

ARTICLE

Towards a practical trap for deer flies (Diptera: Tabanidae): initial tests of a bi-level Nzi trap

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Abstract

A modified Nzi trap was tested at a residence and at a farm in eastern Ontario, Canada to better capture high-flying tabanids (Diptera) such as *Chrysops* Meigen. A new upper trap entrance was created to provide a higher and larger opening by reducing the front blue top shelf to half its height. To minimise escape of low-flying tabanids, a vertical inner baffle was added to direct low-flying tabanids up into the cone. Half of the tests of 18 new designs caught 1.5–2.7 times more deer flies than the Nzi trap did, with the other trap designs being as effective as the Nzi trap. The optimal design that maintained equal catches of other biting flies relative to the Nzi trap was one with a phthalogen inner horizontal shelf and a netting inner vertical baffle. This design is defined in the present as the “bi-level Nzi trap.” *Chrysops* entered the trap mostly through the top (88%; 17 spp.), along with *Hybomitra* Enderlein (94%; 12 spp.). *Tabanus* Linnaeus (9 spp.) entered through both entrances. The most abundant *Tabanus*, *T. quinquevittatus* Wiedemann, entered mostly through the bottom (70%), whereas *Stomoxys calcitrans* Linnaeus entered mostly through the top (92%).

Résumé

Un piège Nzi modifié a été mis à l'essai dans une résidence et dans une ferme de l'est de l'Ontario, Canada pour mieux attraper les tabanidés (Diptera) volant à haute altitude comme les *Chrysops* Meigen. Une nouvelle entrée supérieure au piège a été créée pour fournir une ouverture plus haute et plus grande en réduisant simplement l'étagère supérieure bleue avant à la moitié de sa hauteur. Pour minimiser la fuite des tabanidés volant à basse altitude, un déflecteur intérieur vertical a été ajouté pour diriger les tabanidés volant à basse altitude dans le cône. La moitié des tests de 18 nouveaux modèles ont capturé 1,5 à 2,7 fois plus de mouches à chevreuil que le piège Nzi; les autres modèles de piège étaient aussi efficaces que le piège Nzi. Le modèle optimal qui maintenait des captures égales d'autres mouches piqueuses par rapport au piège Nzi était celui avec une étagère horizontale intérieure en phthalogène et une chicane verticale intérieure en filet. Ce modèle est défini comme « le piège Nzi à deux niveaux ». *Chrysops* sont entrées principalement par le haut (88 %, 17 spp.), ainsi que *Hybomitra* Enderlein (94 %, 12 spp.). *Tabanus* Linnaeus (9 spp.) est entré par les deux entrées. La *Tabanus* la plus abondante, *T. quinquevittatus* Wiedemann, est entrée principalement par le fond (70 %), tandis que *Stomoxys calcitrans* Linnaeus est entré principalement par le haut (92 %).

Introduction

Deer flies (Diptera: Tabanidae) are an annoying pest in recreational areas in North America. They are blood-feeding ectoparasites that can disperse over several kilometres over a long time (Sheppard and Wilson 1976) and can fly among hosts over tens of metres in a short time

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(Foil 1983). A few tropical species are active in the forest canopy, but most species are active within only a few metres of the ground (de Souza Amorim *et al.* 2022). In Canada, flies typically ambush people in open areas along forest trails and at picnic areas near lakes. In areas with many flies, such as Algonquin Park in Ontario, Canada (Ossowski and Hunter 2000), flies swarm around people's heads during mid- to late summer. They are also a nuisance in residential settings and are aggressive biters of dogs. They feed on wild mammals such as white-tailed deer, *Odocoileus virginianus*, and moose, *Alces americanus* (Cervidae) (Smith *et al.* 1970), and are common pests of livestock (Teskey 1960; Lewis and LePrince 1981). During peak seasonal abundance, maximum catches up to 1000 per hour (mostly *Chrysops vittatus* Wiedemann) were obtained in a sweep net survey in Michigan, United States of America (Strickler and Walker 1993). *Chrysops vittatus* is particularly attracted to people, accounting for 78% of tabanids caught in sweep nets *versus* only 5% in Malaise traps in New Jersey, United States of America (Tallamy *et al.* 1976).

In Africa, *Chrysops silacea* (Austen) and *Chrysops dimidiata* (Wulp) are of medical significance as vectors of *Loa loa* Cobbold, a filarial parasite responsible for the disease loiasis (Kelly-Hope *et al.* 2017). Catches of *Chrysops* Meigen as a whole are often 1% or less of all tabanids in all but a few African trapping studies (Sinshaw *et al.* 2006; Koné *et al.* 2011; Acapovi-Yao *et al.* 2017). Hence, sweep nets are used for critical sampling in the study of loiasis (Pryce *et al.* 2022). In the absence of convenient sampling methods, the biology of deer flies is not well documented, with most studies recording only their presence and species composition.

Researchers have explored only a few alternatives other than traps for sampling deer flies – for example, sticky weather balloons (Snoddy 1970), trolling with mobile sticky objects (Mizell *et al.* 2002), and use of sticky patches on clothing (Cilek 2000). More practical collection methods have received little attention other than the development of the large two-tier box trap for two salt marsh species (French and Hagan 1995). The upper tier of the box trap, at 1.8 m, is very effective for *Chrysops atlanticus* Pechuman and *Chrysops fuliginosus* Wiedemann. Mean catches of up to about 600/day were achieved in both tiers in 1-octen-3-ol-baited traps. Unfortunately, this large and heavy trap is cumbersome and is practical only for fixed applications. *Chrysops atlanticus* can also be captured in the large Townsville Malaise trap (Schreck *et al.* 1993).

Two-tier box traps do not catch other species of deer flies in large numbers; for example, means of approximately seven per day in unbaited traps in Vermont, United States of America (Freeman 2017) and similar numbers in traps baited with CO₂ in Florida, United States of America (Cilek and Olson 2008), are common. Overall, in 80 representative tabanid studies reviewed by the author, low catches in any trap are the norm, including those in Malaise traps (Skvarla *et al.* 2021). The one exception is for another coastal species, *Chrysops flavidus* Wiedemann. One hundred or more per day can be captured in the large Stoneville Malaise trap in Mississippi, United States of America, especially with CO₂ (Roberts 1972, 1975, 1976, 1978). Catches of deer flies are increased by baiting traps with CO₂ or 1-octen-3-ol (Wilson and Richardson 1970; Cilek and Olson 2008) but not with other baits targeting tsetse or horse flies (Mihok and Lange 2012).

To address the need for a simple and portable trapping device, I have revisited the basic design of the multipurpose blue–black Nzi trap (Mihok 2002) for the high-flying behaviour of deer flies. The experiments reported here may also be applicable to tabanids with similar behaviour, in particular *Haematopota* Meigen in Europe (Thomson and Saunders 1986). A typical example of the high-flying behaviour of *Chrysops* is from Algonquin Park (Bennett and Smith 1968). In that study, equal numbers of *Chrysops* were caught in CO₂-baited cage traps with entrances at 36 or 86 cm above ground level, whereas most *Hybomitra* Enderlein (84%) were captured at the lower height. A similar contrast in height of landing has also been documented for *Chrysops versus Tabanus* Linnaeus landing on a sticky enclosure surrounding a Nzi trap (Mihok and Carlson 2007).

The Nzi trap has been useful for sampling biting flies worldwide (Mihok *et al.* 2022), with 129 studies in 43 countries, 93 of which have included data on tabanids and 60 of which have included data on stable flies (*Stomoxys* Geoffroy). The Nzi trap catches *Chrysops* well in Canada (maximum

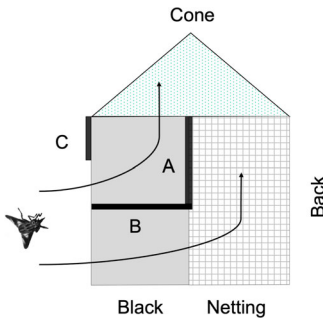


Figure 1. Side view of the layout of a modified Nzi trap with an additional upper front entrance and a new vertical inner baffle: **A**, new vertical inner baffle, **B**, existing horizontal netting shelf, and **C** reduced front horizontal blue shelf.

catches of up to 190 per day; 8987 trap-days to date). Capture efficiency has not been measured in detail but appears high (81%; 51/(50+13); Mihok *et al.* 2007). This has made it a useful tool for testing odour baits (Mihok and Lange 2012) relative to more cumbersome fabric traps – for example, the 6-m Gressitt Malaise trap (Ringrose *et al.* 2014). The Nzi trap is also unique in catching *Stomoxys* while maintaining equitable catches of tabanids (Tunnakundacha *et al.* 2017), a feature that was incorporated in its development (Mihok 2002). Hence, biting fly surveys have often included Nzi traps in addition to *Stomoxys*-specific traps such as the Vavoua trap (Mihok *et al.* 1995; Onju *et al.* 2020).

Here, I report on a simple modification to the design of the Nzi trap that improves the catch of deer flies and that does not require traps to be set at an inconvenient height, without unduly affecting the catch of horseflies and stable flies.

Material and methods

Experiments were conducted in 2020 in a residential turfgrass setting on the outskirts of Russell, Ontario, Canada (Mihok *et al.* 2006), and at a hobby farm with cattle, pigs, and poultry 6.5 km north (a new location) of the residence. A standard Nzi trap (Supplementary material, Fig. S1A) in phthalogen blue (TDV Industries, Laval, France; type IF3GM, copper phthalocyanine, CuPc) or phthalogen turquoise cotton (home-dyed 5% sulphonated CuPc) was included in each experiment as the control (Mihok and Carlson 2021). Turquoise traps (Supplementary material, Fig. S1K) were used at the farm because this colour is particularly effective for *Hybomitra*, which was expected to be common at the farm. Modified traps were in the same colour within experiments as the control was. Sunbrella marine acrylic (Pacific Blue; Glen Raven Inc., Glen Raven, North Carolina, United States of America) or Top Notch polyester (Blue #563; Marlen Textiles, New Haven, Missouri, United States of America) was used for black. Ultraviolet light-resistant white polyester mosquito netting (Mosquito Curtains, Alpharetta, Georgia, United States of America) was used.

Experiments were mostly replicated Latin squares with treatments rotated among sites separated by 10–25 m. The exception was when a Plexiglas® trap (at a fixed location) was compared to a fabric Nzi trap set nearby in experiments P1–P3. All traps were baited with an octenol lure (1-octen-3-ol; Biosensory Inc., Putnam, Connecticut, United States of America) and were set 5–10 cm above ground level. Other baits were added in some experiments to provide contrasts in how different designs performed for ways in which Nzi traps are sometimes baited. These included household ammonia (changed every few days; 5%), aged cattle urine (Mihok and Lange 2012), and fresh cattle manure (faeces intermingled with some straw; 2 kg). At the farm, livestock were near traps and sometimes roamed freely.

Experiments focused on testing a modified Nzi trap by increasing the entrance area, as in the multiple entrance tetra trap (Dia *et al.* 2008), but keeping the existing layout for simplicity. To do this, the bottom half of the blue front horizontal panel was removed (Fig. 1). The horizontal inner

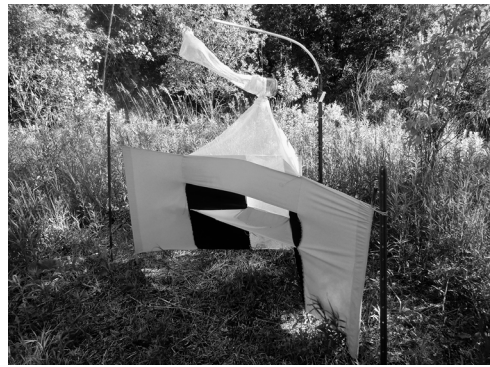


Figure 2. Photograph of a phthalogen blue cotton bi-level Nzi trap with a flexible plumbing pipe used to suspend the cone. Note the netting sleeve and plastic chamber collection system. This is an example of the “horizontal” configuration.

shelf was retained and was connected at the back to a new inset vertical baffle. This was done to limit escape as in the final design phase of the Nzi trap. These modifications partitioned the trap into a lower compartment and an upper compartment. A photo of this new design is provided in Fig. 2.

The trap is best set with bamboo poles inserted into corner sleeves with guy wires or metal poles used for obtaining a symmetrical shape. These stretch the top of the trap and result in a smooth cone. The cone can be suspended from a sapling or any flexible pole (13-mm-diameter CPVC plumbing pipe). A practical collection system consists of a sturdy 2-L plastic juice bottle cut to provide a short funnel exit (32 mm diameter) and an intermediate chamber (the back should be cut off and closed with netting for less wind resistance and increased drainage of water and to keep flies in good shape for identification). A white netting collection sleeve (50 cm long) is attached to the chamber with light sewing elastic.

The designs tested are summarised in Table 1 (H for Home (or residence); F for Farm) with a breakdown of the detransformed mean catches by genus in the standard Nzi trap provided for each experiment. At the farm, three experiments were also conducted with a special Plexiglas trap set next to a pig pen (coded as P1–P3; Supplementary material, Fig. S1AD). The trap was made from blue (#2114) and black Plexiglas; the Plexiglas was previously used for two seasons in Mihok and Carlson (2021). The trap was modified to have two netting cones so that flies entering through either the high or low entrances could be enumerated (Supplementary material, Fig. S1AF). Catches were compared with a standard fabric Nzi trap rotated among nearby locations in experiments F2–F4. In 2021 and 2022, this trap was also operated in a variety of alternative materials to supplement 2020 data. Data are reported in the present study only for the heights at which tabanids entered.

The logic of changing the Nzi trap design for high flyers originated in the tabanid literature and from an observation that some deer flies likely approach Nzi traps above a height of 1 m (Mihok *et al.* 2007). I report further on this phenomenon in a test of trap height at the residence (H0) in 2001. In that test, an unbaited Nzi trap was alternately set at ground level or at 50 cm off the ground for 34 days.

The goal of the design changes was to find an entrance configuration that would provide a representative sample of species flying at various heights, while minimising escape of high- and low-above-ground flyers. The choice of what to test was empirical and incremental, with promising designs retested as the tabanid fauna changed. Work focused on opaque or transparent panels of phthalogen blue, phthalogen turquoise, black, or netting for the horizontal shelf, the vertical inner baffle, or both. The starting point was based on insights gained during the design phase of the Nzi trap (Mihok 2002). Those experiments showed that transparency in the centre of the trap was the key factor leading to improved performance. However, little attention was paid to incorporating blue or black materials for a small “target” area to improve trap entry.

Table 1. Experimental summary with the detransformed mean catches for the control Nzi trap. Catch indices for experimental traps in tables and figures are relative to this standard trap within each experiment.

Experiments				ANOVA design				Biting fly fauna		Means in standard fabric Nzi trap				
Code	Location	Colour	Baits	Traps	Sites	Rep	<i>N</i>	Total	Species	Tabanids	Tab	Chr	Hyb	Sc
H0	Home	blue	None	2	1	17	17	421	Tq,Ca,Ts	12.0	6.0	2.8		
H1	Home	blue	O	4	4	4	16	459	Cc,Ts,Ca	4.7	1.4	2.3		
H2	Home	blue	O	4	4	4	16	813	Ca,Cu,Tq	10.0	3.6	5.1		
H3	Home	blue	OUA	4	4	4	16	698	Cu,Ca,Tl	4.9	1.5	3.0		0.8
F1	Farm	turquoise	OMA	3	3	4	12	1020	Hl,Ts,Hi	18.8	3.0	1.0	13.7	
F2	Farm	turquoise	OMA	5	5	3	15	3220	Tq,Ts,Hl	47.2	40.0	1.3	3.8	1.8
F3	Farm	turquoise	OMA	5	5	3	15	1601	Tq,Ts,Tl	13.0	11.6	1.0		1.6
F4	Farm	turquoise	OUA	5	5	4	20	1059	Tq,Tl,Ts	9.7	9.2			3.7
F5	Farm	blue	O	6	6	4	24	687	Sc					4.1
F6	Farm	blue	O	4	4	4	16	679	Sc					7.5
P1	Farm	blue	OMA	2	2	8	8	797	Tq,Ts,Tl	59.1	55.3	1.1		
P2	Farm	blue	OUA	2	2	8	8	408	Tq,Ts,Tl	20.3	19.0	1.2		
P3	Farm	blue	OUA	2	2	16	16	612	Tq,Tl,Cu	10.9	9.8	0.5		

Note: *N*, sample size for each trap (e.g., 4 traps rotated among 4 sites × 4 replicates are *N* = 16 per trap).

Means for each genus of biting flies are the detransformed $\times \log(x + 1)$ values.

Total, catch of all tabanids in all traps, except for two entries for total catches of stable flies in F5 and F6.

Species, top three species by raw catch, typically accounting for about 90% or more of the total.

An extra trap set for other purposes in experiments H3 and F1 is not reported in this paper.

Empty cells represent no or few catches.

ANOVA, analysis of variance; Rep, replicates (number of days each trap was set); Tabanids/Tabanidae, Tab, *Tabanus*; Chr, *Chrysops*; Hyb, *Hybomitra*; Sc, *S. calcitrans*; Cc, *C. cincticornis*; Ca, *C. aberrans*; Cu, *C. univittatus*; Hi, *H. illota* (Osten Sacken); Hl, *H. lasiophthalma*; Tl, *T. lineola*; Ts, *T. similis*; Tq, *T. quinquevittatus*; O, octenol lure; U, cattle urine; M, cattle manure; A, household ammonia.

The position of opaque materials was the focus of many comparisons, given how tabanids behave towards horizontal and vertical features of objects at different angles and heights (Horváth *et al.* 2020). Lastly, a glossy phthalogen blue vinyl or black spray-painted ball (33 cm diameter) was tested in place of an opaque vertical baffle. This was done to test whether specular reflection inside the trap would improve catches by exploiting the attraction of tabanids to polarised light (Egri *et al.* 2012). The balls used moved freely in the wind and would also have provided a random movement cue. Each experiment tested a theme of simple contrasts for clarity in choosing what to test next. For reporting, three configurations are referred to as horizontal, vertical, or “both”, reflecting where an opaque object (fabric or ball) was used in place of transparent netting. Figure 3 shows these configurations. Table 2 summarises all configurations by experiment. Supplementary material, Fig. S1 provides photos of all traps with detailed descriptions.

Sixty half-hour high-definition or 4K videos were also taken at the front of traps to document the kinds of behaviour the author has observed over the last two decades. A representative clip of deer fly behaviour is presented for a trap baited with octenol at the residence (Supplementary material, Video S1). Horse fly behaviour varied among species and was difficult to document well. Hence, a few videos were taken in an area with many species (White Lake, Ontario, 45° 15.8 N, 76° 41.1 W). A representative clip is presented for a trap baited with octenol, dry ice, ammonia, and cattle urine (Supplementary material, Video S2).

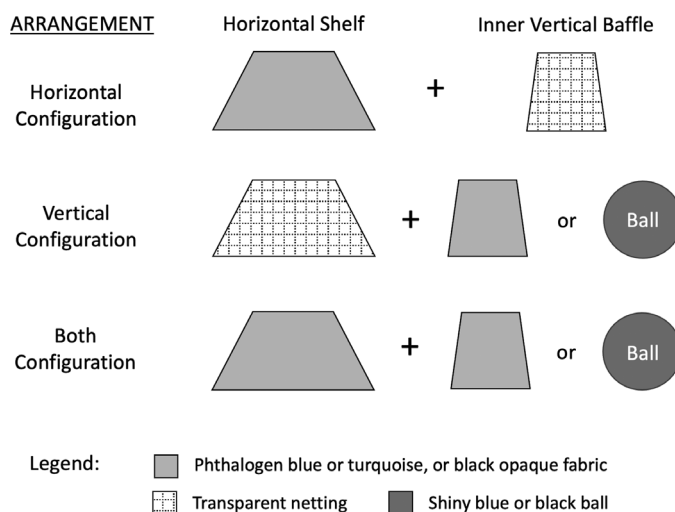


Figure 3. Schematics of the three arrangements of opaque materials used to group experimental traps in Figs. 4, 5, and 6. The configurations are based on where an opaque material (or ball) was used for the horizontal shelf or the vertical inner baffle or both.

Statistical analyses

Analyses of variance were performed for $\log(x + 1)$ -transformed catches (Mihok and Lange 2012). Results are summarised as detransformed mean catches per day, with relative catch indices and 95% confidence intervals provided for cross-comparison, as in many previous experimental studies (Vale 1993). The catch index is the ratio of the detransformed mean of the test trap to the standard; it is more explicitly defined as a response ratio in the statistical literature (Hedges *et al.* 1999). The outcome was an *a priori* test for a significant difference relative to a standard Nzi trap ($P < 0.05$ based on the analysis of variance mean square error), considering sites and days. A detailed example of a statistical analysis for a typical Latin square experiment is in Mihok and Carlson's (2021) Supplemental material, Appendix S2.

Results

The total catch was 10 132 female tabanids (66% *Tabanus*, 23% *Chrysops*, 11% *Hybomitra*) and 21 males. The tabanid fauna was similar to that caught in past work, with 28 species recorded. Mean catch was 12.9 tabanids/trap/day, with a maximum of 121. In addition, 1859 stable flies were captured (83% males), most of these catches occurring at the farm. As an example of the power of experiments, the average least significant difference in H and F experiments translated into being able to detect a 42% ($1.42 \times \textit{Tabanus}$) or 52% ($1.52 \times \textit{Chrysops}$) change in catch for a test trap relative to the control at $P < 0.05$. Log transformation typically normalised data (Shapiro–Wilk test; *e.g.*, Tabanidae in H2: $W = 0.99$, $P = 0.67$; F2: $W = 0.97$, $P = 0.07$).

In experiment H0, a Nzi trap set 50 cm above ground caught as many tabanids as a trap set at ground level in a late-season experiment dominated by *Tabanus quinquevittatus* Wiedemann and *Chrysops aberrans* Philip (catch index = 0.94, 95% confidence interval 0.52–1.73). A nonsignificant shift in fauna occurred, with higher captures of *Chrysops* (index = 1.54) and lower captures of *Tabanus* (index = 0.73) at 50 cm.

Over 12 experiments in 2020, useful data were obtained for 29 comparisons of catches by genus (Table 1) for 18 modifications of Nzi traps (Table 2). Two representative experiments at high numbers are presented in full for phthalogen blue and turquoise traps, followed by a graphical summary across all experiments.

In experiment H2 at the highest mean catch of *Chrysops* (Table 3), traps with a blue horizontal shelf (Supplementary material, Fig. S1D) or a blue vertical baffle (Supplementary material,

Table 2. Summary of the trap configurations in experiments H1–3, F1–6, and P1–3.

Type	Configuration of materials				Home			Farm						Plexiglas		
	Entrance	Horizontal	Vertical	Variation	1	2	3	1	2	3	4	5	6	1	2	3
Vertical	Rectangle	Netting	Phthalogen		x	x		x					x	x		
Horizontal	Rectangle	Phthalogen	Netting		x	x				x		x	x			
Both	Rectangle	Phthalogen	Phthalogen	Slope	x	x										
Both	Rectangle	Phthalogen	Phthalogen				x		x	x	x	x				
Both	Rectangle	Phthalogen	Black ball	Slope			x									
Both	Rectangle	Phthalogen	Blue ball				x									
Both	Rectangle	Black	Phthalogen						x							
Both	Rectangle	Phthalogen	Black						x							
Both	Rectangle	Black	Black	Phthalogen sides at top					x							
Vertical	Rectangle	Netting	Phthalogen	Phthalogen sides at top						x						
Both	Triangle	Phthalogen	Phthalogen						x							
Both	Triangle	Phthalogen	Black ball							x		x				
Both	Triangle	Phthalogen	Blue ball								x					
Vertical	Rectangle	Netting	Blue ball								x					
Both	Rectangle	Phthalogen	Blue ball	Clear PVC front							x					
Vertical	Rectangle	Netting	Plexiglas	Black floor or grass											x	
Both	Rectangle	Plexiglas	Plexiglas	Black floor												x
Both	Rectangle	Plexiglas	Plexiglas	Opaque or clear front												x

Note:

Type: Whether an opaque material (phthalogen or black cotton/suspended ball or phthalogen Plexiglas) was used for the horizontal inner shelf, the vertical inner baffle, or both panels, for clarity, and for grouping data in presentations.

H1: Simple contrasts, slope trap had a single phthalogen panel extending from the middle of the trap at the front to the middle of the trap sides at the bottom of the cone.

H2: Same traps as H1 but with a clear, reflective piece of PVC placed over the netting at the front of the cone.

H3: Slope trap modified to a shallower panel extending to 23 cm from the bottom of the cone, balls also tested.

F1: Only one trap tested with a vertical phthalogen baffle and a netting horizontal shelf as in H1 and H2.

F2: Variations on black instead of turquoise or netting, one trap had turquoise top sides, and one had a triangular entrance.

F3: Retesting simple designs, also testing a triangular front entrance with a black ball.

F4: Exploring the use of a blue ball instead of a baffle, first test of a transparent front shelf as a physical barrier only.

F5: Autumn test at seasonal peak of stable flies, retesting some designs and inclusion of a pyramidal trap as a 2nd control.

F6: Repeat test of the simplest designs for stable flies at the highest numbers to confirm best design.

P1: Plexiglas trap with horizontal netting shelf and vertical blue baffle, a triangular black Plexiglas “floor” was set inside the trap on the ground on alternate days; presence/absence of this target did not affect the catch.

P2: Plexiglas trap retested from P1 with a blue horizontal shelf, black floor used on alternate days (not significant).

P3: Plexiglas trap retested from P2 with ground level black target now present throughout, front blue Plexiglas shelf alternated with clear Plexiglas (not significant).

Fig. S1C) caught significantly more *Chrysops* than the standard Nzi trap did, with similar patterns among species (Table 3). Catches of *Chrysops* with a single sloping shelf (Supplementary material, Fig. S1E) were equal to the control. Catches of *Tabanus* were equal to the control for two variations and slightly significantly lower for one variation (index = 0.62, confidence interval 0.40–0.94; Table 3). *Tabanus quinquevittatus*, *T. similis* Macquart, and *T. lineola* Fabricius

Table 3. Experiment H2 at the residence in Russell, Ontario, Canada in early July 2020 ($N = 16$ per trap) in phthalogen blue traps. Total catches per trap, detransformed mean catches (flies/trap/day), and catch indices with 95% confidence intervals for two genera of Tabanidae relative to a standard phthalogen blue Nzi trap.

Traps	Total	Mean	Index	95% confidence interval
<i>Chrysops</i> (6 spp., 57% <i>C. aberrans</i> , 34% <i>C. univittatus</i>)				
Horizontal blue	172	9.2*	1.67	1.21–2.31
Vertical blue	173	8.6*	1.58	1.15–2.18
Nzi trap control	103	5.1	1.00	Standard
Single blue sloping shelf	94	5.0	0.98	0.71–1.36
<i>Tabanus</i> (4 spp., 61% <i>T. quinquevittatus</i> , 22% <i>T. similis</i>)				
Single blue sloping shelf	76	3.8	1.03	0.68–1.57
Nzi trap control	71	3.6	1.00	Standard
Horizontal blue	78	3.2	0.91	0.60–1.39
Vertical blue	39	1.9*	0.62	0.40–0.94

Note: All traps had a clear PVC panel covering the front of the netting cone.

Vertical refers to a blue baffle with a horizontal netting shelf; horizontal refers to a horizontal blue shelf with a netting baffle; the single sloping shelf was blue.

Index: ratio of detransformed mean catch in the experimental trap to the detransformed mean catch in the standard trap.

*Significant at $P < 0.05$ in *a priori* comparison versus the standard trap.

accounted for most of the catch. A sloping shelf was not explored further because this configuration caught many nontarget Diptera (muscsids, Syrphidae) and Hymenoptera (especially Vespidae).

Experiment F2 was conducted at the farm at high numbers of *Tabanus* and was run at the same time as experiment H2. It explored black for one or both inner panels, relative to a trap with turquoise for both (with a triangular rather than a rectangular entrance). The trap with turquoise for both panels (Supplementary material, Fig. S1M) caught significantly more *Chrysops* (index = 1.58; Table 4) than the control did while catching equal numbers of *Tabanus* (index = 0.88) as the control. Catches of *Tabanus* were lower in traps with any inner black (Supplementary material, Figs. S1N–P; index 0.48–0.69), with catches of *Chrysops* equal to those of the control. Because of this, use of black for inner panels was not explored further.

To simplify cross-comparisons, catch indices are presented over all experiments by genus for meaningful data in Figs. 4, 5, and 6, with an index to photos in Supplementary material, Fig. S1 provided in each caption. Significant increases in catch relative to the control are green, equal catches are blue, and lower catches are red. All traps with an upper entrance performed well for *Chrysops*, with 11 of 24 tests resulting in significantly increased catches (index 1.5–2.7; Fig. 4) and with 13 tests resulting in catches that were equal to those of the control. Addition of a polarising feature to the trap did not improve catches (Supplementary material, Fig. S1F, clear UV-absorbing vinyl attached to the cone in H2; Supplementary material, Fig. S1J, blue or black balls suspended in H3, F3). The highly reflective Plexiglas trap in P1–3 (Supplementary material, Fig. S1A) was nevertheless one of the best traps.

Catches of *Tabanus* were equal to those of the control in 15 of 25 tests and lower than those of the control in 10 tests (Fig. 5). Results were similar for analyses of variance conducted separately for the three common *Tabanus* (data not shown). Lower catches occurred when a reflective object or black was used, with a few exceptions. For example, use of a shiny black ball (Supplementary material, Figs. S1Q and S1AC, experiments H3 and F3) did not affect catches; catches were lower than that of the control when the substitution was a shiny blue ball (Supplementary material, Fig. S1I and S1W, experiments H3 and F4). Lower catches also occurred when the vertical baffle

Table 4. Experiment F2 at the farm in Russell, Ontario, Canada in early July 2020 ($N = 15$ per trap) in phthalogen turquoise traps. Total catches per trap, detransformed mean catches (flies/trap/day), and catch indices with 95% confidence intervals for two genera of Tabanidae relative to a standard phthalogen turquoise Nzi trap.

Traps	Total	Mean	Index	95% confidence interval
<i>Chrysops</i> (seven spp., 31% <i>C. aberrans</i> , 31% <i>C. univittatus</i> , 18% <i>C. vittatus</i>)				
Both (triangular entrance)	65	2.6*	1.58	1.01–2.47
Both (vertical black)	35	2.0	1.54	0.83–2.04
Both (horizontal black)	37	2.0	1.29	0.82–2.01
Both (black) (turquoise top sides)	42	1.9	1.26	0.80–1.97
Nzi trap control	24	1.3	1.00	Standard
<i>Tabanus</i> (four spp., 77% <i>T. quinquevittatus</i> , 21% <i>T. similis</i>)				
Nzi trap control	688	40.0	1.00	Standard
Both (triangular entrance)	757	35.1	0.88	0.69–1.12
Both (black) (turquoise top sides)	465	27.3*	0.69	0.54–0.88
Both (vertical black)	486	24.5*	0.62	0.49–0.80
Both (horizontal black)	353	18.6*	0.48	0.38–0.61

Note: All trap variations had turquoise or turquoise and black panels, with the position of any black panels noted in parentheses along with a variation with turquoise top sides.

Index: ratio of detransformed mean catch in the experimental trap to the detransformed mean catch in the standard trap.

*Significant at $P < 0.05$ in *a priori* comparison versus the standard trap.

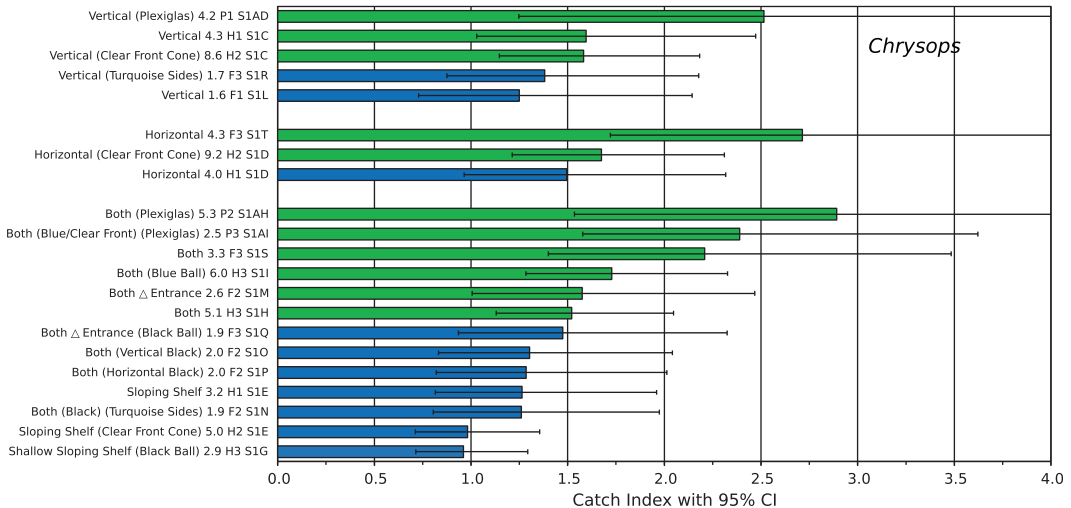


Figure 4. Catch indices with 95% confidence intervals for *Chrysops*. Indices are relative to the catch in a standard Nzi trap within experiments. Trap designs are grouped in terms of the three configurations for the position of opaque materials: vertical, horizontal, or both. Substitutions of the phthalogen panel for black or other minor variations noted. For example, vertical (black) refers to a trap with an opaque black vertical baffle instead of a phthalogen baffle, and with a netting horizontal shelf. Green bars represent catches significantly greater than the control, blue bars represent equal to the control, and red bars represent lower than the control. The detransformed mean catch in the modified trap is provided in each label. The last element of the caption is a reference to a photograph of the trap in Supplementary material, Fig. S1.

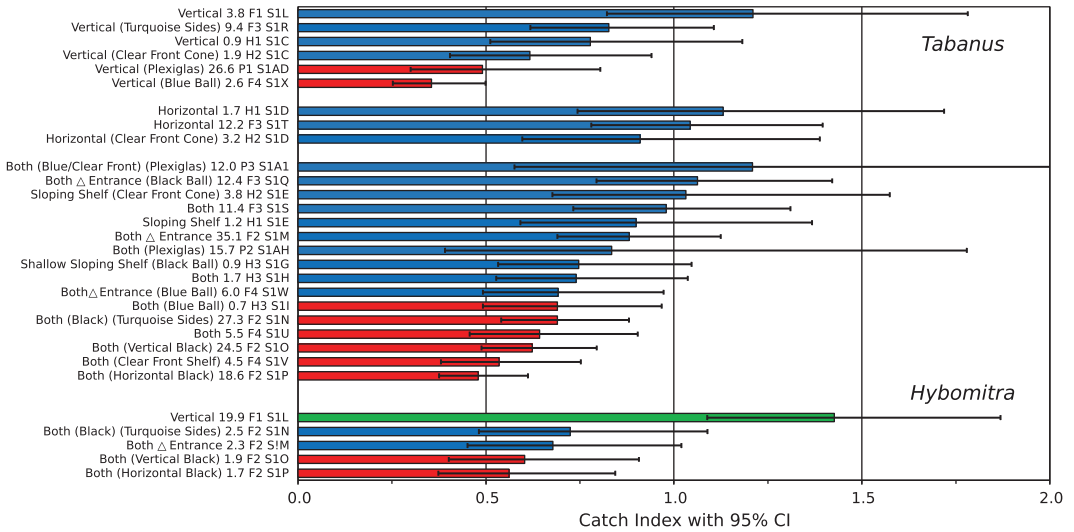


Figure 5. Catch indices with 95% confidence intervals for *Tabanus* and *Hybomitra*. See Fig. 4 caption for an explanation of the Y-axis legend and the colours.

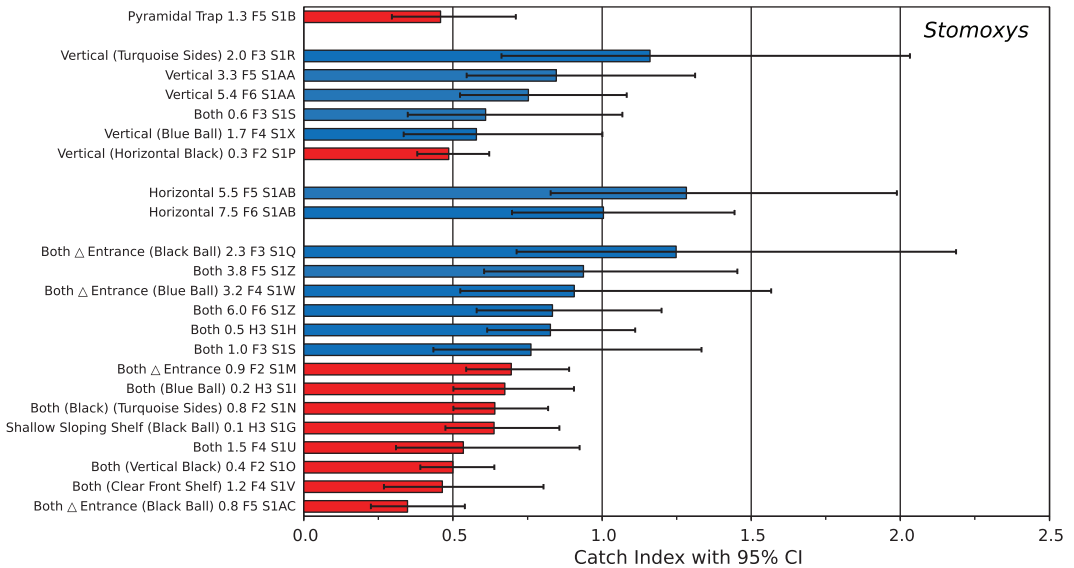


Figure 6. Catch indices with 95% confidence intervals for *Stomoxys*. See Fig. 4 caption for an explanation of the Y-axis legend and the colours.

was blue Plexiglas (Supplementary material, Fig. S1AG; experiment P1) at high numbers of *T. quinquevittatus* and *T. similis*. The Plexiglas trap performed well with both inner panels in blue (Supplementary material, Fig. S1AH, experiments P2, P3). Catches were also nearly the same when the front shelf was blue or clear Plexiglas (experiment P3, Supplementary material, Fig. S1AH, 12.3 blue, Supplementary material, Fig. S1Ai, versus 11.8 clear, NS, index 0.96, confidence intervals 0.37–2.48). This option was retested in experiment F4 using a turquoise fabric trap with a clear vinyl front shelf (Supplementary material, Fig. S1V). Substitution of the shelf for

PVC reduced catches. This result could have been related to morning condensation on the PVC (Supplementary material, Fig. S1Y); this rarely occurred with Plexiglas.

Hybomitra (89% *H. lasiophthalma* (Macquart)) was caught mainly in experiments F1 and F2 (Fig. 5). In experiment F1, a trap with a turquoise vertical inner baffle (Supplementary material, Fig. S1L) caught more *Hybomitra* than the control did (index 1.43, confidence intervals 1.09–1.87). Catches were equal to or lower than those of the control in four comparisons in experiment F2 when the focus was on testing black (Supplementary material, Fig. S1N–P).

Stable flies (*Stomoxys calcitrans* Linnaeus) were present during experiments, with two tests conducted at their seasonal peak in autumn (F5, F6). Overall, catches were equal to the control in 13 of 22 tests and were significantly lower in nine, with no clear optimal design (Fig. 6). The pyramidal trap included in experiment F5 (Supplementary material, Fig. S1B) did poorly (index 0.46), despite being similar to the Vavoua trap.

Behaviour

The use of two collectors in the Plexiglas trap set from 2020 to 2022 provided data on how 7399 tabanids partitioned by height over 159 days. *Chrysops* (17 spp.) mainly entered through the top (88%, range among species 80–100%, $N = 3173$). They landed on and investigated mostly the middle and upper front blue surfaces of a trap (Supplementary material, Video S1, *Chrysops cincticornis* Walker only present). After entering, deer flies typically flew up and were captured within several minutes.

Tabanus (9 spp.) entered the trap through both entrances, with various patterns among species. *Tabanus quinquevittatus* (70%, $N = 771$), *T. similis* (78%, $N = 107$), *T. reinwardtii* Wiedemann (77%, $N = 13$), and *T. novaescotiae* Macquart (83%, $N = 6$) entered mostly through the lower entrance. *Tabanus marginalis* Fabricius entered mostly through the upper entrance (87%, $N = 30$), and *Tabanus lineola* entered evenly through both entrances (54% top, $N = 109$). *Tabanus*, like *Chrysops*, transferred to the cone quickly and were caught within minutes. *Hybomitra* (12 spp.) entered mainly through the upper entrance, with little variation among species (94%, range 87–97%, $N = 3167$). *Hybomitra* was the most reluctant tabanid to fly directly up after entering the trap, with prolonged flight inside the cone and upper body of the trap, and some downward flight leading to escape.

Horse flies (*Tabanus* and *Hybomitra*) mostly landed on and investigated the lower front blue surfaces of traps (Supplementary material, Video S2), often focusing activity on the lower outside blue corners. Some horse flies were also observed landing on the underside of the inner horizontal shelf or on the inner black side walls. Horse flies also flew in between the blue–black panels and entered or collided with the back netting before flying up into the cone.

Stomoxys calcitrans entered mainly through the top (92%, $N = 65$). Flies rested on and investigated all of the blue surfaces of traps for long periods on sunny days. Flies also engaged in chasing behaviour. *Stomoxys* was almost never observed landing on blue surfaces inside the body of the trap, although flies often crawled along the very front edge of the new horizontal blue shelf before flying away. On cool mornings, flies would rest on the back of the trap on the black or blue panels when the sun was to the east. Numbers of flies investigating traps at any one time were sometimes higher than those captured, in agreement with a low estimate of trap efficiency for this species (Mihok *et al.* 2006).

Discussion

The goal of increasing catches of deer flies in a modified trap design was successfully met. Fifty per cent of the modified designs caught 1.5–2.7 times significantly more deer flies than the standard Nzi trap did; catches in the remaining designs were equal. Some of the common species at the residence site were *C. aberrans*, *C. univittatus*, and *C. cincticornis*. Other common species

around livestock at the farm were *Chrysops frigidus* Osten Sacken, *C. indus* Osten Sacken, and *C. vittatus*. All of these species bite people, livestock, or wildlife (Teskey 1960; Smith *et al.* 1970; Lewis and LePrince 1981). In surveys in Ontario and Québec, Canada with Malaise or Manitoba traps (Golini and Wright 1978; Baribeau and Maire 1983a), other *Chrysops* spp. are also often captured in high numbers, but they were not common in this study. The actual species diversity of *Chrysops* is not well known, with hardly any comparative data for flies caught in traps *versus* larvae in the environment (Baribeau and Maire 1983b).

This positive outcome does not appear to have any caveats in terms of whether traps are made from phthalogen blue or turquoise fabric or blue Plexiglas or of how they are baited. It was achieved through opening the front of the trap to provide a larger and higher entrance up to a height of 75 *versus* 50 cm. This was done to address the natural tendency of deer flies to swarm around the head and to explore the logic of traps with a very high entrance (Schreck *et al.* 1993; French and Hagan 1995). A higher entrance was also tested in combination with a new inner baffle to minimise escape of low-flying species (Bennett and Smith 1968). This was a critical point in lessons learned during refinement of the Nzi trap relative to similar blue–black traps, such as the epsilon trap (Mihok 2002).

In contrast to the high-flying behaviour of *Chrysops*, *T. quinquevittatus* and *T. similis* entered traps mainly through the lower entrance. Other common tabanids, such as *T. lineola*, partitioned evenly. Mullens and Gerhardt (1979) also found that *T. lineola* alighted higher on cattle than *T. quinquevittatus* did. Species differences in behaviour imply that a universal trap for all tabanids may be an elusive goal. A new high-flying *Tabanus*, *T. marginalis*, was recorded in the present study, along with a few captures of *T. atratus* Fabricius. These *Tabanus* species are uncommon in most trapping surveys (Smith *et al.* 1970; Matthyse *et al.* 1974; Baribeau and Maire 1983b; Thibault and Harper 1983; McElligott and Galloway 1991b; Freeman 2017).

An unexpected finding was the bias for entry through the high *versus* low entrance in *Hybomitra* spp. This occurred despite the ease of capture of thousands of *Hybomitra* in Nzi traps with only a low entrance and their preference for landing on the lowest tier of a sticky blue–black target (Mihok and Lange 2012). The present finding was also odd given that the most common species (*H. lasiophthalma*) alights low on the abdomen of cattle (Mullens and Gerhardt 1979). Tabaninae (mainly *Hybomitra*) also bite the legs or abdomen of moose and deer, whereas Chrysopsinae tend to bite the head or neck (Smith *et al.* 1970).

Hybomitra is nevertheless readily caught in traps with a high entrance. For example, the Manitoba trap catches many *Hybomitra* (Hanec and Bracken 1964; McElligott and Galloway 1991a); the trap has an entry height of roughly 1 m, based on the photo in Thorsteinson *et al.* (1965). Above this height, *Hybomitra* activity declines considerably; for example, 12 times fewer flies landed at 3 m *versus* 1 m on Tanglefoot-coated balls, with none recorded at 6 m (Thorsteinson *et al.* 1965). Similarly, no captures were recorded in CO₂-baited cage traps at about 2 m in an area with many *Hybomitra* (Bennett and Smith 1968). *Tabanus lineola* (high and low entry) and *T. similis* (low entry) can also be captured in Manitoba traps (Bracken *et al.* 1962). Behavioural data from wind tunnels on how other biting Diptera orient towards objects (Gurba *et al.* 2012) could be explored for further design insights, but similar insights from the field are few (Phelps and Vale 1976; McElligott and McIver 1987). This makes it difficult to speculate on attraction distances and close-range behaviour of tabanids towards artificial objects *versus* hosts (Phelps and Holloway 1990; Hribar *et al.* 1992; Muzari *et al.* 2010; Odeniran and Ademola 2018).

The bi-level Nzi trap

The choice of an opaque or transparent material for the inner panels in a modified Nzi trap appears to be somewhat arbitrary, as is choosing between a triangular or a rectangular upper entrance. A triangular entrance was explored to test a slightly higher entry point at 1 m while still

closing off the corners of the cone. This shape was tested after observing horse flies often resting at these corners once inside the trap. The simplest option for the inner baffle to optimise entry *versus* escape appears to be to maintain high transparency inside the trap. This may confound exit routes, as noted in Mihok (2002). Adding more black to the inside of a trap as a landing “target” (Vale 1993) was found to be detrimental, as is rearranging the blue and black outside features (Mihok and Carlson 2021). Increasing visual complexity seems to be a poor overall strategy, given how tabanids react to solid objects broken up by stripes or spots (Blahó *et al.* 2012; Vaduva 2020). Unlike canopy traps, the use of shiny decoys was also not useful (Egri *et al.* 2012), as was noted in previous tests of Nzi traps with larger decoys (Mihok *et al.* 2006).

Observations of horse flies landing on an inner phthalogen blue horizontal shelf suggest that this feature combined with a vertical netting baffle is best. I define this design for future reference as the “bi-level Nzi trap”, with an optimal width for the upper entrance to be determined for a wider variety of species. This design provided equitable captures of tabanids and stable flies. It remains easy to sew. An opaque rather than a netting horizontal shelf also has some support in how tabanids may perceive a trap (Horváth *et al.* 2017; Vaduva 2020). An opaque shelf could be perceived as the lower abdomen of a host, given the outline of the blue front of the trap, which resembles a torso and legs, despite the fact that animals are not blue (Santer *et al.* 2023). Interception of circling tabanids through a transparent centre is also an important feature of the design, as was determined in the evolution of a flanking net in place of black fabric in tsetse targets (Esterhuizen *et al.* 2011).

Although improving the Nzi trap for stable flies was not an objective of this study, practical devices for the sampling or control of the many species of *Stomoxys* are of keen interest globally (Baldacchino *et al.* 2018; Duvallet and Hogsette 2023). The new bi-level Nzi trap design was tested in an area with *Stomoxys calcitrans* only and performed as well as a standard Nzi trap. Captures were nevertheless quite low relative to what is possible with sticky traps such as the Alsynite trap (Mihok *et al.* 2006). Sticky targets on the whole “capture” exceptional numbers of *Stomoxys* (Sharif *et al.* 2020), especially when baited with m-cresol (Zhu *et al.* 2021). Researchers, however, continue to explore simple traps (Duvallet 2022).

Further tests of a bi-level Nzi trap in a variety of materials have since been completed in Ontario at much higher numbers and diversity of tabanids to expand on these initial results (90 919 tabanids caught in 2021–2022, with up to 417 *Chrysops* and 532 *Hybomitra* per trap per day). The bi-level Nzi trap also performed well for other species of tabanids and the common African stable fly *Stomoxys niger niger* Macquart in Malawi in 2023. Researchers interested in nonbiting flies may also wish to test a version of the Nzi trap with a single phthalogen blue sloping shelf. That option collected many nontarget insects, as noted in the occasional use of Nzi traps for Syrphidae (Vezsenyi *et al.* 2019) and Hymenoptera (Vizza *et al.* 2021).

Supplementary material. The supplementary material for this article can be found at <https://doi.org/10.4039/tce.2023.26>.

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