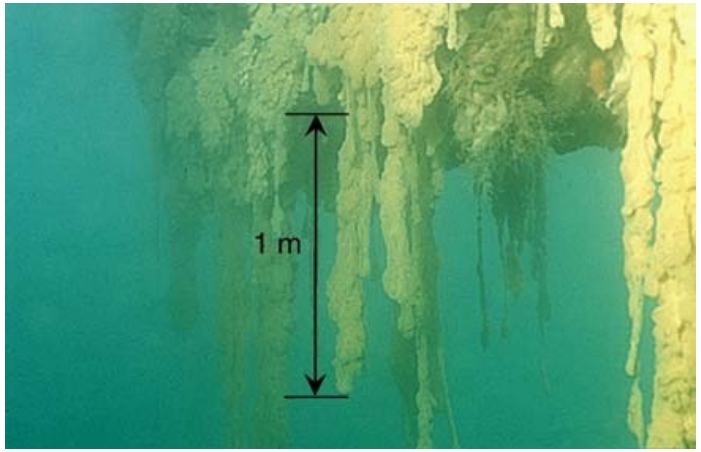
The non-native invasive sea squirt *Didemnum vexillum* – Guernsey surveillance and detection

Luke Rigby and Francis Russell



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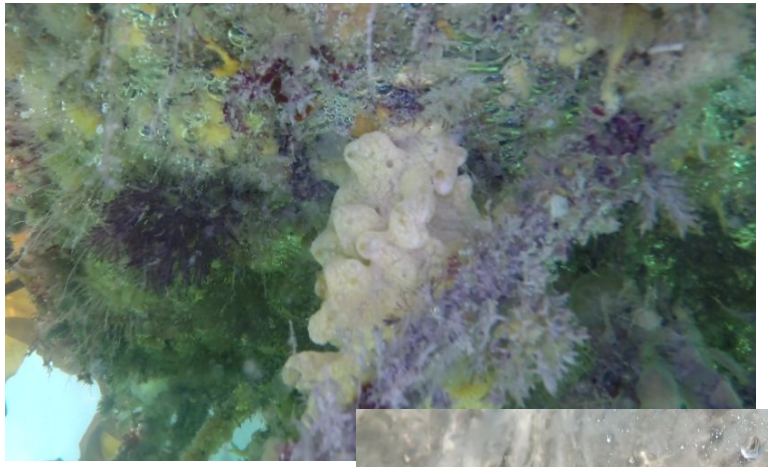
Introduction

- What is *Didemnum vexillum?*
  - A high-impact, globally-invasive, colonial tunicate, native to Japan.
- Why is *D.vex* important?
  - A successful fouling organism, grows on many natural and artificial structures. Coastal and offshore environments, from 1-65m
  - Reproduces rapidly, outcompetes native species, causes environmental degradation & significant economic harm
  - Management – some success at control/management, biosecurity protocols – long term containment
- Surveillance
  - Forms encrusting mats or lobate masses
  - Traditional surveys of marinas, wrecks, shoreline
  - Fragmentation/larval dispersal when + how far?
  - Novel techniques – eDNA seawater?
- Channel island status/Guernsey context
  - 'near miss' – hull fouled vessel GSY harbours March 2022
  - *D.vex* undetected in CI prior to 2023.



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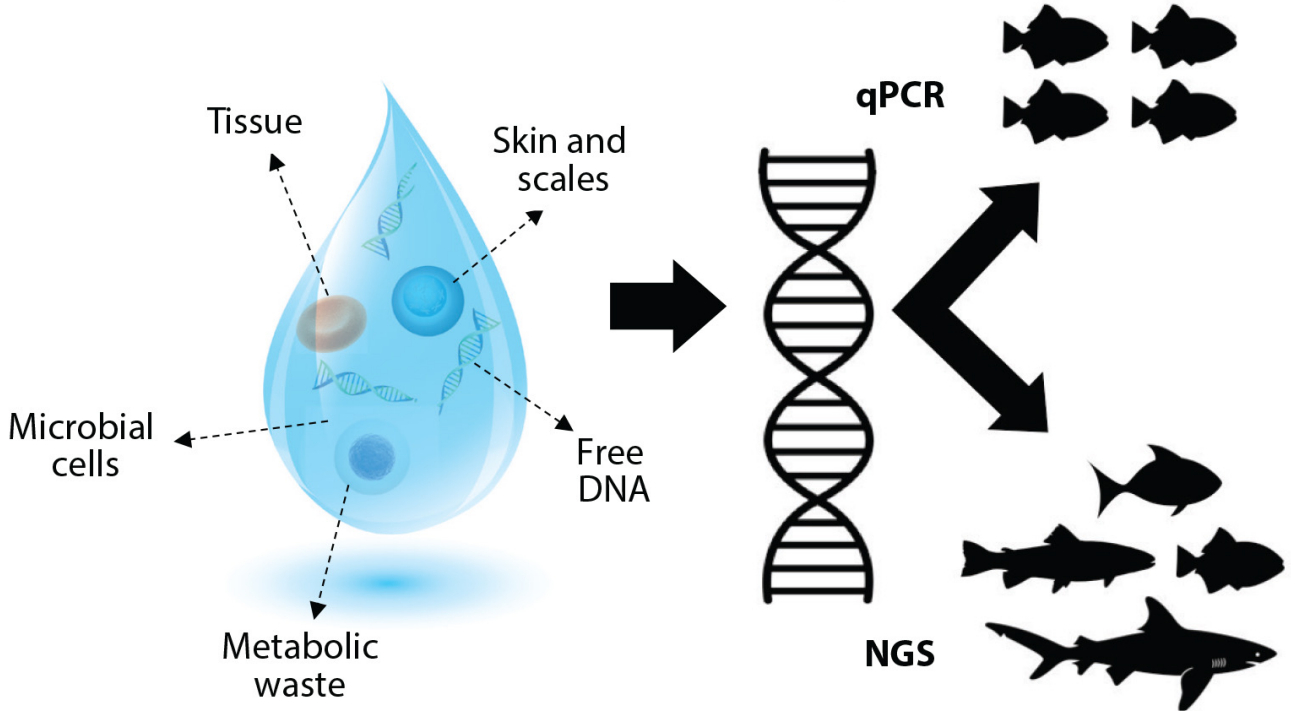
Environmental DNA (eDNA)

- What is Environmental DNA (eDNA)?
- Average eDNA half-life is ~48 hours but has been known to last for weeks
- Why is eDNA useful?
- Main analysis routes: qPCR and sequencing
- Mention examples of application e.g. Great crested newt populations UK ponds



Implementing a *D.vex* surveillance programme on Guernsey

- Phase 1 Settlement tiles – St Peter Port harbour (Aug-Sept. 2022)
- Phase 2 Scrape + seawater samples (CEFAS* & SAL) – St Peter Port harbour (Sept. 2022)
- Phase 3 Seawater samples (SAL) - St Peter Port harbour, St Sampsons Marina and Beaucette Marina (May + Aug, 2023)

*Centre for Environment, Fisheries and Aquaculture Science - Weymouth

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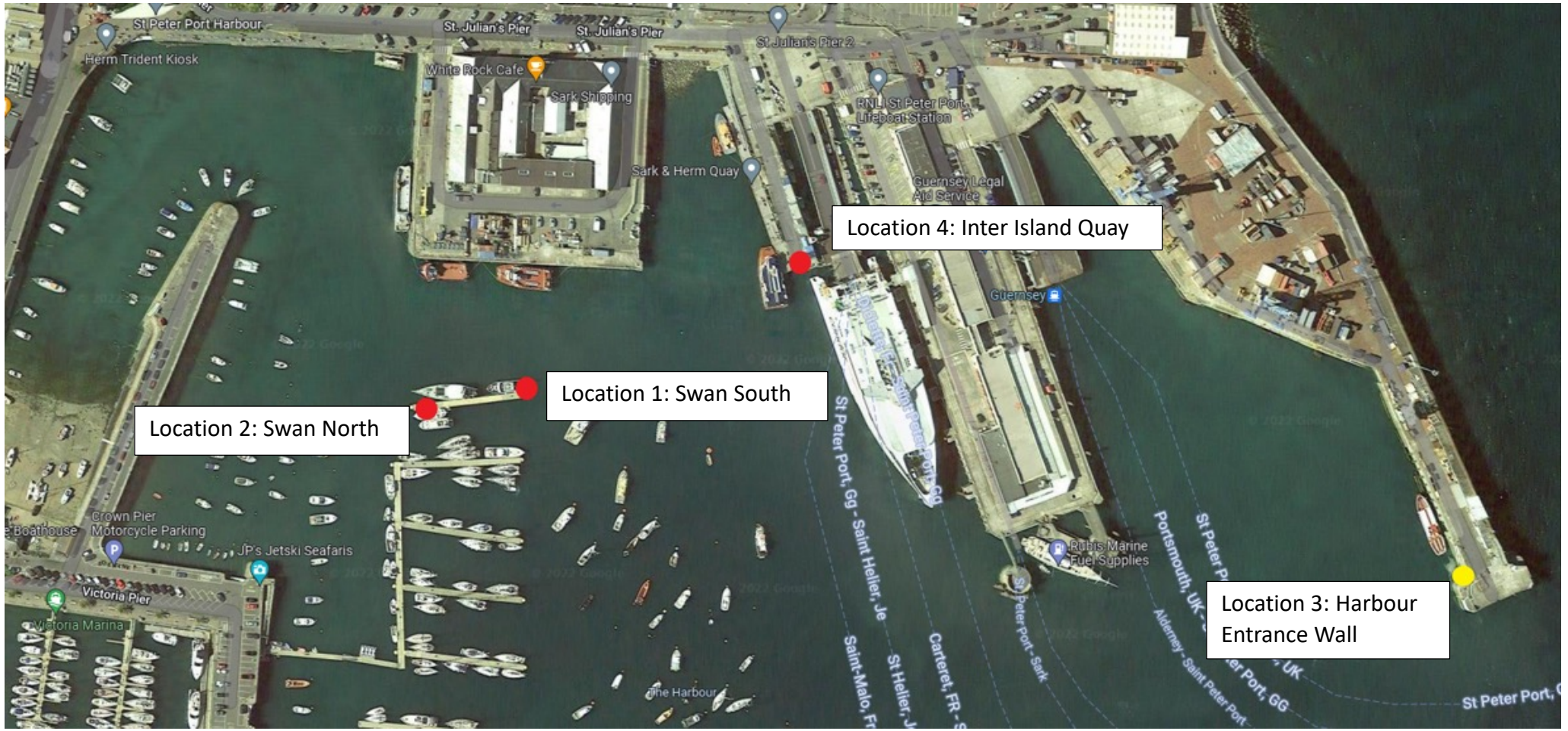
Settlement tile placement - sample collection points, 2022



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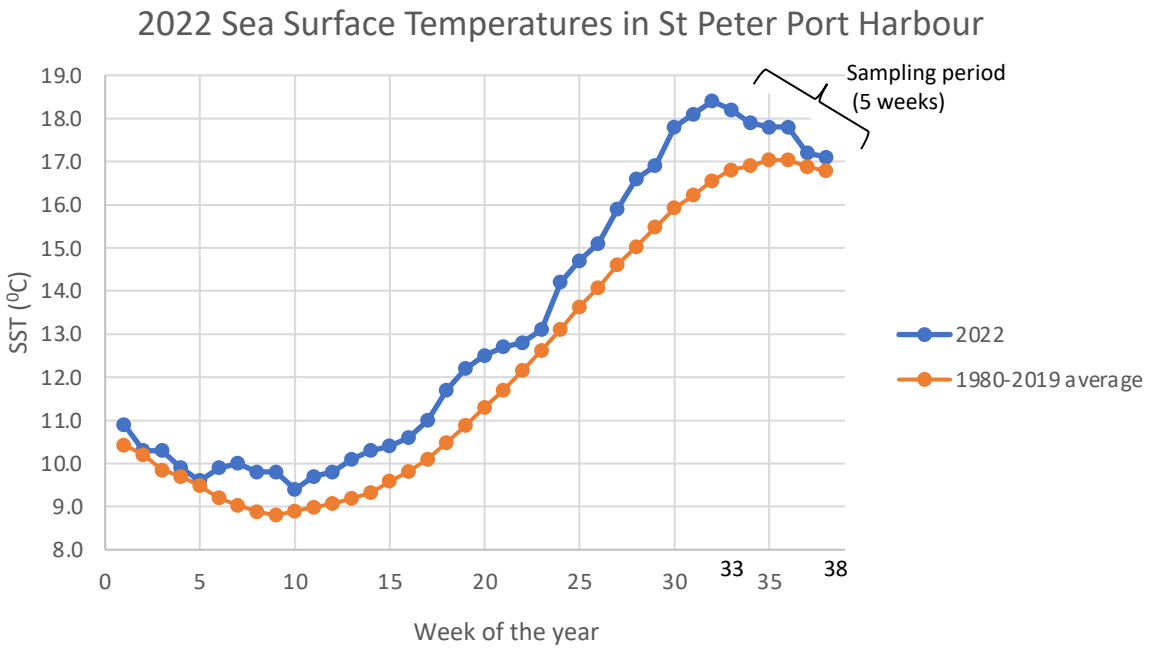
Settlement tiles – after 2 month colonisation period



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2023. Seawater sample collection points – St Peter Port

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Seawater sample collection points – St Sampsons

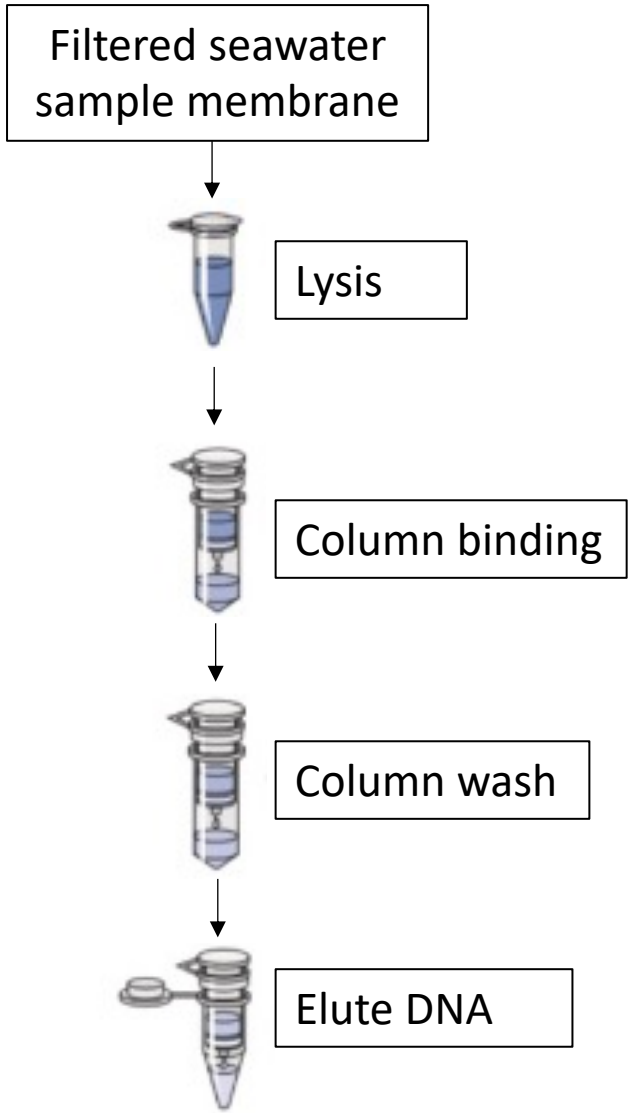
Seawater sample collection point– Beaucette marina

SAL Molecular Facilities

- The States Analytical Lab (SAL) initially built a molecular biology facility for SARS-CoV-2 variants of concern detection in August 2021
- Molecular facilities included three labs; DNA extraction, PCR and sequencing
- SARS-CoV-2 qPCR equipment kindly donated to SAL by PEH Pathology department

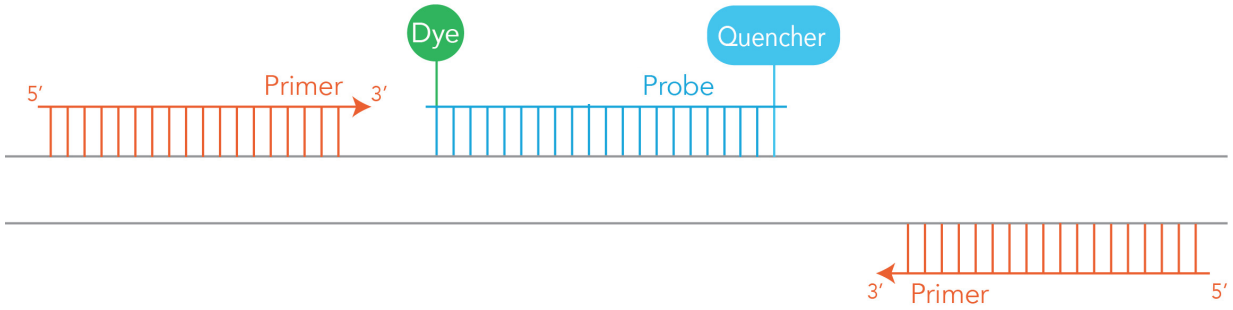
DNA Extraction

- Environmental samples can be dirty and contain high levels of PCR inhibitors
- DNA purified from sample matrices and PCR inhibitors removed using DNA extraction methods
- Various DNA extraction methods
- Spin-column DNA extraction used for SAL eDNA analysis



D.vex qPCR Analysis

- Quantitative Polymerase Chain Reaction (qPCR)
- D.vex specific forward and reverse primers
- D.vex specific fluorescent probe



Target	Primer/probe	Sequence	Product size (bp)
D. vexillum COI	D. vex Forward primer	5’- CGACTAATCATAAAGATATTAGAACA -3’	111
	D. vex Reverse primer	5’- TTCTTGTAGAACTTAATTCTATTCG -3’	
	D.vex LNA probe	FAM 5’-ATAGT{T}{A}GAGCT{A}G{A}TTTAGT{A}TA{A} -3’ IBFQ	

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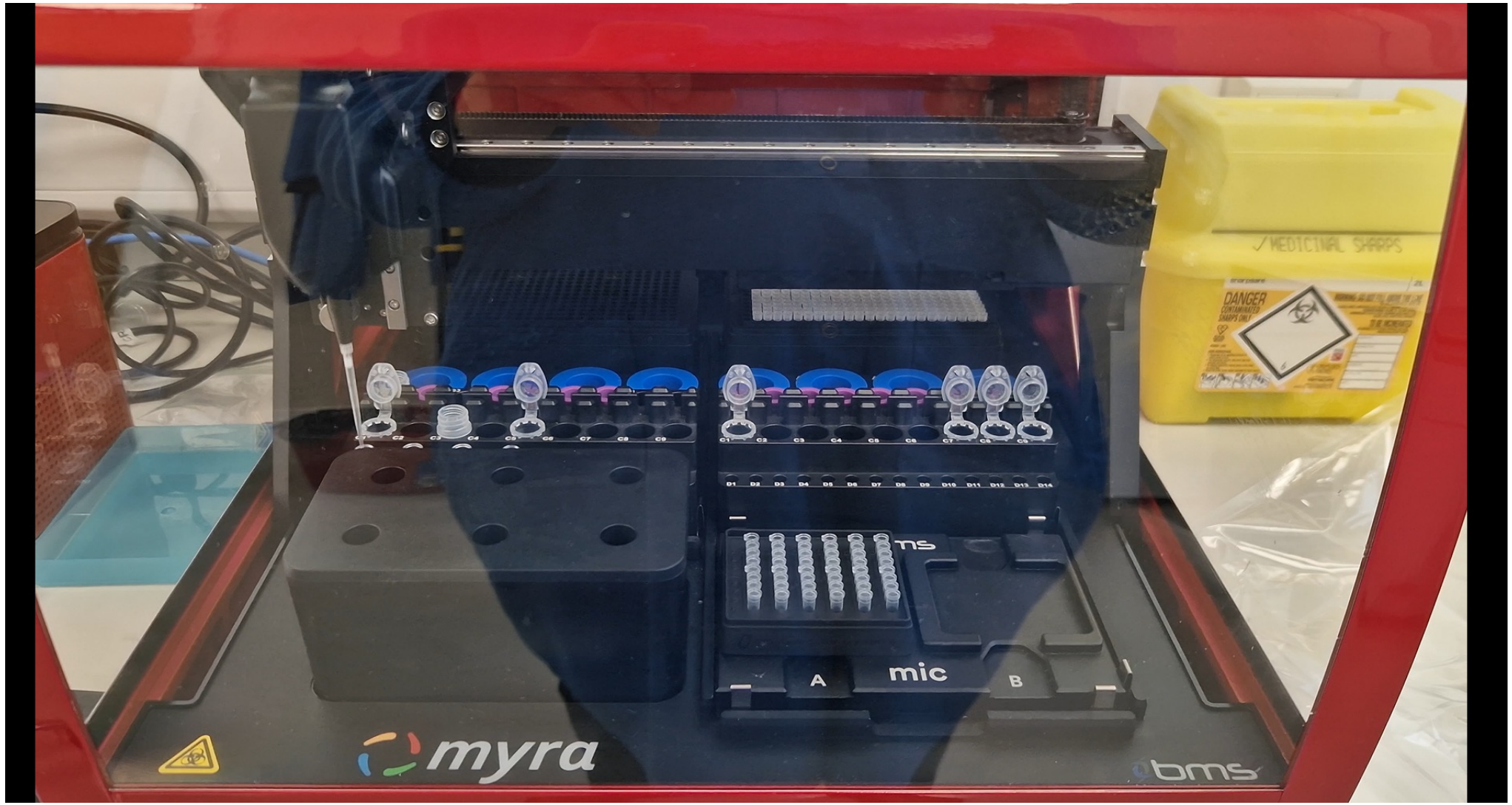

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MEDICINAL SHARPS
DANGER
CONTAMINATED
SHARPS ONLY
C1 C2 C3 C4 C5 C6 C7 C8 C9
D1 D2 D3 D4 D5 D6 D7 D8 D9 D10 D11 D12 D13 D14
TS
A
mic
B
myra
bms

2022 Settlement tile & seawater qPCR results

- All settlement tiles qPCR results negative
- Portion of settlement tile samples also sent to CEFAS with all settlement tiles negative
- All St Peter Port harbour seawaters qPCR results negative

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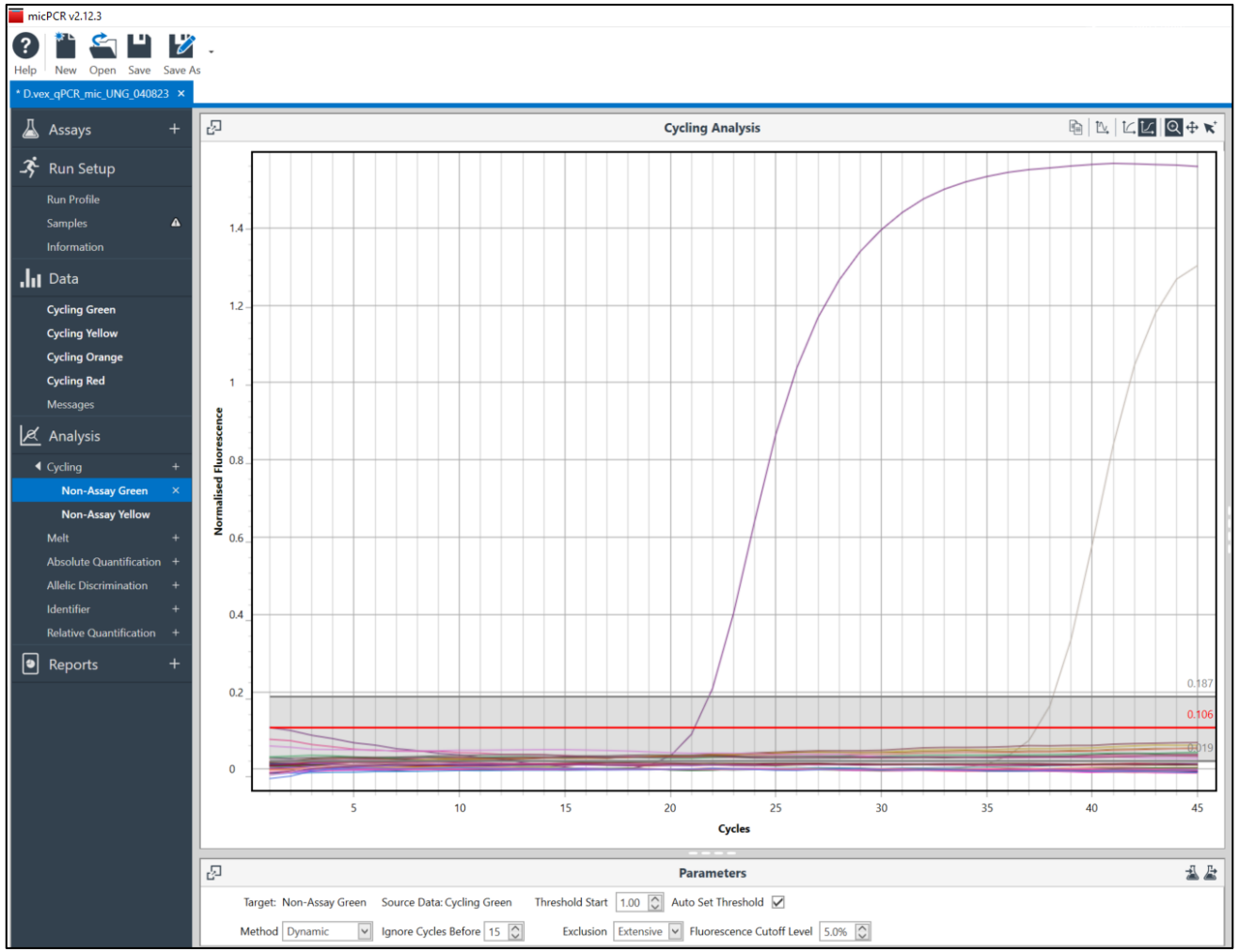
2023 Seawater eDNA qPCR Results

- 23rd May 2023 Expanded Surveillance – St Peter Port Harbour, St Sampsons Marina and Beaucette Marina seawater samples. St Peter Port Harbour - Victoria Marina seawater sample suspected positive

- 30th May 2023 St Peter Port Harbour - Victoria Marina seawater resampled and confirmed as positive result with Marine Scotland PCR product sequencing

- No settlement tiles deployed



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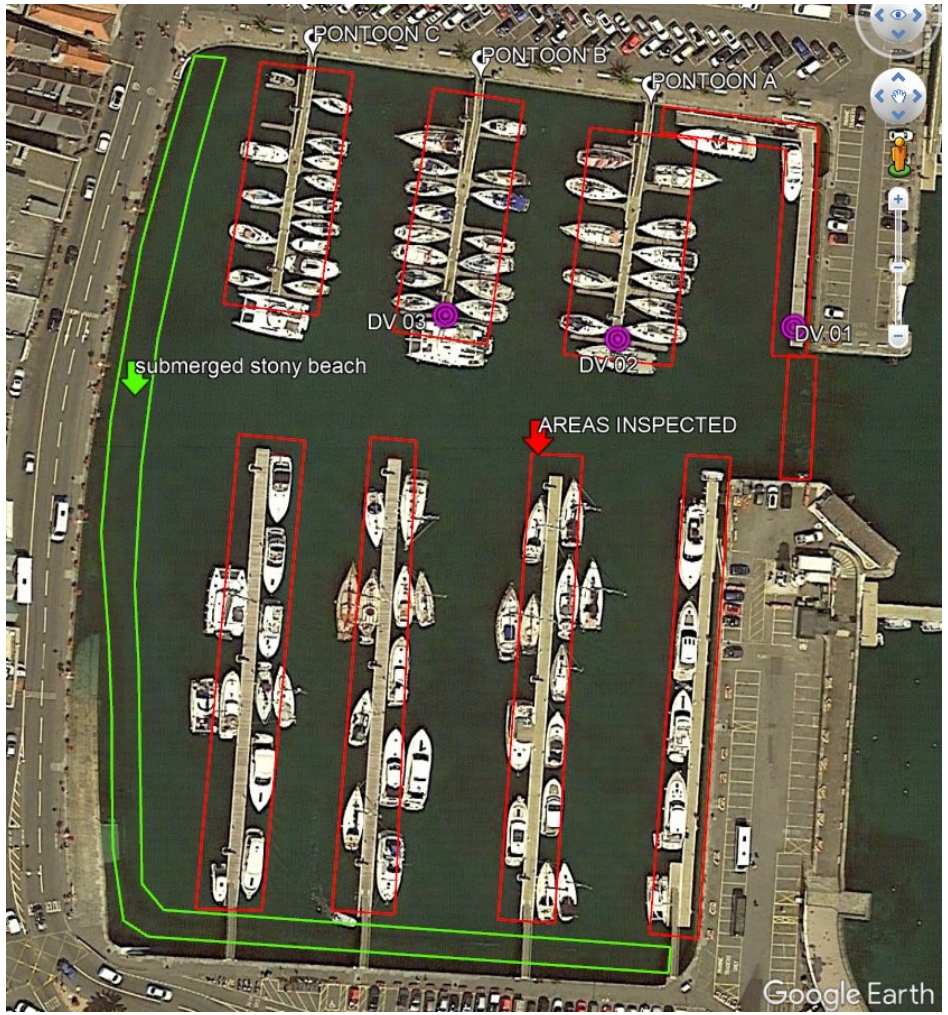
Victoria Marina - physical survey undertaken June 15/16th, 2023

- In response to the positive test confirming the presence of *D.vex* in the marina, ACLMS commissioned a physical survey.
- Chris Isaacs – marine INNS expert from Jersey, completed his survey over two days in June 2023.
- A combination of visual inspection, scrape samples and underwater video camera was used to locate larger colonies.
- Chris' survey identified the presence of *D.vex* on 3 pontoons on the north side of the Victoria Marina.



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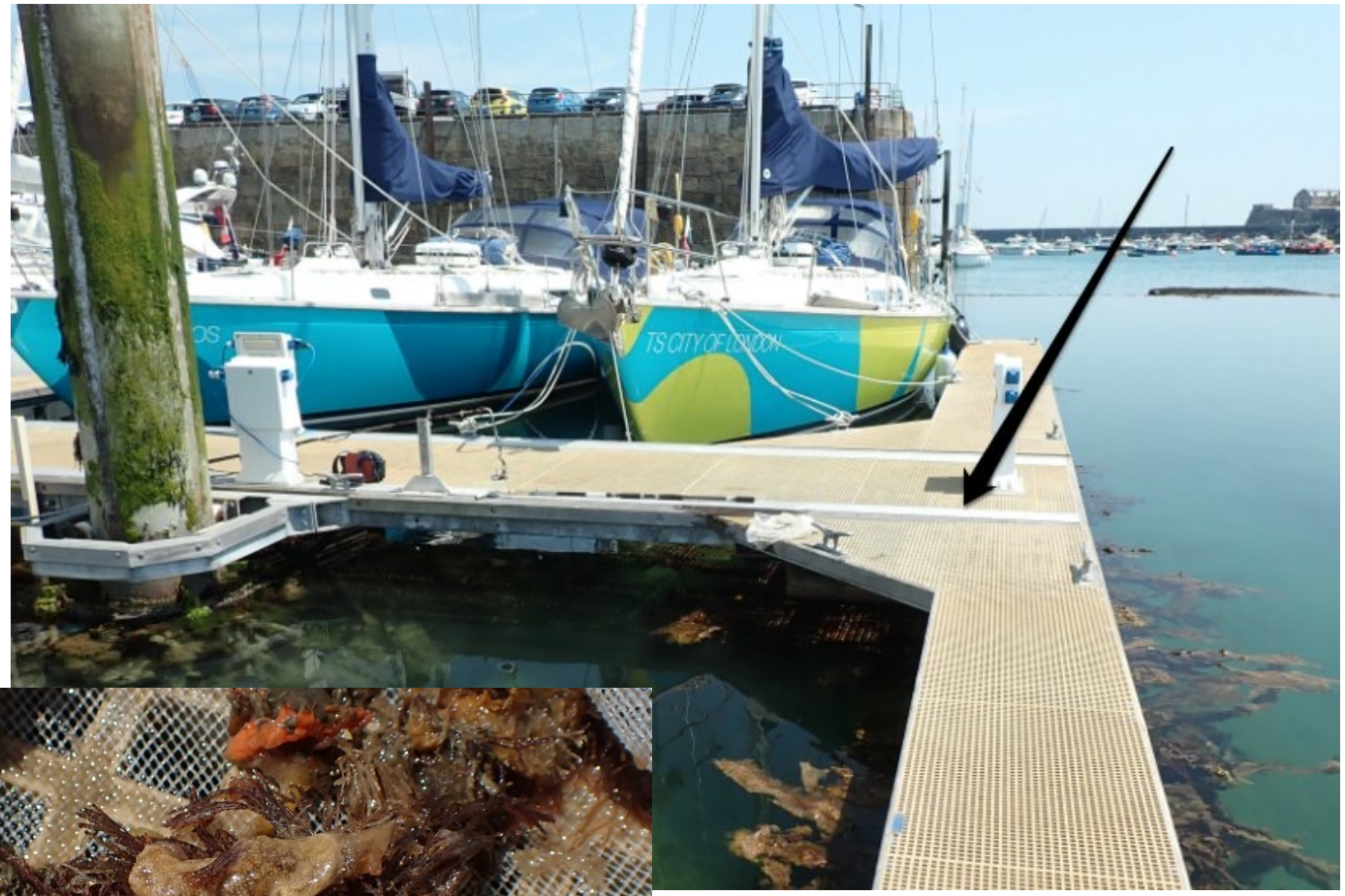

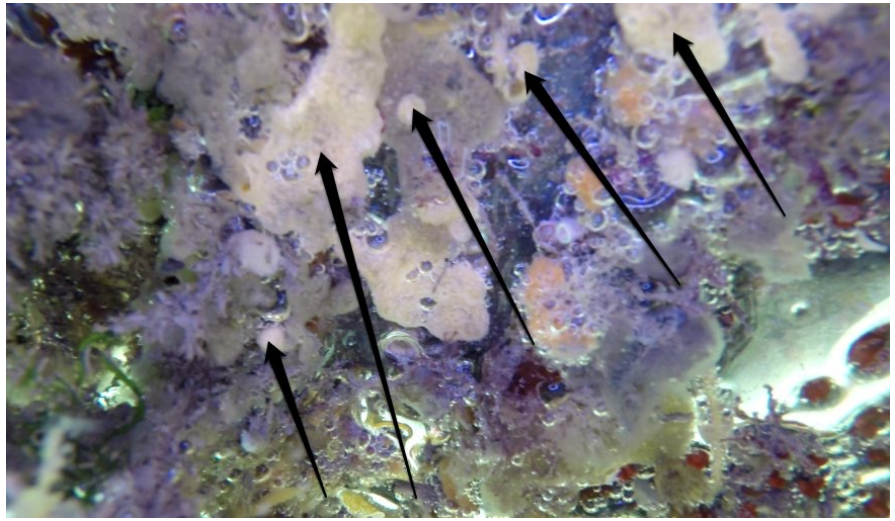

qPCR August 2023 Results

- August 2023 (sea temp. 17°C - optimal for larval dispersal) - St Peter Port harbour, St Sampsons marinas and Beaucette marina seawater samples analysed again

- D.vex seawater eDNA still present Victoria Marina seawater but no sign of spreading into the harbour outside of the marina, or evidence it may be present at any of the other sample locations

- Victoria Marina physical survey tunicate tissue sample collected in June confirmed as *D.vex* through qPCR

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Future *D.vex* surveillance monitoring

- Annual testing of seawater for *D.vex* is to be included as part of the wider annual monitoring programme of marine INNS in Guernsey's waters

- This should include better education and information to increase public awareness on the importance of biosecurity measures to limit the further introduction and spread of *D.vex* and other INNS

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Other current eDNA qPCR Projects - ATM

Asian Tiger Mosquito (ATM) eDNA pilot study 2023

- Trap water eDNA qPCR detection
- Mosquito adult qPCR detection
- Mosquito egg/larvae qPCR detection

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Potential future projects

- Guernsey quarry water sequencing surveillance for non-native species e.g. fish & reptiles
- Signal crayfish sequencing, island distribution from water samples (douits)
- Bat roost faeces sequencing for species and prey identification
- Oak and Pine Processionary Moth (OPM/PPM) trap surveillance
- Asian Hornet air eDNA surveillance?

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- SAL Laboratory Manager - Michael Hughes

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References

- D.vex survey official report Chris Isaacs 15-06-23
- Iveta Matejusova, Jennifer Graham, Fiona Bland, Jean-Pierre Lacaze, Guillaume Herman, Lyndsay Brown, Eric Dalgarno, John D. Bishop, Jenni E. Kakkonen, Kirsty F. Smith and Alex Douglas (2021). Environmental DNA Based Surveillance for the Highly Invasive Carpet Sea *Squirt Didemnum vexillum*: A Targeted Single-Species Approach. *Frontiers in Marine Science*, vol. 8, article 728456.
- Johan Spens, Alice R. Evan, David Halfmaerten, Steen W. Knudsen, Mita E. Sengupta, Sarah S. T. Mak, Eva E. Sigsgaard and Micaela Hellstrom (2017). Comparison of capture and storage methods for aqueous macrobial eDNA using an optimized extraction protocol: advantage of enclosed filter. *Methods in Ecology and Evolution*, 2017, 8, 635–645.
- Centre for Environment, Fisheries and Aquaculture Science (CEFAS)
- Qiagen DNeasy Blood & Tissue Handbook
- https://eu.idtdna.com/pages/products/qpcr-and-pcr/gene-expression/primetime-qpcr-probes
- https://en.wikipedia.org/wiki/Aedes_albopictus
- https://www.google.com/maps
- https://tos.org/oceanography/article/observing-life-in-the-sea-using-environmental-dna