VALIDATION

Complete eDNA sampling set

SYL009

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1. Experimental set-up

The eDNA dual filter capsules were compared with commonly used filtering methods used in the eDNA research, namely the Sterivex ™ filter units and PES filter funnels (**Table 1**). Quantitative detection of the fish species *Rutilus rutilus* (SYL139) was used to compare the recovery of eDNA with these filter systems.

Table 1. Filters used in this study.

	eDNA Dual Filter ™	Sterivex ™	Filter funnel
Manufacturer	Sylphium	Merck	Sarstedt
Filter area (cm^2)	69	10	65
Material	PES	PES	PES
Pore seize (µm)	0.8	0.22	0.22
Closed capsule	Yes	Yes	No

2. Filtration

Water was collected with barrels from the river “De beek”, Drenthe, Netherlands. After collection, the vessels were taken to the laboratory and filtered. Each filter system was tested in triplicate. A vacuum pump with a vacuum of 0.85 bar was used during the filtration. Filtration was stopped after 10 minutes. The maximum volume of filtered water was recorded per filter (**Table 1**). The water was stirred with a magnetic stirrer during the filtration to avoid sedimentation. The filter capsules are filled with Sample preservation solution according to the Sylphium manuals (SYL001 and SYL009). The filter membrane of the filter funnel was cut with a scalpel from the funnel and transferred with all residues to a 15 mL tube containing 2 mL of Sample preservation solution.

3 DNA isolation

eDNA was isolated from the samples with the Environmental DNA isolation kit (Sylphium, SYL002) according to the protocol. One mL of each isolate was processed, the remaining isolate was kept as a backup.

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4. Analysis

The efficiency of the filters was compared with a quantitative eDNA analysis (SYL139) targeting the common fish species *Rutilus rutilus*. The analysis was performed according to the protocol associated with this qPCR kit. Each filter was determined 8-fold compared to a simultaneously analyzed calibration line with known target DNA concentrations.

5. Results

5.1. Filtration volume

All filters were clogged within 10 minutes and the maximum filtration volume was reached for each type of filter. The mean maximum filtration volumes were; 2333 mL for the eDNA dual filter capsule, 95 mL for the Sterivex ™ and 277 mL for the filter funnel (**Figure 1**).

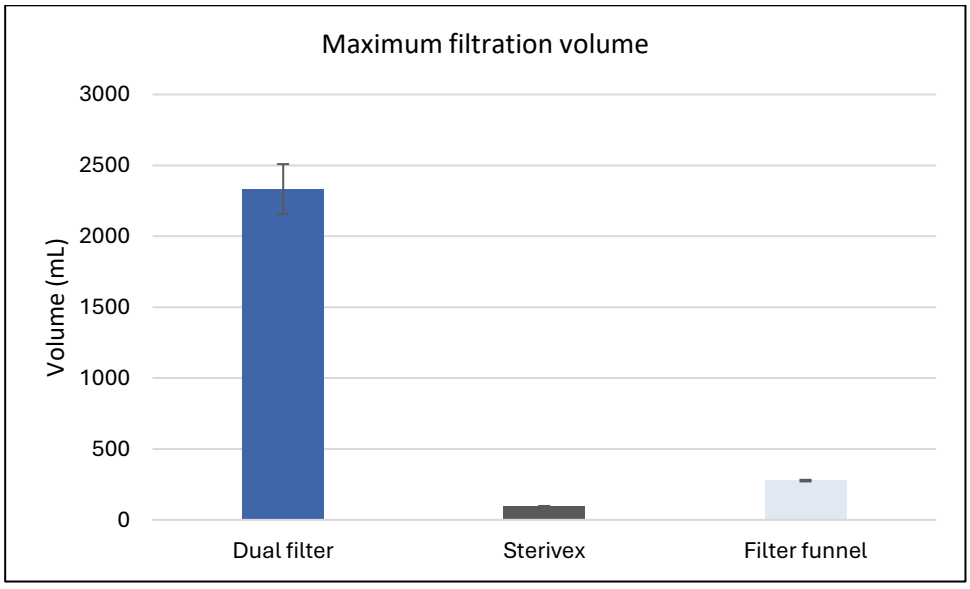


Figure 1. Maximum filtration volume of the eDNA Dual filter capsule, Sterivex ™ and filter funnel determined in triplicate. The error bars represent the standard deviation.

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5.2. Quantitative eDNA analysis

The absolute amount of eDNA collected and isolated from the filter systems was determined with a quantitative eDNA analysis (SYL139) targeting the common fish species *Rutilus rutilus* for each filter type. This resulted in the following values; 1.2 x 10^5 fragments for the eDNA Dual filter capsule, 5.2 x 10^3 fragments for the Sterivex ™ and 1.3 x 10^4 fragments for the filter funnel (**Figure 2**).

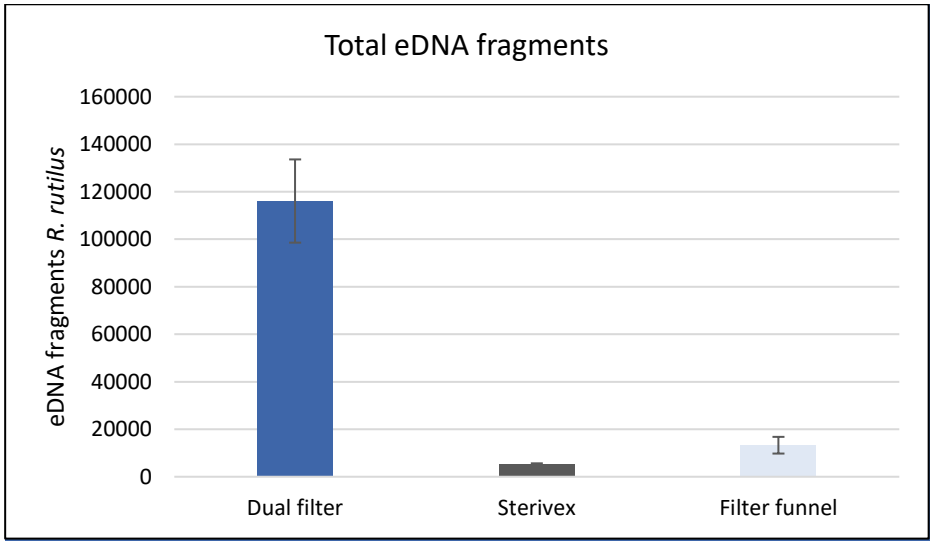


Figure 2. Total eDNA fragments per filter type. The error bars represent the standard deviation.

5.3. eDNA recovery

To determine differences in eDNA uptake and recovery of the filter systems used, the concentration of eDNA fragments was calculated per mL of filtered water and compared between each filter system (**Figure 3**). There were no statistically significant differences, in eDNA uptake and recovery, between filter systems, as determined by a one-way ANOVA (F (2,6) = 0.564, p = 0.596; **Figure 3**).

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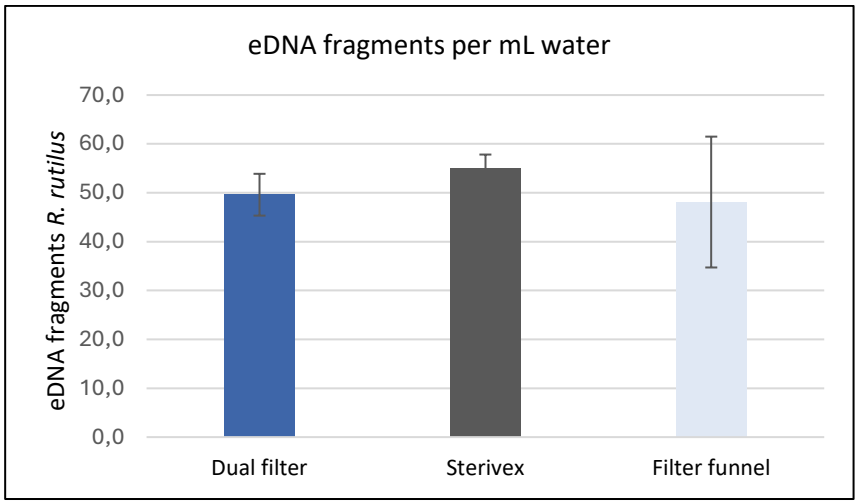


Figure 3. eDNA fragments per mL per filter type. The error bars represent the standard deviation.

4.6. Conclusions

The eDNA dual filter capsule is capable of filtering much more water than competing systems. The maximum filter volume is 10 times as high as a funnel filter and even 20 times as high as a Sterivex ™ capsule. The eDNA dual filter capsule also collects much more eDNA than the competing systems. The eDNA dual filter capsule collects 20 times more eDNA compared to a Sterivex ™ and 10 times more than a filter funnel. There is no difference between the filter systems in the amount of eDNA obtained per unit volume of the sample water. All eDNA is recovered with the used filter systems.

The eDNA double filter capsule and the Sterivex ™ meet all requirements as stated in CEN / TC 230 - Water Analysis - N 1229. Both systems are closed capsules, which prevents cross contamination during sampling. The filter funnel is an open system and requires additional measures to meet all criteria as stated in CEN / TC 230 - Water analysis - N 1229.

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