# Eradication of non-native winter crane fly, *Trichocera maculipennis*, introduced to maritime Antarctica

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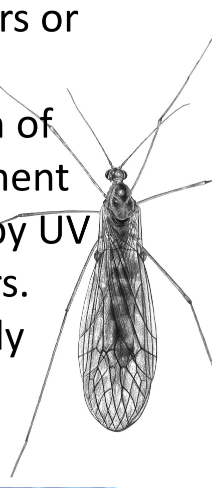
## ABSTRACT

Here we describe our recent efforts to eradicate a non-native insect, winter crane fly (WCF), *Trichocera maculipennis* (Diptera: Trichoceridae), from the King Sejong Station in King George Island. WCF, introduced into King George Island, has spread over several research stations and bases across the island. WCF is cold-tolerant in its native range, and the presence of WCF has been consistent in their invasive habitats over a decade in King George Island, despite some eradication attempts. At the King Sejong Station, systematic efforts have been made to obtain information on WCF's biology in its invasive range and develop an eradication protocol. We have found that embryonic and postembryonic developments of WCF can be successful at least in part under the outdoor conditions in King George Island. We have also found that ultraviolet (UV) light and some habitat-related odorants are attractive to WCF adults. We have been closely monitoring the WCF population at the King Sejong Station using traps over the last ten years or so. It appears that WCF is now being eradicated from the King Sejong Station although the options that can be applied in Antarctic environment are limited. Through the combination of delimitation of the presence of WCF, elimination of WCF adults from its habitats, management of its main habitats, and prevention of its re-entry, the number of WCF adults, monitored by UV trap captures and visual inspection, has been consistently decreased over the last few years. Now, no WCF adults have been captured or detected from the King Sejong Station since July 2022, and we will be able to declare a successful eradication of WCF from the King Sejong Station if no further WCF is detected there for one more year.



## MATRIALS & METHODS

### 01 *Embryonic and postembryonic development of WCF at King Sejong Station*

Oviposition preference of female WCF was investigated in the laboratory at King Sejong Station. Two petri-dishes, one filled with distilled water and the other with freshwater collected from nearby lake (Sejong Lake) containing mosses, were placed in a mesh-cage (30 cm x 30 cm x 30 cm) and adult males and females of WCF collected from the station were released into the cage. The number of eggs oviposited in each petri-dish was counted daily, and petri-dish containers were replaced with new ones at 2 to 3-day interval. The embryonic development of WCF eggs in the petri-dish containers retrieved from the oviposition cage was observed under a microscope until larval hatch. Neonate WCF larvae were then transferred to a petri-dish containing freshwater collected from Sejong Lake with mosses, and their postembryonic development and feeding activities were observed.

### 02 *Visual and olfactory communication and attractants*

**For SEM observation**, male and female WCF collected at King Sejong Station were fixed in 70% ethanol for >24 hrs. Then, heads bearing antennae were isolated from their body with a scalpel, air-dried, and sputter-coated with gold (Q15ORS, Quorum, UK). The samples were then observed with a SEM (FEI Quanta 250 FEG, FEI, USA).

**Electrophysiological recordings** were conducted to investigate the olfactory and visual responses of WCF at the station, using a portable recording system assembled on site (Figure 1). To evaluate the olfactory responses of adult WCF to a panel of volatile compounds produced from their habitat, 17 compounds were selected based on earlier reports (Escalas et al 2003; Kotowska et al 2012; Haider 2022) and used.

**Laboratory bioassay** was conducted to investigate the behavioural responses of WCF to various olfactory and visual stimuli that were identified through EAG and ERG studies. The new trap was constructed with 3D printers (Figure 2A, B & C). A mesh cage (80 x 60 x 120 cm) was constructed at King Sejong Station for the bioassay (Figure 2D). After placing two traps with different combinations of stimuli in the bioassay cage, approximately 20 WCF adults were released into the cage. The number of WCF captured in each trap was then counted after 24 hrs.

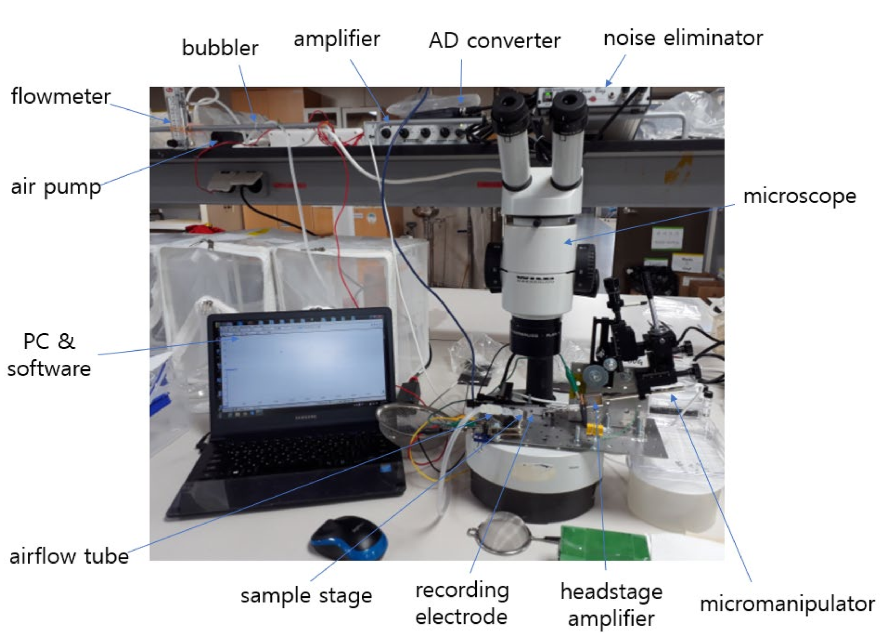



**Figure 1**. An electrophysiological recording system, to measure electroantennogram (EAG) and electroretinogram (ERG) responses of *T. maculipennis* to various olfactory and visual stimuli.

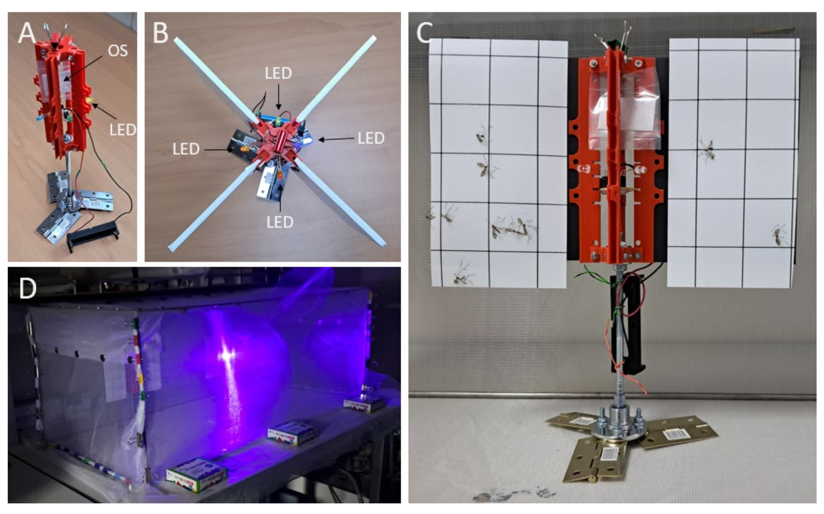



**Figure 2**. Newly developed olfacto-visual trap for *T. maculipennis*. A: visual stimuli (LED) and odor stimulus (OS); B: four LEDs can be placed in a trap so that the visual stimuli can be seen from all direction; C: captured *T. maculipennis* adults on sticky panels; D: a mesh-cage bioassay system was used to evaluate the attractiveness of different visual and olfactory stimuli.

### 03 *Monitoring WCF and Eradication efforts at King Sejong Station*

The population of adult WCF has been continuously monitored with traps at King Sejong Station since 2016. UV traps (Figure 3A, B) and delta traps (Figure C, D) with sticky bases were placed in various buildings including two wastewater treatment facilities, and the number of WCF adults captured in the traps was recorded at 1-to-3-day interval during summer season and biweekly during the rest of the year. Intensive physical removal has also been performed every summer since December 2018; two wastewater treating buildings have been thoroughly inspected almost every day during each summer season, and WCF adults found were removed from the building. Entry point inspection was intensified and tightly controlled to prevent further entry of WCF from Fildes Peninsula.


**Figure 3**. Traps used to monitor and catch *T. maculipennis* adults at King Sejong Station.

## RESULTS & DISCUSSION

### 01 *Embryonic and postembryonic development of WCF at King Sejong Station*

Field-collected males and females of WCF mated successfully in mesh cages under indoor condition at King Sejong Station, and mated females actively oviposited eggs in the cage. Females laid eggs individually or as a mass in a Petri dish containing freshwater with mosses taken from nearby lakes (Figure 4A). It was found that mated females oviposited significantly more eggs in lake water containing mosses than in distilled water (Figure 4B). Most eggs laid in mesh cage underwent embryonic development, and many of them successfully completed embryonic development within a few days under indoor condition (Figure 4C). Neonate larvae from the eggs showed continuous postembryonic development with active feeding on organic matter on the surface of mosses taken from freshwater lakes (Figure 4D).

The results indicate mated female WCF are likely to lay eggs in freshwater lakes around King Sejong Station and the eggs can undergo successful embryonic development in the freshwater lakes at least during summer period. The results also suggest that the freshwater lakes can be a good habitat for WCF larvae, supplying organic matter for larval feeding and growth.

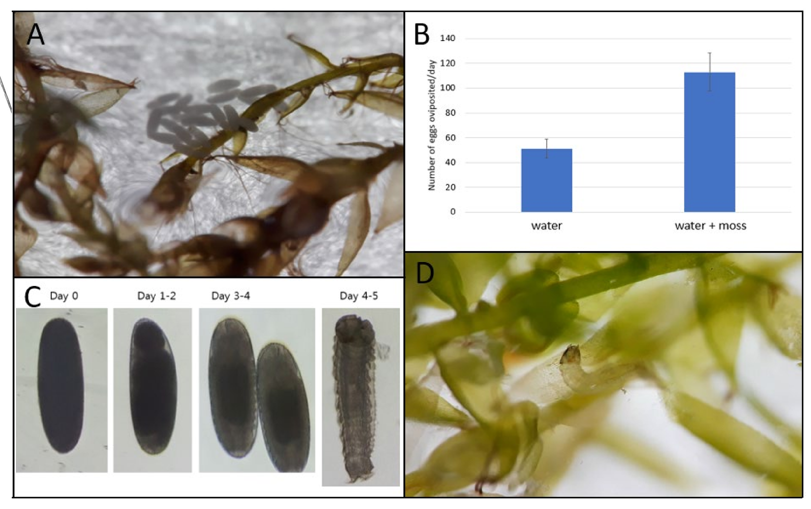



**Figure 4.** Oviposition, embryonic development and postembryonic feeding activity of *T. maculipennis*. A: A cluster of eggs were deposited around moss and algae collected from a freshwater lake in King George Island; B: Significantly more eggs were oviposited when moss was present in the oviposition container; C: Embryonic development was successful in the media collected from a Antarctic freshwater lake; D: Larvae of *T. maculipennis* actively fed on organic matters in freshwater lake.

### 02 *Visual and olfactory communication and attractants*

Our SEM observation exhibited that the antennae of WCF contain well-developed olfactory sensilla. Numerous olfactory sensilla were identified in the flagella subsegments of the antennae of male and female WCF, showing that trichoid sensilla (Figure 5A) and basiconic sensilla (Figure 5B) are two major types of olfactory sensilla in WCF antennae. The presence of multiple nanoscale cuticular pores indicated the olfactory function of these sensilla (Figure 5).

When the behavioral attraction of WCF to EAG-active olfactory stimuli and ERG-active visual stimuli was evaluated in a net cage (Figure 2), it was found that UV LED light and green LED light were significantly more attractive to WCF males and females and 3 volatile compounds, 2-phenylethanol, Z3-hexenol and 1-hexanol, were behaviorally attractive to WCF adults. However, linalool did not show any behavioral attraction to WCF although this compound elicited the largest EAG responses.

The results of our SEM observation, EAG tests and behavioural bioassay clearly indicate that olfaction plays an important role in the life of WCF adults, which in turn suggests that olfactory cues can be developed as new attractants for WCF. Currently, UV traps are used as a monitoring tool for WCF at various stations in King George Island. However, UV and other light-based trapping systems are unlikely to be useful in Antarctic outdoor conditions during their summer breeding season because of long daytime. In this context, odour-based trapping system can be an effective tool for monitoring and controlling WCF in outdoor environment. Effective odor bait can also enhance the attractiveness of UV trap when used in combination with the visual attractant. It would also be interesting to see if green LED light exhibiting strong behavioural attraction to WCF can enhance the attractiveness of UV light.

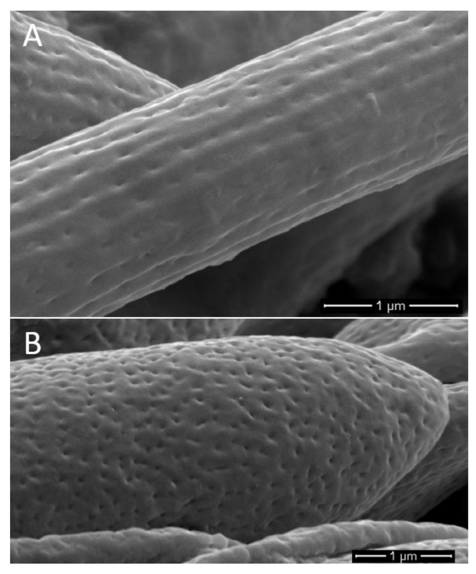



**Figure 5**. Two major types of olfactory sensilla identified in *T. maculipennis*. A: trichoid sensilla; B: basiconic sensilla.

### 03 *Monitoring WCF and Eradication efforts at King Sejong Station*

UV traps captured significant number of WCF at King Sejong Station. In contrast, most delta traps with sticky bases did not catch any WCF and only a small number of WCF were captured in some delta traps. When annual total trap catches were compared, the number was maintained around or over 200 individuals per year from 2016 through 2020, with one peak of adult emergence during summer season (Dec. – Feb.) each year. However, the trap catches were continuously dropped after 2019, reaching zero trap catch for more than a year since July 2022 (Figure 6). Considering that large populations of WCF have been maintained at other stations in Fildes Peninsula during this period, the rapid decrease of WCF in King Sejong Station is likely to be achieved through our systematic efforts to control this species from the station. This seems to suggest that the population of this species is under effective control around the King Sejong Station.

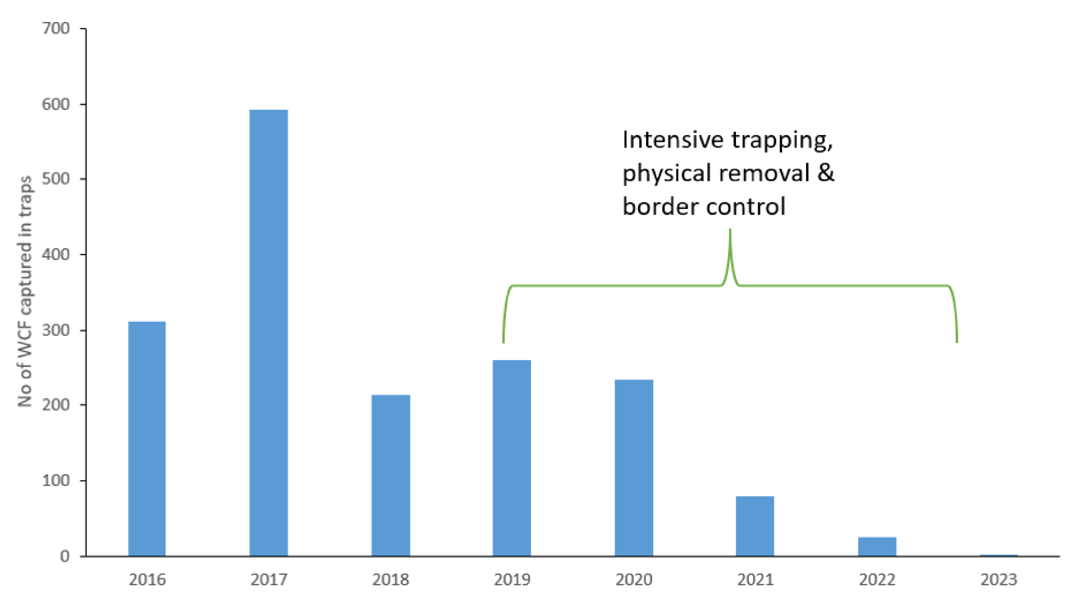



**Figure 6**. Total number of adult *Trichocera maculipennis* captured in traps placed at King Sejong Station in each year. The annual trap catches of *T. maculipennis* has been continuously decreased since intensive trap catch, visual inspection and physical removal.

## CONCLUSION

Our study indicates that WCF can oviposit and develop in Antarctic freshwater lakes during summer period, and WCF has well developed and selective olfactory and visual communication system. WCF are currently being eradicated from King Sejong Station through enhanced trapping, border control, and intensive physical removal. If successful eradication is declared, preventing re-entry of WCF from Fildes Peninsula would be important.