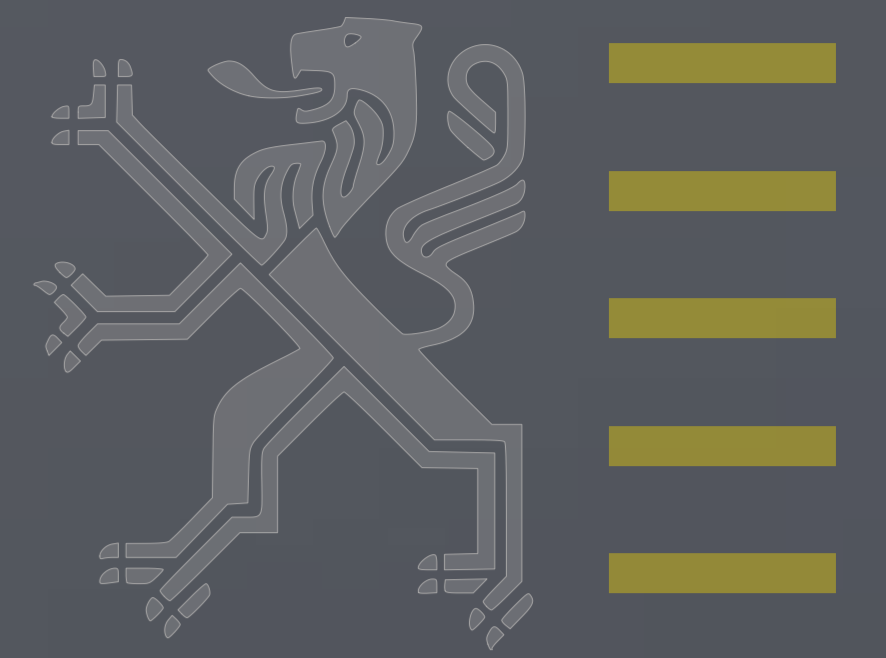


Risk assessment of non-indigenous marine invaders: A combined approach of morphological and molecular analysis allowed unambiguous identification of the comb jelly *Mnemiopsis leidyi* in the Belgian part of the North Sea

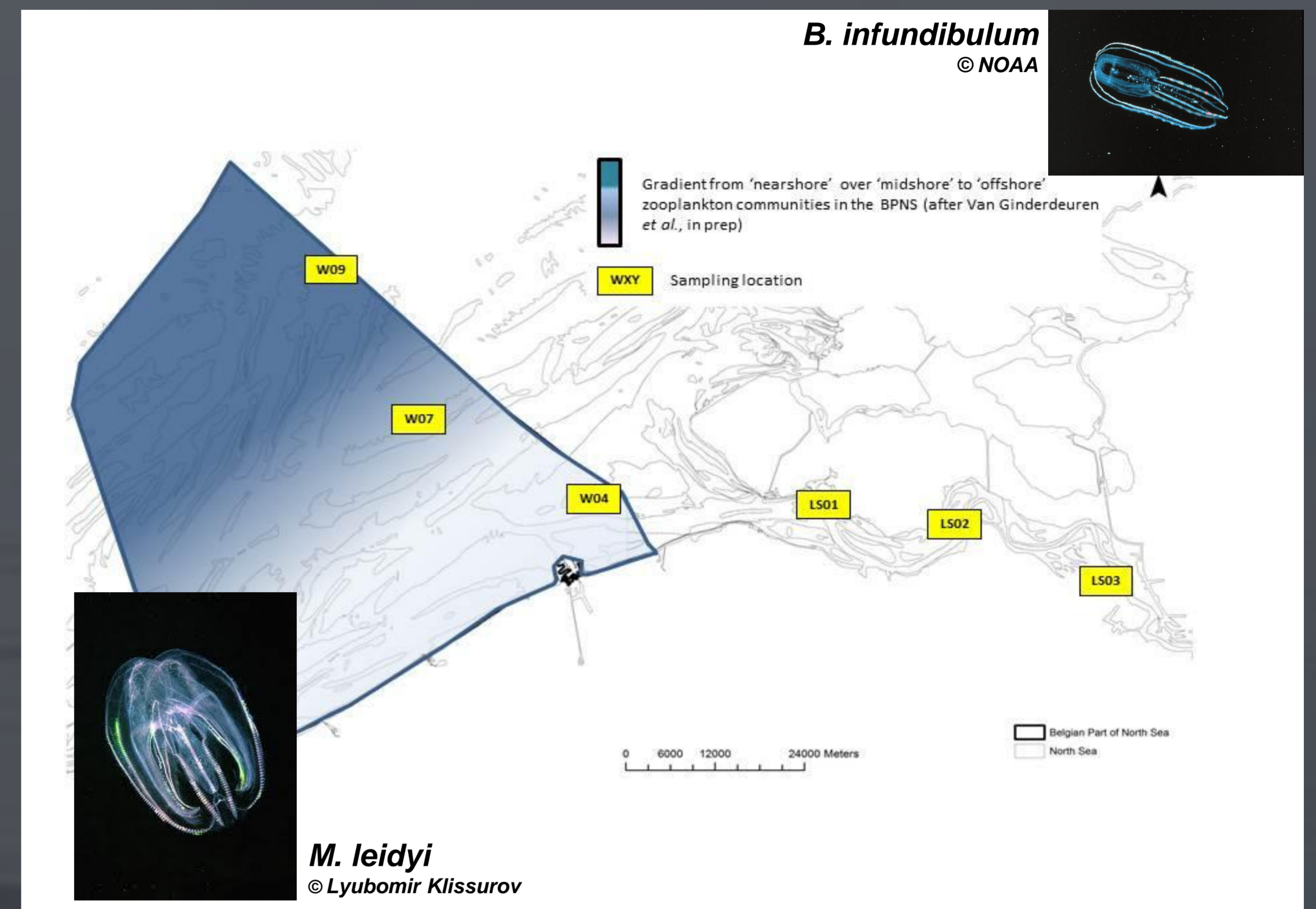


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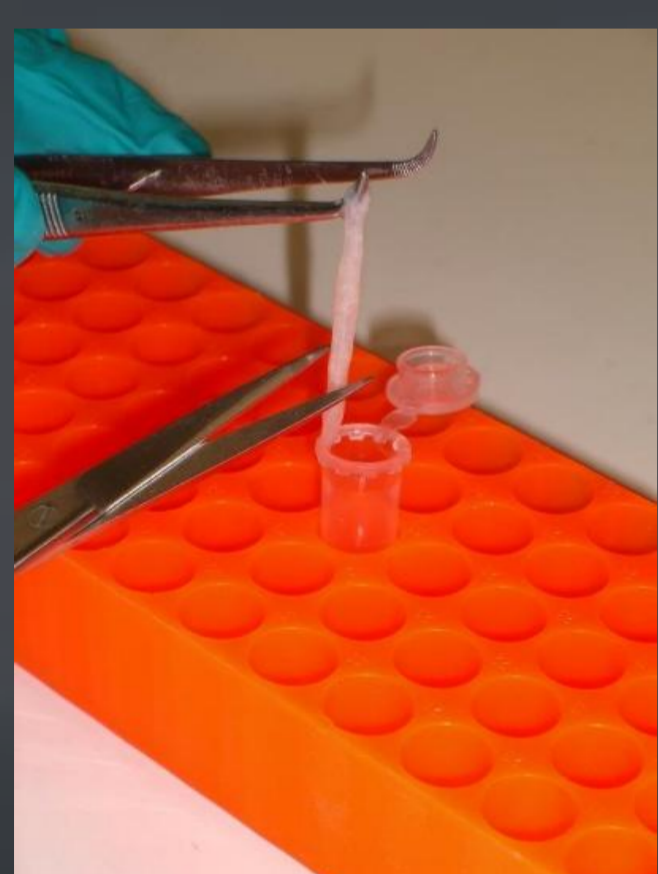
Risk assessment of non-indigenous marine invaders in the Belgian part of the North Sea (BPNS):

- *Mnemiopsis leidyi* is a well-known notorious predator presenting great risks to the marine environment.
- Detection of *M. leidyi* at an early phase of a possible invasion is necessary.
- Unambiguous identification of *M. leidyi* in bulk plankton samples is difficult due to the fragile nature of jellyfish that causes loss of structure during sampling.
- The existence of morphologically very similar species such as *Bolinopsis infundibulum* can lead to misidentification.
- Need for a protocol that allows visual identification and molecular analysis on individual samples.
- A sampling strategy to confirm the presence of *M. leidyi* on the BPNS was initiated.



A combined approach of morphological identification and molecular analysis using genetic markers:

- Combined morphological identification and molecular analysis of individual jellyfish samples is needed in studying biological invasions.
- A fixative that preserves morphology and allows DNA extraction and marker amplification can overcome sample disintegration problems that occur when using conventional preservation methods based on ethanol 70-100%.



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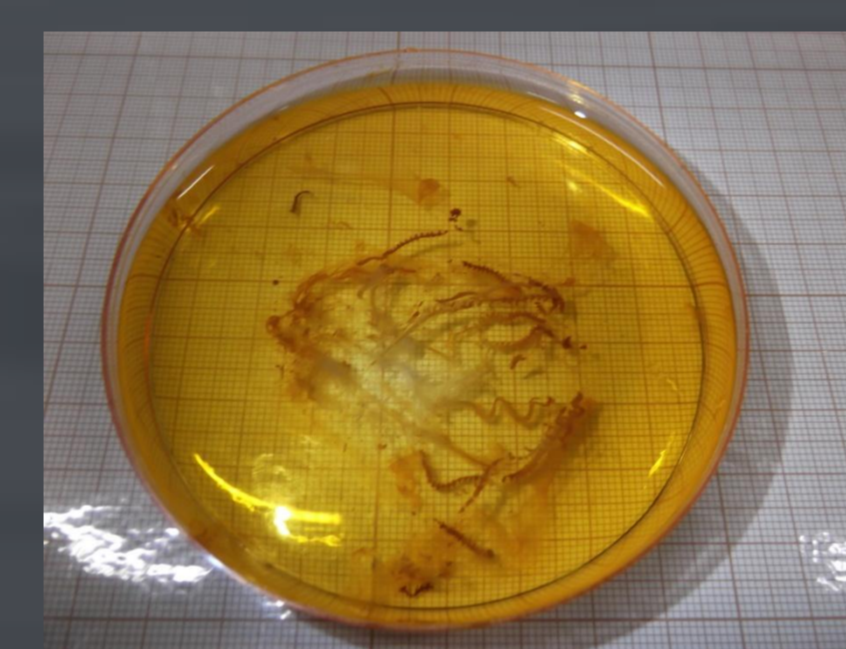


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- Acid Lugol's solution (Engell-Sørensen et al, Journal of Plankton Research, 2009), prepared by mixing 1L of ultrapure water containing 100g potassiumiodide and 50g of crystalline iodine dissolved in 100ml glacial acetic and subsequently diluted to different working concentrations with seawater was tested.

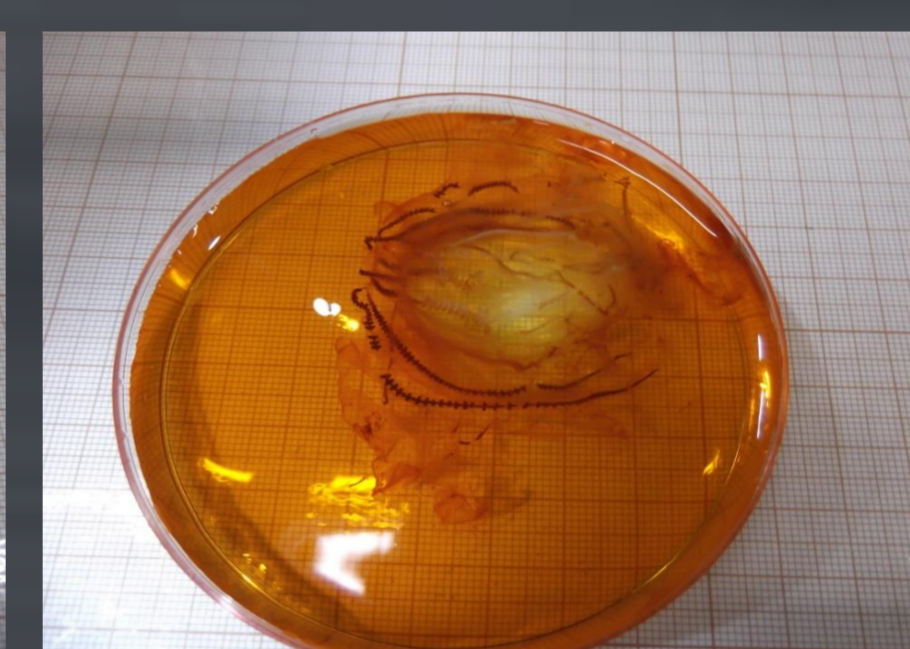
Acid Lugol's solution – fixation and ITS1 marker amplification on *M. leidyi*.

Visual identification:

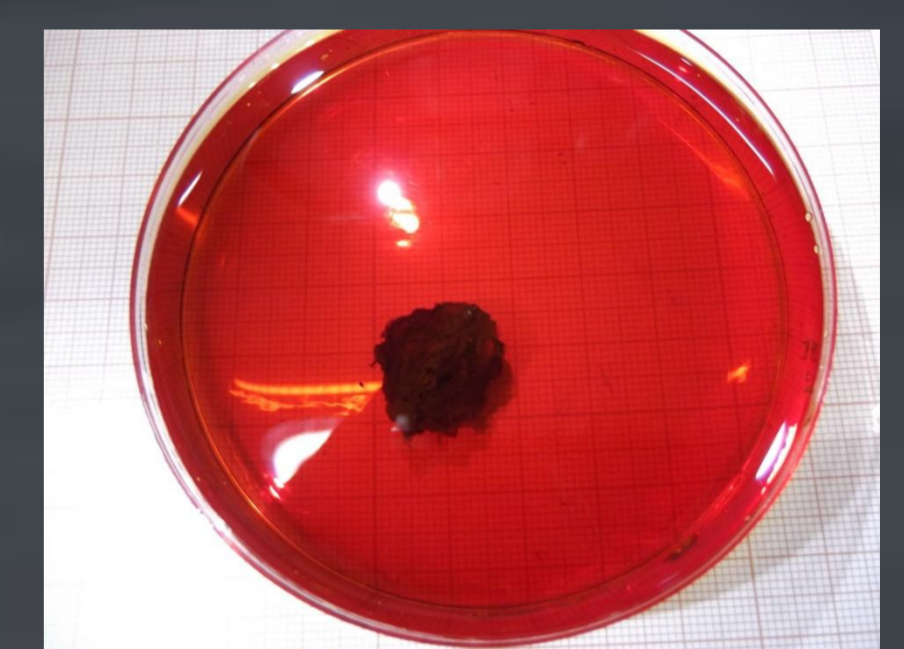


M. leidyi in 1% Lugol

© pictures: ILVO

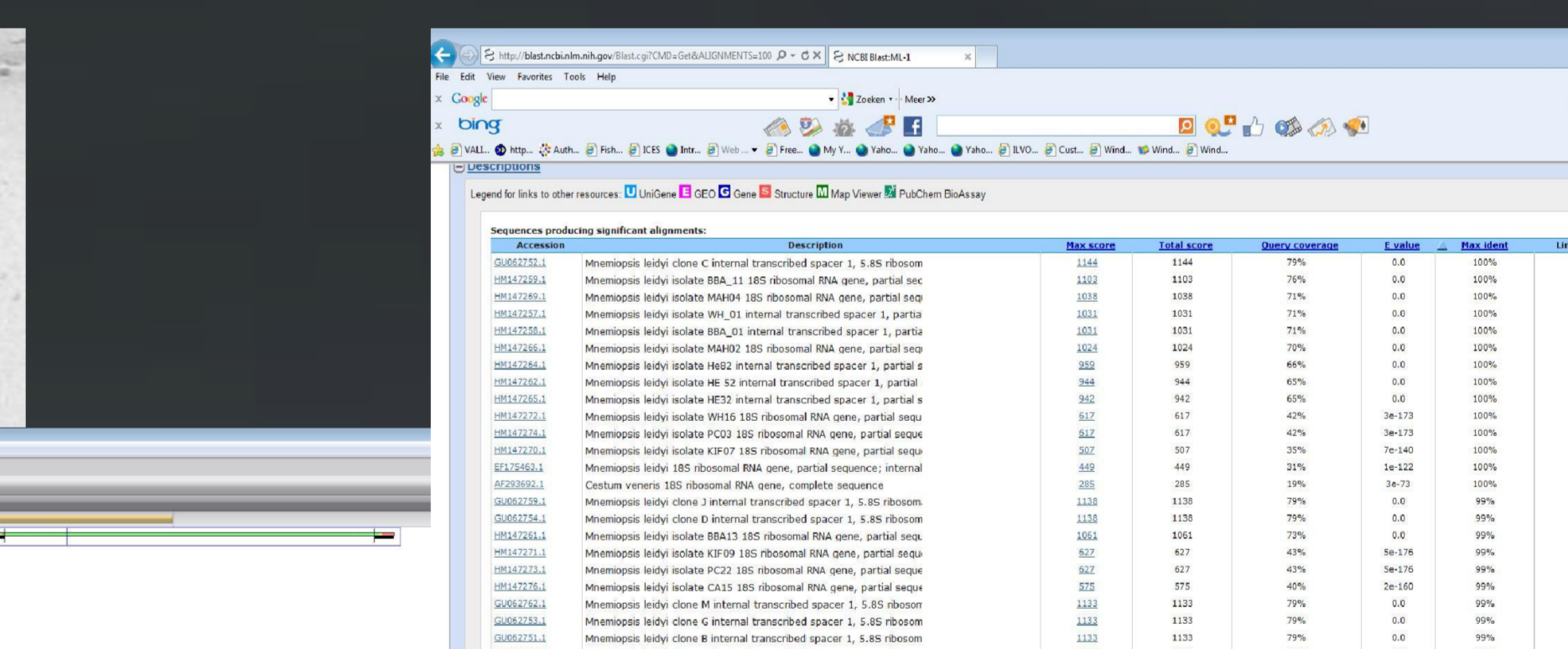
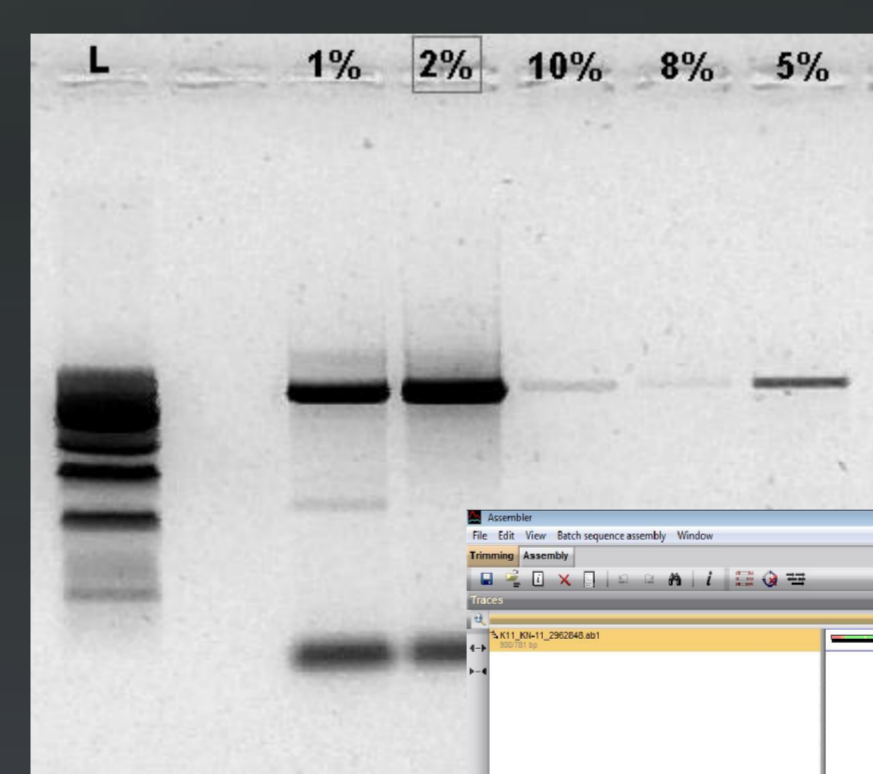


M. leidyi in 5% Lugol



M. leidyi in 10% Lugol

Amplification of the Internally transcribed spacer 1 region (ITS1):



Conclusion:

- The presence of *M. leidyi* in the BPNS could be confirmed by a combination of visual identification and molecular analysis of the ITS1 marker. ("Distribution of the invasive ctenophore *Mnemiopsis leidyi* in the Belgian part of the North Sea", Van Ginderdeuren et al., 2012, Aquatic invasions records, accepted).
- Acid Lugol's fixative solution used for this study is not optimal as high concentrations and long storage times (>24h) lead to considerable shrinkage which hamper visual identification and give DNA extracts of insufficient quality for ITS1 marker amplification.
- A combination of visual and molecular analysis prevents misidentification of non-indigenous invaders but testing of other fixation methods and new commercially available fixatives is needed.