

# New technologies and concepts in emerging disease detection

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Together we are working for  
a **sustainable blue future**

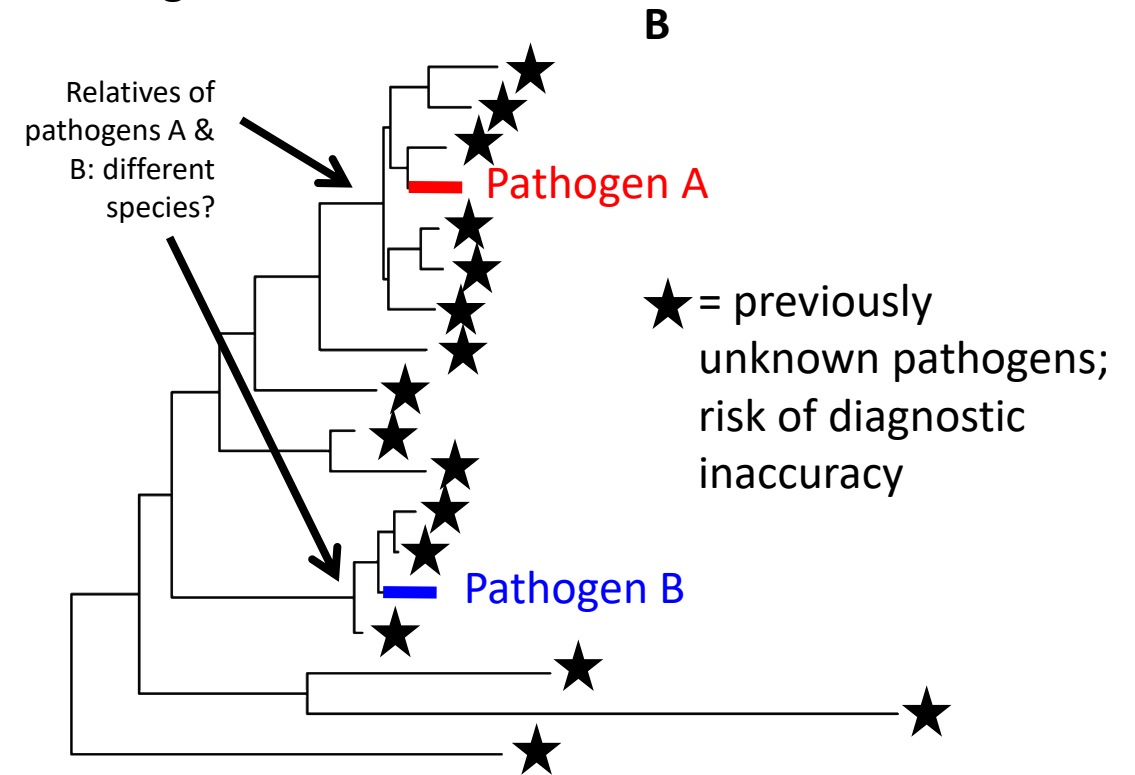
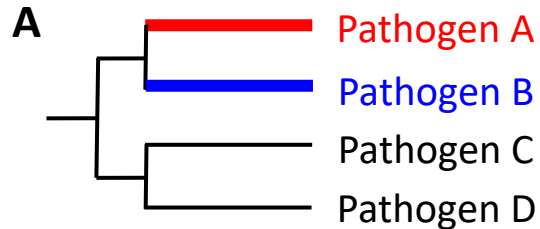
# Outline of presentation

1. Pathogen diversity and implications for diagnostics, surveillance, and emerging diseases
2. eDNA for pathogen disease surveillance and early warning
3. Portable and rapid molecular technologies
4. Syndromic disease and the pathobiome



## Pathogen diversity and accurate diagnostics

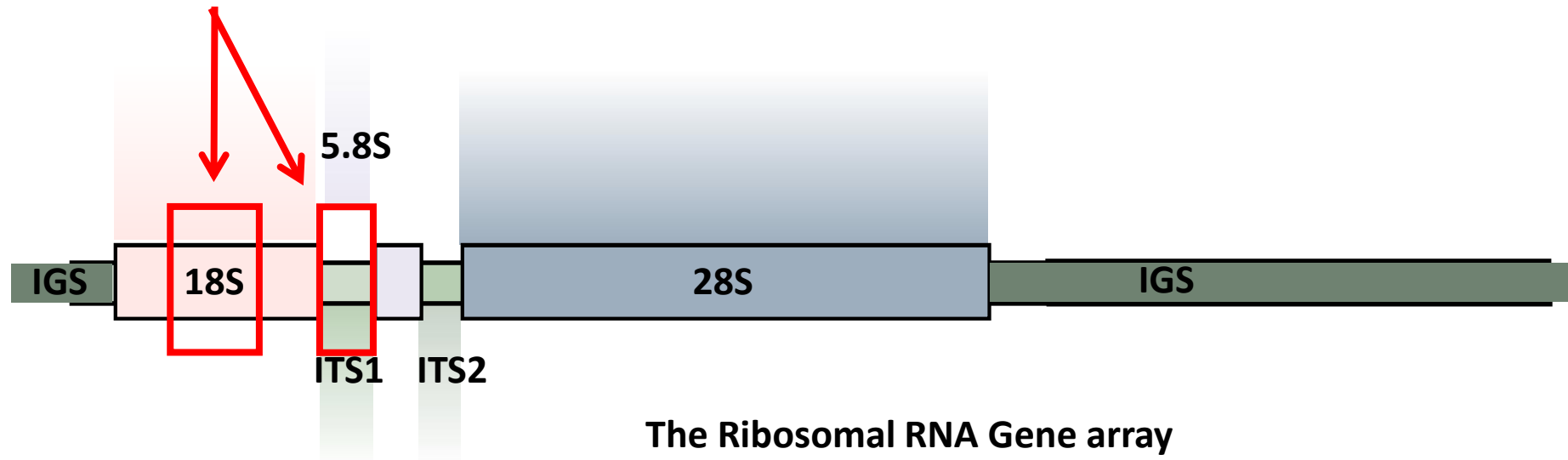
- Pathogen diversity is much greater than previously realised, and more widely distributed
- Many diagnostic assays were designed before this knowledge



- Lack of diagnostic tools to cope with this greater diversity, leading to risk of inaccurate assays and non-specific identification

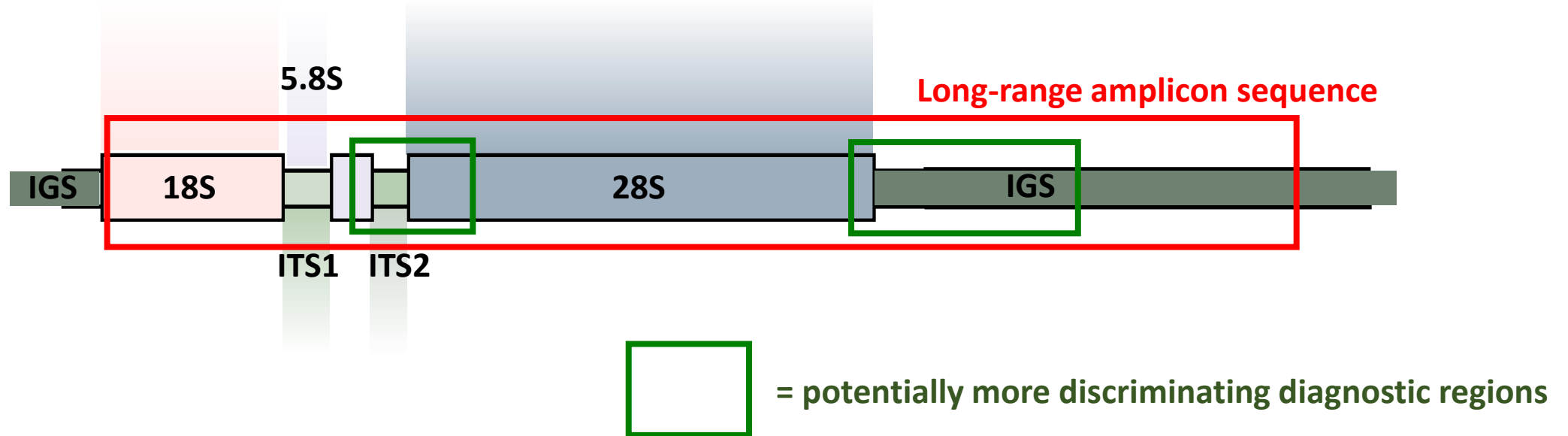
## Pathogen diversity and accurate diagnostics: ways forward

Most current diagnostic assays target **short regions** of a **limited number** of genes, often 18S or ITS1



## Pathogen diversity and accurate diagnostics: ways forward

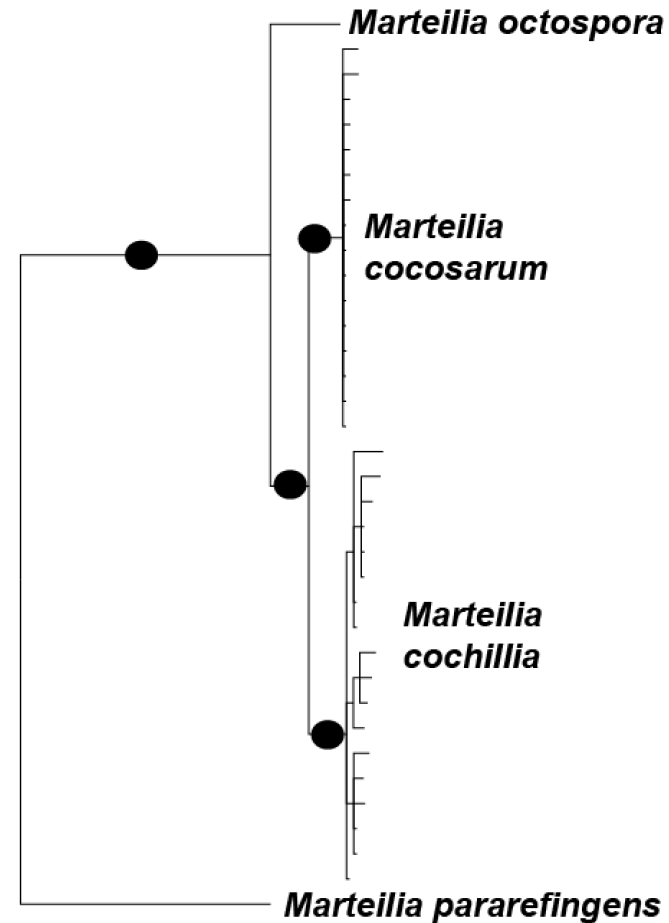
Long-range sequencing (using Nanopore/Illumina) of more of the array provides access to **more discriminating diagnostic** marker regions, and **greater phylogenetic resolution** than diagnostic regions used so far.



## The importance of phylogenetic interpretation

**Greater phylogenetic resolution is necessary**

- 1) for accurate and reliable interpretation of new lineages (e.g. emerging diseases),
- 1) to discriminate between closely related species, and
- 1) Define taxonomic units and pathological characteristics of related species



- *Marteilia* (parasite of bivalves) species phylogeny based on **long-range amplicon sequences** and good **phylogenetic methods**
- Robustly supported, monophyletic taxa
- Facilitates **design of accurate diagnostic markers**

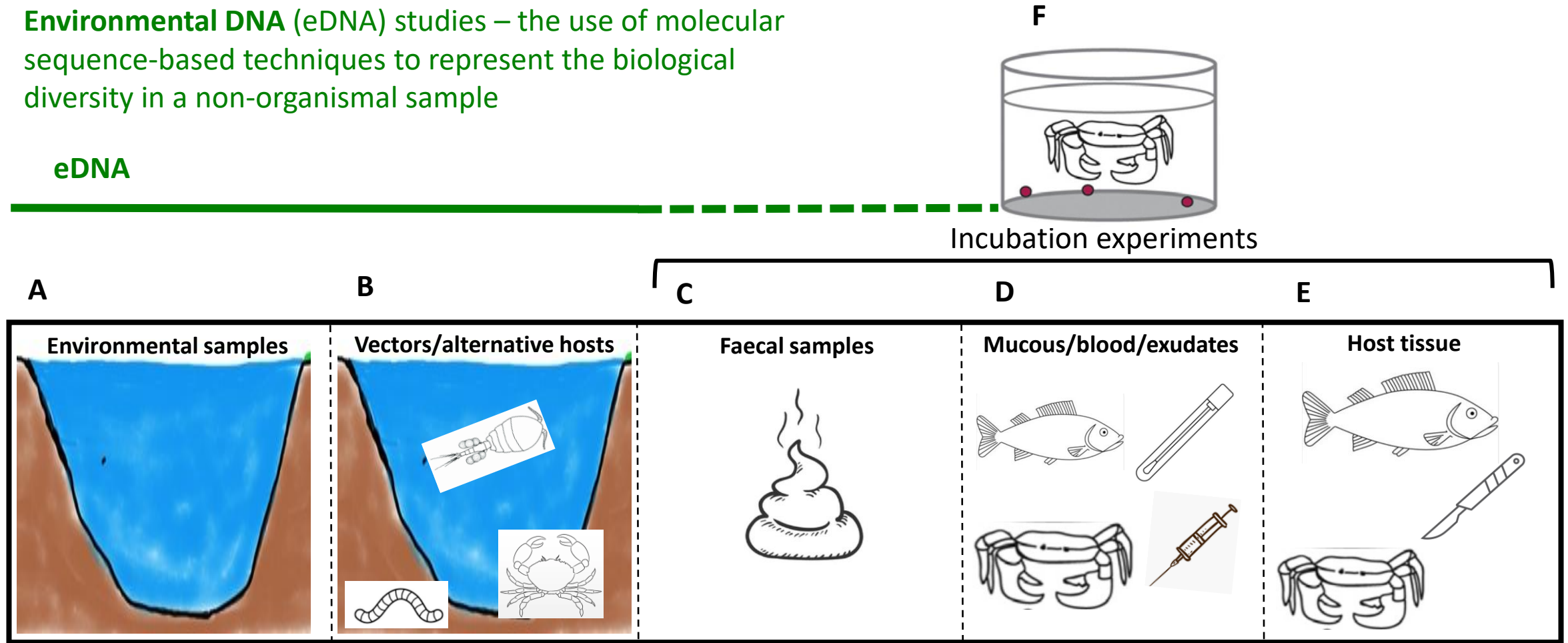




# The eDNA sampling continuum

**Environmental DNA (eDNA)** studies – the use of molecular sequence-based techniques to represent the biological diversity in a non-organismal sample

eDNA





## Advantages of eDNA approach:

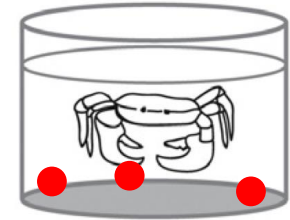
- Low cost, rapid, easy sampling, non-invasive, comprehensive
- Environmental surveillance and screening for trade
- Risk assessment and early warning of disease
- Identification of vectors and reservoirs; transmission pathways
- Understanding pathogen ecology (e.g. lifecycle) & occurrence
- Basis for disease management and control
- Monitor other causes of disease e.g. algal blooms

● = target pathogen

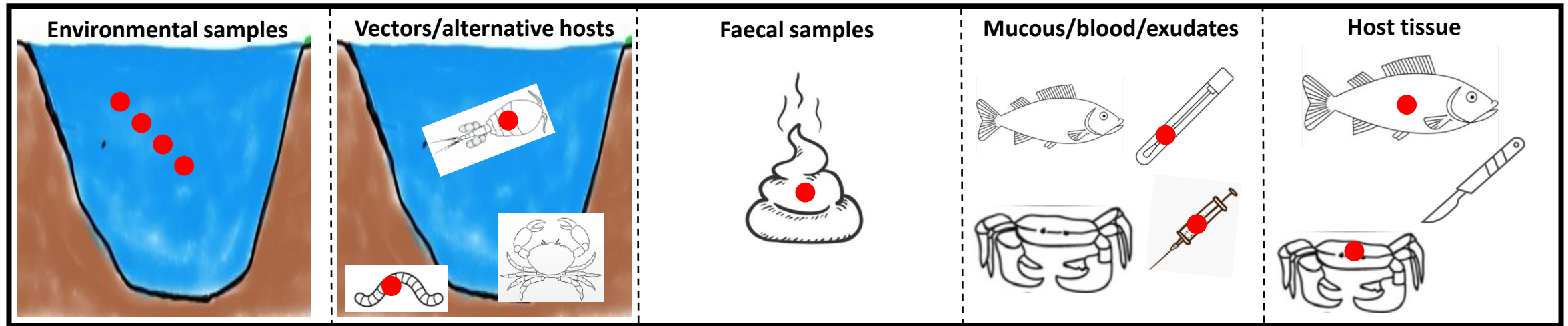
➤ Many pathogens are more widely distributed in environmental and non-host compartments than previously recognised

**Risk:** increased detection of politically sensitive pathogens

**Imperative:** R&D to understand significance and meaning of eDNA detection of pathogens



Incubation experiments



# Workflow for design, validation, and standardisation of 'Pathogen eDNA'

**Define question**



**Select target pathogen(s)**



**Experimental design:**

Sampling strategy  
Sample processing  
Nucleic acid extraction method



**Choice of molecular assay:**

Specificity  
Sensitivity



**eDNA analysis**



**Interpretation**



**Validation**



# Portable/in-field technology can be optimised for 'Pathogen eDNA'

Define question



Select target pathogen(s)



Experimental design:

Sampling strategy  
Sample processing  
Nucleic acid extraction method



Choice of molecular assay:

Specificity  
Sensitivity



eDNA analysis



Interpretation



Validation



LamPORE COVID-19



Loop-mediated isothermal amplification (LAMP/RT-LAMP)

Recombinase polymerase amplification (RPA)

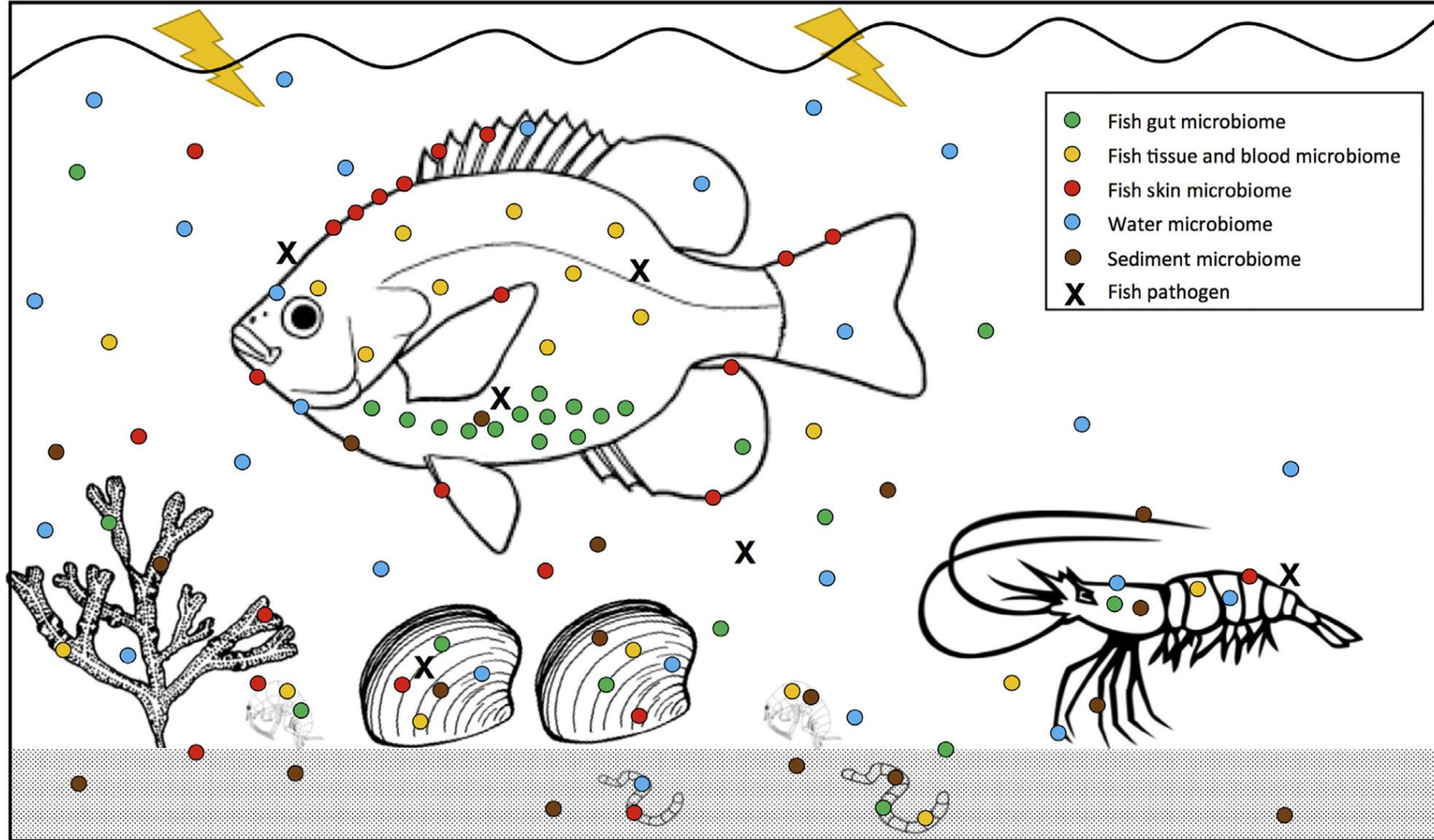


WSSV: Minardi et al 2018

## Pathobiome:

set of host-associated organisms (prokaryotes, eukaryotes, and viruses) associated with reduced health status, as a result of interactions between symbionts, host, and environment.

**Significance for disease/pathogen management:** a single aetiology may result from interactions of multiple agents, which may differ between cases



Trends in Ecology & Evolution

The pathobiome in animal and plant diseases *Trends in Ecology and Evolution* 2019



## The rise of the syndrome – sub-optimal growth disorders in farmed shrimp

Rajendran Kooloth Valappil<sup>1</sup> , Grant D. Stentiford<sup>2,3</sup> and David Bass<sup>2,3,4</sup>

### Sub-optimal growth syndromes in farmed shrimp

Associated with infections with several viruses, the microsporidian *Enterocytozoon hepatopenaei* (EHP), and various bacteria (e.g. *Vibrio*)

Concept of individual pathogens as ‘necessary but insufficient’ cause of disease

How can legislation account for and accommodate multi-agent diseases and syndromes?



## In summary...

1. Recognition of **greater diversity of pathogens** relevant to aquatic animal health (not just listed pathogens) is essential
1. Legislation needs to account for the facts that pathogens can be **widely distributed** ecologically and geographically
1. More genetic data and phylogenetic frameworks are needed for **accurate diagnostics** and **characterisation of emerging diseases**
1. eDNA methods have **great potential for pathogen surveillance and early warning of disease**, but:
  - Require **validation**
  - Need to understanding **significance of eDNA detection**

### 2. Research priorities:

More advanced **diagnostics** (genomics-based, high precision, rapid & in-field applications)

Knowledge of **distribution and diversity** of pathogens/symbionts and **associated risk of disease** (e.g. eDNA)

Improved ability to characterise emerging and **multi-factorial/syndromic diseases**

Better understanding of **host-symbiome-environment** relationship in relation to disease **AND** health

- Manage and limit incompatibility between emerging scientific evidence/approaches and current legislative framework