Detection of Four Invasive Crayfish Species in Brussels Waterbodies Using eDNA and qPCR

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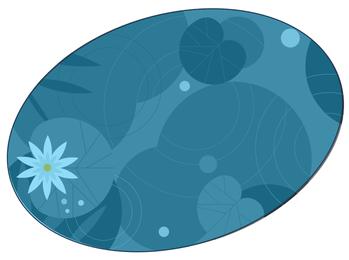
INTRODUCTION

Environmental DNA (eDNA) sampling has emerged as a powerful method for detecting and monitoring aquatic populations. In freshwater environments across Europe, invasive crayfish species pose a significant ecological threat due to their ability to establish and spread rapidly. Traditional methods of tracking their presence, such as live captures, are often time-consuming and cost-prohibitive. Therefore, the potential of eDNA assays as a non-invasive monitoring tool has gained interest. In this study, we investigated the utility of eDNA sampling to detect the presence of four invasive crayfish species (*Procambarus clarkii, Procambarus virginalis, Faxonius limosus, and Astacus leptodactylus*) in fifty locations across Brussels, Belgium. In this study, performed for Brussels Environment as part of the LIFE RIPARIAS project (LIFE19 NAT/BE/000953), we investigated the utility of eDNA sampling to detect the presence of four invasive crayfish species.

MATERIAL & METHODS

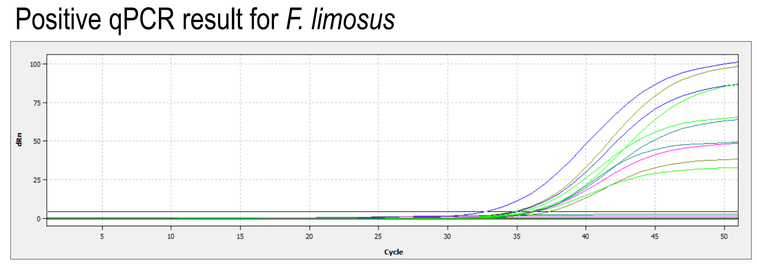


SAMPLING: 50 ponds were selected by Brussels Environment (see map below). From each pond 2 x 1L was sampled.

FILTRATION: Water from both replicates was filtered using two different filtration methods (Whatman syringe filters + Nalgene filters vacuum filters)

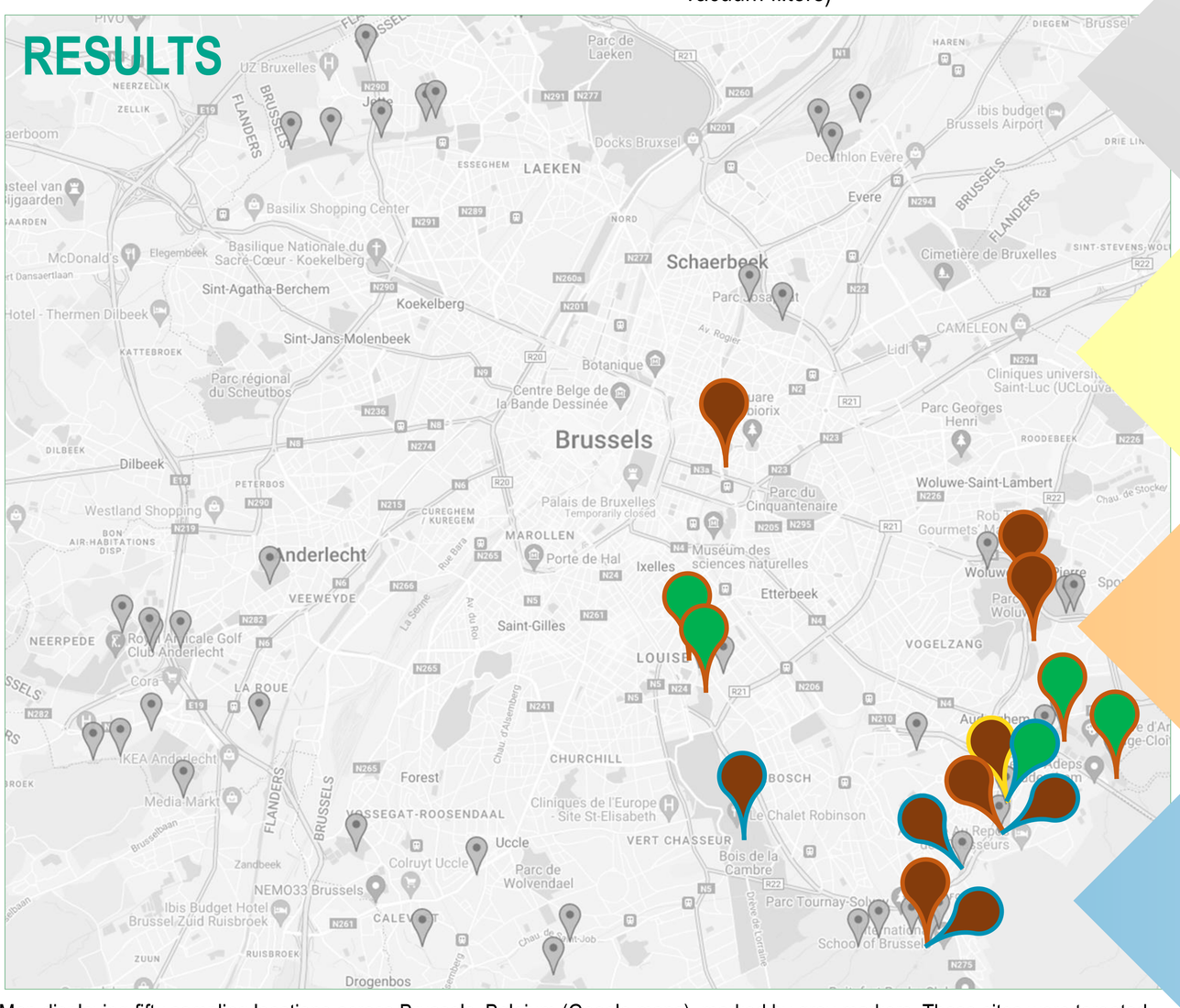
eDNA EXTRACTION: Filters were extracted using eDNA Nucleospin kit

Positive qPCR result for *F. limosus*



qPCR ANALYSIS: For each pond a four qPCR-assays (species specific probe) were run. If at least one qPCR replicate was found to have a strong positive result this was deemed as "Species Detected" for that specific pond.

RESULTS



Map displaying fifty sampling locations across Brussels, Belgium (Google maps), marked by grey markers. These sites were targeted for environmental DNA (eDNA) sampling to detect invasive crayfish species in freshwater environments.

Procambarus virginalis (Marbled crayfish/ Marmorkrebs)

0 — No live-capture locations were reported by Brussels Environment

0 — No eDNA for this species was detected in any of the locations.

Procambarus clarkii (Louisianna crayfish)

1 — One live capture location was reported by Brussels Environment

0 — No eDNA for this species was detected in any of the locations.

Faxonius limosus (Spinycheek crayfish)

9 — Nine live capture locations were reported by Brussels Environment

4 — eDNA for this species was detected in four locations.

Astacus leptodactylus (Turkish crayfish)

5 — Five live capture locations were reported by Brussels Environment

1 — eDNA for this species was detected in one location.

CONCLUSION

While progress has been made in testing eDNA protocols for detecting four invasive crayfish species, results indicate an underrepresentation when eDNA data was compared to live capture data (Brussels Environment). Most likely the presence of qPCR inhibitors caused the underperformance of the qPCR protocols. To improve future projects, we will aim to 1) increase sampling efforts, 2) implement pre-filtration steps and 3) counter qPCR inhibitors.

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