Developing multiplex assays for crayfish detection using eDNA and ddPCR

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Background

Impacts of introduction of invasive alien crayfish species (IACS) include:
Extinction of (native) species. Loss of macrophyte vegetation.
Change in macroinvertebrate community. Habitat competition.

The early detection of IACS can reduce the impact/spread of the species.

eDNA is a molecular technique that can be used for early detection and monitoring/control programs, however more DNA information (BARCODING, PCR and genomics) is needed.

Objectives

Barcoding
Using COI, 16S, and 18S markers.

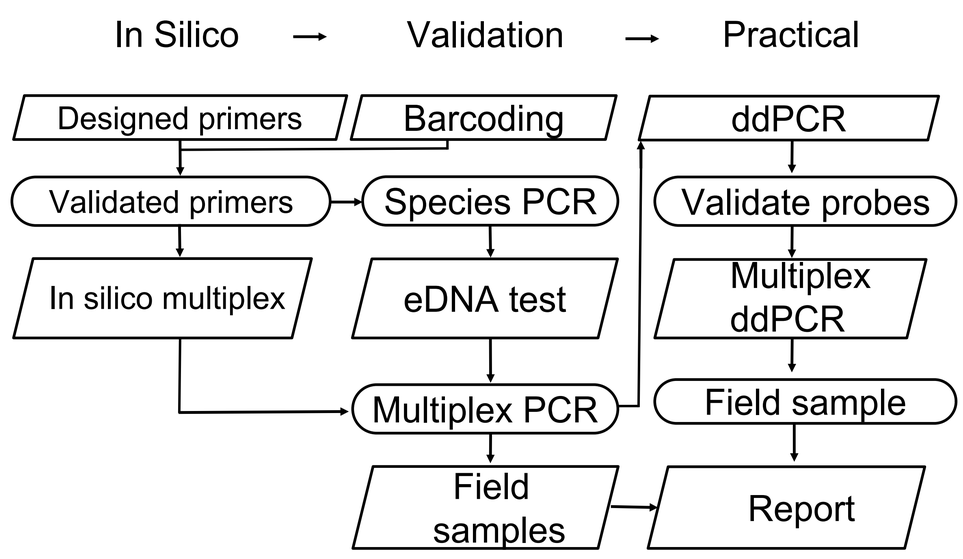
Primer design
Testing existing and design new primers/probes.

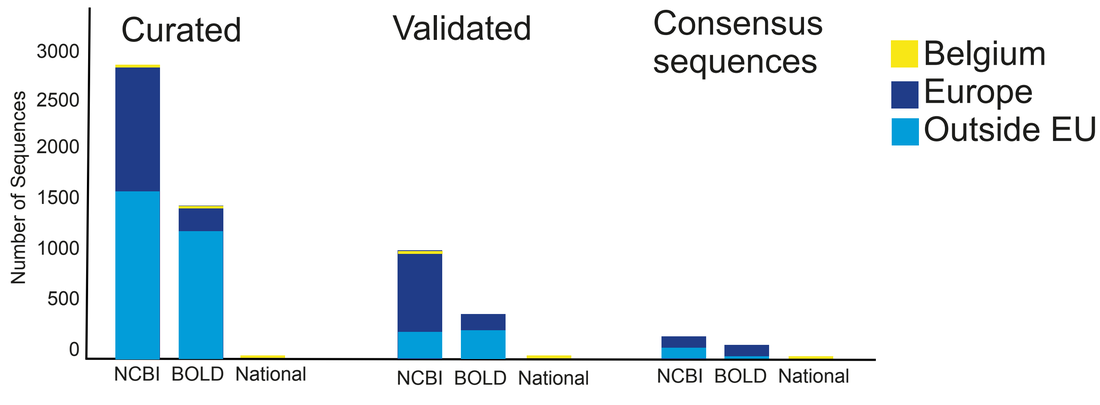
Multiplex Assay
Develop a (dd)PCR multiplex assay

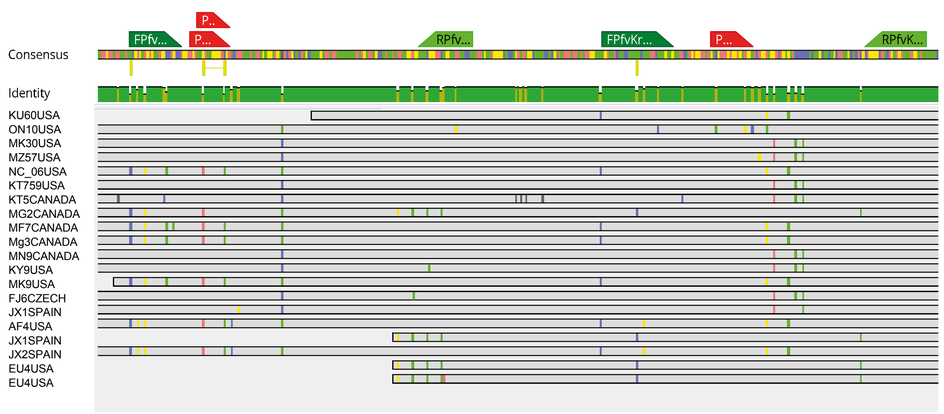
Species target

1 *Procambarus acutus*
2 *Procambarus clarkii*
3 *Procambarus virginalis*
4 *Pontastacus leptodactylus*
5 *Pacifastacus leniusculus*
6 *Faxonius virilis*
7 *Faxonius rusticus*
8 *Faxonius limosus*
9 *Astacus astacus*

Methods







Lessons learned

Techniques and complexity depend on local conditions, resources, and study objectives

Local sequence validation is keystone for primer design.

Primers from the literature often fail in different regions due to sequence variation.

Using multiple genes (COI, 16S, 18S) may improves detection accuracy.

ddPCR is sensitive but expensive

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