

NAQS QLD, WA and NT report Entomology

RECOMMENDATION

1. That the NAMP Technical Committee

NOTE: The detection of exotic vector species *Culicoides nudipalpis* on Cobourg Peninsula, Northern Territory

NOTE: For the financial year 2022/23 there were no identification of vector species outside the bluetongue virus transmission zone.

BACKGROUND

1. Monitoring for biting midges in NT and WA is conducted jointly by NT and WA Govts and NAQS.
 - NT Govt manages the collection of specimens at 14 official NAMP sites
 - WA Govt manages the collection of specimens at 18 official NAMP sites
 - NAQS manages collection at seven sites (WA:1, NT:3, QLD:3)
2. Northern Territory Department of Industry Tourism and Trade (DITT) Berrimah Veterinary Lab sorts *Culicoides* from by catch from all samples from WA and NT
3. NAQS Darwin entomology lab provides species identification for all specimens from NT and WA.
4. NAQS contracts sorting and identification of traps from north Queensland to a private contractor (Bill Doherty)
5. This report includes the 36 official NAMP entomology sites in the Northern Territory & Western Australia, and three NAQS sites in Northern Queensland (Image 1)

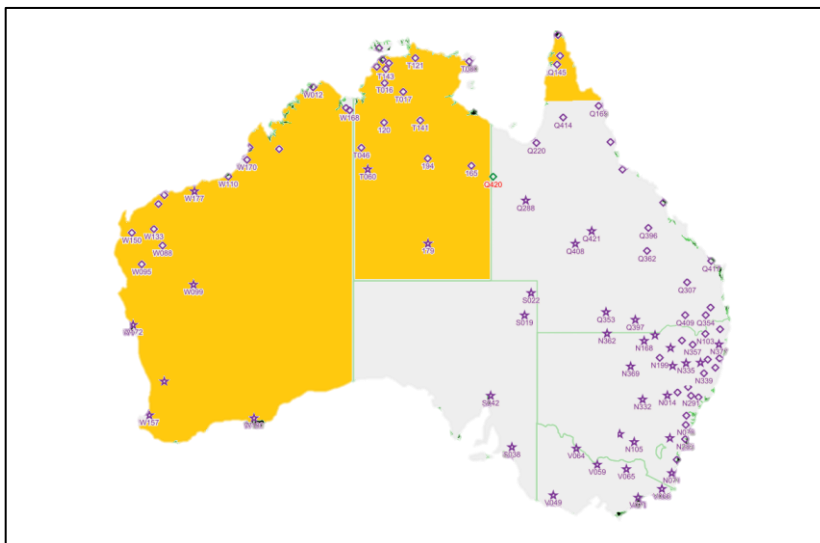


Image 1: NAMP entomology sites (shaded yellow) included in this update.

DISCUSSION

1. Due to logistics of collection, transport, sorting and ID there is often several months delay, hence reporting on 2022/23 financial year are incomplete.
2. There are a total of 256 budgeted collections across WA and NT. 165 trap collections have been received (65% of totally budgeted collections), Of those only 19 traps remain to be sorted presently (88% completed with the remainder to be completed as the NAQS lab receives them)
3. WA has 124 budgeted with the NAQS lab receiving 89 collections so far, 71%
4. NT has 132 budgeted with the NAQS lab receiving 76 collections so far, 57%
5. A total of 34,017 midges were sorted and identified from WA and NT in 2022-23 season at present.
6. The numbers of sites at which key vector *Culicoides* species were identified decreased compared to the previous years across NT and WA
7. The number of key vector *Culicoides* species in the NT is significantly lower than previous years (Table 6)
8. The number of key vector *Culicoides* species in the WA has shifted from previous years, *C. actoni* & *C. wadai* has decreased compared to last year, *C. brevitarsus* has slightly increase, *C. fulvus* has remained the same (table 7)
9. No vector species have been recorded outside the BTV transmission zone
10. Detection of *exotic* vector species (Appendix 1)
 - *Culicoides nudipalpis* was detected on 01/07/2022 (2 specimens) and 29/08/2022 (1 specimen) at Black Point on the Cobourg Peninsula
 - These detections follow detections of *C. nudipalpis* in northwestern parts of the Northern Territory in 2019 and 2020
 - *Culicoides nudipalpis* is a sister species to *Culicoides imicola*, which is the primary vector of Bluetongue and African Horse Sickness viruses (AHSV) in Africa and, more recently, of AHSV in Thailand. Evidence linking the transmission of BTV or AHSV to *C. nudipalpis* is lacking although BTV has been detected in field specimens of *C. nudipalpis* from Timor. Nevertheless, the close relationship with *C. imicola* suggests that *C. nudipalpis* may be a competent vector that may impact the ecology of BTV in Australia and alter susceptibility to AHSV.
 - To investigate potential establishment of *C. nudipalpis* NAQS entomologists conducted an intensive survey (i.e. trapping) of Cobourg Peninsula in June 2023. Trapping will occur on nearby Croker Island in September 2023. Trap sorting and identification is ongoing.
11. The DAFF sponsored, Biosecurity Innovation Program, project “An efficient DNA metabarcode platform for rapid identification of bulk sampled pest biting midges” being undertaken by NSW DPI continues. A draft report is due 31 August 2023. A final report is due 16 October 2023. Project scope and progress update are provided in Attachment C.

FINANCIAL IMPLICATIONS

12. *Nil*

CONSULTATION/COMMUNICATION

13. *Nil*

NEXT STEPS/ACTIONS

14. Further investigation (trapping on Croker Island) and diagnosis (sorting and ID of traps) of *Culicoides* in response to the *Culicoides nudipalpis* detection will be completed in the coming months. Any further samples of *C. nudipalpis* will be reported to NAMP
15. The final report of the metabarcoding project by NSW DPI will inform further steps (e.g. Publication of research, need for further validation and testing, implementing this technology and techniques, requirements for NAMP policies).

Attachment A: *Entomology report details, NT, WA and NAQS QLD*

Attachment B: *Detection and investigation of *Culicoides nudipalpis* in the Cobourg Peninsula and Croker Island, Northern Territory*

Attachment C: *An efficient DNA metabarcode platform for rapid identification of bulk sampled pest biting midges*

ATTACHMENT A: ENTOMOLOGY REPORT DETAILS, NT, WA AND NAQS QLD

Submissions and identifications:

WA

- 15 of 19 budgeted sites active
- One hundred and twenty-four (124) budgeted with the NAQS lab receiving 89 collections so far, 71%
- A total of 22,774 *Culicoides* specimens were identified in 22/23 FY
- Further submissions and identifications are expected, and those missing have yet to be investigated

Northern Territory

- 13 of 17 budgeted sites active
- One hundred and thirty-two (132) budgeted with the NAQS lab receiving 76 collections so far, 57%.
- A total of 11,243 *Culicoides* specimens were identified in 22/23 FY
- Further submissions and identifications are expected, and those missing have yet to be investigated

NAQS QLD

- Two of three budgeted sites active
- Thirty (30) budgeted with 21 received (70%)
- A total of 924 *Culicoides* specimens were identified

Vector summary:

- The numbers of sites at which key vector *Culicoides* species were identified decreased compared to the previous years across NT and WA.
- The number of key vector *Culicoides* species in the NT is significantly lower than previous years (Table 4).
- The number of key vector *Culicoides* species in the WA has shifted from previous years, *C. actoni* & *C. wadai* has decreased compared to last year, *C. brevitarsus* has slightly increased, *C. fulvus* has not been recorded for several years. (table 7)
- A single collection was made of *C. actoni* last year at Glenflorrie station, we have not seen any this year.
- *C. brevitarsus* is the most abundant vector found across NT & WA.
- No vector species have been recorded outside the transmission zone

Table 1: Counts of vector species by quarter - NT

NT	Jul-Sep 22	Oct-Dec 22	Jan-Mar 23	April-Jun 23	Total
<i>C. actoni</i>	4	150	10	15*	179
<i>C. brevitarsis</i>	1167	572	243	95*	2077
<i>C. fulvus</i>	0	4	3	4*	11
<i>C. wadai</i>	28	3	56	1*	88
Total	1199	729	312	115	2355

Table 2: Counts of vector species by quarter - WA

WA	Jul-Sep 22	Oct-Dec 22	Jan-Mar 23	April-Jun 23	Total
<i>C. actoni</i>	0	2	0	0*	2
<i>C. brevitarsis</i>	12	95	28	0*	135
<i>C. fulvus</i>	0	0	0	0*	0
<i>C. wadai</i>	0	0	0	0*	0
Total	12	97	28	0	137

Table 3: Counts of vector species by quarter – NAQS Qld

NAQS QLD (Seisia, Berthiaugh & York Downs)	Jul-Sep 22	Oct-Dec 22	Jan-Mar 23	April-Jun 23	Total
<i>C. actoni</i>	0	0	0	0*	0
<i>C. brevitarsis</i>	3	8	145	0*	156
<i>C. fulvus</i>	0	0	0	0*	0
<i>C. wadai</i>	0	0	17	0*	17
Total	3	8	162	0	173

*Data incomplete

Table 4: Number of vector specimens, by month; 2019 – 2023 - Northern Territory

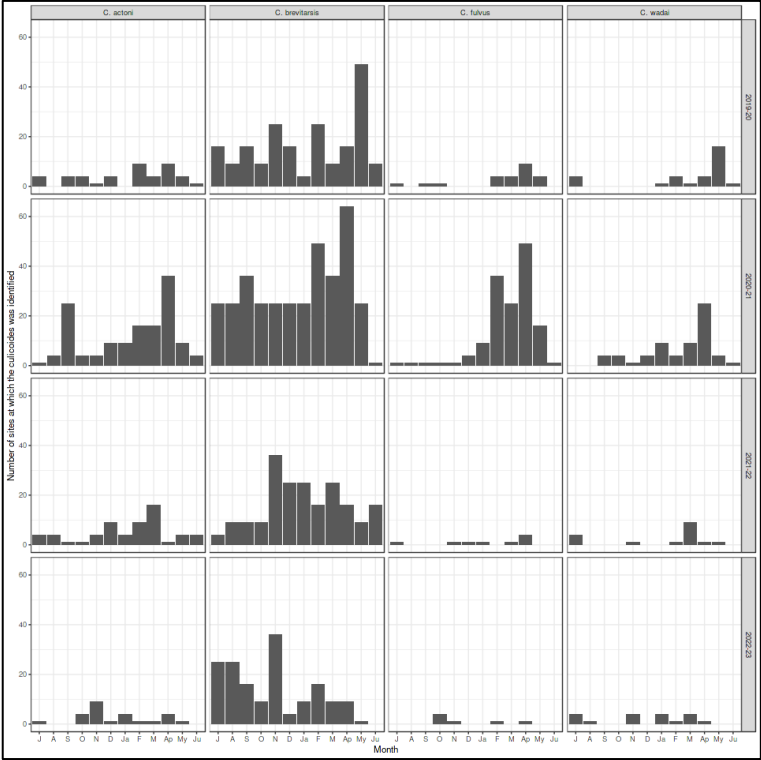


Table 5: Number of vector specimens, by month; 2019 – 2023 – Western Australia

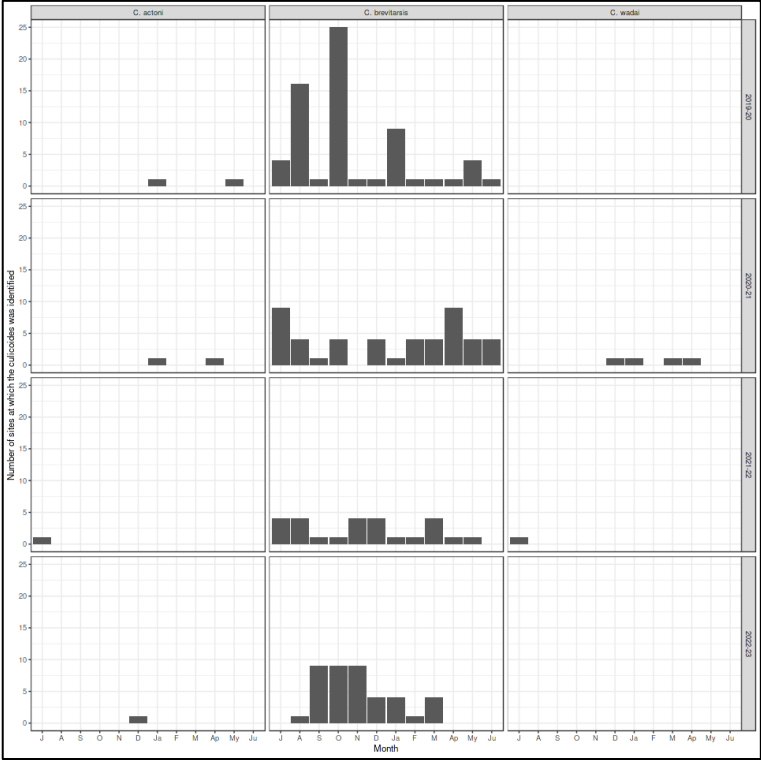


Table 6: Abundance of vector *Culicoides* 2019 – 2023 - Northern Territory

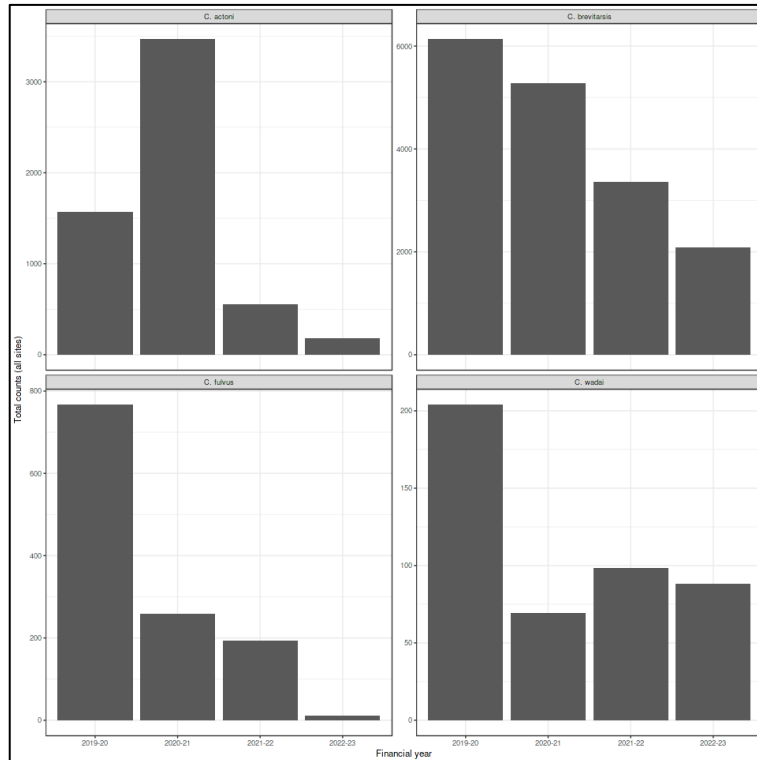


Table 7: Abundance of vector *Culicoides* 2019 – 2023 – Western Australia

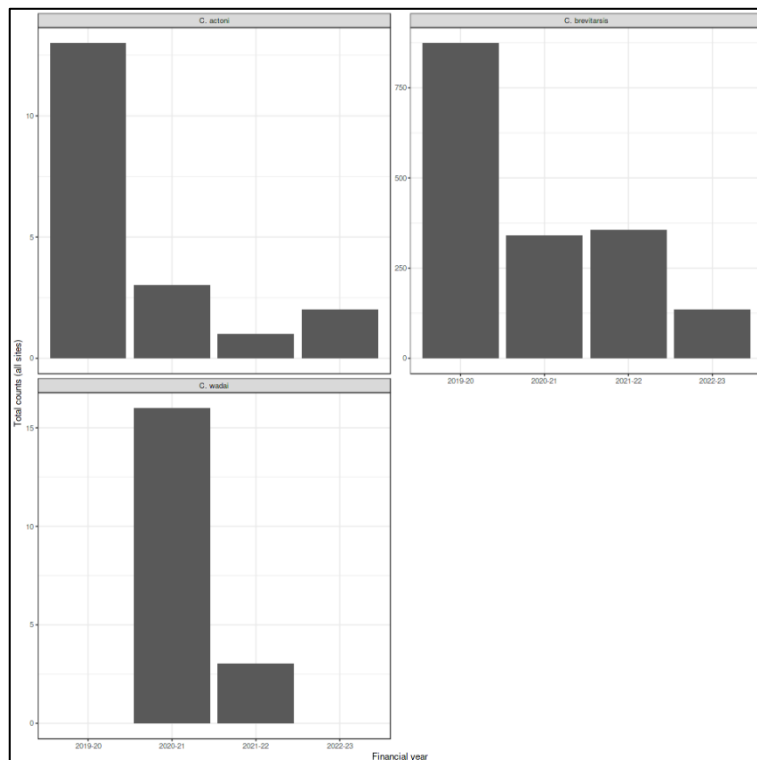


Table 7: Culicoides species diversity: Northern Territory - 2022/23

C. actoni	C. nudipalpis
C. austropalpis	C. obscurus
C. brevipalpis	C. ornatus
C. brevitarsis	C. oxystoma
C. bundyensis	C. pallidothorax
C. bunrooensis	C. peregrinus
C. clavipalpis	C. wadai
C. clavipalpis group	C. williwilli #4
C. clavipalpis group #8	C. zentae/kayi
C. dycei	C. ornatus group #6
C. fulvus	C. victoriae group
C. histrio	C. victoriae group #6
C. marksi	C. williwilli group
C. narrabeenensis	C. williwilli group #19

Table 8: Culicoides species diversity: Western Australia - 2022/23

C. actoni	C. oxystoma
C. austropalpis	C. pallidothorax
C. brevipalpis	C. peregrinus
C. brevitarsis	C. pseudopalpis
C. bundyensis	C. zentae/kayi
C. bunrooensis	C. ornatus group #6
C. clavipalpis group #8	C. victoriae group
C. dycei	C. victoriae group #6
C. marksi	C. victoriae group #14
C. nattaensis	C. williwilli
C. ornatus	C. williwilli group
C. ornatus group	C. williwilli group #3
C. ornatus group #6	Undescribed species
C. ornatus group #8	

Table 9: Culicoides species diversity: NAQS Queensland - 2022-23

C. molestus
C. antennalis group
C. austropalpis
C. brevitarsis
C. bundyensis
C. clavipalpis group
C. mackerrasi
C. marksi
C. minimus
C. ornatus group
C. oxystoma
C. victoriae
C. victoriae group
C. wadai

ATTACHMENT B: DETECTIONS AND INVESTIGATION OF *CULICOIDES NUDIPALPIS* IN THE COBOURG PENINSULA AND CROKER ISLAND, NORTHERN TERRITORY

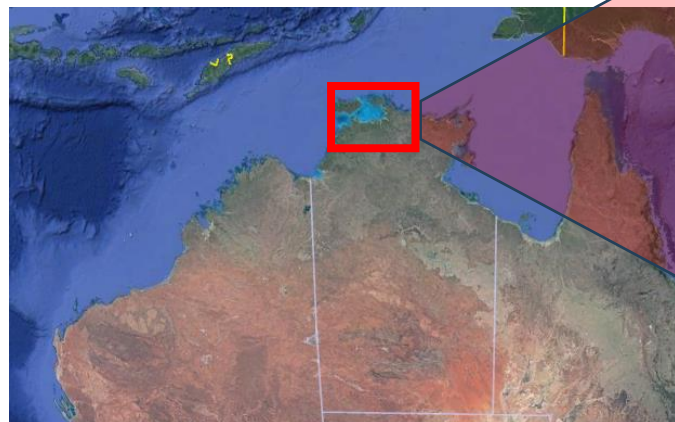
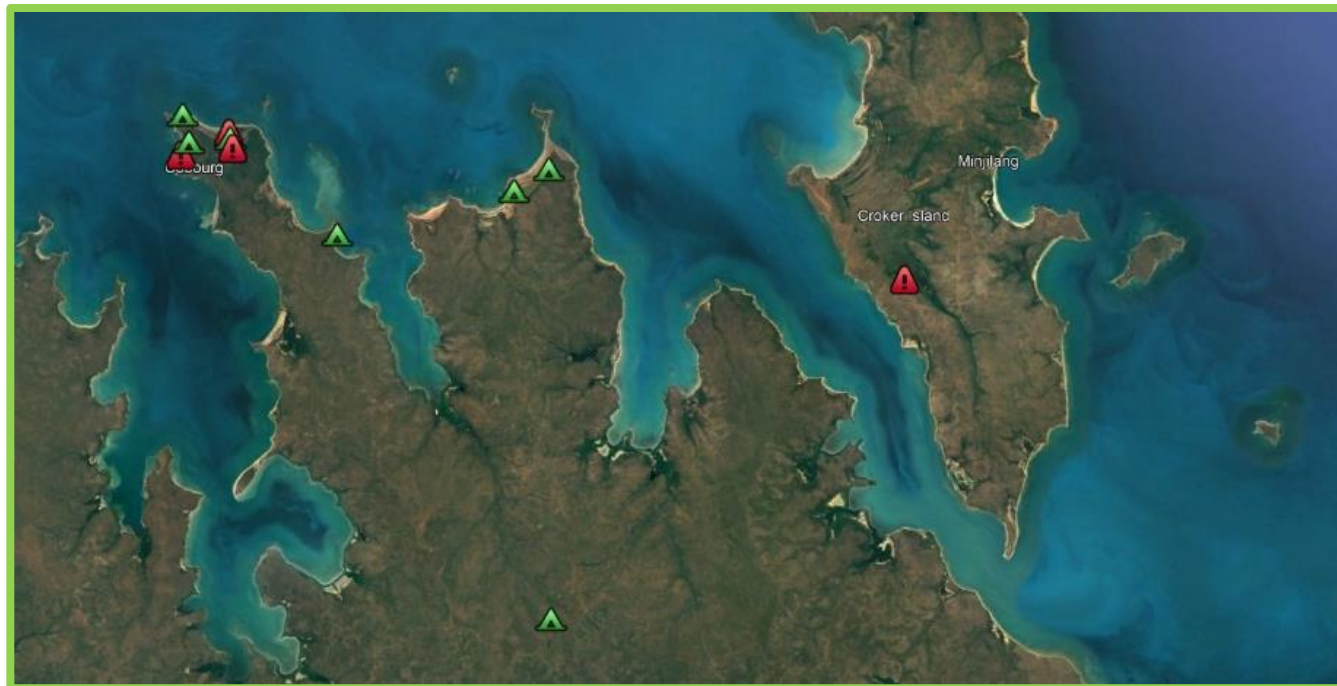
Legend



Culicoides nudipalpis detection



Trapping - 2023



ATTACHMENT C: AN EFFICIENT DNA METABARCODE PLATFORM FOR RAPID IDENTIFICATION OF BULK SAMPLED PEST BITING MIDGES

Description of the project and its scope.

We propose metabarcoding hundreds of pooled specimens to provide a fast (48 hour) and accurate listing of biting midge (*Culicoides*) species and cryptic varieties captured in a NAMP trap, without a need to sort midges from unwanted by-catch. Our published research demonstrates COI DNA barcoding is accurate for species level identifications and DPI NSW extensive library of validated sequences can also identify cryptic species and populations. Design and validations of our metabarcoding platform will primarily use MiSeq™ (Illumina) sequencing technology available to us, but we will also explore use of evolving platforms available from Oxford Nanopore Technologies to facilitate less expensive and potentially portable metabarcoding capacity

We will validate non-destructive DNA digestion from pooled midges whilst maintaining the features used for visual identification. This will allow critically required retrospective taxonomic examination of specimens when there is a need for confirmation or expert examination.

We will develop a Standard Operating Procedure and metabarcoding bioinformatics pipeline linked to our online DNA barcode library as an evolving legacy for molecular identification of Australasian biting midges.

Finally, we will report the cost-effectiveness of our developed meta-barcoding platform to determine its relative costs and savings to taxonomic identification services used by NAMP.

ACTIVITY	PROGRESS AT 11/08/ 2023
Activity 1. Inventory / curate a biting midge sample collection.	completed
Activity 2. Develop DNA digestion methods – (refinements through project)	completed
Activity 3. DNA barcode library (N=500 biting midges).	completed
Activity 4. Develop preferential biting midge COI amplification – (refinements through project).	completed
Activity 5. Metabarcoding trial 01: mixed species DNA pool (N=100 midges + single replicates of notifiable vector species) sequenced using MiSeq (illumina platform).	completed

Activity 6. Metabarcoding trial 2: Digest pool (N=100 midges) + single rep notifiabes, MiSeq sequenced.	completed
Activity 7. Metabarcoding trial 3: Four indexed digest pools (4 pools with N = 100, 250, 500, 1000 midges) + single rep notifiabes sequenced using MiSeq; incidental DNA barcoding as required.	initiated and in advanced progress
Activity 8. Metabarcoding trial 4: comparison of pooled specimen metabarcoding and taxonomic assessment of a wild trap midge pool sequenced using MiSeq; incidental DNA barcoding as required.	initiated and in advanced progress
Activity 9. Metabarcoding trial 5: repeat wild trap trial using ONP Minlon sequencing platform.	delayed till completion of activity 8 & 9
Activity 10. Prepare SOP biting midge metabarcoding & completed Bioinformatics pipeline.	initiated and in advanced progress
Activity 11. Prepare scientific draft for peer review.	initiated and in advanced progress