design point is modelled by a probability distribution. A generalised linear model framework then permits prediction at other combinations of generation or backcrosses,

$$\theta = G(\eta) \tag{A.1}$$

$$\eta = X\beta, \tag{A.2}$$

where $G(\cdot)$ applies a monotonic link function $g(\cdot)$ to each entry in the $d$-dimensional vector $\eta$. The model design matrix $X$ is derived from a log-linear model with predictors that allow for interactions between Ac(WT) and Ac(DSM)2 through different levels of backcrossing and different levels of laboratory adaptation. The unknown coefficients $\beta$ are assumed to have a multivariate normal distribution.

The human feeding rate and the extrinsic incubation period parameters have positive support, $(0, \infty)$, and use the log link,

$$\eta_i = g(\theta_i) = \log \theta_i, \tag{A.3}$$

where $\theta_i$ is the target parameter assessed for the $i^{\text{th}}$ design point. The inverse link is then given by, $\theta_i = g^{-1}(\eta_i) = e^{\eta_i}$, which is an exponential relationship between the target parameter and the linear predictor.

All other parameters (transmission efficiency and the probability of daily mortality) are bounded $(0, 1)$ and use the complementary log-log link function,

$$\eta_i = g(\theta_i) = \log(-\log(1 - \theta_i)), \tag{A.4}$$

**Table A.2:** Elicitation design points presented to the experts for parameterisation of the first-order model Each column heading identifies the covariate to which it is associated in Eq. (A.5) and Table A.1.

| G3 | GE | WT | B | F |
|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 |
| 0 | 0 | 1 | 0 | 0 |
| 0 | 0 | 1 | 0 | 70 |
| 0 | 1 | 0 | 0 | 0 |
| 0 | 1 | 0 | 70 | 70 |
| 0 | 1 | 0 | 5 | 70 |
| 0 | 1 | 0 | 35 | 35 |

where $\theta_i$ is the probability of transmission or daily mortality. The complementary log-log function assumes an exponential relationship between the hazard rate[14] and the linear predictor.

On the link-transformed scale, the model is given by

$$\begin{aligned}\eta =&\beta_{G3} + \beta_{WT} u_{WT} + \beta_{GE} u_{GE} \\ &+ \beta_F u_F + u_{GE} \left(\beta_{GE:F} u_F + \beta_B u_B + \beta_{F:B} u_F u_B\right)\end{aligned} \tag{A.5}$$

The first line of Eq. (A.5) addresses the main effects of the three source strains: G3, Ag(DSM)2 and Ac(WT). The second line of Eq. (A.5) addresses the effect of laboratory habituation and backcrosses. The model allows for interactions between Ac(WT) and Ac(DSM)2 through different levels of backcrossing and laboratory adaptation by Ac(WT). This model has 7 unknown parameters.

The number of laboratory generations for the wild type lineage extends from the source wild population ($F = 0$) to the 70$^{\text{th}}$ generation in the laboratory ($F = 70$). The feasible region of the full factorial obtained from Table A.1 (considering as feasible points every 5th generation or backcross) has the following constraints. There is no genetically engineered wild-type lineage. For Ac(DSM)2 the number of backcrosses cannot exceed the number of generations of Ac(WT) because the wild-type colony was established before backcrossing began (Figure 1.4). Also, if there are no backcrosses then there is no contribution of the wild-type strain to G3 and so $u_F = 0$ in that case.

With these constraints, a D-optimal design is given by Table A.2. With a generation time of 3 weeks, the 17$^{\text{th}}$ generation is about a year's duration for a laboratory lineage, the 35$^{\text{th}}$ generation about two years, and the 70$^{\text{th}}$ generation about four years. 100 generations is about 5 years and 9 months.

The model design matrix $X$ is given in Table A.3. Letting $X_s$ denote the design matrix with columns normalised to unit length, the condition number of $X_s^\top X_s$ is 217.86. An elaborated

[14] Also known as the hazard function or force of mortality, the hazard rate is roughly interpretable as the probability of an event, such as death or transmission, occurring given a small increase of the linear predictor. For example, the increment may be due to a small increase in the number of generations for Ac(WT) and/or number of backcrosses for Ac(DSM)2.

**Table A.3:** Model matrix for the elicitation design. Each column heading identifies the covariate to which it is associated in Eq.(A.5).

| (Intercept) | WT | F | GE | B | F:GE | F:GE:B |
|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 70 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1 | 0 | 70 | 1 | 70 | 70 | 4900 |
| 1 | 0 | 70 | 1 | 5 | 70 | 350 |
| 1 | 0 | 35 | 1 | 35 | 35 | 1225 |

design matrix was required for mortality under the lymphatic filariasis model, where mortality was allowed to vary depending on whether or not the vector was infected. To account for this variation, a full interaction model was specified with model matrix given by Table A.4.

The elicited priors at the design points are not sufficient for the purpose of the risk analysis, hence a model-based approach that allows for predictions at different levels of backcrossing and laboratory generation is developed (Hosack et al., 2017). The model adopts a conditional mean prior approach wherein the elicited responses contributed by an expert for a given parameter are assumed conditionally independent given the covariate values (Bedrick et al., 1996).

The model construction (Section A.1.1) assumes that on the linear predictor scale all parameters are adequately described by a Gaussian distribution, and so a Gaussian distribution on this scale is elicited at each design point (see Hosack et al., 2017, for methodological details). A vector of elicited means $m$ and diagonal covariance matrix $V$ are thereby obtained on the linear predictor scale.

The elicited location parameter and covariance matrix that model the expert's opinion for the unknown coefficients $\beta$ is given by,

$$\mu = \left(X^\top V^{-1} X\right)^{-1} X^\top V^{-1} m,$$
$$\Sigma = \left(X^\top V^{-1} X\right)^{-1},$$

with $p(\beta) \sim N(\mu, \Sigma)$.

The risk assessment endpoint is a comparison of the vectorial capacity of Ac(DSM)2 at backcross 29 with the local wild population Ac(WT) at generation $F = 0$. If the predictive design point for this comparison is given by the matrix $U$, then the predictive prior assessment of the parameter $\theta'$ is obtained by,

$$p(g(\theta')|U) = N(U\mu, U\Sigma U^\top),$$

where $g(\cdot)$ is the vectorised link function.

**Table A.4:** Model matrix for the elicitation design of mortality in the lymphatic filariasis analysis. This design matrix includes an interaction between the base design (Table A.4) and the factor $L$ that denotes lymphatic filariasis infection.

| (Intercept) | WT | F | GE | B | F:GE | F:GE:B | (Intercept):L | WT:L | F:L | GE:L | B:L | F:GE:L | F:GE:B:L |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 70 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 70 | 1 | 70 | 70 | 4900 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 70 | 1 | 5 | 70 | 350 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 35 | 1 | 35 | 35 | 1225 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 0 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 0 | 0 | 0 | 0 | 0 | 1 | 1 | 0 | 0 | 0 | 0 | 0 |
| 1 | 1 | 70 | 0 | 0 | 0 | 0 | 1 | 1 | 70 | 0 | 0 | 0 | 0 |
| 1 | 0 | 0 | 1 | 0 | 0 | 0 | 1 | 0 | 0 | 1 | 0 | 0 | 0 |
| 1 | 0 | 70 | 1 | 70 | 70 | 4900 | 1 | 0 | 70 | 1 | 70 | 70 | 4900 |
| 1 | 0 | 70 | 1 | 5 | 70 | 350 | 1 | 0 | 70 | 1 | 5 | 70 | 350 |
| 1 | 0 | 35 | 1 | 35 | 35 | 1225 | 1 | 0 | 35 | 1 | 35 | 35 | 1225 |

### A.1.2 Predictive posterior distribution and model evidence

If data $y$ is collected at a particular design point defined by the matrix $W$, then the posterior assessment is obtained by,

$$p(\theta|W,y) = \frac{p(y|\theta)p(\theta|W)}{p(y|W)},$$

where from the previous equation, $p(g(\theta)|W) = N(W\mu, W\Sigma W^{\top})$.

The model evidence $p(y|W) = \int p(y|\theta)p(\theta|W)d\theta$, is the probability of the observed data at design point $W$ arising from the expert's predictive assessments assumed conditionally independent given the design matrix $X$.

The model's link functions are non-linear (Section A.1.1), so a straightforward analytical expression for the above integral is unobtainable. Instead a Monte Carlo method is used. An importance sampler generates $j = 1,\ldots,J$ draws from the posterior $p(\theta|W,y)$ and additionally estimates the model evidence $p(y|W)$.

The predictive posterior assessment of the target $\theta'$ at design point $U$ conditional on the observed data $y$ at design point $W$ is given by,

$$p(\theta'|U,W,y) = \int p(\theta'|\theta,U,W)p(\theta|W,y)d\theta.$$

The integral is approximated by first drawing $J$ posterior samples $\left\{\theta_j\right\}$ from $p(\theta|W,y)$, then drawing $J$ predictive posterior samples $\left\{\theta'_j\right\}$ from $p(\theta'|\theta_j,U,W)$, where $\eta = g(\theta)$ and $\eta' = g(\theta')$ with $p(\eta'|\eta_j,U,W)$ conditionally Gaussian.

A priori, each expert's contributed probability model $M_i$, $i = 1,\ldots,I$, is weighted equally, $P(M_1) = P(M_2) = \ldots = P(M_I)$. The prior prediction is the mixture distribution,

$$p(\theta|I) = \sum_{i=1}^{I} p(\theta|M_i)P(M_i).$$

The posterior mixture is given by the Bayesian model average (BMA),

$$p(\theta|y,I) = \sum_{i=1}^{I} p(\theta|y,M_i)P(M_i|y), \tag{A.6}$$

with

$$P(M_i|y) = \frac{p(y|M_i)P(M_i)}{\sum_{i=1}^{I} p(y|M_i)P(M_i)}, \tag{A.7}$$

where $p(y|M_i)$ is the model evidence for the $i^{\text{th}}$ expert.

Similarly, the predictive posterior mixture for unobserved $\theta'$ is given by,

$$p(\theta'|y,I) = \sum_{i=1}^{I} P(M_i|y) \int p(\theta'|\theta)p(\theta|y,M_i)d\theta.$$

### A.1.3 Predictive posterior distribution for daily probability of mortality

In the first Mark Release Recapture experiment three groups of female mosquitoes were collected in the wild, raised in the laboratory, marked with three different coloured dyes, and then concurrently released and monitored for recaptures using pyrethioid spray catches (PSC) inside houses. Swarm sampling and clay pot monitoring stations were also deployed but these methods recovered too few recaptures to include in this analysis.

To analyse the female PSC catch data we developed a simple mark recapture model that assumes a binomial observation error and age independent exponential decay,

$$y_k(t) \sim \text{Binomial}(\gamma, \lfloor n_k(t) \rfloor)$$
$$n_k(t) = n_k(t-1)p,$$

with unknown recapture probability $\gamma$, unknown daily survival probability $p$, $k = A, B, C$ (the three different dyes) and initial release sizes $n(0) = [1197, 1158, 1182]^\top$. The expert elicitations provide the priors for $p$. The exponential decay model is a simple model that is appropriate given the sparse recaptures and cleanly maps into the assumptions of the Ross-Macdonald model. Clearly relevant population dynamic mechanisms such as negative density dependence and environmental stochasticity are not represented by the exponential decay model or the most common formulations of the Ross-Macdonald model. As such, the models are viewed as abstract, measurable representations of complex biological processes.

A logit normal prior was placed on the recapture probability $\gamma$ that allowed 0.25 probability that the recapture probability was below 1% and 0.25 probability that the recapture probability was greater than 10%. This prior matches the median and the interquartile range for *Anopheles* summarised in a recent survey of Mark Release Recapture experiments (Guerra et al., 2014). Aspiration and human baited catches predominated in the studies surveyed by Guerra et al. (2014), and it is possible that the prior for the PSC method may be overly optimistic. The recapture probability of each method could incorporate context-specific expert opinion if this were made available.

An importance sampling estimator generated 1 million samples from the posterior distribution associated with each expert. The minimum effective sample size was $5.79 \times 10^5$. The importance sampling estimate also approximated the model evidence (Section A.1.2). The mixture prior and posterior for the daily probability of survival of Ac(WT) at $F = 0$ are plotted in Figure 3.10. The prior and mixture posterior for the recapture probability are plotted in Figure A.1. The posterior is tightly concentrated relative to the prior. The posterior estimates for the daily probability of survival and the recapture probability are dependent, but the posterior of the recapture probability remains concentrated despite the difference in opinion expressed by the experts for the probability of daily mortality, a priori. The estimated posterior population size by day is shown in Figure A.2.

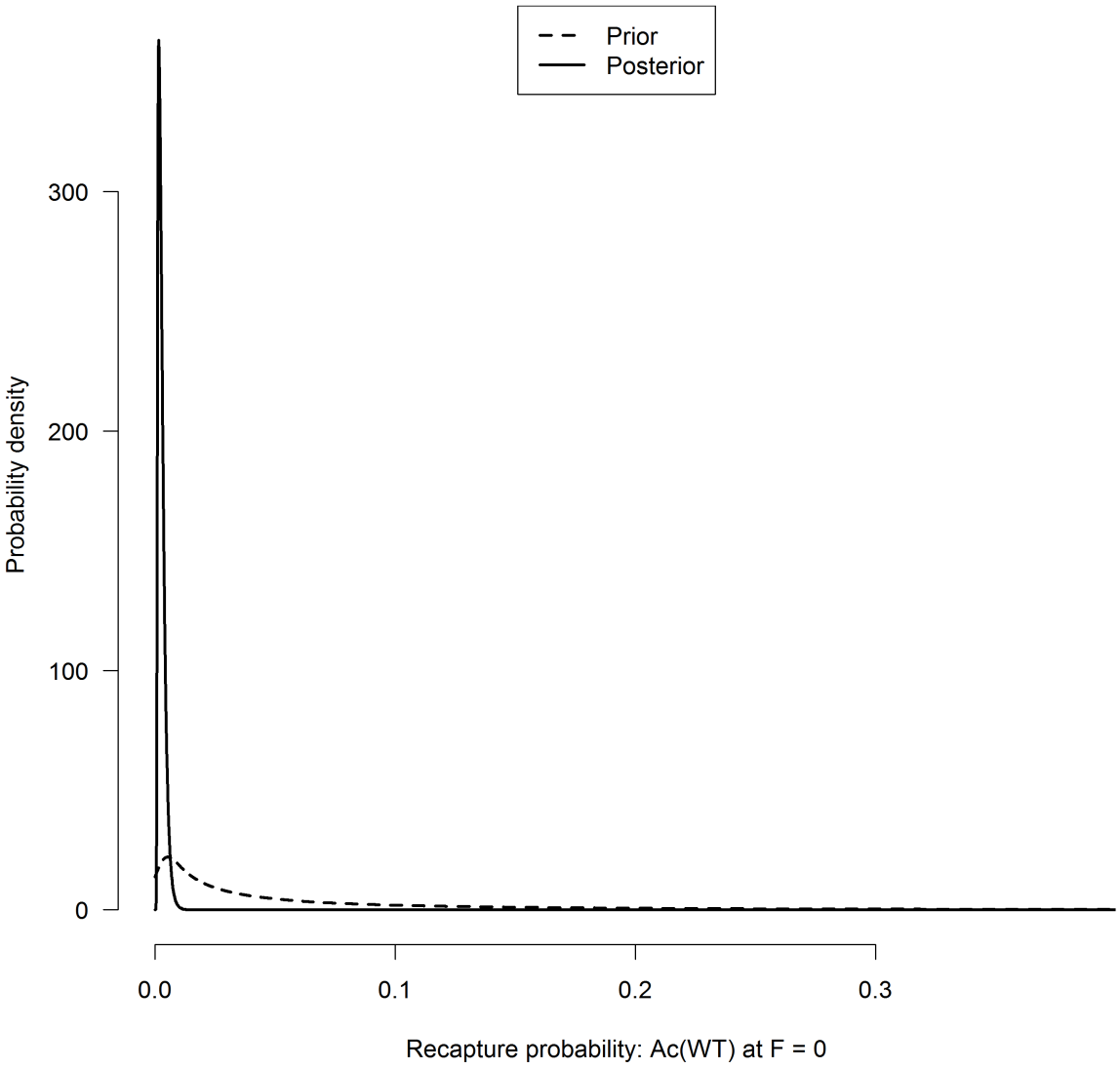


**Figure A.1:** Prior and mixture posterior probability of recapture probability for Ac(WT) at $F = 0$.

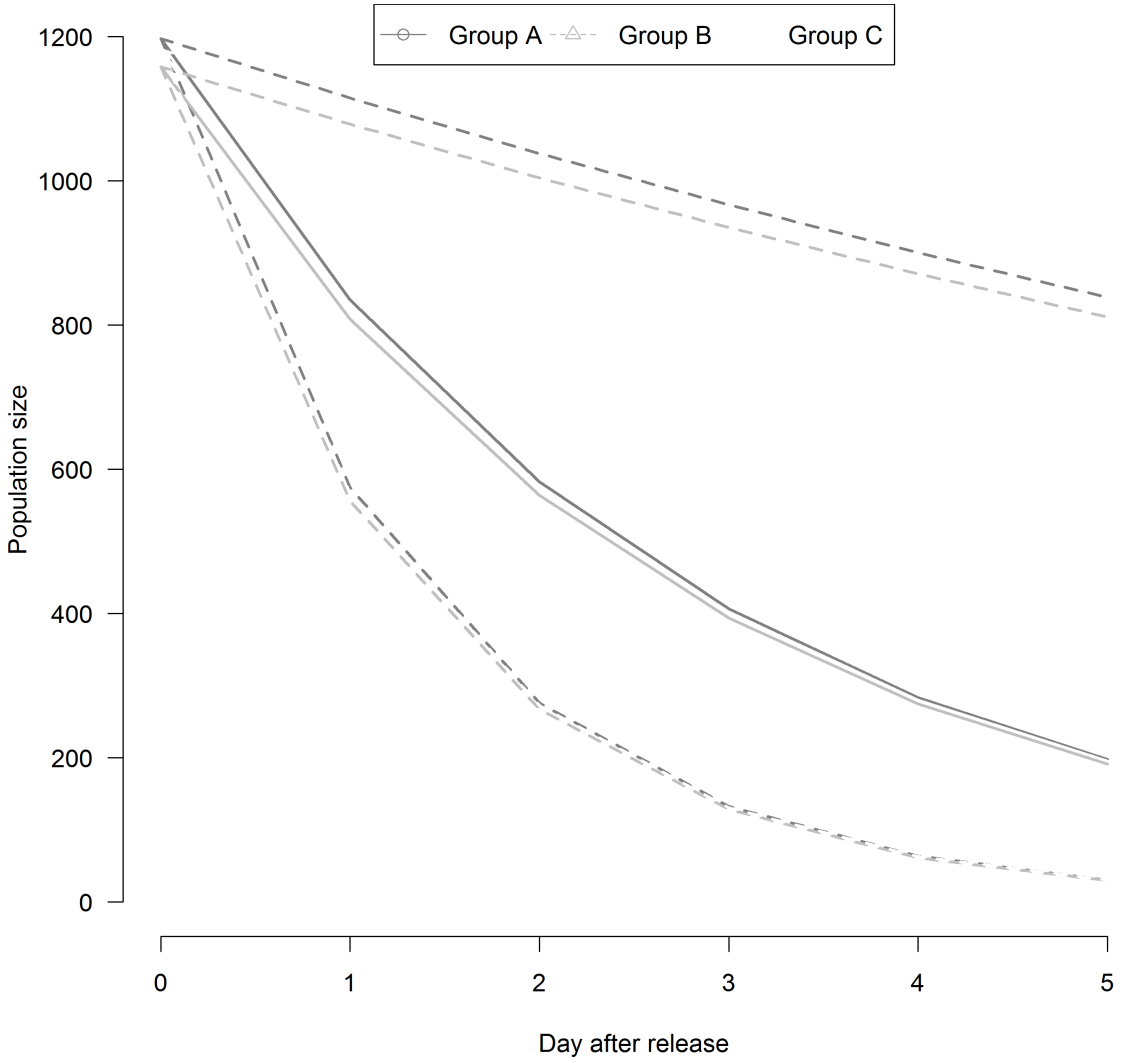


**Figure A.2:** Posterior estimated median (solid lines) and central 90% CI (dashed lines) for the populations of released Ac(WT) at $F = 0$ for three concurrent releases marked by red dye (Group A), blue dye (Group B) and green dye (Group C) by day.

## A.2 Female survival

The analysis adopts a simple model of Ac(DSM)2 females population dynamics following the field release:

$$\frac{dN(t)}{dt} = (\nu(t) - \mu)\, N(t) \implies \frac{dN(t)}{dt} - (\nu(t) - \mu)\, N(t) = 0 \tag{A.8}$$

where $N(t)$ is the female population size at time $t$, $\nu(t)$ is the time-dependent female birth parameter, and $\mu$ is the time-constant female mortality rate. Again we recognise that the model is simple and does not represent population dynamic mechanisms such as negative density dependence, Allee effects, environmental stochasticity or demographic stochasticity. The birth rate in the model is allowed to vary with time in order to mimic the observed seasonal fluctuations in mosquito abundance. In particular, the female birth-rate is defined to be a cyclical parametric function of time:

$$\nu(t) = \alpha_0 + \sum_{j=1}^{M} \left[ \alpha_j \cos\left(\frac{2\pi}{365} j t^*\right) + \beta_j \sin\left(\frac{2\pi}{365} j t^*\right) \right]$$

where $M$ is finite (and typically of the order of 1, 2, or 3), $t^*$ is the day-of-the-year $(1 \ldots 365)$, and $\alpha_j$ and $\beta_j$ are regression coefficients to be estimated from available data. This function is cyclic as $\nu(t) = \nu(t + 365)$, which is necessary for multi-year processes and $\alpha_0$ can be considered to be an 'average' birth rate for the entire year (as all the sine and cosine terms sum to zero over the year).

The model – that is Equation (A.8) – is a first-order, linear, inhomogeneous, function with the following analytical solution:

$$\log\left(N(t)\right) = \log(N_0) + (\alpha_0 - \mu)t + \sum_{j=1}^{M} \left[ \frac{365}{2\pi} \alpha_j \sin\left(\frac{2\pi}{365} j t^*\right) + \frac{365}{2\pi} \beta_j \left( \cos\left(\frac{2\pi}{365} j t^*\right) - 1 \right) \right] \tag{A.9}$$

and on the log scale, is a linear function of the coefficients $\alpha$ and $\beta$. This function can therefore be fit to entomological survey data (Figure 4.1) as a generalised linear model with log-link function and a suitable observation model.

To perform this regression we assume that the mosquito population is stable – that is there is no net annual population growth or decline over yearly and longer time scales – hence the average birth rate for the entire year is equal to the time constant mortality $\alpha_0 = \mu$ and the term $(\alpha_0 - \mu)t$ in (A.9) disappears.

The regression analysis explores two alternative observation models

$$\begin{aligned} N(t) &\sim \text{Poisson}\left(\lambda_t\right) \\ N(t) &\sim \text{NegBinomial}\left(\lambda_t, k\right) \end{aligned} \tag{A.10}$$

and five models for the unobserved (latent) process

$$\log\left(\lambda_t\right) = \log\left(\eta_0\right) + \sum_{j=1}^{M} \left[ \frac{365}{2\pi} \alpha_j \sin\left(\frac{2\pi}{365} j t^*\right) + \frac{365}{2\pi} \beta_j \left( \cos\left(\frac{2\pi}{365} j t^*\right) - 1 \right) \right] \tag{A.11}$$

where $M = 1, \cdots, 5$ and the intercept $\eta_0$ is an unknown function of population size, survey effort and detection probability. AIC and BIC indicate that the best fitting model is the Negative Binomial observation model, with three cyclical latent components ($M = 3$) (Figure A.3).

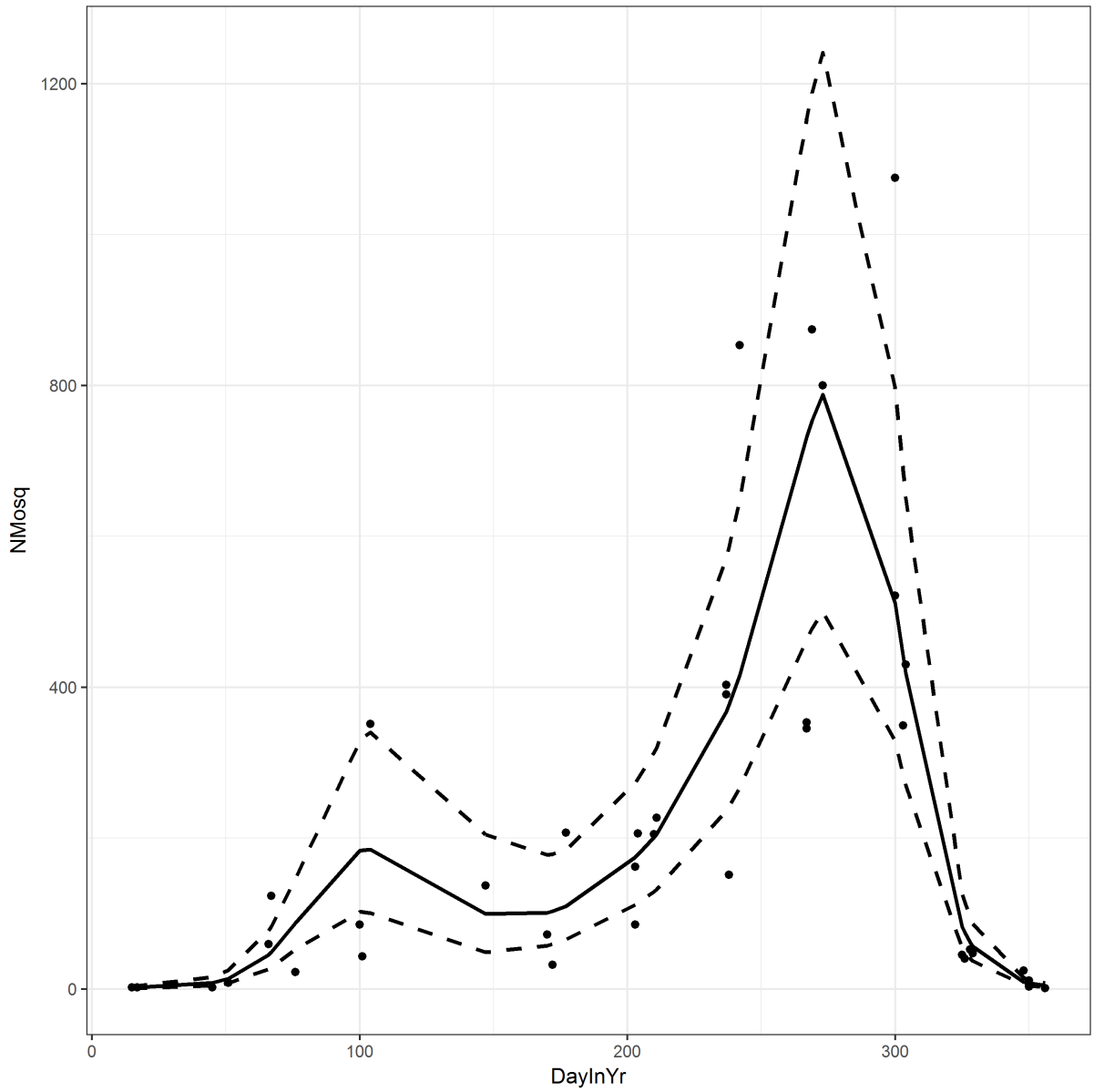


**Figure A.3:** Predicted mean (black line) and approximate 95% confidence interval (grey polygon) for the Negative Binomial regression model fitted to the monthly entomological survey data from Bana Village (black dots).

In the absence of any information on the birth rate of Ac(DSM)2 females in the field, we assume that the average birth rate of accidentally released Ac(DSM)2 females in the field is one half of the Bayesian updated mortality rate of wild type females (Figure 3.10). This approach assumes that all accidentally released Ac(DSM)2 females mate only with Ac(WT) males, and imposes a fitness cost due to the transgene of 0.5 relative to 1 for wild type females.

The analysis simulates the survival of Ac(DSM)2 females following the field release using model (A.9) with $\mu = \mu_{Ac(DSM)2}$ given by the posterior distribution for Ac(DSM)2 at backcross 29 (Figure 3.10), $\alpha_{0Ac(DSM)2} = -\mu_{WT}/2$ (as described above) and the mean and standard deviation of the regression coefficients $\alpha$ and $\beta$ estimated with (A.11). The model does not account for any density dependent effects on birth or death rates. The simulation is seeded with an initial population size $N_0 = 25$, released on the 1st July 2018.

## A.3 Insecticide resistance

The data, number of dead mosquitoes $y$ out of $N$ tested, and structure of the susceptibility tests suggest a hierarchical Binomial model, which we estimate by introducing weakly informative priors

$$\begin{aligned} y_i &\sim \text{Binomial}\,(N_i, p_i) \\ \text{logit}(p_{ijk}) &= \alpha_i + \beta_i x + \eta_{jk} \\ \alpha_i &\sim \text{Normal}\,(0, 10) \\ \beta_i &\sim \text{Normal}\,(0, 2) \\ \eta_{jk} &\sim \text{Normal}\,(0, 2)\,. \end{aligned} \tag{A.12}$$

Here $x$ is an indicator function taking the value 1 for the Ac(DSM)2 strain mosquitoes, $\alpha_i$ is a treatment level effect for the $i = 1, \cdots, 8$ treatments (including the control), $\beta_i$ is the transgene interaction coefficient and $\eta_{jk}$ is random effect for the $j = 1, \cdots, 6$ replicates within batches $k = 1, \cdots, 7$.

We use Hamiltonian Monte Carlo methods to draw samples from the posterior distribution of the parameters given the data. This was performed using the R packages rstan and rethinking, with 2 independent chains of 100,000 iterations each (the first 2,000 of which are warm up) to derive the joint posterior of the model coefficients conditional on the observations $p\left(\alpha_i, \beta_i, \eta_{jk} | y\right)$.

Table A.5 summarises the posterior distribution of the treatment effect and transgene interaction coefficients. Figure A.4 and Figure A.5 show the prior and posterior distributions for the treatment and transgene effect coefficients respectively. Table A.6 summarises the posterior distribution of the random effect coefficients.

Diagnostics for the treatment effect and transgene interaction coefficients (Table A.5), and the random replicate effect coefficients (Table A.6) indicate that the number of effective samples (n_eff) is sufficient for reliable inference, and the Rubin convergence diagnostic (Rhat) does not indicate signs of non-convergence in the MCMC chains.

The posterior distributions of the transgene interaction coefficients suggest that the Ac(DSM)2 strain experienced lower mortality (better survival) in the control than the Ac(WT) strain (although the evidence for this is not strong), but equal or higher mortality in all of the other treatments, particularly DDT, Deltamethrin and Permethrin. There is no suggestion within the posterior distributions that Ac(DSM)2 strain has lower mortality (higher resistance) to insecticides than Ac(WT).

The summary statistics of the posterior distributions of the replicate effect coefficients do not indicate any significant bias in any of the replicates except for first replicate of the Permethrin test ($w[25]$). Here the model suggests some significant additional mortality occurred beyond that expected due to the treatment or transgene effect.

To calculate the probability of a change in insecticide resistance attributable to the genetic construct (the risk endpoint $\mathcal{R}$) we calculate the difference in the posterior probability of

**Table A.5:** Posterior treatment and transgene effect coefficient summary

| | Mean | StdDev | lower 0.95 | upper 0.95 | n_eff | Rhat |
|---|---|---|---|---|---|---|
| a[1] | -4.88 | 0.75 | -6.38 | -3.44 | 93502 | 1 |
| a[2] | 1.14 | 1.03 | -0.87 | 3.16 | 61722 | 1 |
| a[3] | 5.55 | 1.52 | 2.64 | 8.60 | 117061 | 1 |
| a[4] | 1.15 | 1.03 | -0.89 | 3.15 | 59414 | 1 |
| a[5] | 0.02 | 1.02 | -2.00 | 2.00 | 60578 | 1 |
| a[6] | 12.75 | 5.18 | 4.40 | 23.27 | 129809 | 1 |
| a[7] | 2.15 | 1.05 | 0.11 | 4.25 | 65702 | 1 |
| a[8] | 1.00 | 1.03 | -1.02 | 3.02 | 62405 | 1 |
| bGMOT[1] | -1.29 | 0.77 | -2.83 | 0.18 | 196000 | 1 |
| bGMOT[2] | 0.59 | 0.38 | -0.15 | 1.35 | 196000 | 1 |
| bGMOT[3] | 0.11 | 1.26 | -2.41 | 2.59 | 196000 | 1 |
| bGMOT[4] | 1.06 | 0.42 | 0.24 | 1.91 | 196000 | 1 |
| bGMOT[5] | 1.27 | 0.34 | 0.61 | 1.94 | 196000 | 1 |
| bGMOT[6] | 0.24 | 1.92 | -3.58 | 3.98 | 196000 | 1 |
| bGMOT[7] | 0.45 | 0.50 | -0.54 | 1.41 | 196000 | 1 |
| bGMOT[8] | 1.70 | 0.49 | 0.77 | 2.67 | 196000 | 1 |

**Table A.6:** Posterior random effects coefficient summary

| | Mean | StdDev | lower 0.95 | upper 0.95 | n_eff | Rhat |
|---|---|---|---|---|---|---|
| w[1] | 0.72 | 1.20 | -1.71 | 3.0 | 196000 | 1 |
| w[2] | -0.94 | 1.58 | -4.06 | 2.1 | 196000 | 1 |
| w[3] | -0.04 | 1.03 | -2.05 | 2.0 | 62627 | 1 |
| w[4] | 1.13 | 1.05 | -0.93 | 3.2 | 64289 | 1 |
| w[5] | -0.95 | 1.03 | -2.95 | 1.1 | 62342 | 1 |
| w[6] | -0.15 | 1.03 | -2.15 | 1.9 | 62488 | 1 |
| w[7] | -0.93 | 1.59 | -4.12 | 2.0 | 196000 | 1 |
| w[8] | -0.94 | 1.58 | -4.15 | 2.0 | 196000 | 1 |
| w[9] | 0.13 | 1.95 | -3.68 | 4.0 | 196000 | 1 |
| w[10] | 0.12 | 1.94 | -3.62 | 4.0 | 196000 | 1 |
| w[11] | 0.13 | 1.94 | -3.67 | 4.0 | 196000 | 1 |
| w[12] | 0.14 | 1.95 | -3.66 | 4.0 | 196000 | 1 |
| w[13] | -0.96 | 1.58 | -4.18 | 1.9 | 196000 | 1 |
| w[14] | 0.90 | 1.22 | -1.55 | 3.2 | 196000 | 1 |
| w[15] | -0.52 | 1.05 | -2.59 | 1.5 | 61902 | 1 |
| w[16] | 0.80 | 1.09 | -1.32 | 2.9 | 64608 | 1 |
| w[17] | 0.35 | 1.07 | -1.75 | 2.4 | 63974 | 1 |
| w[18] | -0.61 | 1.05 | -2.64 | 1.5 | 61758 | 1 |
| w[19] | 0.89 | 1.22 | -1.57 | 3.2 | 196000 | 1 |
| w[20] | -0.94 | 1.58 | -4.11 | 2.0 | 196000 | 1 |
| w[21] | -0.45 | 1.04 | -2.50 | 1.6 | 63978 | 1 |
| w[22] | -0.18 | 1.05 | -2.21 | 1.9 | 64806 | 1 |
| w[23] | 0.52 | 1.06 | -1.57 | 2.6 | 65687 | 1 |
| w[24] | 0.15 | 1.05 | -1.91 | 2.2 | 64793 | 1 |
| w[25] | 2.23 | 0.93 | 0.38 | 4.0 | 131151 | 1 |
| w[26] | 0.84 | 1.22 | -1.59 | 3.2 | 196000 | 1 |
| w[27] | -0.07 | 1.06 | -2.13 | 2.0 | 65873 | 1 |
| w[28] | 0.45 | 1.08 | -1.65 | 2.6 | 67627 | 1 |
| w[29] | -0.67 | 1.06 | -2.76 | 1.4 | 65459 | 1 |
| w[30] | 0.34 | 1.08 | -1.78 | 2.4 | 68065 | 1 |
| w[31] | -0.96 | 1.58 | -4.10 | 2.0 | 196000 | 1 |
| w[32] | 1.80 | 1.03 | -0.22 | 3.8 | 196000 | 1 |
| w[33] | 0.06 | 1.10 | -2.09 | 2.2 | 72834 | 1 |
| w[34] | 0.43 | 1.12 | -1.73 | 2.6 | 75453 | 1 |
| w[35] | -0.95 | 1.07 | -3.08 | 1.1 | 68856 | 1 |
| w[36] | 0.52 | 1.11 | -1.66 | 2.7 | 72940 | 1 |
| w[37] | -0.91 | 1.59 | -4.11 | 2.0 | 196000 | 1 |
| w[38] | -0.99 | 1.57 | -4.14 | 1.9 | 196000 | 1 |
| w[39] | -0.91 | 1.46 | -3.77 | 1.9 | 196000 | 1 |
| w[40] | 0.95 | 1.66 | -2.24 | 4.2 | 196000 | 1 |
| w[41] | -0.79 | 1.46 | -3.64 | 2.1 | 196000 | 1 |
| w[42] | 0.95 | 1.66 | -2.25 | 4.3 | 196000 | 1 |

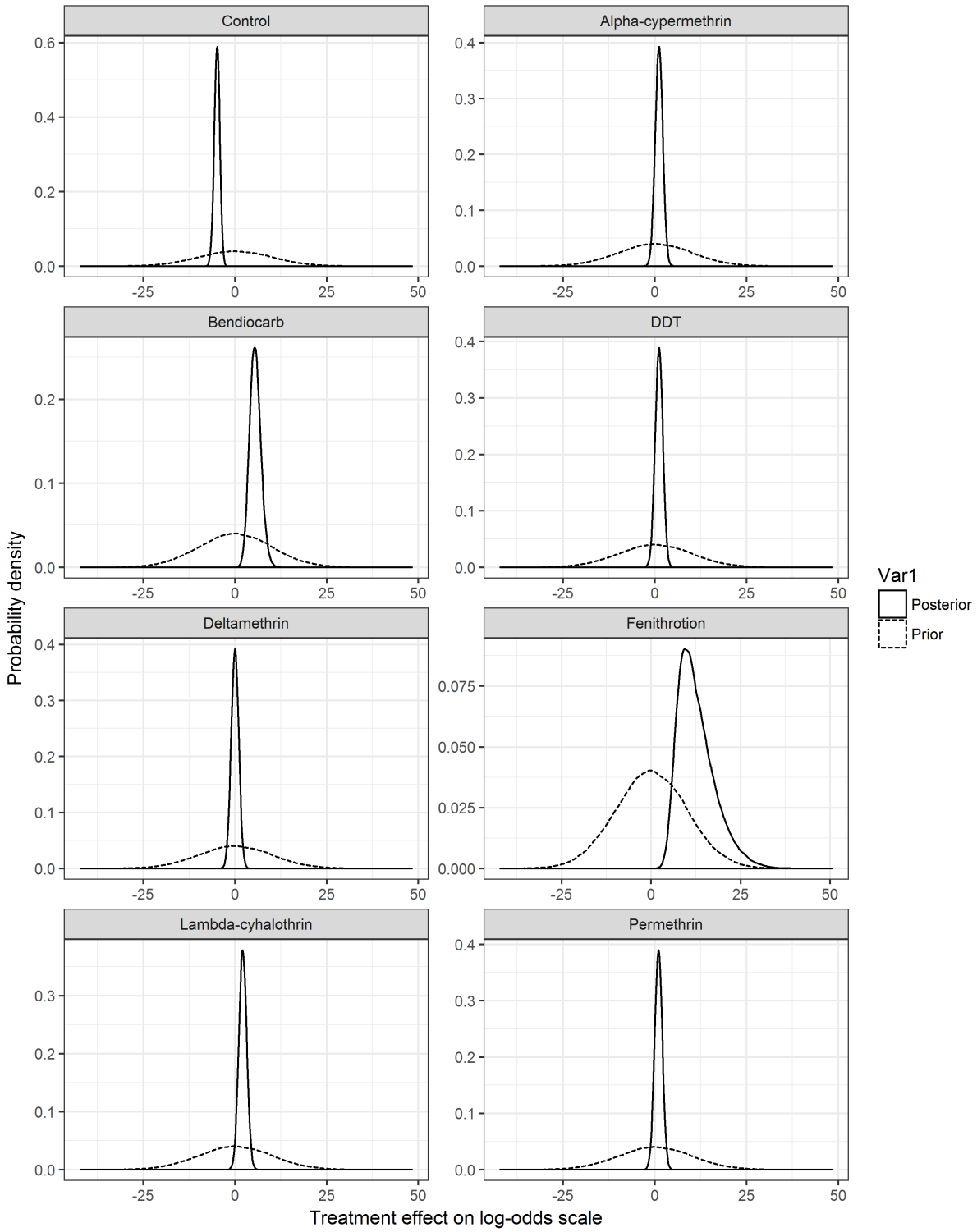


**Figure A.4:** Posterior probability of insecticide treatment coefficients $\alpha_i$ (relative effect on log odds scale)

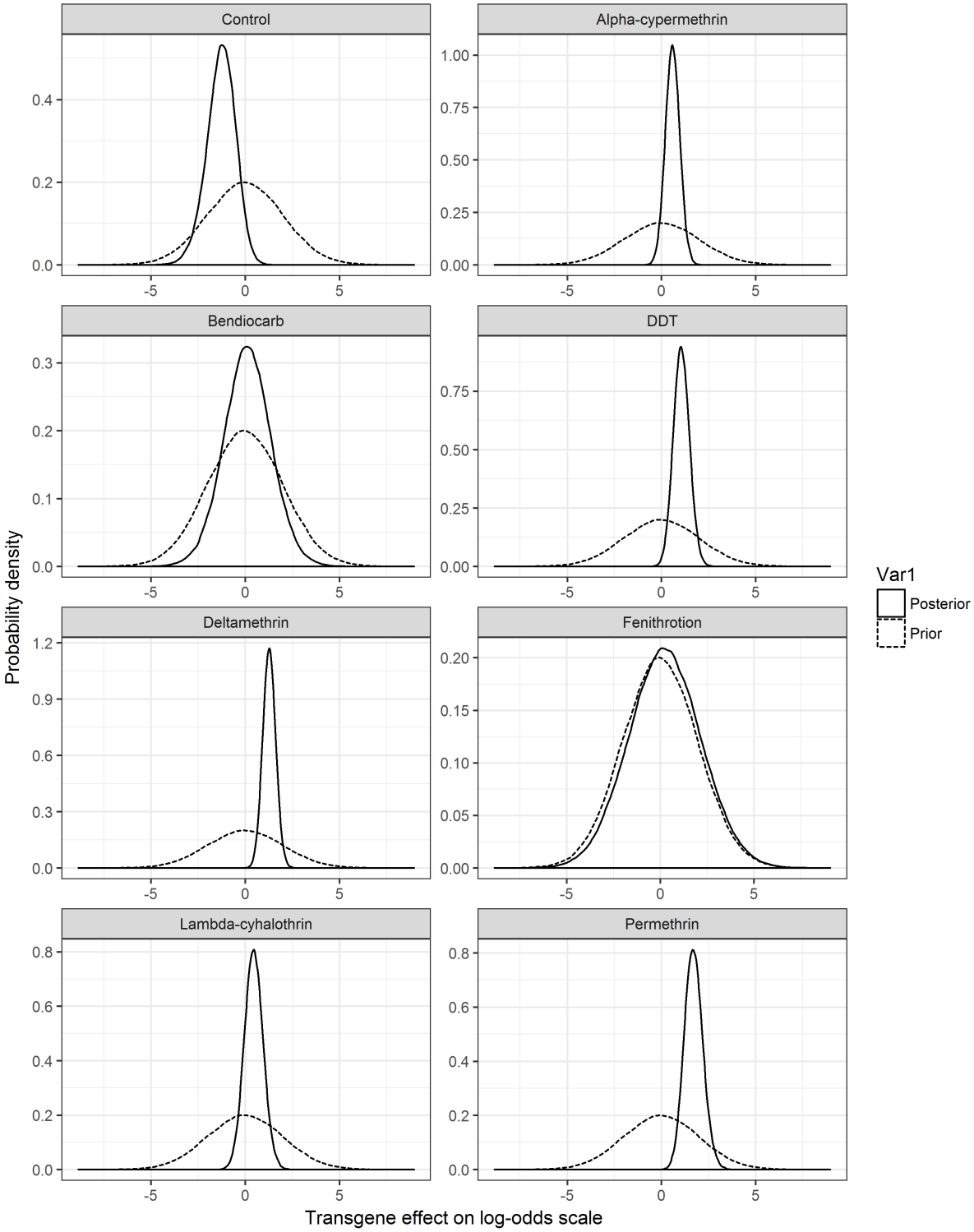


**Figure A.5:** Posterior probability of transgene interaction coefficients $\beta_i$ (relative effect on log odds scale)

mortality between Ac(DSM)2 and Ac(WT)

$$\begin{aligned} \mathcal{R} &= \left(p_i^{GE} - p_i^{WT}\right) \\ p_i^{GE} &= g^{-1}\left(\alpha_i + \beta_i + \eta_{jk}\right) \\ p_i^{WT} &= g^{-1}\left(\alpha_i + \eta_{jk}\right) \end{aligned} \tag{A.13}$$

where $g^{-1}(.)$ is the inverse link function (inverse logit), and $\alpha_i$,$\beta_i$ are drawn from the joint posterior distribution for each treatment.

## A.4 Spread and survival of sterile males

### A.4.1 Latent process model

The spread and survival model is a partial differential equation that combines reaction terms (death of male mosquitoes) with advection and diffusion terms (attraction to swarm locations and random dispersal)

$$\lambda_t = \boldsymbol{\delta}^2\Big[\lambda_{ss} - \boldsymbol{\alpha} \cdot \nabla_s \left(\lambda U(\boldsymbol{s})\right)\Big] - \boldsymbol{\mu}\lambda. \tag{A.14}$$

with the following boundary conditions (Neumann reflecting conditions):

$$\frac{\partial \lambda}{\partial s} = 0 \tag{A.15}$$

$\nabla_s$ is the spatial gradient, and the model parameters are:

- $\lambda$: The expected number of mosquitoes present at time $t$ at location $\boldsymbol{s} = (\text{easting}, \text{northing})$. This is the solution to the partial differential equation. The partial derivatives are denoted $\lambda_t$ (with respect to time) and $\lambda_{ss}$ (with respect to location).
- $\boldsymbol{\delta}^2$: The diffusion coefficient ($m^2$.day$^{-1}$), assumed to be the same in all directions.
- $\boldsymbol{\alpha}$ The relative importance of chemotaxis ($m^{-1}.R^{-1}$). Male mosquitoes are assumed to be attracted to swam locations in order to breed with female mosquitoes who are attracted to residences possibly by $CO_2$. The $R$ in the units is a measure of reward and for female mosquitoes could be related to a concentration of $CO_2$. Large values of $\boldsymbol{\alpha}$ imply that the movement of males mosquitoes is dictated mostly by chemotaxis whereas a zero value implies that all movement is diffusion.
- $\sigma$: The range of attraction of the swarm sites (m). The parameter measures the distance at which the male mosquitoes notice (or "smell") a particular swarm site.
- $\boldsymbol{\mu}$: The daily mortality rate of male mosquitoes (day$^{-1}$), related to the daily probability of mortality $q$ by the expression $\boldsymbol{\mu} = -\log(1-q)$

The term $U(\boldsymbol{s}) \triangleq \boldsymbol{x}(\boldsymbol{s})^\top \boldsymbol{\beta}$ in Equation (A.14) is a utility function that describes how attractive a location $\boldsymbol{s}$ is to male mosquitoes in terms of the environmental covariates $\boldsymbol{x}(\boldsymbol{s})$ and a coefficient $\beta$. The model uses the known swarm locations in Bana village as the center of the attraction area. In general $\beta$ is the relative weight of the advection fields – that is the relative influence of multiple attractants. Here we assume only one attractant (swarm sites), hence the advection field is unidimensional and $\beta = 1$.

The attraction to swarms site is assumed to decrease exponentially with distance, and is modelled using a squared-sum exponential decay kernel with range parameter $\sigma$:

$$\boldsymbol{x}(\boldsymbol{s}) = \sum_{\boldsymbol{s}_\ell \in \mathcal{L}} \exp\Big[-\frac{(\boldsymbol{s}-\boldsymbol{s}_\ell)^2}{\sigma}\Big] \tag{A.16}$$

where $\mathcal{L}$ lists the known swarm locations.

The observations (counts) of male mosquitoes is modelled as a two step process that reflects variation in counts given probability of capture and variation in the probability of capture given the number of mosquitoes in the vicinity of a capture device (trap). The counts in a trap are assumed to follow a Binomial distribution:

$$\mathrm{y}_{\mathrm{r,c}} | n_r, \theta_c \sim \text{Binomial}(n_r, p_c) \tag{A.17}$$

where $n_r$ is the number of trappable mosquitoes in the vicinity of a trap, and $p_c$ represents the trap's "efficiency" - that is what proportion of trappable mosquitoes it actually captures, which will be device dependent (and also dependent on the process model's grid resolution). The number of mosquitoes in the vicinity of a trap is assumed to follow a Poisson distribution

$$n_r|\lambda_t(\theta_d) \sim \text{Poisson}(\lambda_t(\theta_d)) \tag{A.18}$$

where $\lambda_t(\theta_d)$ is the expected number of mosquitoes given by Equation A.14. The model is deterministic so its parameters $\theta_d$ entirely define $n_r$.

The posterior distribution of the process ($\theta_d$) and observation model ($\theta_c$) parameters given the observations from the Mark Release Recapture experiments is obtained by integrating over the possible Poisson outcomes (and their respective probabilities):

$$\pi(\theta_d, \theta_c|\text{y}) \propto \prod_c \prod_r \Big[ \sum_{n_r} \underbrace{\pi(\text{y}_{\text{r,c}}|n_r, \theta_c)}_{data} \underbrace{\pi(n_r|\lambda_t(\theta_d))}_{process} \Big] \underbrace{\pi(\theta_d), \pi(\theta_c)}_{priors} \tag{A.19}$$

The prior distribution for the observation model parameters $\pi(\theta_c)$ - the trap device specific efficiency $p_c$ in Equation (A.17) - are difficult to specify because catchability in the context of the model is related to the resolution of the model grid over which numerical solutions to Equation (A.14) are calculated. The analysis used a uniform distribution of the range $[0, 1]$ for PSC and pots, and a beta distribution with parameters 5, and 10 for swarm netting.

The prior distributions for the dispersion parameter ($\delta^2$) is based on expert knowledge, and has been obtained through expert elicitations for *An. gambiae* (see Hayes et al., 2015, for details). The model's parameter was not directly elicited. The experts all indicated that a log-normal distribution was appropriate for dispersal. We standardised the time frame (to a seven day period), so that all experts' opinions could be combined. Overall, the idea is to use the relationship between the dispersal distance ($r$) and the diffusion ($\delta$):

$$\delta^2 = \frac{r^2}{4t} \tag{A.20}$$

### A.4.2 Chemotaxis parameters

There are two chemotaxis related parameters. The first relates to the relative attractiveness of a zone of interest ($\alpha$, units being $m^{-1}R^{-1}$, where $R$ is a measure of reward, typically a $CO_2$ concentration level). The second relates to the distance at which a mosquito "smells" that particular area (also called range, denoted $\sigma$, units being $m$). Finding prior information on these parameters proved difficult for the particular setting of this model, in particular for $\alpha$. For that reason we chose a distribution spanning multiple orders of magnitude, from $1\%$ (1st percentile) to $140\%$ (99th percentile).

For the range $\sigma$, the searched the published literature for chemotaxis observations with mosquitoes and other arthropods. Values cited in the literature range from $3$m (McIver and McElligott, 1989) to $18$m for single baiting experiments, to values over $36$m for double baiting experiments (Gillies and Wilkes, 1972), with values as high as $40$m (Zhu et al., 2015, unpublished study results). We fit a log-normal distribution to this prior (Figure A.7), with parameters listed in Table A.7, that provided positive probability across all of these possibilities.

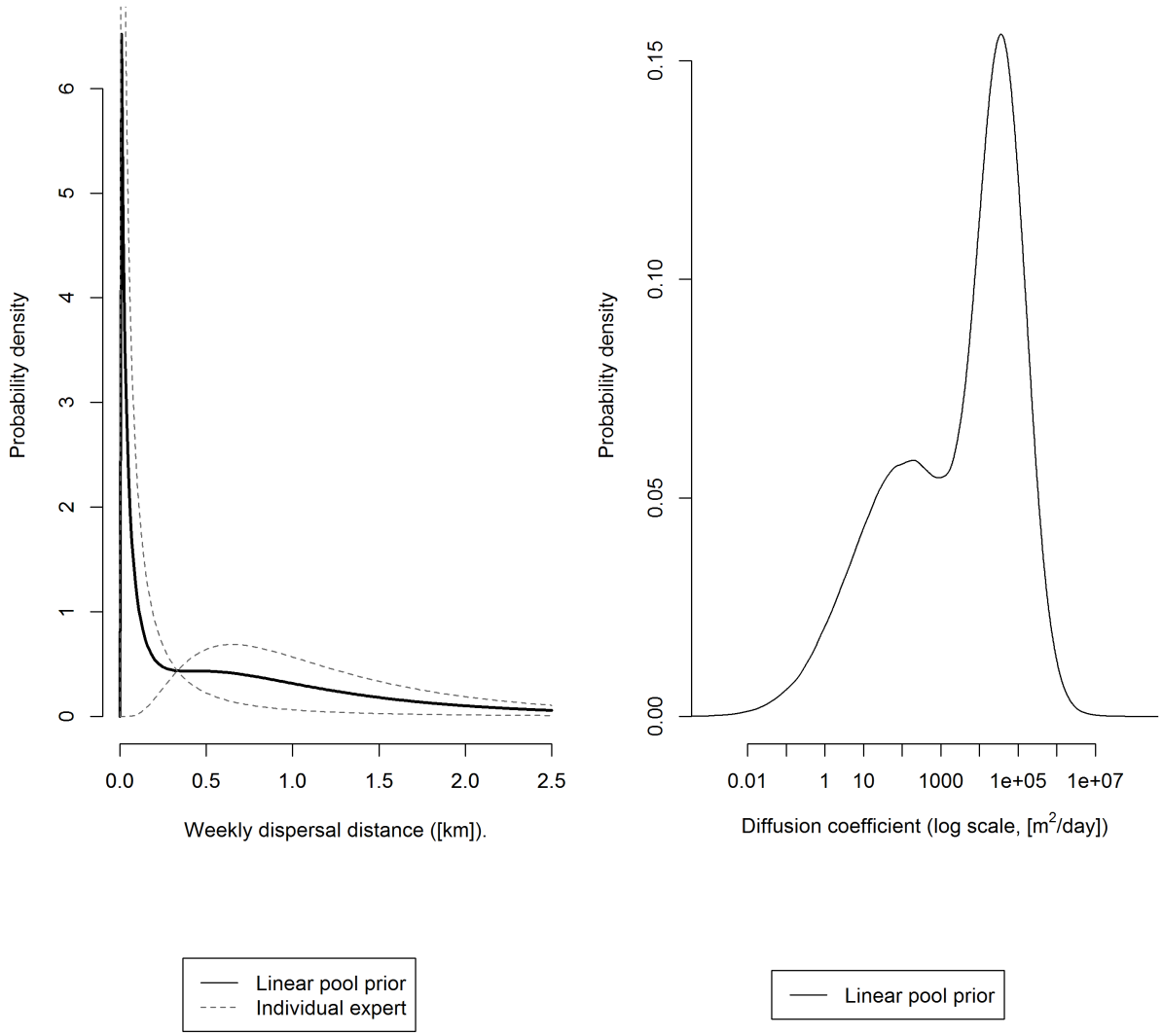


**Figure A.6:** Elicited prior for dispersal (left plot) and resulting linear pool prior for diffusion (right plot) for Ag(WT) male.

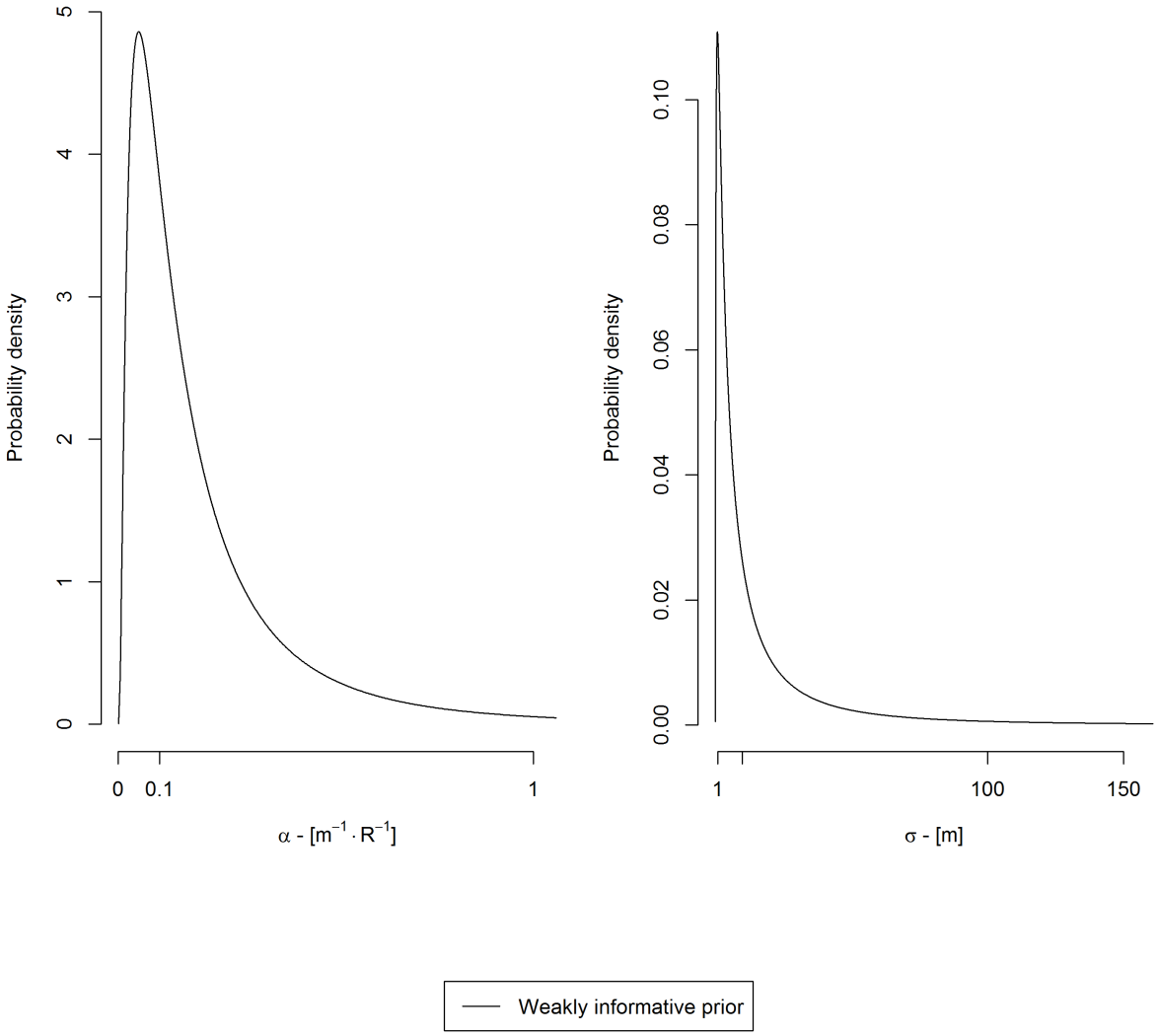


**Figure A.7:** Weakly informative prior distributions for chemotaxis parameters: range (right plot) and attraction (left plot) parameters. The unit R stands for *Reward*, and could be a $CO_2$ concentration or anything susceptible to attract male mosquitoes.

| Name | $\theta_1$ | $\theta_2$ | mean | q01 | q05 | q95 | q99 |
|---|---|---|---|---|---|---|---|
| $\alpha$ | -2 | 1 | 0.22 | 0.01 | 0.03 | 0.70 | 1.39 |
| $\sigma$ | 2 | 1 | 12.18 | 0.72 | 1.43 | 38.28 | 75.67 |

**Table A.7:** Summary statistics of the chemotaxis parameters prior distributions

### A.4.3 Mortality rate

An informative prior distribution for the daily probability of male mortality, from which the diffusion coefficient prior can be derived, was collected by Hayes et al. (2015). During that elicitation, experts answered the mortality question using different metrics. In particular experts provided either a daily probability of mortality, or a daily probability of survival. Both were converted to daily probability of mortality and then converted to the mortality rate parameter used in the PDE model (Figure A.8).

### A.4.4 Catchability parameters

The number of mosquitoes likely to be caught in a trap, for a given a trap effort and release parameters, is unknown because the trap catch rates are unknown. That is, we don't know how many mosquitoes a trap will catch given a known number in the proximity of the trap. This is encompassed in a catchability parameter, defined as the probability of catching a mosquito given that it was in the locality of the trap at the time of sampling. The catchability of a mosquito is sometimes referred to as the detectability (see for example Martin et al., 2005). This is represented in the analysis by the parameters $p_{CFR}$, $p_{PSC}$ and $p_{Swarm}$. The prior and posterior estimates of these parameters are model dependent and grid scale dependent – the values of these parameters will depend on, and vary by, the resolution of the grid used to numerically solve the PDE. As a result it is important that all model predictions on made on the same grid resolution as that used to update the priors distributions. The priors we used for these parameters are uniform between $0$ and $1$ for spray catches and clay pots, and beta with parameters $5$ and $10$ for the swarm catches (meaning on average one third of the mosquitoes in the swarm are caught).

### A.4.5 Bayesian inference

Inference about the parameters can be made using the data from Mark Release Recapture experiments described by Epopa et al. (2017). During the first four experiments mosquitoes were released two hours before swarming (around 4pm), at three different locations (see Table A.9), on 4 different dates (see Table A.8). Swarm sampling subsequently occurred during the evening (not specified, but we assume the sampling occured between 7pm and 9pm). Pyrethroid Spray Catches (PSC) were conducted in compounds in the following morning and Clay pots were laid overnight, and collected in the morning (6am-7am). A fifth experiment was also performed, but only at one location with a much larger number of mosquitoes. We use this experimental data to validate the posterior predictions of the model.

Estimating the parameters of a linear Partial Differential Equation using data can be achieved using frequentists or Bayesian approaches (McGoff et al., 2015; Chkrebtii et al., 2016). Hierarchical Bayesian models (BHM Wikle, 2003; Ruggeri et al., 2017) have been success-

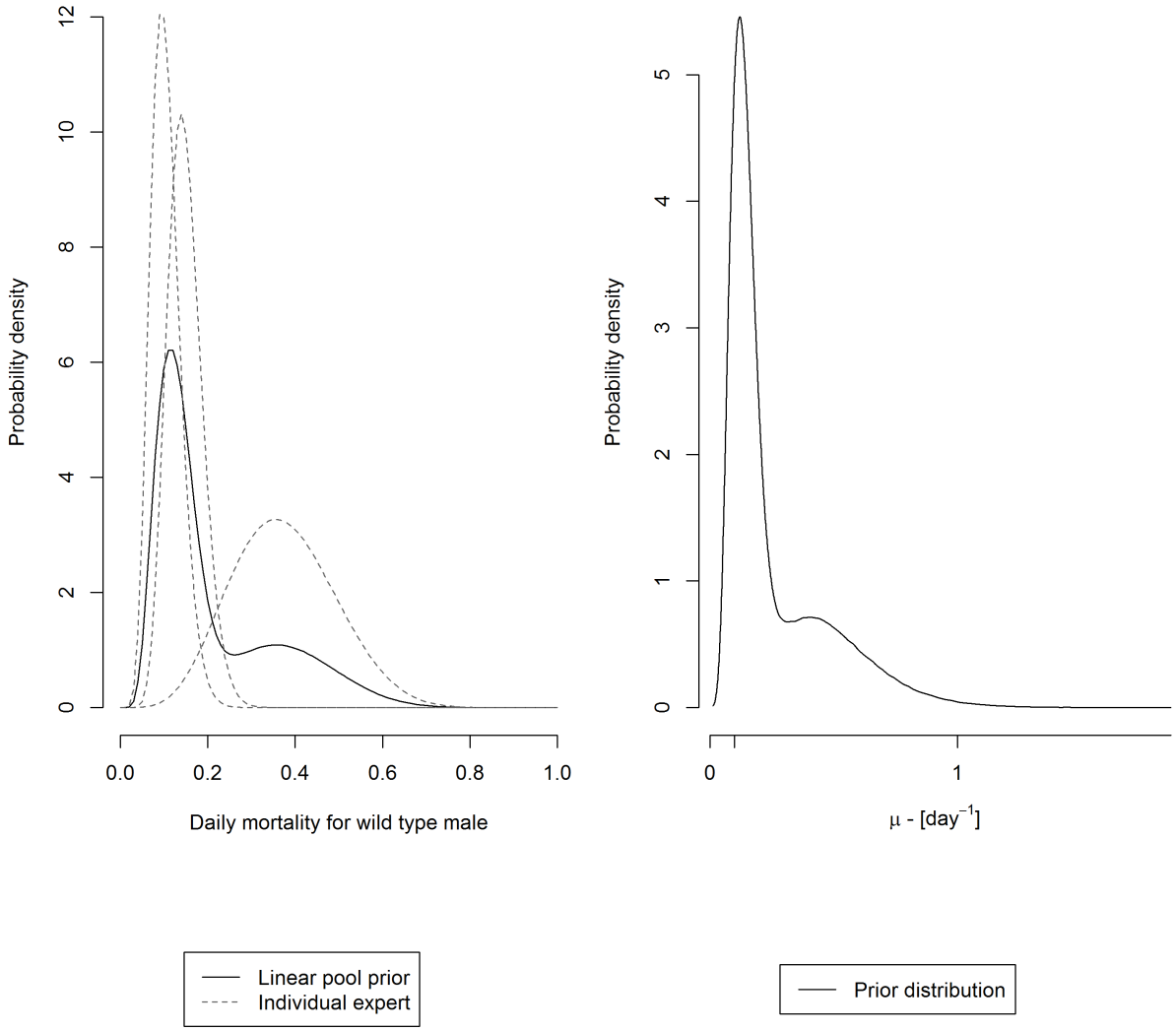


**Figure A.8:** Elicited prior for daily mortality (left plot) and resulting (linear) prior for mortality parameter (right plot).

| MRR | Release date |
|---|---|
| 1 | 2013-10-09 |
| 2 | 2014-05-07 |
| 3 | 2014-09-04 |
| 4 | 2015-04-09 |

**Table A.8:** Summary of the MRR release dates

fully applied to linear systems for many years, and the risk assessment adopts this approach. A Bayesian Hierarchical Model is classically written:

$$\boldsymbol{\pi}(\theta, \lambda|\mathsf{y}) \quad \propto \quad \underbrace{\boldsymbol{\pi}(\mathsf{y}|\lambda)}_{data}\,\underbrace{\boldsymbol{\pi}(\lambda|\theta)}_{process}\,\underbrace{\boldsymbol{\pi}(\theta)}_{prior} \tag{A.21}$$

where $\theta$ refers to the set of parameters of the PDE model and the trapping efficiency, $\lambda$ refers to the output of the PDE model given a set of parameter values, and $y$ refers to the MRR observations. More specifically, the data have been collected out of $12$ releases ($r = 1, \ldots, 12$), and from $3$ different collection techniques ($c = 1, \ldots, 3$). Assuming independence, the posterior distribution can be re-written:

$$\boldsymbol{\pi}(\theta_d, \lambda_r, \theta_c|\mathsf{y}) \quad \propto \quad \prod_c \prod_r \Big[ \underbrace{\boldsymbol{\pi}(\mathsf{y}_{\mathsf{r,c}}|n_r, \theta_c)}_{data}\,\underbrace{\boldsymbol{\pi}(n_r|\lambda(\theta_d))}_{process} \Big]\,\underbrace{\boldsymbol{\pi}(\theta_d)}_{prior} \tag{A.22}$$

In the present situation, we consider y as the observed count in a given trap. Because a trap only catch a portion of the mosquitoes available at a given location, a natural model is the Binomial distribution:

$$\mathsf{y}_{\mathsf{r,c}}|n_r, \theta_c \quad \sim \quad \text{Binomial}(n_r, p_c) \tag{A.23}$$

where $n_r$ represent the number of mosquitoes available in the trap area, and $p_c$ represents the "trap efficiency", which will be catching method dependant. In this study we do not parametrize false positive and false negative probabilities as they are assumed to be equal to $0$ (Epopa et al., 2017).

The analysis subsequently assumes that the number of mosquitoes available to the trap is distributed as a Poisson distribution, with parameter $\lambda_t(\theta_d)$:

$$n_r|\lambda_t(\theta_d) \quad \sim \quad \text{Poisson}(\lambda_t(\theta_d)) \tag{A.24}$$

where $\lambda_t(\theta_d)$ is the output of the PDE model described in Eq. A.14. Because this equation defines a deterministic model, $\theta_d$ entirely defines $n_t$.

To evaluate the log-likelihood, we simply integrate over the possible Poisson outcomes (and their respective probabilities):

$$\boldsymbol{\pi}(\theta_d, \theta_c|\mathsf{y}) \quad \propto \quad \prod_c \prod_r \Big[ \sum_{n_r} \underbrace{\boldsymbol{\pi}(\mathsf{y}_{\mathsf{r,c}}|n_r, \theta_c)}_{data}\,\underbrace{\boldsymbol{\pi}(n_r|\lambda_t(\theta_d))}_{process} \Big]\,\underbrace{\boldsymbol{\pi}(\theta_d)}_{prior} \tag{A.25}$$

| MRR | Release site | Longitude | Latitude | Number released |
|---|---|---|---|---|
| 1 | A | -4.4724 | 11.2347 | 1146 |
| 1 | B | -4.4755 | 11.2342 | 1158 |
| 1 | C | -4.4718 | 11.2318 | 1103 |
| 2 | A | -4.4724 | 11.2347 | 1878 |
| 2 | B | -4.4755 | 11.2342 | 1655 |
| 2 | C | -4.4718 | 11.2318 | 1734 |
| 3 | A | -4.4724 | 11.2347 | 1665 |
| 3 | B | -4.4755 | 11.2342 | 1673 |
| 3 | C | -4.4718 | 11.2318 | 1684 |
| 4 | A | -4.4724 | 11.2347 | 2107 |
| 4 | B | -4.4755 | 11.2342 | 2013 |
| 4 | C | -4.4718 | 11.2318 | 1953 |
| 5 | C | -4.4724 | 11.2347 | 5992 |

**Table A.9:** Summary of the MRR release location coordinates

| | 0 | 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|---|
| PSC | 704 | 8 | 1 | 0 | 1 | 0 |
| Pot | 2483 | 13 | 0 | 0 | 0 | 0 |
| Swarm | 1930 | 67 | 14 | 6 | 1 | 1 |

**Table A.10:** Occurence of number of mosquitoes per trap per catching method

### A.4.6 Evaluating the PDE model

The dispersal and survival model does not have an analytical solution and we therefore evaluate it using numerical integration methods with daily time steps across a grid of locations. Here we used the R-package `ReacTran` (Soetaert and Meysman, 2012) to perform the integration. `ReacTran` uses a finite-differencing method to solve PDE's, which is common and well-established approach (see for example Soetaert and Herman, 2008). The basic premise is that the continuous space, where the PDE is defined, can be well-approximated by a discrete grid. Defining the grid is a compromise between computational efficiency and numerical accuracy. Coarse grid resolution is computationally efficient but numerically inaccurate and fine grids are the reverse. For this analysis, we developed a compromise solution by defining a reasonably fine grid around the release area (where most mosquitoes will stay) whereas outside this area we allow a coarser grid as the boundaries are approached. The grid used for computation is shown in Figure A.1. We define the 'inner' area (where the grid is finest) to be three times the range of the spatial coordinates of the

swarms. The boundary of the outer area is such that each spatial coordinate is seven times the range of the swarms. There are a total of 100 cells in the easting direction and 150 cells in the northing direction, which corresponds to (roughly) square cells in the finest (inner) grid. Figure A.9 presents the used grids in the solver.

### A.4.7 Sampling algorithm

Given the large time required to run a single simulation, parallelization may be considered, in which case rejection or importance sampling methods (see Liu, 2004) can be used. The acceptance rate for these methods, however, is strongly dependent on the choice of proposal distribution, and a better option is often to use a random walk metropolis-hastings algorithm instead (Gelman et al., 2014, describe a number of simulation algorithms). A final improvement to the sampling can be achieved by using adaptive methods such as the adaptive metropolis presented in Haario et al. (2001). The acceptance rate being higher, it does converge in less iteration to the required posterior distribution. Details of the algorithms are presented in the Appendix section.

### A.4.8 Posterior distributions and model evidence

Collecting field data for the wild type male mosquito allows us to update the prior distribution for the different parameters and also allows us to measure how well the prior distribution matches the observed data. Ultimately, this allows us to weight the prior from each expert. The theory is often referred to as model evidence (Robert, 2007) and its measure is called the Bayes factor (Kass and Raftery, 1995). Essentially, if we have a collection of models, $\{\mathcal{M}_1, \ldots, \mathcal{M}_m\}$, and a dataset $D$, then we can calculate the probability of a model given the data:

$$p(\mathcal{M}_k|D) = \frac{p(D|\mathcal{M}_k)p(\mathcal{M}_k)}{\sum_\ell p(D|\mathcal{M}_\ell)p(\mathcal{M}_\ell)} \tag{A.26}$$

The main difficulty is often to calculate the likelihood term:

$$p(D|\mathcal{M}_k) = \int p(\theta|\mathcal{M}_k)p(D|\theta, \mathcal{M}_k)d\theta \tag{A.27}$$

as it requires a fairly large integration over the parameter $\theta$. In our context, each model is a different expert opinion on the prior distribution for a parameter $\theta$, so we can write:

$$p(D|\mathcal{E}_k) = \int \pi_k(\theta)L(D|\theta)d\theta \tag{A.28}$$

where $\pi_k(\theta)$ is the prior distribution of $\theta$ given by expert $\mathcal{E}_k$, and $L()$ is the likelihood of observations $D$ given the parameter $\theta$. A useful approximation to that quantity is proposed in Kass and Raftery (1995):

$$p(D|\mathcal{E}_k) = \sqrt{(2\pi)^d}\sqrt{|\tilde{\Sigma}|}L(D|\tilde{\theta})\pi_k(\tilde{\theta}) \tag{A.29}$$

where $\tilde{\theta}$ is the MAP and $\tilde{\Sigma}$ is the Hessian approximation of the covariance matrix. Using these notations, and assuming a uniform prior on the experts, we finally have:

$$p(\mathcal{E}_k|D) = \frac{p(D|\mathcal{E}_k)}{\sum_\ell p(D|\mathcal{E}_\ell)} \tag{A.30}$$

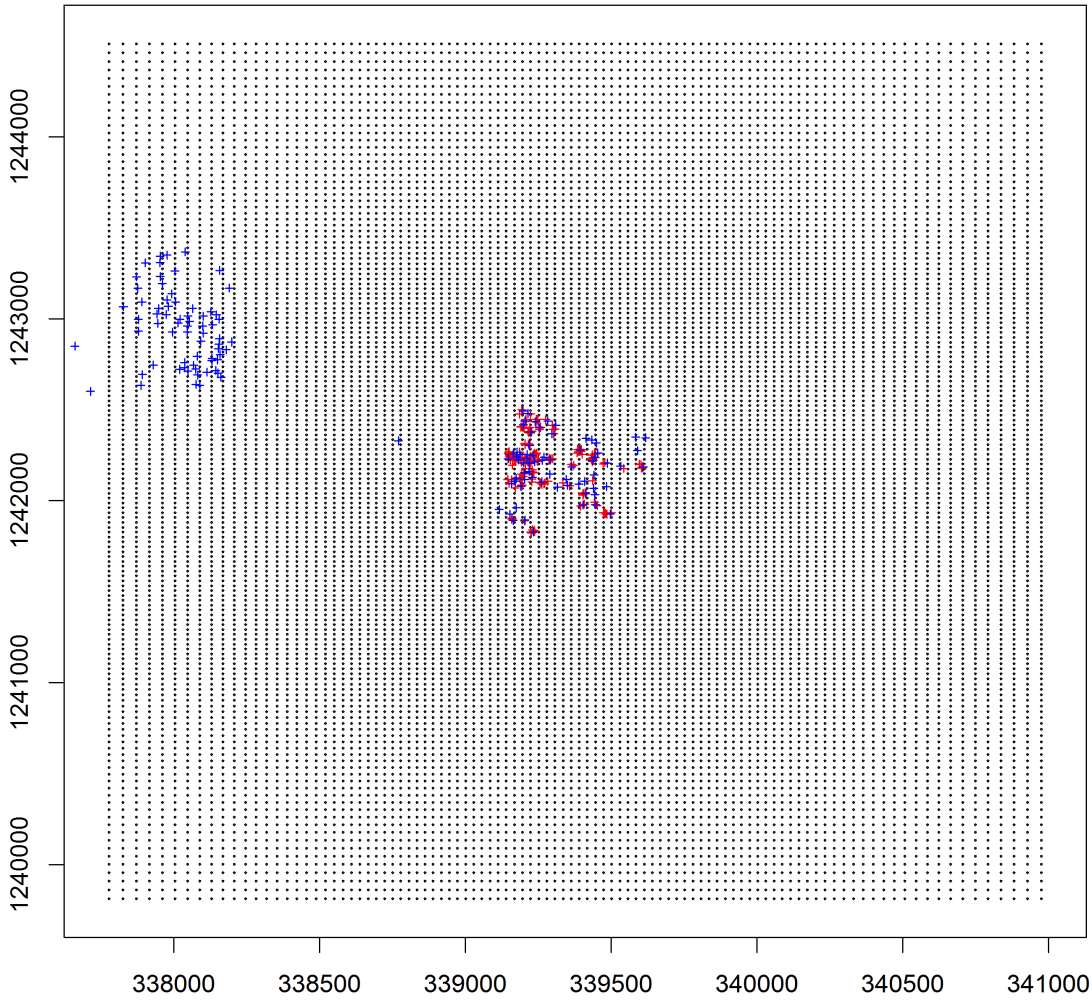


**Figure A.9:** Grid resolution and swarm locations. The black dots are the cell centers (as used in the PDE solver) and the red crosses are the known swarm locations.

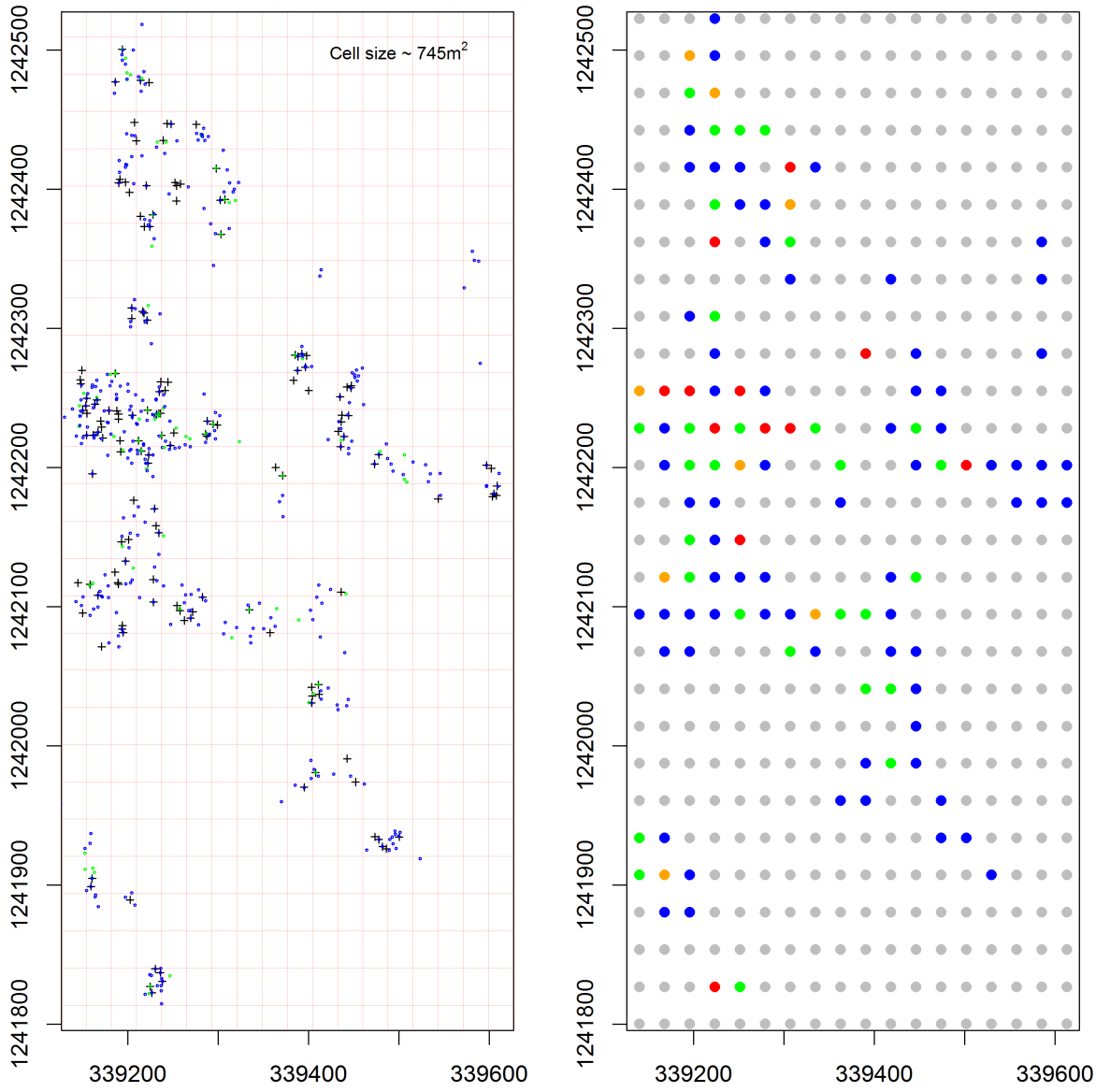


**Figure A.10:** Grid resolution, swarm locations and catches. The panel on the left shows the raw data at GPS scale accuracy. The black crosses on the left hand plot are the known swarm locations. The coloured dots show the location of observed catches (colour codes refer to the total number of observed mosquitoes over time; blue = 0, green = 1, orange = 2, red = 3). The red lines on the left hand plot shows the grid used in the PDE solver. The right hand plot shows the same data aggregated to the spatial grid scale. The grey dots in the right plot represent the grid center in the PDE solver. The coloured dots show the total number of observed mosquitoes over time (with the same colour code as the left hand plot).

#### Mortality parameter

Using the previous equations we can calculate the weight of each expert's mortality prior against the data. The weights are presented in Table A.11. Experts $1$ and $2$ have similar low weights, indicating that the data does not support their priors. The prior from expert $3$, however, is much closer to the observed data and assigned a relatively high weight.

| MortExpID | Weight |
|---|---|
| 1 | $1.22 \times 10^{-22}$ |
| 2 | $2.37 \times 10^{-26}$ |
| 3 | $1.00 \times 10^{0}$ |

**Table A.11:** Weights of the experts' priors for mortality.

#### Dispersal parameter

The same results are presented for the dispersal parameter in Table A.12. With only two experts, we can see that expert $1$'s prior performed better than expert $2$'s.

| DiffExpID | Weight |
|---|---|
| 1 | 0.839 |
| 2 | 0.161 |

**Table A.12:** Weights of the experts' priors for diffusion.

### A.4.9 Posterior and correlation

The posterior distributions presented in this section are the posterior distributions obtained after the model selection step, as described in the previous section.

Figure A.11 presents histograms and correlation plot for the $4$ main PDE parameters (mortality $\mu$, diffusion $\delta^2$, and chemotaxis attraction $\alpha$ and range $\sigma$). Comparing with the priors (see Figure A.13) we see that uncertainty has been reduced and the posterior distribution has mass centred in different location to priors.

#### Diffusion coefficient

Compared to the broad prior elicited, the posterior distribution of the diffusion coefficient has a narrower distribution, center around almost the same mode but with much less variance.

#### Mortality coefficient

The summary statistics for the mortality parameter $\mu$ posterior distribution are given in Table A.14. For consistency with the existing literature, we also report the transformed posterior summary statistics of daily mortality, in A.15. The figures reported in these table are consistent with the figures reported in Epopa et al. (2017).

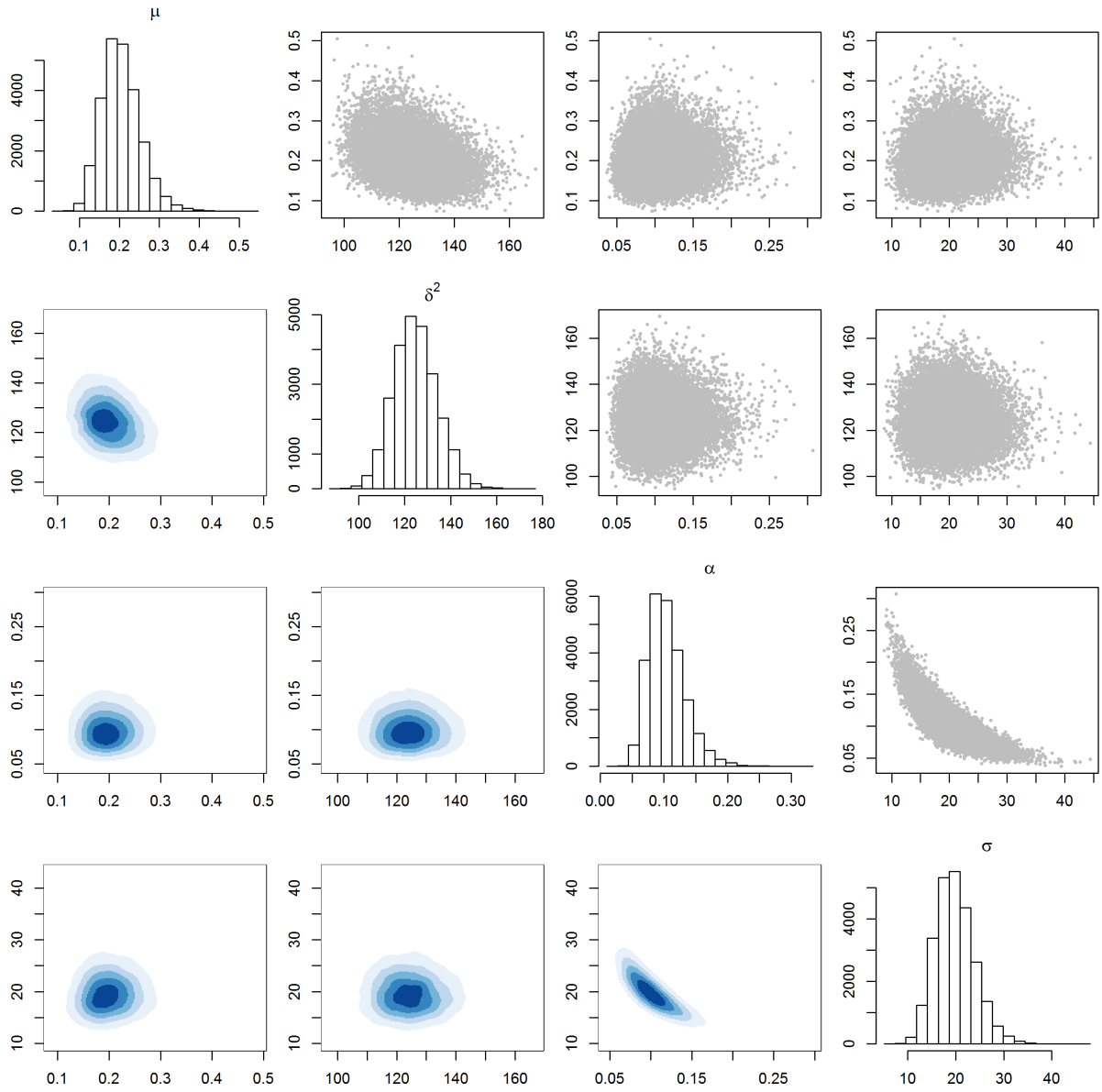


**Figure A.11:** Histograms and correlation plots for the PDE parameters posterior distributions. The bottom left plots density plots of the accepted samples and the top right plot are the accepted sampled coloured by likelihood value.

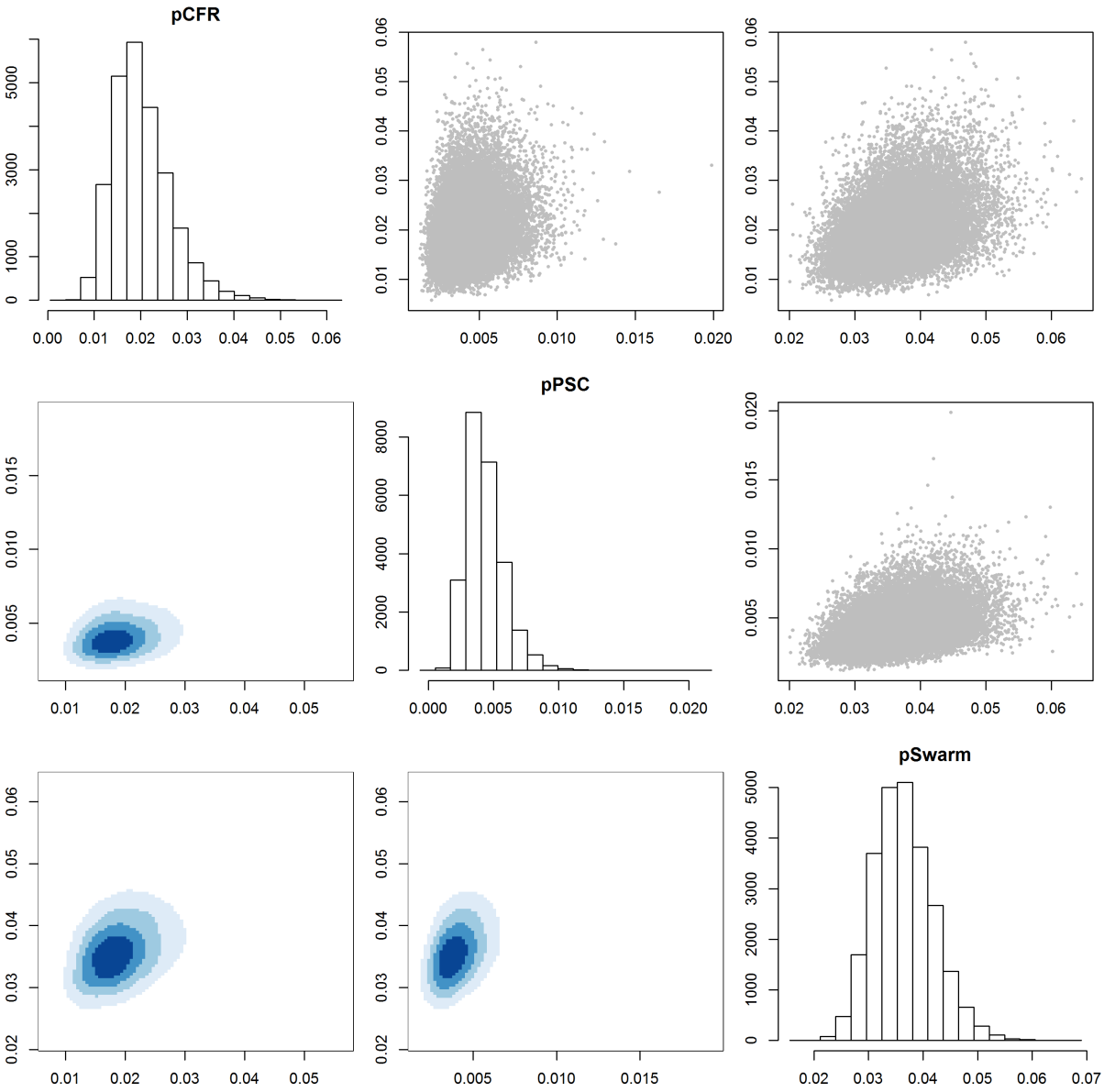


**Figure A.12:** Histograms and correlation plots for the PDE parameters posterior distributions. The bottom left plots density plots of the accepted samples and the top right plot are the accepted sampled coloured by likelihood value.

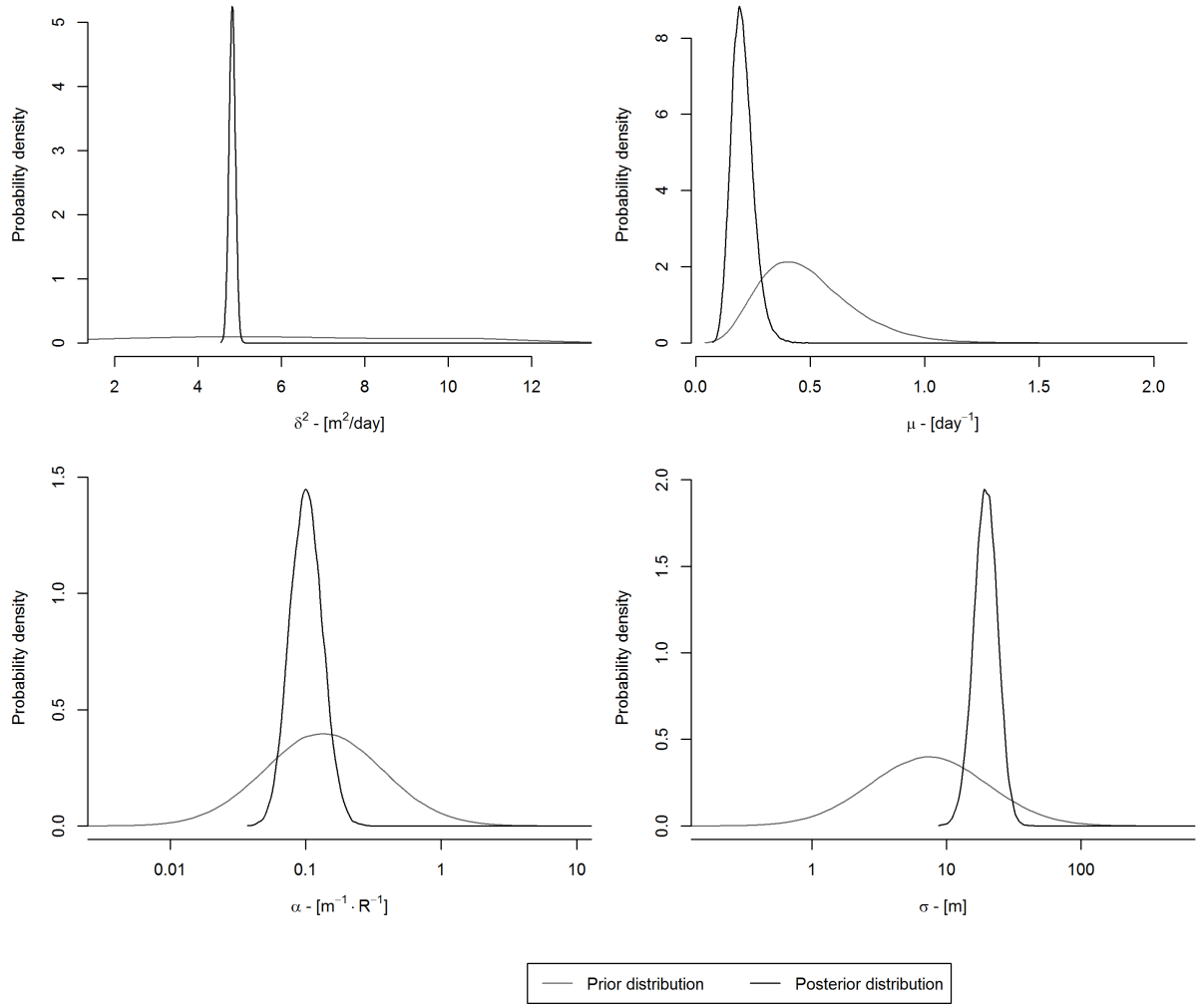


**Figure A.13:** Prior and posterior distributions for the 4 PDE parameters (and their units). The mortality posterior relates to the wild type males captured during the MRR experiments in Bana. We assume that the other parameters are identical for Ac(WT) males and Ac(DSM)2 males.

| Dist | Mean | Pctl5 | Pctl95 |
|---|---|---|---|
| Prior | $7.66 \times 10^{4}$ | $1.79 \times 10^{0}$ | $2.70 \times 10^{5}$ |
| Posterior | $1.25 \times 10^{2}$ | $1.10 \times 10^{2}$ | $1.41 \times 10^{2}$ |

**Table A.13:** Summary statistics of the diffusion.

| Mortality.rate | Mean | Pctl5 | Pctl95 |
|---|---|---|---|
| Prior | $4.88 \times 10^{-1}$ | $2.11 \times 10^{-1}$ | $8.63 \times 10^{-1}$ |
| Posterior | $2.05 \times 10^{-1}$ | $1.34 \times 10^{-1}$ | $2.90 \times 10^{-1}$ |

**Table A.14:** Summary statistics of the mortality.

### A.4.10 Model validation

A classic approach to evaluate the prediction abilities of a model is to test it against existing data that were not used for the inference. We present in Figure A.14 a comparison between the observed recaptures in MRR $5$ and the simulations from the PDE model with the inferred posterior distributions from MRR $1$ to $4$. Contrary to the previous experiments, the release from MRR $5$ occured on $1$ location only (contrary to the 3 release sites of the previous MRRs), and with a much larger number of mosquitoes as seen in Table A.9.

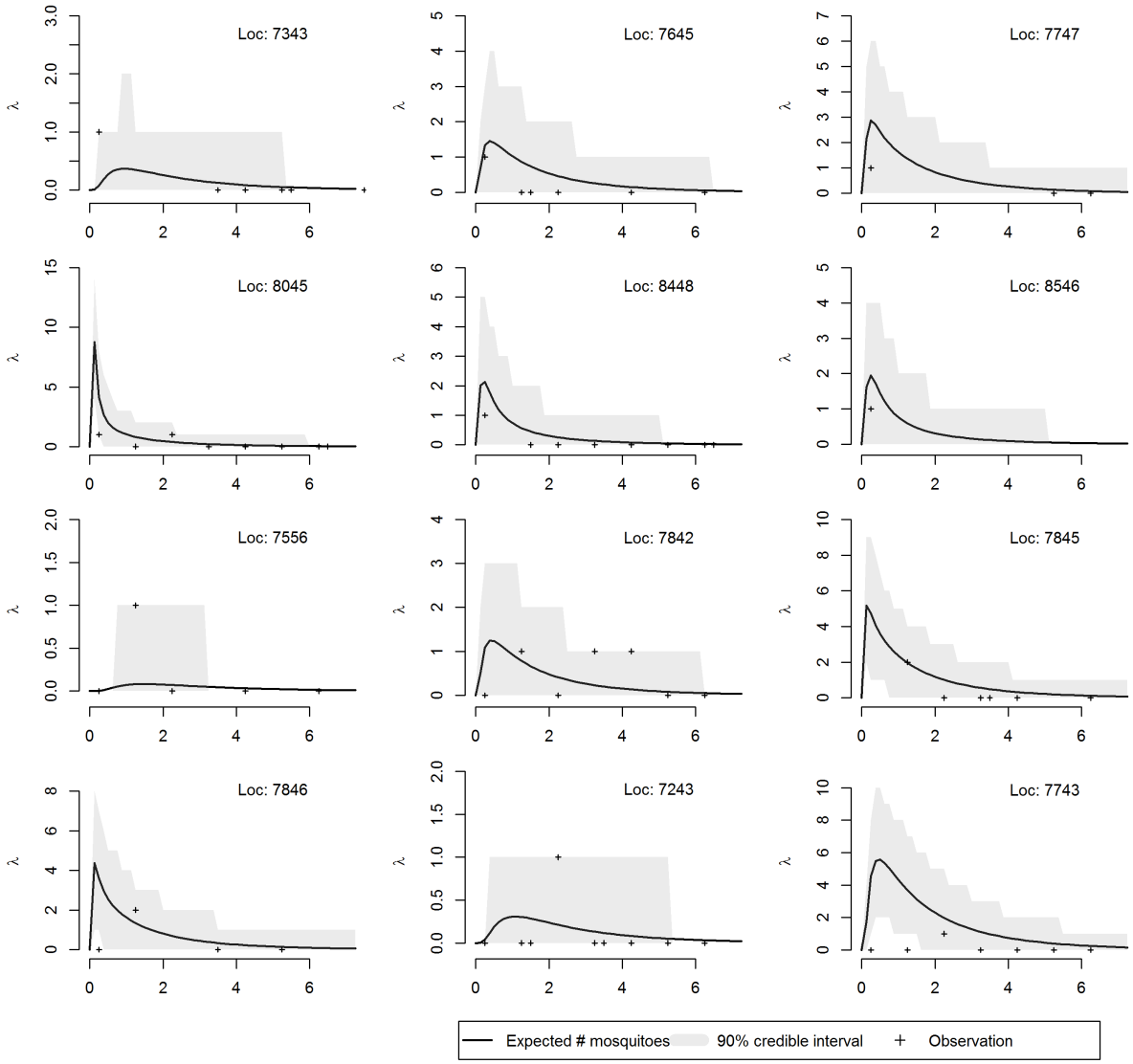


**Figure A.14:** Plot of the true observations (crosses) and the expected number of catches (solid line) from the simulated model. The shaded area represents the $90\%$ credible interval for the number of catches at the specified location. It is expected that the red crosses falls within the orange polygon.

| Daily.survival | Mean | Median | Pctl5 | Pctl95 |
|---|---|---|---|---|
| Prior | 0.63 | 0.63 | 0.42 | 0.81 |
| Posterior | 0.82 | 0.82 | 0.75 | 0.87 |

**Table A.15:** Summary statistics of the daily survival for wild type males in Bana.

| | 0 | 1 | 2 |
|---|---|---|---|
| PSC | 137 | 1 | 0 |
| Swarm | 236 | 13 | 2 |

**Table A.16:** Frequency of number of observed mosquitoes per catching method for MRR experiment 5.

## A.5 Probability of construct failure

In this analysis we treat each mating as an independent trial of the construct and assume that observations of construct failure $y$ has a Binomial distribution with probability of failure per trial $\theta^*$. We assume that all of the females in each experiment were successfully mated and hence use the number of females (N_female in Figure 4.7) as the number $n$ of independent experimental trials. This choice is conservative relative to other possibilities, such as treating the number of eggs (N_eggs in Figure 4.7) as the sample size.

If we aggregate across experiments within each of the two treatments Ag(DSM)2 and Ac(DSM)2 and assume that the efficacy of the construct under field conditions will be the same as that under laboratory conditions then the posterior distribution of the probability construct failure for Ag(DSM)2 and Ac(DSM)2 is given by (Section 2.3 Christensen et al., 2011)

$$\begin{aligned} p\left(\theta_j|y_j,n_j,\alpha_j,\beta_j\right) &= \frac{p\left(y_j|\theta_j,n_j\right)p\left(\theta_j|\alpha_j,\beta_j\right)}{\int p\left(y_j|\theta_j,n_j\right)p\left(\theta_j|\alpha_j,\beta_j\right)d\theta} \\ &= \frac{\Gamma\left(n_j+\alpha_j+\beta_j\right)}{\Gamma\left(y_j+\alpha_j\right)\Gamma\left(n_j-y_j+\beta_j\right)}\theta_j^{y_j+\alpha_j-1}\left(1-\theta_j\right)^{n_j-y_j+\beta_j-1} \end{aligned} \tag{A.31}$$

where $j=1,2$ is an index for Ag(DSM)2 and Ac(DSM)2 respectively, $n_1=2086$, $n_2=888$, $y_1=3$, $y_2=0$ and $p\left(\theta_j|\alpha_j,\beta_j\right)$ are the informative priors collected for *An. gambiae* and *An. coluzzii* by Hayes et al. (2015).

The posterior distribution of the probability of construct failure is available through Monte Carlo methods by accounting for the different unit of analysis between the prior and the likelihood Equation (3.2):

$$\begin{aligned} p\left(\theta^*|y_i\right) &\propto \pi\left(\theta^*\right)p\left(y_i|\theta^*\right) \\ p\left(y_i|\theta^*\right) &\sim \text{Binomial}\left(n_i,\theta^*\right) \\ \pi\left(\theta\right) &\sim \text{Beta}\left(\alpha,\beta\right) \\ \theta &= 1-\left(1-\theta^*\right)^{\frac{1}{5000}} \end{aligned} \tag{A.32}$$

where $\theta^*$ is the probability of a construct failure (fertile male) per individual male mating.

We derive the posterior distribution $p\left(\theta^*|y_i\right)$ for each expert using JAGS implemented through the rjags package, using 2 independent chains of 100,000 iterations each (the first 1,000 of which are warm up). Back transforming the posterior probability of construct failure per mosquito allows enables the analysis to derive a posterior probability (for each expert) of some mosquitoes being fertile given a release of 5,000 Ag(DSM)2 males.

The mixture posterior is again calculated as a Bayesian model average to create a weighted average from each expert's posterior distribution. To calculate the model evidence $p(M_{ij}|y_j)$ requires an estimate of the marginal likelihood (Equation (A.27)) which in this instance is achieved using an importance sample approximation:

$$p(D|\mathcal{E}_k) \sim \frac{1}{N}\sum_{i=1}^{N}\frac{\pi_k(\theta_i^*)L(D|\theta_i^*)}{g_k(\theta_i)} \tag{A.33}$$

**Table A.17:** Model evidence weights for expert's prior distributions for the probability of construct failure per mating for Ac(DSM)2 mosquitoes,

| Expert ID | Weight |
|---|---|
| 1 | 0.34 |
| 2 | 0.33 |
| 3 | 0.33 |

where $g_k(.)$ is a proposal (or instrumental) distribution tailored to provide samples efficiently from each expert's transformed prior, typically a Beta density with a slightly higher mean and variance than their transformed prior, $L(D|\theta_i^*)$ is the likelihood of the binomial model, and $\pi_k(\theta_i^*)$ is the transformed Beta prior for $\theta^*$ given by

$$\frac{c\,(\theta^*-1)^{c(b-1)}}{B(a,b)}\left[1-(1-\theta^*)^c\right]^{\alpha-1}(1-\theta^*)^{(c-1)} \tag{A.34}$$

where $c$ is the conversion factor (in this instance $\frac{1}{5000}$) and $\alpha,\beta$ are the parameters of the Beta distribution fitted to the expert's elicited responses by Hayes et al. (2015).

The weights for the experts who responded to the elicitation for *An. coluzzii* are shown in Table A.17 and the weights for the experts who responded for *An. gambiae* are shown in Table A.18.

**Table A.18:** Model evidence weights for expert's prior distributions for the probability of construct failure per mating for Ag(DSM)2,

| Expert ID | Weight |
|---|---|
| 1 | 3.4e-07 |
| 2 | 6.9e-13 |
| 3 | 2.3e-01 |
| 4 | 1.3e-07 |
| 5 | 1.5e-03 |
| 6 | 3.5e-04 |
| 7 | 1.6e-03 |
| 8 | 5.7e-22 |
| 9 | 4.9e-03 |
| 10 | 9.5e-14 |
| 11 | 9.4e-10 |
| 12 | 9.5e-04 |
| 13 | 7.6e-01 |

# Appendix B Pre-elicitation document

CSIRO Health and Biosecurity
www.csiro.au

## Pre-elicitation Document for Engineered Nucleases Risk Assessment, Part II: Small scale field release of sterile male mosquitoes

**CSIRO Environmental and Ecological Risk**
July 21, 2017

Prepared for the Foundations of the National Institutes of Health
Preparatory material for expert elicitation session

Commercial In Confidence

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## Contents

**Background** . . . . . 1

**1 Welcome to the expert elicitation session** . . . . . 2

1.1 Goal of this elicitation session . . . . . 2

1.2 Session expectations . . . . . 3

**2 Parameter definitions** . . . . . 4

**3 Scope of elicitation** . . . . . 4

**4 Populations and lineages** . . . . . 5

**A Technical details: parametric model** . . . . . 8

**Background**

The Foundation for the National Institutes of Health (FNIH) has commissioned an independent risk assessment of plans for the next stage of field testing by the Target Malaria project, which aims to develop and share new, cost-effective and sustainable genetic technologies to modify mosquitoes and reduce malaria transmission. In February 2014 the Foundation for FNIH engaged the Australian Commonwealth Science and Industrial Research Organisation (CSIRO) to quantitatively assess any potential environmental and human health risks associated with an accidental release of genetically modified mosquitoes from insectaries in three African nations. This work supports a larger international effort, led by Dr. Austin Burt at Imperial College London, to develop genetic methods to eliminate malaria vectors in Africa (http://targetmalaria.org/).

Dr. Burt and his colleagues are investigating the use of nucleases to move newly introduced traits, such as reduced reproductive capacity, through a mosquito population quickly. The project's ultimate goal is to develop nuclease-based constructs as a flexible, robust, powerful, and safe system to drive useful traits through populations of mosquitoes that transmit malaria. As a first step, they have developed a genetically engineered male sterile mosquito line, which they would like to use as a test strain in an African setting.

The CSIRO team recently completed the first part of a quantitative risk assessment procedure that has identified 5 key scientific issues potentially associated with the accidental escape of the sterile male mosquitoes from insectaries within African research facilities:

1. An increase in the incidence of malaria around the insectaries due to the GM construct
2. An increased capacity of mosquitoes to vector a new blood-borne pathogen
3. Spread of the genetic construct in the target mosquito species, *Anopheles gambiae*
4. Spread of the genetic construct in non-target Eukaryotes
5. Spread of the genetic construct in Prokaryotes

This risk assessment was conducted to support an application for permission to import the sterile male line into secure (PC2) facilities in Burkina Faso. A technical report is available on the web[1]. Permission to import the male sterile line for contained use was granted in Burkina and currently is being sought in Mali.

The second part of the risk assessment investigates risk associated with a planned release of the sterile male mosquitoes at defined site(s) in Burkina Faso and potentially Mali. Although the intent is for a potential release of sterile males, with low probability it is possible that some female mosquitoes may also accidentally be released. Any released female is expected to have an equal chance of being a transgenic or non-transgenic individual.

A key aspect of this second part requires incorporation of expert knowledge and empirical data from laboratory experiments about *Anopheles gambiae* sensu lato disease transmission of *Plasmodium falciparum*, lymphatic filariasis and O'nyong'nyong virus. CSIRO will use structured elicitation procedures to quantify the risk associated with each in a way that will coherently assimilate expert knowledge with empirical data, where the latter is available. We are asking you to partake in these risk elicitation exercises in order to provide an independent assessment of risk.

FNIH, CSIRO and Imperial believe that your participation in the risk assessment is an important component of the overall process because you can provide an independent opinion and help guard against the potential for inadvertent motivation bias among the project participants.

[1] http://targetmalaria.org/wp-content/uploads/pdf/target-malaria-risk-assessment-sterile-males-plus-executive-summary.pdf

# 1 Welcome to the expert elicitation session

This document provides more information about the upcoming expert elicitation session. Details are provided here on the overall approach and methods. It should be read in conjunction with the background material document distributed with this Pre-elicitation document. Please contact the CSIRO facilitators if you have further questions or would like to request information about specific details.

## 1.1 Goal of this elicitation session

The goal of this elicitation session is to assess the relative risk of vector borne disease transmission following a planned release of transgenic, dominant male-sterile *Anopheles coluzzii* mosquitoes of the Ac(DSM)2 lineage (Table 1.1). Although the intent is for a potential release of sterile males, with low probability it is possible that some female mosquitoes may also accidentally be released.

This elicitation session addresses the risk of disease transmission for 3 vector-borne diseases:

1. *Plasmodium falciparum*,
2. O'nyong'nyong virus,
3. Lymphatic filariasis.

You will only be asked to address diseases for which you are a recognised expert. Disease risk will be evaluated using well-established parameters of vector-borne disease transmission for the lineages described in the background material that accompanies this document (summarised in Table 1.1). More information about the relationships among these lineages, or strains, will be discussed below.

Table 1.1: Summary table of strains described in the background material.

| Label | Description |
|---|---|
| Ac(WT) | local *A. coluzzii* wild-type |
| G3 | original laboratory strain |
| Ag(DSM)2 | G3 lab strain with GE sterile male construct |
| Ac(DSM)2 | Ag(DSM)2 with Ac(WT) introgressed |

The parameters that will be addressed are those associated with the well-known basic reproduction number ($R_0$) and the closely related vectorial capacity. These parameters underlie some of the most commonly used analyses and modelling approaches applied to vector-borne disease transmission. The list of parameters that will be addressed in this elicitation session will depend on your domain knowledge, expertise and experience. The parameters addressed at your discretion may include those listed in Table 1.2. Please consider which parameters are appropriate for you to assess in the elicitation session. Depending on your experience and background, this may be a subset or all of the transmission parameters.

The elicitation session will use a structured approach to document your expert assessment based on your domain experience, your expertise, theory and scientific literature. We will record any commentary that you wish to document while contributing your assessment. The method deliberately allows for uncertainty that may arise from knowledge gaps or variability in a target parameter. This information will be used to develop a probabilistic (Bayesian) model (see Appen-

Table 1.2: Parameters subject to expert elicitation.

| Parameter | Definition | Varies by disease? |
|---|---|---|
| $a$ | Number of bites on humans per day per mosquito | No |
| $q$ | Daily probability of mortality of female mosquitoes | Yes* |
| $b$ | Transmission efficiency from female mosquitoes to humans | Yes |
| $c$ | Transmission efficiency from humans to female mosquitoes | Yes |
| $\tau$ | Duration of extrinsic incubation period in days | Yes |

*Mortality rate may vary for female mosquitoes infected with lymphatic filariasis.

dix A for technical details about the model structural form). Where empirical data is available, the model will coherently assimilate these data to efficiently update your contributed assessments with this independent source of information.

## 1.2 Session expectations

The elicitation session has been designed to tackle a challenging problem, and it depends on collaboration and cooperation with you, the expert. For this session, you will be asked to contribute your knowledge and expertise while responding to presented scenarios. The quality of the elicitation depends on a collegial, open-minded and focused atmosphere.

The elicitation stages are as follows:

1. Ethics forms (one copy to sign for our records, one copy for your records),
2. Review project brief: scope, description and background,
3. Education on probabilistic risk analysis and expert assessments,
4. Practice examples,
5. Start elicitation session.

The goal is to elicit a probabilistic description that is a reasonable approximation of your assessment. This is a cooperative effort that requires us to work within the confines of a model while seeking to describe your opinion. You will be given opportunity to trial out responses, and revise as necessary given graphical and numerical feedback. We'll always ask for your confirmation before finalising any given response.

A few guidelines to remember while participating in the session:

- Practice patience. The problem is new and challenging. It will take some thought and work to assess and talk about, which can take time. Occasionally, points will need to be considered again (and even again).
- If you feel that you are not sufficiently knowledgeable to address a particular parameter please let the session facilitator know.
- Ask questions of the session facilitators. Assessing expert opinion is a communication exercise, and please ask if you are unsure about what's being asked of you. We much prefer to address any confusion as it arises.

Thank you for agreeing to participate!

## 2 Parameter definitions

The human feeding rate ($a$) and daily probability of mortality ($q$) are assumed independent of disease pathogen (Table 1.2). The extrinsic incubation period ($\tau$), transmission efficiency from vectors to humans ($b$) and transmission efficiency from humans to vectors ($c$) may vary with disease.

**Human feeding rate:** The human feeding rate ($a$) is the average number of bites on humans per day per mosquito.

**Daily probability of mortality:** The daily probability of mortality $q$ is closely related to other traditional indices of mosquito mortality such as the daily probability of survival $p$ or the daily mortality rate $\mu$:

$$p = 1 - q$$
$$\mu = -\log(1 - q).$$

Based on expert feedback from the Part I elicitation sessions and the model structure (Appendix A), this elicitation session will target the daily probability of mortality, $q$, if addressed.

**Transmission efficiency from female mosquitoes to humans:** Probability that an uninfected and susceptible human becomes infectious after being bitten by an infectious mosquito. This parameter may vary with the disease.

**Transmission efficiency from humans to female mosquitoes:** Probability that an uninfected and susceptible mosquito becomes infectious after biting an infectious human. This parameter may vary with the disease.

**Duration of the extrinsic incubation period:** Number of days from when a mosquito ingests an infected bloodmeal to the time that the mosquito becomes infectious. This parameter may vary with the disease.

The parameters are defined with respect to the spatial and temporal scope of the elicitation exercise as described in the background document and Section 3 below. It is assumed that insecticide treated nets have been deployed to the release site.

## 3 Scope of elicitation

The background material that accompanies this document describes the basic set of assumptions that will form the foundation for the elicitation session. Please review these materials carefully. We will also be able to refer to the background material during the course of the session.

#### Spatial scope

The spatial scope of the elicitation considers a release location at one of the sites in either Burkina Faso or Mali as described in the background document.

#### Temporal scope

The parameters in Table 1.2 will be defined as annual averages for non-aestivating mosquitoes. Aestivating mosquitoes are excluded from the scope of the elicitation. Mosquitoes from the insectary will potentially be released at the beginning of the wet/end of the dry season in Burkina

Faso. Released mosquitoes may in theory survive and reproduce into and beyond the following dry season.

## 4 Populations and lineages

For the wild type *An. coluzzii*, Ac(WT), the genetic composition of the local wild type population within the laboratory production facility is expected to diverge from the local field wild-type population with each generation (Figure 4.1). You will be asked how values for the target parameter may depend on the number of generations that the wild type *An. coluzzii*, Ac(WT), is contained in the laboratory.

Figure 4.1: The potential exists for rapid loss of genetic diversity from a colonised wild population. Above figure and caption (redacted for public release) from Aguilar et al. (2005).

The laboratory population with the genetically engineered sterile male construct, Ac(DSM)2, was originally sourced from the G3 strain (Figure 4.2). The genetic composition of the original G3 strain appears to be a mixture of *A. gambiae* and *A. coluzzii*. Prior to backcrossing with Ac(WT), the genetically engineered lineage is referred to as Ag(DSM)2. The Ac(DSM)2 lineage begins by backcrossing Ac(WT) into Ag(DSM)2. For Ac(DSM)2, you will be asked to consider scenarios that vary the number of backcrosses with the Ac(WT) lineage.

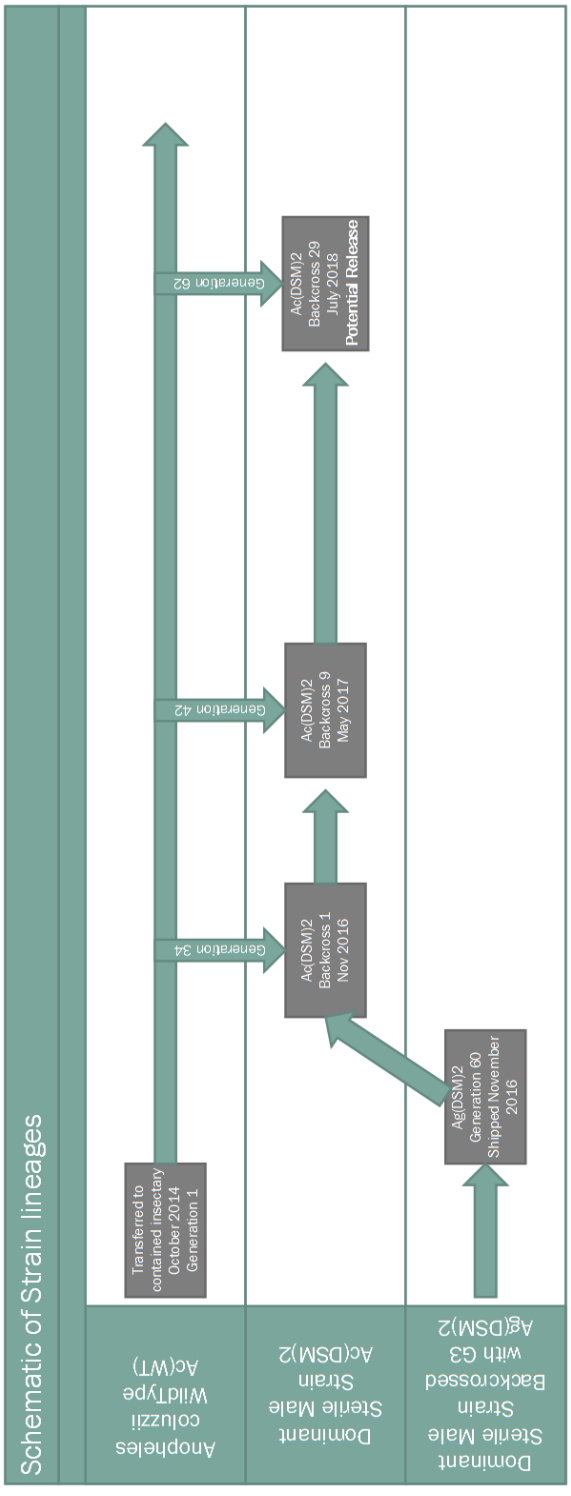


Figure 4.2: From Background material July 2017: "The strain Ac(DSM)2 is maintained in the insectary by introgressive backcrossing of transgenic Ac(DSM)2 females with the wild-type males of the strain names Ac(WT) ... As of May 2017, introgression has occurred in 9 generations ..."

### Inferring risk for potential Ac(DSM)2 release by backcross number

For a given parameter, you will be asked to respond to a series of questions to capture the dependencies among the lineages (Table 1.1). More specifically, you will be asked to assess disease transmission parameters for the following backcross numbers: 0, 5, 35, 70. With a roughly approximated generation time of 3 weeks, then 5 backcrosses corresponds to about 3.5 months of backcrossing in the insectary. 35 backcrosses will be about 2 years and 70 backcrosses about 4 years. Similar numbers of generations will be considered for the Ac(WT) lineage.

Empirical data from ongoing experiments conducted by the Target Malaria project will be included (where available) in the risk analysis as a formal Bayesian update to your assessment out of session. Expert assessments may be otherwise conditioned on empirical data from independent sources. Please note that the information derived from the expert assessment can also assist future predictions based on relevant empirical data, if these were to become available at a later date.

## References

Aguilar, R., Dong, Y., Warr, E., and Dimopoulos, G. 2005. *Anopheles* infection responses; laboratory models versus field malaria transmission systems. *Acta Tropica*, 95:285–291.

# A Technical details: parametric model

For a given target parameter, the elicitations will occur at pre-specified design points. A design point can be thought of as a scenario that is defined by the choice of strain or lineage, for example, G3 or Ag(DSM)2. In some cases, it may include a numeric covariate for number of generations in the lab for the wild type strain, Ac(WT), and the number of backcrosses for Ag(DSM)2. Expert assessments for a target parameter at a given design point is modelled by a probability distribution. A generalised linear model framework then permits prediction at other combinations of generation or backcrosses,

$$\theta = G(\eta) \tag{A.1}$$

$$\eta = X\beta, \tag{A.2}$$

where $G(\cdot)$ applies a monotonic link function $g(\cdot)$ to each entry in the $d$-dimensional vector $\eta$. The model design matrix $X$ is derived from a log-linear model with predictors that allow for interactions between Ac(WT) and Ac(DSM)2 through different levels of backcrossing and different levels of laboratory adaptation by Ac(DSM)2. The unknown coefficients $\beta$ are assumed to have a multivariate normal distribution.

The human feeding rate and the extrinsic incubation period parameters have positive support, $(0, \infty)$, and use the log link,

$$\eta_i = g(\theta_i) = \log \theta_i, \tag{A.3}$$

where $\theta_i$ is the target parameter assessed for the $i^{\text{th}}$ design point. The inverse link is then given by, $\theta_i = g^{-1}(\eta_i) = e^{\eta_i}$, which is an exponential relationship between the target parameter and the linear predictor. All other parameters (transmission efficiencies and the probability of daily mortality) are bounded $(0, 1)$ and use the complementary log log link function,

$$\eta_i = g(\theta_i) = \log(-\log(1 - \theta_i)), \tag{A.4}$$

where $\theta_i$ is the probability of transmission or daily mortality. The complementary log log function assumes an exponential relationship between the hazard rate[2] and the linear predictor.

[2] Also known as the hazard function or force of mortality, the hazard rate is roughly interpretable as the probability of an event, such as death or transmission, occurring given a small increase of the linear predictor. For example, the increment may be due to a small increase in the number of generations for Ac(WT) and/or number of backcrosses for Ac(DSM)2.

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# Appendix C Background material

## Assessing vectorial capacity risks to support the controlled environmental release of a genetically engineered sterile male strain of *An. coluzzii*.

### Background information

#### Research objective

Malaria is a mosquito-borne parasitic disease that continues to exact an enormous public health toll despite ongoing and intensive control efforts. Estimates of malaria-related deaths in 2010 ranged from 655,000 [1] to over 1.2 million [2], with the majority of deaths occurring among African children under 5 years of age. These figures decreased to a range of 235,000 to 639,000 deaths in 2015, a reduction of 22% since 2010 [3]. The international Roll Back Malaria partnership, which includes WHO, UNICEF, UNDP and the World Bank, has pledged a goal to "eradicate malaria worldwide by reducing the global incidence to zero through progressive elimination in countries." Yet it is acknowledged widely that this goal will require developing new tools and control methods [4].

Controlling mosquito vectors is one of the most effective ways to reduce the transmission of disease in endemic areas. Mosquitoes of the *Anopheles gambiae* species complex, found in Africa, are highly efficient malaria vectors. Target Malaria is focusing on the use of enzyme-based genetic approaches to dramatically diminish the population size of *An. gambiae* sensu lato mosquitoes in Africa, starting in three countries; Burkina Faso, Mali and Uganda.

The ultimate aim of the Target Malaria research project is development of a self-sustaining population suppression technology that will reduce numbers of vector mosquitoes over successive generations, until they are unable to sustain malaria transmission [5,6]. Because the nucleases are spread by the mosquitoes themselves, this technology offers potential advantages of being able to target difficult-to-reach segments of the vector population and of offering protection without relying on people to change their behavior or have access to health care for malaria treatment. Such a tool should provide area-wide, durable and low-cost protection, and be a valuable aid for malaria eradication when used in conjunction with other malaria control tools.

## The genetic construct

The genetic construct inserted into the mosquito comprises of a homing endonuclease enzyme and two fluorescent marker proteins. The Homing Endonuclease Gene I-Ppo-I encodes a protein that renders male mosquitoes sterile. The genetic modification also involves genes encoding Green and Red fluorescent proteins inserted in the construct as marker genes to identify those individuals that express the genetic modification. The green fluorescent protein is fused to the I-Ppo-I gene, and driven by the B-tubulin promoter, so that expression is testes specific. The genes are introduced in "trans" in a construct that is bounded by untranslated inverse terminal repeats from the non-autonomous piggyback vector. The figure below shows the linear construct insert in the mosquitoes. The transformation was accomplished via a conventional binary system in which the transposase necessary for the insertion event is supplied in trans and is not in the final insertion.

## The I-PpoI HE protein

The genetic construct that is the subject of this risk assessment uses a naturally occurring HE gene called *I-PpoI* to cleave and inactivate genomic targets in *Anopheles* mosquitoes. The *I-PpoI* HE was first identified as a strain-specific optional intron in the large subunit (28S) rRNA gene of the acellular slime mold *Physarum polycephalum* [7]. The I-PpoI protein is a 163 residue, 17.8 kDa protein with a pI of 8.1 that in its active form is a homodimer of 35.6 kDa [8].

The I-PpoI target gene sequence in *P. polycephalum* is within the large subunit (28S) rRNA gene that is present in ~300 copies and located on extrachromosomal nuclear plasmids [7]. The I-PpoI nuclease binds and cleaves this 15bp target site in *Physarum* to generate cohesive, 4 base pair, 3'OH single-stranded ends [9, 10]. Catalytic activity requires a divalent cation, Mg2+, in each of the two active sites located at the center of the pseudo-palindromic homodimer structure [8, 11]. In contrast to many HE proteins, I-PpoI is an efficient catalyst in that target site cleavage and product release are not rate-limiting as they are for other HE proteins. The target site specificity of I-PpoI has been defined by a combination of site-directed mutagenesis as well as random mutagenesis followed by sequential enrichment [10, 12].

## Heterologous expression of I-PpoI in Anopheles

The 15 bp I-PpoI target site is present in the large subunit rRNA gene of all eukaryotes [7, 13]. I-PpoI has been expressed to generate DNA double strand breaks in the large subunit rRNA genes of a range of organisms including budding yeast [14], human cells [15, 16], *Anopheles gambiae* [17, 18] and *Aedes aegypti* [19]. I-Ppo-I is not a toxin per se, and appears to share high levels of sequence homology with endonucleases from the order *Bangiales*, which contains the highly consumed seaweed, nori [20]. High level continuous expression of I-PpoI

is toxic at a cellular level, which is not surprising as many simultaneous DNA breaks are likely to lead to chromosomal fragmentation, loss and cell death even in organisms with extremely efficient DNA double strand break repair. This is the most likely explanation for the local toxicity of I-PpoI when expressed in *Anopheles* cell lines [7]. Transient expression of I-PpoI is better tolerated, as human and other eukaryotic cells have an efficient DNA break repair machinery, and can repair small numbers of DNA double strand breaks to restore chromosomal integrity regardless of whether a repair template is present. A majority of these repair events are likely to be error-free, and the result of simple re-ligation of the 4 bp overhanging cohesive ends generated by I-PpoI cleavage. This "regenerates" the I-PpoI target site which is again cleavage sensitive and restores chromosomal integrity.

The Target Malaria project uses I-PpoI to disrupt the *An. gambiae* s.l. large subunit rDNA genes, which in *An. gambiae s.l.* are found on the X chromosome (Figure 1) [17, 18]. In some approaches under exploration by the project, the desired activity of the HE depends on its ability to home. However, in the subject of this risk assessment, homing is not expected to occur, nor is it required to achieve the desired effect. Rather, the sterile male technology described here depends solely upon the ability of the I-PpoI HE to cleave its target sequence within the rDNA repeats on the X chromosome.

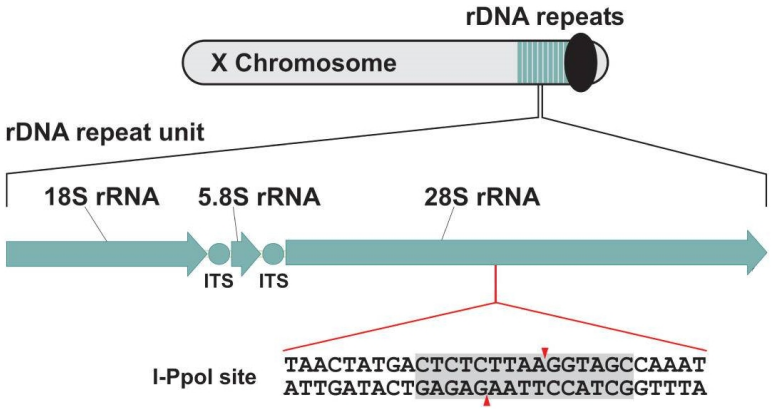



***Figure 1: Location of the ribosomal DNA repeats in the Anopheles gambiae s.l. genome.*** Each of approximately 350 rDNA repeats consists of the 18S, 5.8S and 28S rDNA genes. Each 28S gene copy contains an I-PpoI target site sequence. The location of the rDNA cluster relative to the centromere (black oval) is indicated. However, the exact location and nature of the centromere as well as the second heterochromatic and poorly defined arm of the X chromosome are currently unknown. Evidence exists that in some populations and/or members of the *An. gambiae* complex rDNA genes exist on the Y chromosome [P. Papathanos, personal communication, and 21].

## Testing pathway

The Target Malaria project is proceeding in a stepwise manner with safety as a top priority, beginning with a 1st generation version of the technology that renders male mosquitoes sterile (and thus unable to pass the new transgene into the local mosquito population). An intermediate, or 2nd generation, version has been developed that will pass the nuclease gene on to future generations in a self-limiting manner (i.e., without recurrent releases the gene is expected to gradually disappear from the population). The ultimate desired product is a 3rd generation version that will be self-sustaining (i.e., the nuclease gene will spread rapidly

through contiguous interbreeding *Anopheles* populations). Testing is initially conducted within standard laboratory cages in an insectary at Imperial College London. Promising strains and lines are subsequently tested in large indoor environmentally-controlled cages at the Polo d'Innovazione di Genomica Genetica e Biologia, Perugia, Italy. Contingent upon regulatory approval, those that continue to show promise are tested first in containment (laboratories and purpose-built insectaries) in Africa, prior to small-scale controlled field releases.

The subject of this risk assessment exercise is the 1st generation of the technology: dominantly sexually sterile male *An. gambiae s.l*. The sterile male construct has several genetic elements including the I-PpoI HE fused to a green fluorescent marker gene (*eGPF::I-PpoI*), a second fluorescent marker gene, *DsRed,* on a promotor(*3x3P*) which is expressed in the eye tissue, and the inverted terminal repeats of the non-autonomous *piggyBac* vector. The creation of the strain is further described in Windbichler et al, 2008 [22]. The strains developed and tested in London and Perugia was created in an *An. gambiae* G3 genetic background (https://www.vectorbase.org/organisms/anopheles-coluzzii/g3). Two transgenic male sterile lines, *β*2-Ppo1 (called Ag(DSM)1) and *β*2-Ppo2 (called Ag(DSM)2), were created.

### Importation of the sterile males in Africa

In October 2015, Target Malaria submitted a regulatory application requesting the importation and contained use of the Ag(DSM)2 sterile male strain into ACL2 facilities in a partner institution, the Institut de Recherche en Sciences de la Santé (IRSS) in Burkina Faso. Approval of the dossier was granted and since November 2016 until the present (May 2017), the Burkina team has been introgressing the Ag(DSM)2 strain into the local wild-type background (*An. coluzzii,* termed Ac(WT), rather than G3 which is an *An. gambiae* x *An. coluzzii* hybrid) and repeating studies previously performed in Polo-GGB and Atlanta at the CDC. The introgressed strain is known as Ac(DSM)2 to reflect the *An.coluzzii* background. The next stage in the step-wise approach is to submit a regulatory application for a small-scale controlled field release. The purpose of this controlled field release is to transfer knowledge and build operational and technical capacity in Africa.

### Maintenance of male sterile strains

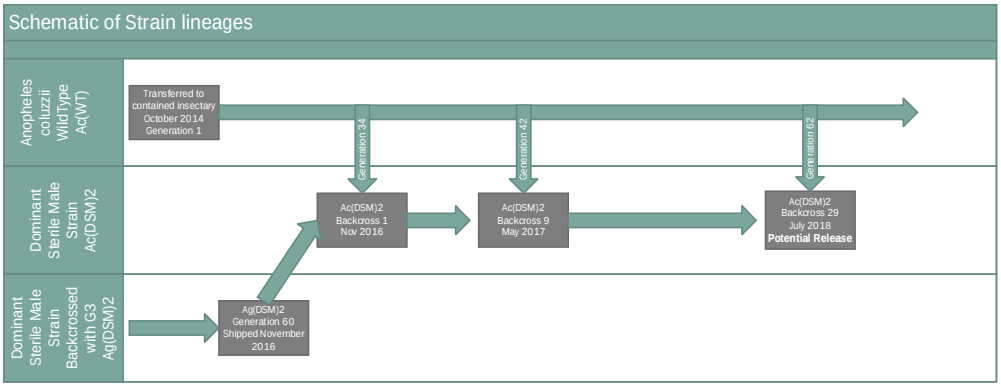
Because males that carry the transgene are sexually sterile, the transgene must be maintained by selecting transgenic females and backcrossing them every generation to wild type males. Often this is done in a two-step process; selecting all transgenic individuals in the larval stage and then, from among these, selecting females in the pupa stage. The resulting progeny in each generation consist of a Mendelian ratio of 50% males and 50% females. In addition, since mothers are hemizygous for the transgene, 50% of the progeny inherit the transgene and 50% will not. Thus, in each generation transgenic females (identified via the fluorescent maker), which make up 25% of the progeny, are identified to be again crossed to wild-type males to parent the following generation.

To avoid inbreeding depression, the wild males to which transgenic females are crossed come from an independent wild-type colony. In London and Perugia, the wild-type strain is G3, a widely used laboratory strain. In African countries, each laboratory has developed their own wild-type strain from mosquitoes that are collected in the vicinity of the potential release sites. These colonies will be subject to genetic analysis to ensure that they are of only one

species/type and material will be preserved for more detailed genetic analysis. A Standard Operating Procedure (SOP) has been developed for both the maintenance of the sterile male strain and the wild-type colonies. Repeated backcrossing of the Ag(DSM)2 transgenic strain shipped to African collaborators with the local strain will gradually replace the G3 genetic background with that of the locally originated strain.

### Lineage of the Burkina Faso mosquitoes

The contained use experiments that have been conducted in the laboratory at IRSS all use the Ac(DSM)2 strain. The strain Ac(DSM)2 is maintained in the insectary by introgressive backcrossing of transgenic Ac(DSM)2 females with the wild-type males of the strain names Ac(WT) as described above. This backcross (BC) leads to a mixture of transgenic offspring (characterized by the presence of the transgene with DsRed detectable marker) and non-transgenic offspring (with absence of the DsRed marker). While both male and female DsRed mosquitoes contain the same transgene, sterility is only expressed in the testis of the male mosquitoes whose sperm is expected to be non-fertile. As of May 2017, introgression has occurred in 9 generations (see schematic below).




## Contained release of the sterile males in Africa

Two villages in western Burkina Faso, and two villages in Mali have been selected as potential sites for this study due to accessibility to partner facilities, ecological confinement, entomological and stakeholder engagement characteristics. These villages have been entomologically characterised by Target Malaria teams since 2012.

### Spatial Scope Burkina Faso

The villages are located within 30 km of Bobo Dioulasso in the Sudanian zone of Burkina Faso. This region has two contrasting seasons: a dry season from November to April and a rainy season from May to October. Total annual rainfall is approximately 1000 mm and temperature varies between 22°C and 42°C. Two winds are dominant in the region: the harmattan, a hot dry wind that blows from the Sahara and dominates between January and April, and the monsoon, a cool wet wind that dominates between May and October. The area has mostly savanna vegetation; predominantly wooded savanna with variation depending on

human agricultural occupation. The main crops are maize, sorghum, millet and ancillary cash crops such as sesame and peanuts. Vegetable growing (onion, cabbage, tomato, eggplant, carrot, chili, lettuce, potato and green bean) is practiced along rivers and adjacent to natural ponds. For the most part, agricultural production is dependent on rainfall, which results in wide inter-annual fluctuations in yields.

There is also pastoral livestock grazing with moderate numbers of cattle, sheep, goats, pigs and poultry. Animals are kept both in the village and in the peripheral area where there are seasonally-inhabited mixed agro-pastoral outlying farms.

The lay-out of each village has been well-documented by the Target Malaria project with the positions of compounds, houses and larval habitats mapped using Geographic Information Systems. The locations of male swarms within the village areas have also been recorded as part of the regular baseline surveys, which will ensure efficient recapture data from a field release study.

*Anopheles coluzzii*, formerly known as the M form of *An. gambiae* and locally as 'Mopti,' occurs in the same habitat and regions as *An. gambiae* and overlaps substantially with *Anopheles arabiensis*. It has been reported that *An. arabiensis* occurs in Bobo-Dioulasso where the Target Malaria insectary is located [23].

Detailed molecular analysis of the mosquitoes captured in both villages by the regular surveys has revealed that occasional hybridisation between *An. coluzzii* and *An. gambiae* does occur. These hybrids have only been found in the dry season when mates are likely to be hard to find. In the season proposed for release studies no hybrids have been found and mates of either species are available.

### Spatial Scope Mali

The villages in Mali are located in the Sudano-Guinean area, to the north of the capital Bamako. The climate is of Sudanese type, with high variability of temperatures during the year, including the lowest (18ºC) are observed in January and highest in May (38ºC). The monsoon, sea wind, and the harmattan, continental wind, follow one another throughout the year. There was observed a rainy season (May to October) and a dry season divided into: cool dry season (November to January) and hot dry season (February to May). The vegetation is the type of grassland with some gallery forests along the Niger River. Large trees such as shea (Vitellariaparadoxa), the Néré (Parkiabiglobosa), mango, orange, and shrubs cover an herbaceous layer. The fauna mainly consists of small mammals including rabbits, hedgehogs (hérissons) etc. Reptiles are represented by lizards, scorpions and a few venomous species (vipers, cobras, snake etc.)

Regular surveys are being conducted in the villages, similar to those in Burkina Faso.

### Planned Release

Contingent upon regulatory and ethical approvals plus community and individual consents (as appropriate), MRR studies will be performed to demonstrate the ability to estimate the daily survival rate of males of the Ac(DSM)2 sterile male strain and to assess their movement within the area of the 'release village'. MRR studies are standard entomological tools to estimate

population size. MRR studies have been conducted with wild-type mosquitoes in one of these villages for 2 years.

MRR with male Ac(DSM)2 will be performed during the rainy season, when conditions are likely to be more favourable for the survival of mosquitoes reared under laboratory conditions (June-October). There is a preference for the earlier part of the rainy season during which there is less plant cover and a below-peak natural mosquito population size which may facilitate discrimination of released individuals in recapture efforts. Exact timing will be dependent on when permission is given by the regulators.

The aim is to release up to a maximum of 5,000 transgenic Ac(DSM)2 male mosquitoes over the study period. All released males will be laboratory-produced at IRSS in Burkina Faso through mass-rearing of the Ac(DSM)2 strain. To ensure adequate numbers and improve survival and dispersal estimates, hemizygous transgenic Ac(DSM)2 males will be released with a known proportion of their sibling males from the back cross that do not carry the sterile trait i.e. a total release of up to 10,000 males with approximately 50% transgenic. The output of insectary rearing is a naturally occurring 50:50 ratio of transgenic and non-transgenic siblings. Handling (which may damage the mosquitoes) is reduced by only sorting for sex (male) and not for positive transgene. The released individuals are all marked externally with fluorescent dust, so the non-transgenic siblings provide a comparator in the release that can be discriminated from both the released transgenic mosquitoes and the local unmarked wild population. Whilst the aim is to release only males, there is a small potential that some females may be released alongside the males (<5 females per 1,000 adults released). It is expected that any such females would consist equally of transgenic and non-transgenic individuals.

## Safety considerations

### Persistence of sterile male construct

Experiments and modeling are being conducted to determine whether the male sterile HE transgene disappears as predicted in the absence of positive selection. This will also be a primary objective of the sterile male strain field releases, when approved. This anticipates risks that might result from the accidental release of hemizygous transgenic females into the environment. It also assumes that short persistence of the transgene in the environment decreases risk.

### Life history – Avidity and oviposition

Mosquitoes held in the contained laboratories in Burkina Faso are being tested for blood-feeding avidity and oviposition characteristics (including numbers of eggs laid and egg hatching rate). Previous work on the effect of the transgene in the G3 background showed no significant differences in egg laying or egg hatching observed between transgenic and the G3 background strain, but reduced adult male emergence, longevity and competitiveness in the transgenic strain was observed [24].

### Insecticide resistance

Mosquitoes held in the contained laboratories in Burkina Faso are being tested for insecticide resistance and compared to wild population comparators from the potential release area and the vicinity of the insectary, using standard WHO insecticide resistance test kits. There is no expectation that rearing procedures would provide selective pressures for insecticide

resistance. Further tests are planned covering the full range of insecticide groups for which resistance has been reported in each country.

## Persistence

In studies conducted in Perugia using Ag(DSM)1 and Ag(DSM)2, transgenic females were released into populations containing wild-type (*An. gambiae* G3 strain) comparator females and males at rates of 100%, 50% and 20%. Eggs were collected weekly from these continuous populations (i.e. not discrete generations) and random samples of larvae were scored for the presence of the transgene and returned to the cage. Experiments were discontinued when two successive ovipositions contained no transgenic individuals in a sample of 200. Data for each line are shown in Figure 2 in comparison with predictions of a model of the decline in frequency of the transgene.

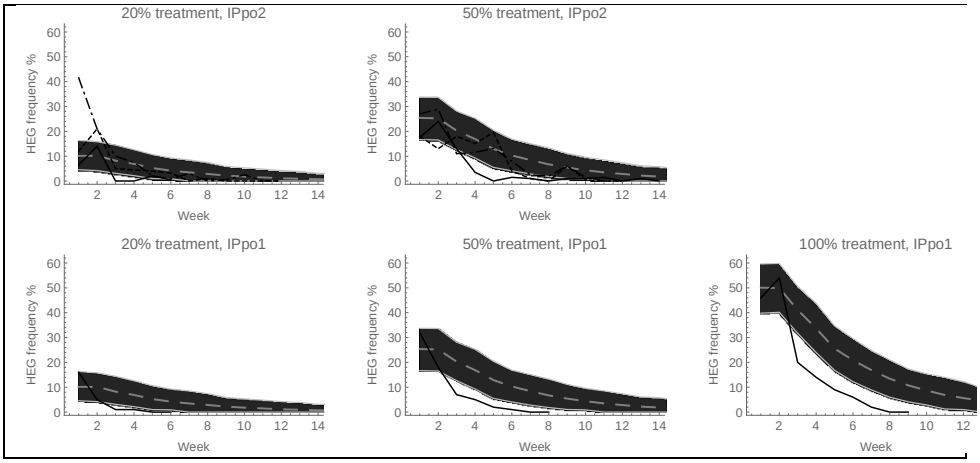



***Figure 2.*** **Model predictions and observations of transgene frequency in unselected populations**. The modeled frequency expected of the transgene in the experimental populations when seeded at different frequencies and the observations for either GMM line. The gray bands show the 95% central quantile intervals of the model trajectories. The dashed and solid black lines represent the experimental results for three replicates of two treatments with Ag(DSM)2 and the single experiments at three treatment levels with Ag(DSM)1. http://www.mdpi.com/2075-4450/7/4/47.

## Vector competence for ONNV and P. falciparum

Studies are being performed in the US to compare the ability of the Ag(DSM)2 and wildtype G3 strains to transmit *Plasmodium falciparum* (at NIH in Bethesda) and O'nyong'nyong virus (ONNV; at the University of Texas). *P. falciparum* is the malaria parasite relevant to the release areas in Africa and ONNV is the most significant of the few arboviruses transmitted by *Anopheles* species. The *Anopheles gambiae* complex is also a primary vector of Lymphatic filariasis in West Africa, however studies [25] have shown very high variability of transmission from infected mosquitoes and therefore provided no meaningful metric to provide a reliable indication of filarial transmission in testing.

## Risk assessment

The sterile male construct in *An. gambiae* has been the subject of a formal hazard analysis, complemented by an extensive literature review, to identify potential hazards associated with an accidental release from the African insectaries. From this analysis a set of high priority hazards have been taken forward into a quantitative risk assessment (report available from http://targetmalaria.org/resources/).

CSIRO has now been engaged to update this risk assessment to support an application to the relevant African authorities to permit a small-scale field release of the Ac(DSM)2 mosquitoes. As a member of the independent expert group, you are being asked to contribute to certain parts of this risk assessment as detailed in the letter and documentation that accompanies this report. Your independent contribution forms an important part of the overall risk assessment methodology.

In this instance, CSIRO is requesting your contribution to account for any potential changes in vectorial capacity of female transgenic *An. colluzzi* mosquitoes – i.e. female Ac(DSM)2 mosquitoes that may be accidentally released along with the planned release of male Ac(DSM)2. You will be asked to participate in a formal indirect elicitation methodology that has been specifically designed to account for potential differences between Ag(DSM)2 and Ac(DSM)2 mosquitoes, and for any potential 'habituation' or 'domestication' that occurs as a result of the insectary rearing processes.

The elicitation will begin with a formal period of training followed by a series of questions directed at vectorial capacity parameters in relation to three blood-borne diseases: malaria, ONNV and Lymphatic filariasis. You will only be approached to answer in relation to the diseases in which you are a recognised expert.

## References cited:


1. World Malaria Report 2011 http://www.who.int/malaria/world_malaria_report_2011/WMR2011_factsheet.pdf
2. Murray CJ, Rosenfeld LC, Lim SS, Andrews KG, Foreman KJ, Haring D, Fullman N, Naghavi M, Lozano R, Lopez AD (2012) Global malaria mortality between 1980 and 2010: a systematic analysis. Lancet. 379:413-431.
3. World Malaria Report 2016 http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf?ua=1
4. Alonso PL, Tanner M (2013) Public health challenges and prospects for malaria control and elimination. Nature Med 19:150-155.
5. Deredec A, Godfray HC, Burt A. (2011) Requirements for effective malaria control with homing endonuclease genes. Proceedings National Academy of Sciences U S A. 108:E874-80.
6. North A, Burt A, Godfray HCJ (2013) Modelling the spatial spread of a homing endonuclease gene in a mosquito population. Journal of Applied Ecology 50:1216-1225.
7. Muscarella DE, Vogt VM (1989) A mobile Group I intron in the nuclear rDNA of Physarum polycephalum. Cell 56: 443-454.
8. Flick KE, Jurica MS, Monnat RJ, Jr., Stoddard BL (1998) DNA binding and cleavage by the nuclear intron-encoded homing endonuclease I -Ppo I. Nature 394: 96-101.
9. Muscarella DE, Ellison EL, Ruoff BM, Vogt VM (1990) Characterization of I- Ppo , an intron-encoded endonuclease that mediates homing of a group I intron in the ribosomal DNA of *Physarum polycephalum* Molecular and Cellular Biology 10: 3386-3396.
10. Ellison EL, Vogt VM (1993) Interaction of the intron-encoded mobility endonuclease I-Ppo I with its target site. Molecular and Cellular Biology 13: 7531-7539.
11. Galburt EA, Chevalier B, Tang W, Jurica MS, Flick KE, et al. (1999) A novel endonuclease mechanism directly visualized for I- Ppo I. Nature Structural Biology 6: 1096-1099.
12. Argast GM, Stephens KM, Emond MJ, Monnat RJ, Jr. (1998) I-PpoI and I-CreI homing site sequence degeneracy determined by random mutagenesis and sequential in vitro enrichment. Journal of Molecular Biology 280: 345-353.
13. Johansen S, Johansen T, Haugli F (1992) Structure and evolution of myxomycete nuclear group I introns: a model for horizontal transfer by intron homing. Current Genetics 22: 297-304.
14. Muscarella DE, Vogt VM (1993) A mobile Group I intron from Physarum polycephalum can insert itself and induce point mutations in the nuclear ribosomal DNA of Saccharomyces cerevisiae Molecular and Cellular Biology 13: 1023-1033.
15. Monnat RJ, Jr., Hackmann AFM, Cantrell MA (1999) Generation of highly site-specific DNA double-strand breaks in human cells by the homing endonucleases I- Ppo I and I- Cre I. Biochemical and Biophysical Research Communications 255: 88-93.
16. Berkovich E, Monnat RJ, Kastan MB (2007) Roles of ATM and NBS1 in chromatin structure modulation and DNA double-strand break repair. Nat Cell Biol 9: 683-690.

17. Windbichler N, Papathanos PA, Catteruccia F, Ranson H, Burt A, et al. (2007) Homing endonuclease mediated gene targeting in Anopheles gambiae cells and embryos. Nucleic Acids Research 35: 5922-5933.
18. Klein TA, Windbichler N, Deredec A, Burt A, Benedict MQ (2012) Infertility resulting from transgenic I-PpoI male *Anopheles gambiae* in large cage trials. Pathogens and Global Health 106: 20-31.
19. Traver BE, Anderson MAE, Adelman ZN (2009) Homing endonucleases catalyze double-stranded DNA breaks and somatic transgene excision in Aedes aegypti. Insect Molecular Biology 18: 623-633.
20. Kirsten M. Müller, Jamie J. Cannone, Robin R. Gutell, Robert G. Sheath; A Structural and Phylogenetic Analysis of the Group IC1 Introns in the Order Bangiales (Rhodophyta). *Mol Biol Evol* 2001; 18 (9): 1654-1667. doi: 10.1093/oxfordjournals.molbev.a003954

21. Wilkins EE, Howell PI, Benedict, M Q (2007) X and Y chromosome inheritance and mixtures of rDNA intergenic spacer regions in *Anopheles gambiae*. Insect Molecular Biology, 16:, 735–741.

22. Windbichler N, Papathanos PA, Crisanti A. (2008) Targeting the X chromosome during spermatogenesis induces Y chromosome transmission ratio distortion and early dominant embryo lethality in Anopheles gambiae. PLoS Genetics 4:e1000291
23. Dabire R, Sawadogo S, Diabate A, Toe, H, Kengne P, Ouasi A, Constantini C, Gouagna C, Pennetier, C, Simard F, Baldet T, Lehman T, Gibson G (2014) Mechanisms of reproductive isolation between incipient specieis in the anopheles gambiae species complex Malaria Journal 13(Suppl 1):O4 **DOI:** 10.1186/1475-2875-13-S1-O4

24. Klein TA, Windbichler N, Deredec A, Burt A, Benedict MQ (2012) Infertility resulting from transgenic I-PpoI male Anopheles gambiae in large cage trials. Pathogens and Global Health 106:20-31.

25. de Souza DK, Koudou B, Kelly-Hope LA, Wilson MD, Bockarie MJ, Boakye DA (2012) Diversity and transmission competence in lymphatic filariasis vectors in West Africa, and the implications for accelerated elimination of Anopheles-transmitted filariasis Parasite & Vectors 5:259 DOI: 10.1186/1756-3305-5-259

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