Rapid detection of invasive species in ballast water using molecular microfluidic technology

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Goal

– To develop a real time, portable microfluidic chip-based detection platform that is capable of detecting the presence of invasive species in ballast water with high sensitivity
**Target organisms**

- *Carcinus maenas*
  - Europe and N. Africa

- *Limnoperna fortunei*
  - China and Southeast Asia

- *Eriocheir sinensis*
  - China

- *Dikerogammarus villosus*
  - Ponto-Caspian region

- *Dreissena polymorpha*
  - Eastern Europe

- *Dreissena bugensis*
  - Eastern Europe
Methods

Sample collection → DNA extraction → Amplification → Hybridization and detection
Polymerase Chain Reaction (PCR) of ballast samples

• Target for amplification
  – Species specific
  – Cytochrome c oxidase subunit I (COI)
Hybridization and detection

- Two methods
  - Fluorescent bead based detection system
  - Carbon nanotube (CNT) detection system
Detection:
Fluorescent bead based detection
Detection:
Fluorescent bead based detection

Sample injection port

Bead chamber
Carcinus maenas
Green crab target

Carcinus aestuarii
Congeneric species
Detection: Carbon nanotube (CNT) based system

- Methods:
  - Amplify (PCR) DNA as before
  - Apply PCR to chip containing tagged carbon nanotubes
  - Detection through electrochemical resistance rather than fluorescence
    - Quantitative measure
Carbon nanotube (CNT) based system

~ 5 centimeters in length

Made on a standard microscope slide
Carbon nanotube (CNT) based system

Current applied through this circuit
Carbon nanotube (CNT) based system

Sample introduced here
Carbon nanotube (CNT) based system

Sample flows through this channel
Looking through the CNT channel

Channel wall

Channel wall

Oligonucleotide probe
Detection:
Carbon nanotube (CNT) based system

• How it works….
  – Samples passed through a channel containing carbon nanotubes that are tagged with a species specific (a.k.a. target species) primer
  – If the sample contains the specific DNA sequence that will bind to the species specific tag, it will cause the resistance to increase.
  – If the DNA is not the target species, it will not bind and no resistance increase will be detected.
Looking through the CNT channel:
Target species

Target species DNA
Looking through the CNT channel:
Target species

Non-target species DNA
Detection:
Carbon nanotube (CNT) based system results

\[ z = R + i \omega C \]

\[ |z| = \sqrt{R^2 + \omega^2 C^2} \]
Carbon nanotube (CNT) based system: Detection of multiple species on a single chip
Results to date

- Target tag successfully developed for 5 of 6 target species

- Successful detection using fluorescent bead technology

- Successful detection using CNTs to 1/500,000 of an individual larvae
Results to date (cont.)

- Timing
  - Ballast filtering and DNA extraction = ~25 min

- PCR amplification: ~30 mins

- Hybridization and detection: ~15 minutes

- Total time from sample collection to detection:
  70 minutes