LIFE18 NAT/IT/000806 – LIFE+ CLAW:

Conservation of *Austropotamobius pallipes* in North-Western Apennine

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LIFE18 NAT/IT/000806 – LIFE+ CLAW

- European LIFE project
- Start 10.2019 and end 09.2024
- Co-financed by EU for the 60%
- Total value: 3,711,742 €
- 10 Partners:

Parco Nazionale dell'Appennino tosco-emiliano

Consorzio di Bonifica di Piacenza

Costa Edutainment

Ente di Gestione per i parchi e la biodiversità dell'Emilia occidentale

Comune di Fontanigorda

Istituto Zooprofilattico Sperimentale delle Venezie

Parco naturale Regionale dell'Antola

Università Cattolica del Sacro Cuore

Università degli studi di Pavia

Comune di Ottone

Areas of the project

Aims of the project

- **Protect and increase the stocks of** white-clawed crayfish ***Austropotamobius pallipes*** **populations** suitable for the conservation of the biodiversity of the species.
- Establish a "**Crayfish zonation map**“ to identify the watercourses suitable for crayfish.
- **Establish four ex situ breeding facilities for restoration** of *Austropotamobius pallipes* populations.
- **Counteract the dispersal of invasive alien crayfish** species (IAS: *Procambarus clarkii*, *Pacifastacus leniusculus*, *Faxonius limosus*) and **crayfish plague**.

Crayfish plague monitoring

- Previous LIFE project (LIFE RARITY) **evidences the presence a low pathogenicity strain of *A. astaci*** (Genotype A) in *A. pallipes* populations (carrier status) in north-eastern Italy.
- **Stressing environmental conditions** (i.e. high densities, water parameters) in breeding facilities can **trigger the outbreak** of crayfish plague.

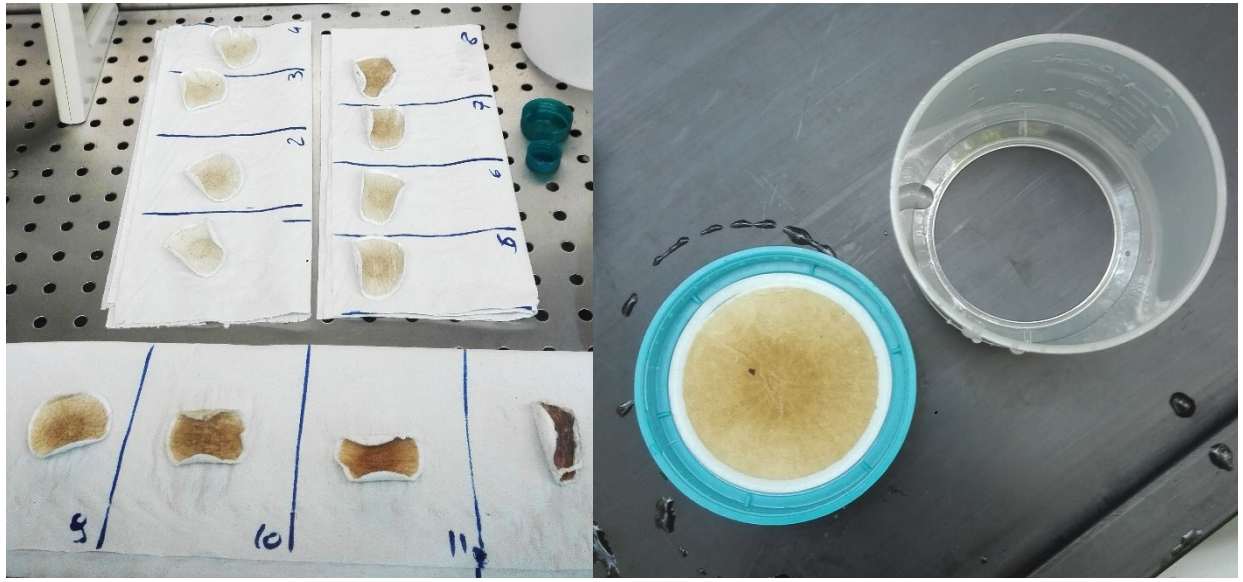
- Introduction in breeding facilities of *A. pallipes* broodstock tested by non invasive method and collected form *A. astaci* negative populations.

Monitoring and diagnosis of *Aphanomyces astaci*

- Cuticular swabs
  - 30-40 populations *A. pallipes*
  - 10 populations IAS
  - 30 specimens/populations

1200-1500 swabs

- eDNA (environmental DNA)
  - 30-40 populations *A. pallipes*
  - 10 populations IAS
  - 4 filter downstream the population (5L / filter)

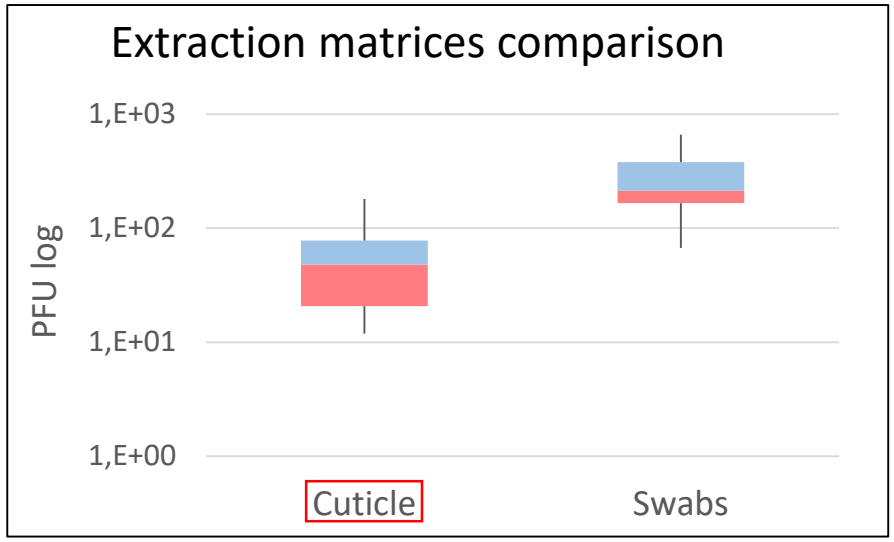


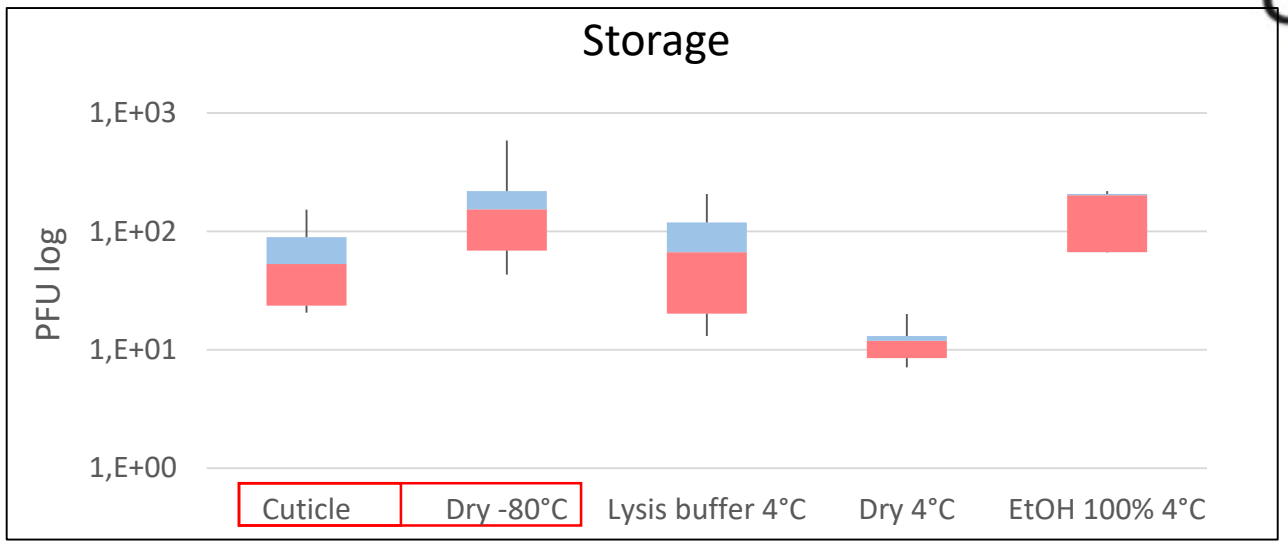
160-200 filter from the project's areas

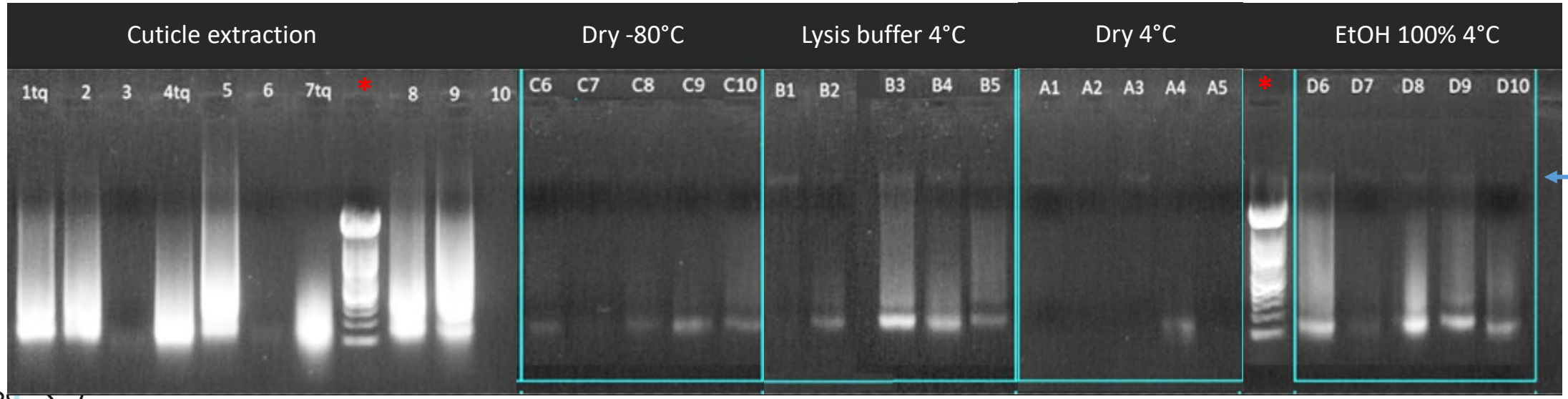
Preliminary tests: Cuticular swabs evaluations

- Matrices extractions: cuticle and swabs collected from carrier IAS (*Procambarus clarkii*).
- Reproducibility test in Real Time PCR (Strand, 2013) for the quantification.
- Classic PCR test (Oidtmann *et al.*, 2006) to confirm the *A. astaci* presence through sequencing.
- Comparative test on storing solution (EtOH 100%; lysis buffer; dry) stored at different temperatures (+4°C; -80°C) to maintain swabs suitable for the molecular analyses.

Cuticular swabs tests







High molecular weight DNA

*, markers (100-1500 bp)

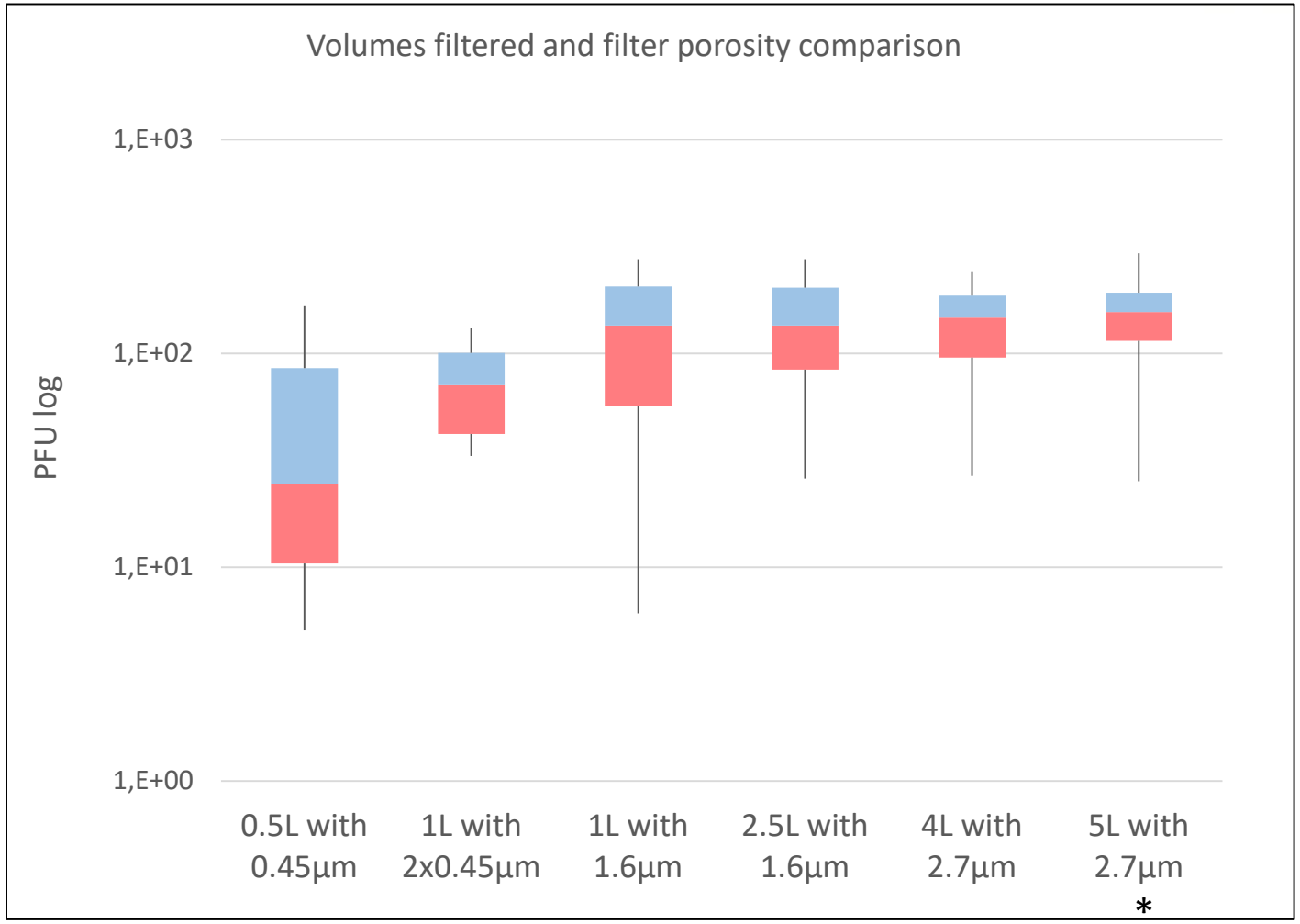
Preliminary tests: eDNA tests

- Filter porosity (0.45µm, 1.6µm, 2.7µm).
- Volumes water filtered (0.5L, 1L, 2.5L, 4L and 5L).
- Reproducibility test in Real Time PCR (Strand, 2013) for the quantification.
- Comparative tests on storing solutions (EtOH, lysis buffer, silica gel) and extraction protocols (kit and CTAB protocol).

- Best conditions tested firstly into IZSVe facility and then “in field”

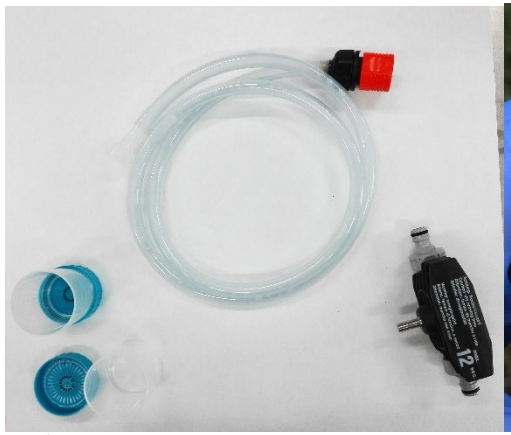
eDNA tests

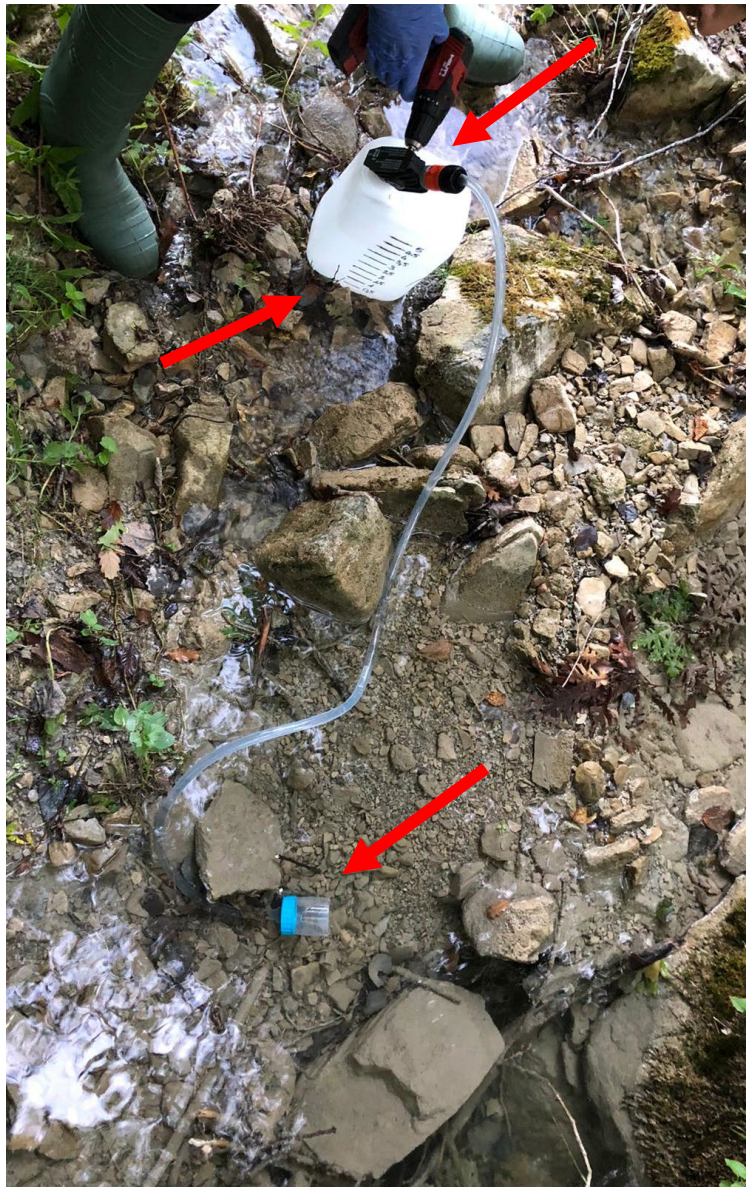
P. clarkii stabulation



Field Equipment

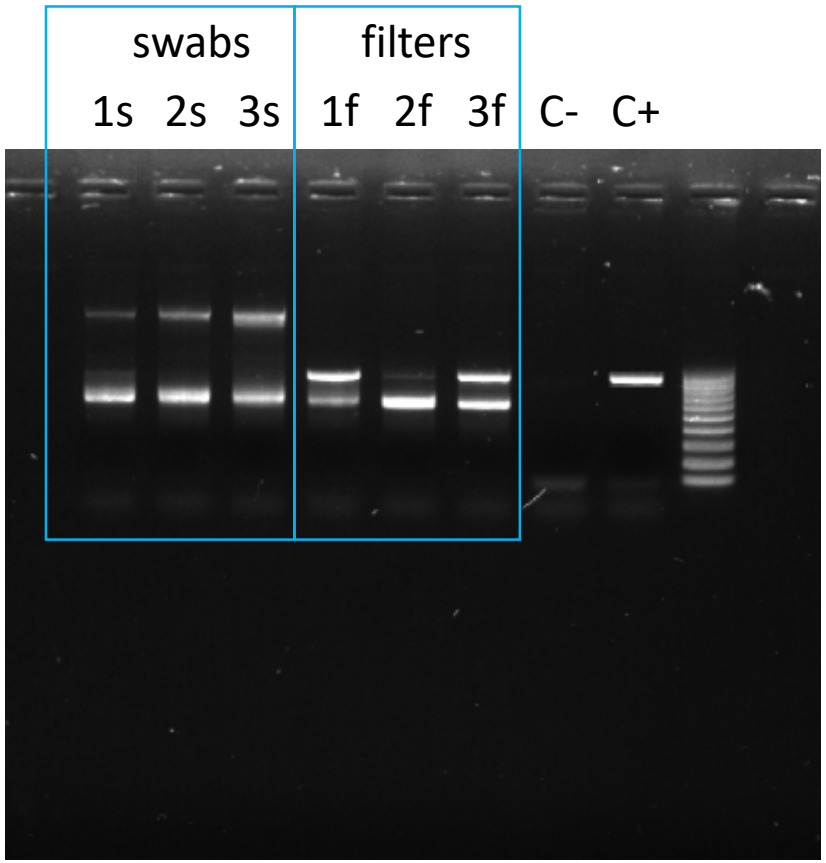
- Battery drill
- Peristaltic pump for gardening
- Gardening pipes
- «Cup» e and filter (2.7 µm)
- 5L tank
- Sterilized tweezers
- Falcon 15ml with EtOH 100%
- Sterilizing solutions



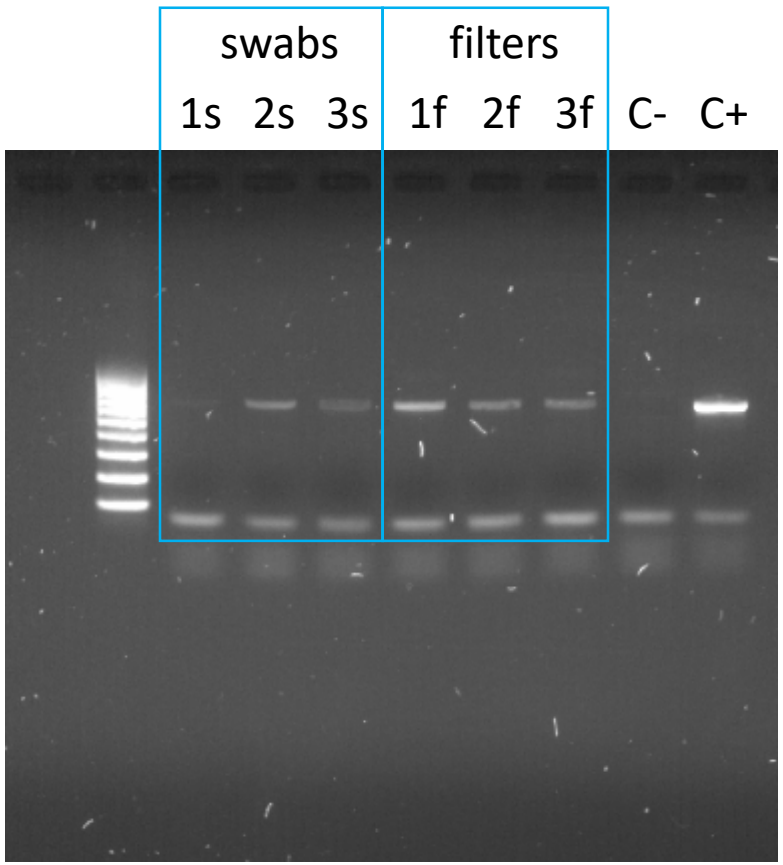


Nested PCR

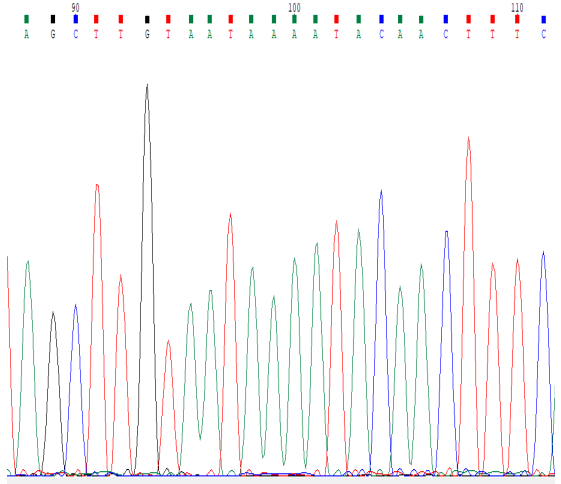
Outer ITS-1 / ITS-4 primers
(White *et al.*, 1999)



Inner Bo42 / Bo640 primers
(Oidtmann *et al.*, 2006)



Sequencing



To distinguish *A. astaci* from other congeneric species (*A. fennicus*).

To summarize:

- Application of non-invasive *A. astaci* analyses:
  - On wild white-clawed crayfish populations.
  - On broodstock before their introduction in the facilities.
  - On broodstock and juveniles during their housing, and before their release in the natural habitat.

Source: LIFE - RARITY project

Thank for your attention

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Partner