

## Protocol & Techniques

# Pan Traps as an efficient and low cost method for sampling *Corethrella* Coquillet, 1902 (Diptera: Corethrellidae)

Eduardo F. Geisler<sup>1✉</sup>, André P. Amaral<sup>2</sup>, Leonardo L. F. Campos<sup>3</sup>, Luiz C. Pinho<sup>3</sup>, Selvino Neckel-Oliveira<sup>3</sup>

<sup>1</sup>Instituto Nacional de Pesquisas da Amazônia, Manaus, AM, Brazil. <sup>2</sup>Ludwig-Maximilians-Universität München, München, BAV, Deutschland. <sup>3</sup>Universidade Federal de Santa Catarina, Florianópolis, SC, Brazil.

✉ Corresponding author: [eduardo.geisler@posgrad.inpa.gov.br](mailto:eduardo.geisler@posgrad.inpa.gov.br)

Edited by: Danilo P. Cordeiro<sup>1</sup>

Received: July 15, 2022. Accepted: October 04, 2022. Published: October 18, 2022.

**Abstract.** The genus *Corethrella* Coquillet, 1902 are known as a group of nocturnal frog-biting-midges (Diptera: Corethrellidae) which locate their frog-hosts through the sound emitted by their conspicuous calls. The classical method to capture these midges consists in using modified Center Disease Control (CDC) traps in which speakers emitting frog calls replace the light bulb. However, the high cost, volume and weight of CDC traps hampers several studies in remote areas and in countries with low research investments. Therefore, the main objective of this study was to compare the capture rate of the classic modified CDC trap with a new trap recently developed, here referred as PTM. We conducted an experiment at flooded areas in the vicinity of a lake in Florianópolis, Santa Catarina State, southern Brazil in which we arranged PTM and modified CDC traps emitting frog calls of two species commonly found in the area, *Physalaemus nanus* (Boulenger, 1888) and *P. cuvieri* Fitzinger, 1826. In a total of 34,5h sampling hours per trap (one trial per night for five days), the PTM traps captured over 20 times more individuals than modified CDC traps (n= 1594; n=72, respectively) and the same number of species (3). Those results demonstrate that the PTM method had a higher capture rate, are more practical and has a lower cost than modified CDC traps. We suggest further studies to investigate which aspects could explain the differences between capture rates among PTM, BG sentinel, bottle and CDC modified methods.

**Keywords:** Ectoparasite, Frogs, Midges, Eavesdropper, Host.

The family Corethrellidae comprises a group of nocturnal frog-biting-midges in which females are hematophagous (McKeever & French 1991). Several studies shown that Corethrellidae females are phonotactic dipterans, attracted to the conspicuous calls emitted from male frogs (e.g., McKeever 1977; McKeever & Hartberg 1980; Bernal et al. 2006; Borkent 2008; Virgo et al. 2019). Since these auditory cues are the main source for host detection, modified Center Disease Control (CDC) miniature light traps, in which the light bulb is replaced for a speaker broadcasting frog calls (McKeever & Hartberg 1980), are the most widespread method used to capture *Corethrella* females (see Halfwerk et al. 2019; Virgo et al. 2019).

Nevertheless, in countries such as Brazil, the severe cuts in research fundings over the last years (Galvão-Castro et al. 2022) hampered the use of CDC traps due to their high cost of importation and maintenance. Furthermore, the CDC modified method is a double battery dependent trap (due to the speakers and the fan battery) and has the necessity of supports to stand the traps off the ground, which may not be present at certain locations as grasslands and anthropized regions (*pers. observation*).

However, practical and low-cost methods such as pan traps (or modified pan traps), which are also useful to capture flying insects (Calor & Mariano 2012), might be effective as a new approach to capturing *Corethrella*. The pan traps consist basically of trays filled with water and detergent (to decrease water's surface tension) or a fixating solution, and because of its simplicity and effectiveness, it has turned into a convenient method for a variety of sampling designs (Calor & Mariano 2012). A modified pan trap method (Amaral & Pinho 2015), referred here as PTM, in which a speaker is placed in the center of the tray broadcasting frogs calls, were used to capture corethrellid females. Despite having used this method, no tests were conducted to verify its

comparative capture rate with classical methods as the CDC modified traps firstly proposed by McKeever & Hartberg (1980). In the present study, we evaluate the capture rate of the newly developed pan trap method (PTM) in comparison to the CDC modified trap, the classic sound trap used for sampling Corethrellidae.

Traps were set between 21 to 25 February 2018 in flooded areas in the vicinity of a lake named "Lagoa do Peri", one of the last remnants of the Atlantic Forest in Florianópolis, Santa Catarina State, southern Brazil (27° 43.214' S, 48° 30.518' W, WGS84 Datum) (Sbroglia & Beltrame 2012).

The CDC modified traps consist of a battery-powered fan trap (6V - 5AH batteries) installed 1.5-2.0 m of the ground, with speakers attached next to the trap entrance (Fig. 1A). The CDC traps weigh about 2,0Kg total (batteries + fan trap) and 45cm height (collecting chamber + fan trap). The CDC trap entrance is 9 cm diameter, but our speakers covered half of the trap entrance in the experiments. Our PTM traps consist of a simple tray (8 x 27 x 40 cm) filled with 500 mL of water, approximately 5 mL of detergent and a speaker suspended by a support into the center of the tray (Fig. 1B).

We delimited a 150 m transect in which six traps were placed (three CDC and three PTM, alternately) 25 m apart from each other to avoid pseudoreplication (Trillo et al. 2016) and each day we rotated the traps position to diminish microhabitat effects. Traps were placed at 1.5-2.0 m height following methods conducted by Borkent (2008) and Ambrozio-Assis et al. (2018). Traps were activated from around 20:00 until 22:30 in a total of one trial per night for five days. Some speakers ran out of battery and daily rain minimized sampling hours through the experiment, but we conservatively considered a total of 69 sampling hours (34.5 h for each method). The speakers played a mixed looping record broadcasting of the natural calls of *Physalaemus*



**Figure 1.** A illustrative photo of the traps used in the experiments. (A) CDC modified trap; (B) PTM trap.

*cuvieri* Fitzinger, 1826 and *Physalaemus nanus* (Boulenger, 1888), two frog species found at the study area. The mixed looping records were generated with the software Audacity® and the frog calls used are available at Haddad et al. (2005). We measured the sound pressure levels (dB) broadcasted by the speakers at 0.5m distance using the software Sound Meter - Abc apps®, resulting in *P. cuvieri* calls at 34-40 dB and *P. nanus* calls at 18-30 dB. We assess the normality of the data visually and proceed using a Wilcoxon signed-rank test.

Individuals caught by the PTM traps were filtered from the solution with a funnel and a thin net and relocated into alcohol 70%. Midges captured by the CDC traps were collected from the chambers, refrigerated for 12 hours and relocated into alcohol 70%. We utilized the procedures for slides mounting from Amaral et al. (2019) and followed the keys to the Neotropical Corethrellidae (Borkent 2008) and further descriptions (Amaral & Pinho 2015; Caldart et al. 2016; Amaral et al. 2019; Amaral et al. 2021) for *Corethrella* midges identification. Detergent does not damage important morphological characters, such as scales, setae and pigmentation (Virgo et al. 2019).

The most abundant species captured in our study were *Corethrella amazonica* Lane, 1939 (n = 1,642), followed by *Corethrella selvicola* Lane, 1939 (n = 9) and *Corethrella peruviana* Lane, 1939 (n = 4). From those, the PTM method captured a total of 1,582 individuals of three species (*C. amazonica* = 1580; *C. peruviana* = 1; *C. selvicola* = 1), resulting in a capture rate of 45.85 corethrellids/hour. Conversely, the CDC traps captured 73 specimens total, also belonging to the three species found (*C. amazonica* = 62; *C. selvicola* = 8; *C. peruviana* = 3). The capture rate of CDC traps were 2.11 corethrellids/hour. The boxplot indicating the difference in logarithmic scale between CDC and PTM methods (Fig. 2). Wilcoxon signed-rank test indicates a significant difference between treatments, with  $p < 0.001$ .

Our results have shown that the PTM method captured more corethrellids per hour and the same species as the modified CDC

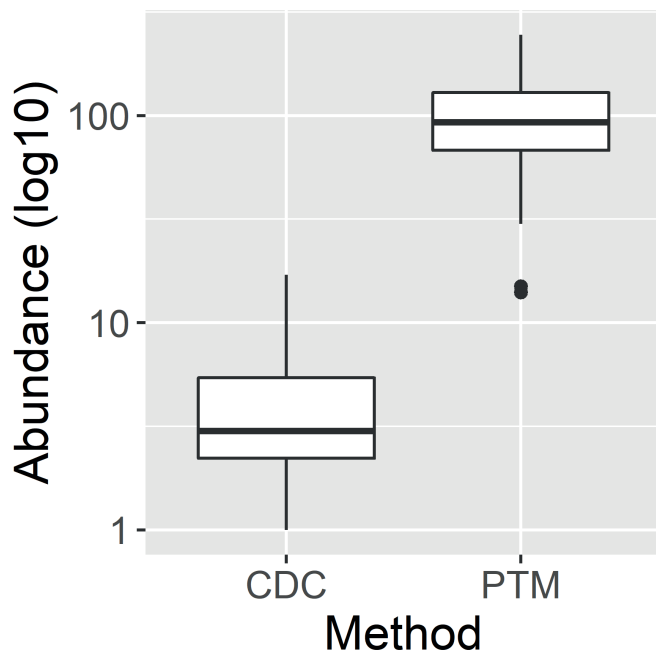
modified method. Even though our results had a huge dominance towards *C. amazonica*, the PTM method were also capable to capture other corethrellids species in high numbers in different studies (Amaral & Pinho 2015; Amaral et al. 2019), indicating its potential to be used in future researches with Corethrellidae.

Virgo et al. (2019) published a new method to capture Corethrellidae comparing the capture rate of similar methods as presented here, but instead of using a conventional CDC and a pan trap, they used speakers attached to BG Sentinel fan-operated traps and bottle traps with water and detergent. Their results showed that the bottle traps had a lower capture rate compared to the BG Sentinel, but the relative abundance remained the same. Instead, we demonstrate that our PTM method, in comparison to CDC modified method, had a higher capture rate and capture the same number of species, corroborating partially with the results found in Virgo et al. (2019).

Until now, there is no explanation for those differences found in our data, but our hypothesis points to the joint effect of the larger landing area of PTM and the partial obstruction of the CDC trap entrance by the speaker. Studies conducted by Bernal et al. (2006) and Virgo et al. (2021) have shown that the mean landing of some *Corethrella* species stands between 7-20 cm from the speaker. These results might explain the higher efficiency of PTM method, which has a larger area of landing compared to the CDC, BG Sentinel fan-trap and the bottle trap method. We suggest new research to unravel the causes of the differences of capture rate between these methods presented and its relation with Corethrellidae biological features.

Currently, the PTM can be considered as a new method with huge potential to be used in various taxonomic and ecological studies in the near future. Traps such as CDC and BG Sentinel can be of high cost, making their use impossible in countries with low research investments. Moreover, CDC traps dimensions and battery dependence make them heavier and larger in comparison to PTM and other alternative

methods. Its low cost, weight, volume and higher capture rates makes the PTM an effective method for sampling Corethrellidae and we suggest its use in further studies.



**Figure 2.** Abundance of corethrellids captured by PTM (n = 15) and CDC (n = 15) traps in puddle areas at the Atlantic Forest, southern Brazil (log<sub>10</sub> for better visualization).

## Acknowledgments

This work was supported by Fundação de Amparo à Pesquisa de Santa Catarina, Programa de Pesquisa em Biodiversidade rede Mata Atlântica (CNPq 457451/2012-9), CNPq/Capes/FAPs/BC-Fundo Newton/PELD nº 15/2016 and FAPESC/2018TR0928.

## Authors' Contributions

EFG conceived the fieldwork expeditions and wrote the manuscript. APA and LFLC identified midges specimens and carried out the data analyses. SN-O and LCP idealized the study and provided equipment and funding support. All authors revised the manuscript.

## Conflict of Interest Statement

The authors declare no conflict of interest.

## References

- Amaral, A. P.; Bello-González, O. C.; Pinho, L. C. (2021) *Corethrella stenostyla* sp. n., a new frog-biting midge (Diptera: Corethrellidae) from Cuba. *Zootaxa*, 5060(4): 595-598. doi: [10.11646/zootaxa.5060.4.9](https://doi.org/10.11646/zootaxa.5060.4.9)
- Amaral, A. P.; Mariano, R.; Pinho, L. C. (2019) Four new species and some new records of Brazilian frog-biting midges (Diptera: Corethrellidae). *Zootaxa*, 4706(1): 103-120. doi: [10.11646/zootaxa.4706.1.4](https://doi.org/10.11646/zootaxa.4706.1.4)
- Amaral, A. P.; Pinho, L. C. (2015) New species and records of frog-biting midges from southern Brazil (Diptera: Corethrellidae). *Zootaxa*, 3946(2): 274-284. doi: [10.11646/zootaxa.3946.2.9](https://doi.org/10.11646/zootaxa.3946.2.9)
- Ambrozio-Assis, A.; Lopes, B. C.; Amaral, A. P.; Pinho, L. C.; Peeters, E. T. H. M.; Neckel-Oliveira, S. (2018) Preferences for anuran calls in hematophagous corethrellids (Diptera: Corethrellidae) from Southern Brazil. *Austral Entomology*, 58(3): 622-628. doi: [10.1111/aen.12376](https://doi.org/10.1111/aen.12376)

- Bernal, X. E.; Rand, A. S.; Ryan, M. J. (2006) Acoustic preferences and localization performance of blood-sucking flies (*Corethrella* Coquillett) to tungara frog calls. *Behavioral Ecology*, 17(5): 709-715. doi: [10.1093/beheco/arl003](https://doi.org/10.1093/beheco/arl003)
- Borkent, A. (2008) The frog-biting midges of the world (Corethrellidae: Diptera). *Zootaxa*, 1804(1): 1-456. doi: [10.11646/zootaxa.1804.1.1](https://doi.org/10.11646/zootaxa.1804.1.1)
- Caldart, V. M.; Santos, M. B.; Iop, S.; Pinho, L. C.; Cechin, S. Z. (2016) Hematophagous flies attracted to frog calls in a preserved seasonal forest of the austral Neotropics, with a description of a new species of *Corethrella* (Diptera: Corethrellidae). *Zoological Science*, 33: 527-536. doi: [10.2108/zs150173](https://doi.org/10.2108/zs150173)
- Calor, A. R.; Mariano, R. (2012) UV Light Pan Traps for Collecting Aquatic Insects. *EntomoBrasilis*, 5(2): 164-166. doi: [10.12741/ebrasilis.v5i2.187](https://doi.org/10.12741/ebrasilis.v5i2.187)
- Galvão-Castro, B.; Cordeiro, R. S. B.; Goldenberg, S. (2022) Brazilian science under continuous attack. *The Lancet*, 399(10319): 23-24. doi: [10.1016/s0140-6736\(21\)02727-6](https://doi.org/10.1016/s0140-6736(21)02727-6)
- Haddad, C. F. B.; Giovanelli, J. G. R.; Giasson, L. O. M.; Toledo, L. F. (2005) *Guia Sonoro de Anfíbios Anuros da Mata Atlântica*. São Paulo: Biota-FAPESP.
- Halfwerk, W.; Blaas, M.; Kramer, L.; Hijner, N.; Trillo, P. A.; Bernal, X. E.; Page, R. A.; Goutte, S.; Ryan, M. J.; Ellers, J. (2019) Adaptive changes in sexual signalling in response to urbanization. *Nature Ecology & Evolution*, 3: 374-380. doi: [10.1038/s41559-018-0751-8](https://doi.org/10.1038/s41559-018-0751-8)
- McKeever, S. (1977) Observations of *Corethrella* feeding on tree frogs (*Hyla*). *Mosquito News*, 37(3): 522-523.
- McKeever, S.; French, F. E. (1991) *Corethrella* (Diptera: Corethrellidae) of eastern North America: laboratory life history and field responses to anuran calls. *Annals of the Entomological Society of America*, 84(5): 493-497. doi: [10.1093/aesa/84.5.493](https://doi.org/10.1093/aesa/84.5.493)
- McKeever, S.; Hartberg, W. K. (1980) An effective method for trapping adult female *Corethrella* (Diptera: Chaoboridae). *Mosquito News*, 40(1): 111-112.
- Sbroglia, R. M.; Beltrame, A. V. (2012) Zoning, conflicts and reclassification of Lagoa do Peri County Park, Florianópolis/SC. *Boletim de Geografia*, 30(1): 5-18. doi: [10.4025/bolgeogr.v30i1.11542](https://doi.org/10.4025/bolgeogr.v30i1.11542)
- Trillo, P.; Bernal, X. E.; Caldwell, M.; Halfwerk, W.; Wessel, M.; Page, R. (2016) Collateral damage or a shadow of safety? The effects of signalling heterospecific neighbours on the risks of parasitism and predation. *Proceedings of the Royal Society B*, 283(1831): 20160343. doi: [10.1098/rspb.2016.0343](https://doi.org/10.1098/rspb.2016.0343)
- Virgo, J.; Ruppert, A.; Lampert, K. P.; Grafe, T. U.; Eltz, T. (2019) The sound of a blood meal: acoustic ecology of frog-biting midges (*Corethrella*) in lowland Pacific Costa Rica. *Ethology*, 125(7): 465-475. doi: [10.1111/eth.12871](https://doi.org/10.1111/eth.12871)
- Virgo, J.; Ufermann, L.; Lampert, K. P.; Eltz, P. (2021) More than meets the eye: decrypting diversity reveals hidden interaction specificity between frogs and frog-biting midges. *Ecological Entomology*, 47(1): 95-108. doi: [10.1111/een.13095](https://doi.org/10.1111/een.13095)