Investigating disease dynamics in shore crabs, *Carcinus maenas*, including two new Haplosporidia species.

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Introduction

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Common shore crab

Carcinus maenas

Introduction

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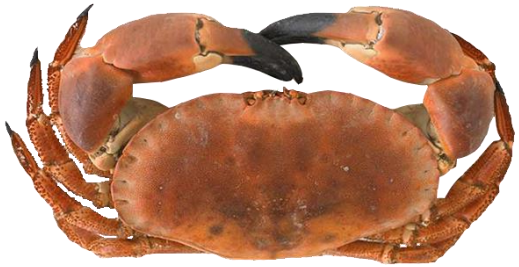
Parasitic dinoflagellate *Hematodinium* sp.



Brown crab (*Cancer pagurus*)

Velvet swimming crab (*Necora puber*)

Tanner crab (*Chionoecetes bairdi*) and snow crab (*C. opilio*)

Norwegian lobster (*Nephrops norvegicus*)

Blue crab (*Callinectes sapidus*)

the sand crab *Portunus pelagicus*

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Questions:

- **Where are the reservoirs of shellfish diseases in the aquatic environment?**
  - Do common shore crabs, *Carcinus maenas*, harbour pathogens potentially dangerous to crustaceans of commercial interest?
  - Can we 'track' pathogens, such as *Hematodinium*, in the water column and into the host using eDNA approaches?

Location

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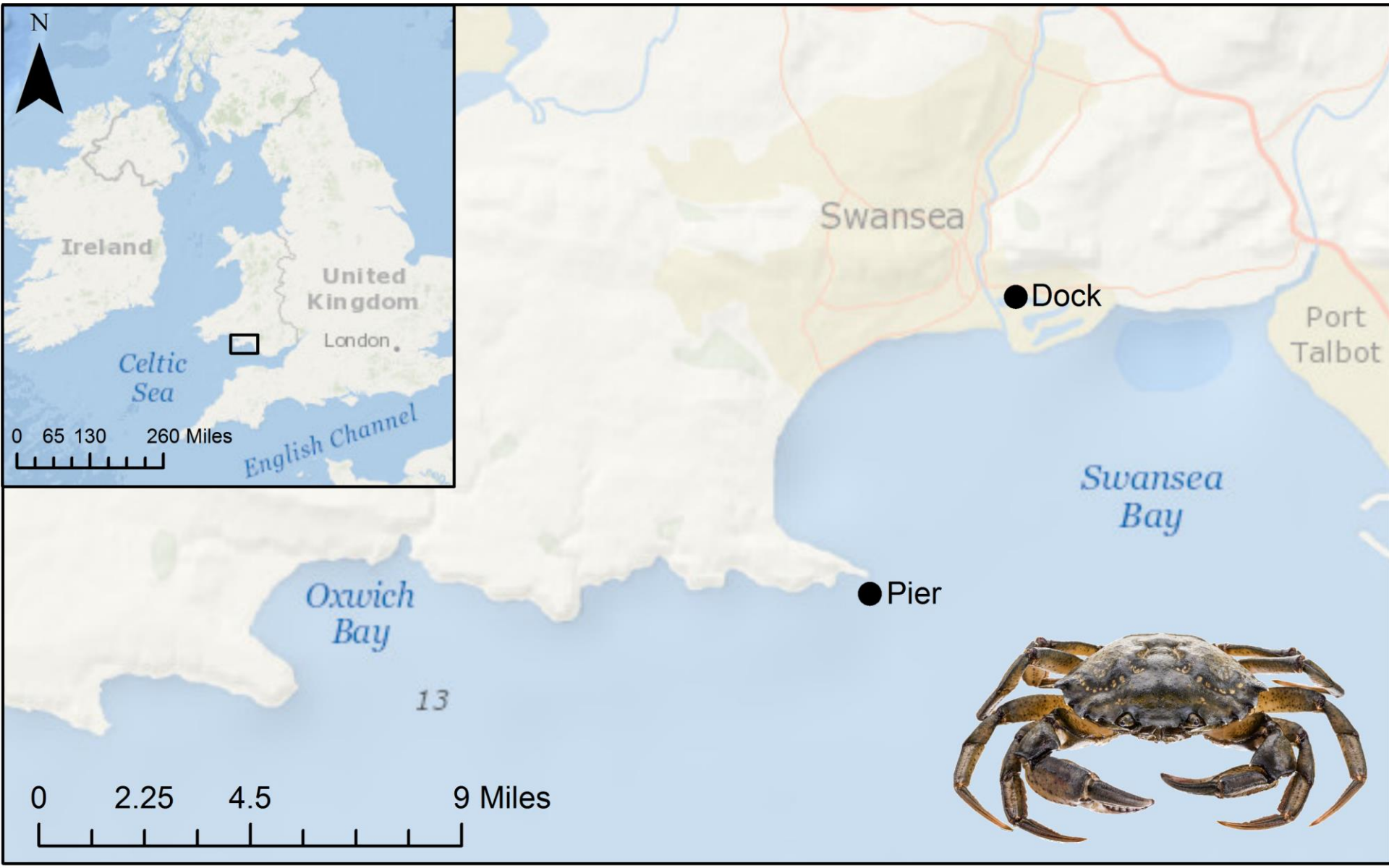

Methods

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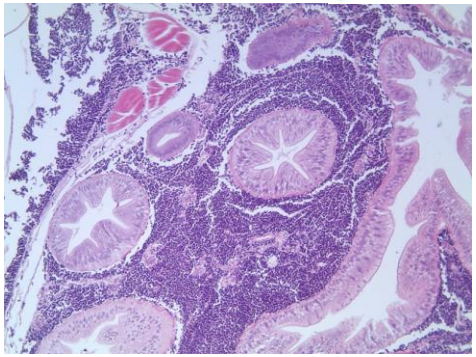
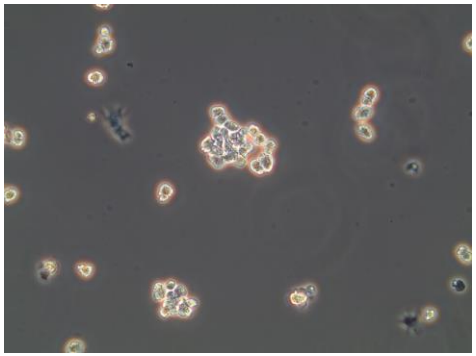


Histology of gill & hepatopancreas

Crabs

50/ month / location

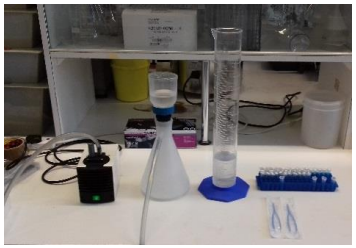
Haemolymph



Blood smears

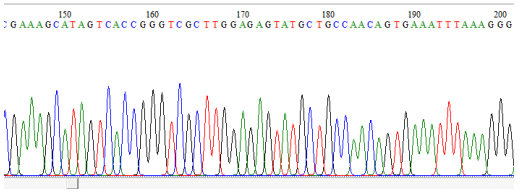
Seawater

1 L / 3 replicates / month / location



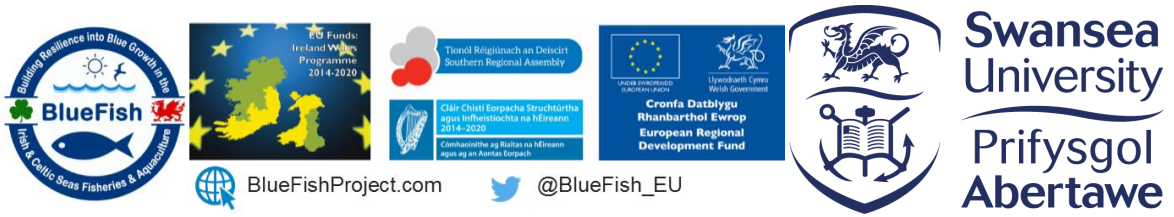
Filtration

DNA extraction and PCR with *Hematodinium* specific primers



Positives sequenced via Sanger and deposited in GenBank

Results - PCR



Tested effects of the following on *Hematodinium* presence:

- Season (winter [Dec, Jan, Feb], spring [Mar, Apr, May], summer [Jun, Jul, Aug], autumn [Sept, Oct, Nov])
- Size (Carapace width, CW [continuous number])
- Sex (male or female)
- Colour (green, yellow or orange)
- Pigment loss (0 or 1)
- Haemolymph opacity (milky or clear)
- Fouling (presence of epibionts, 0 or 1)
- Limb loss (0 or 1)
- Location (Pier or Dock)

Davies et al. (2019) *Parasites & Vectors*
doi: 0.1186/s13071-019-3727-x

Results - PCR

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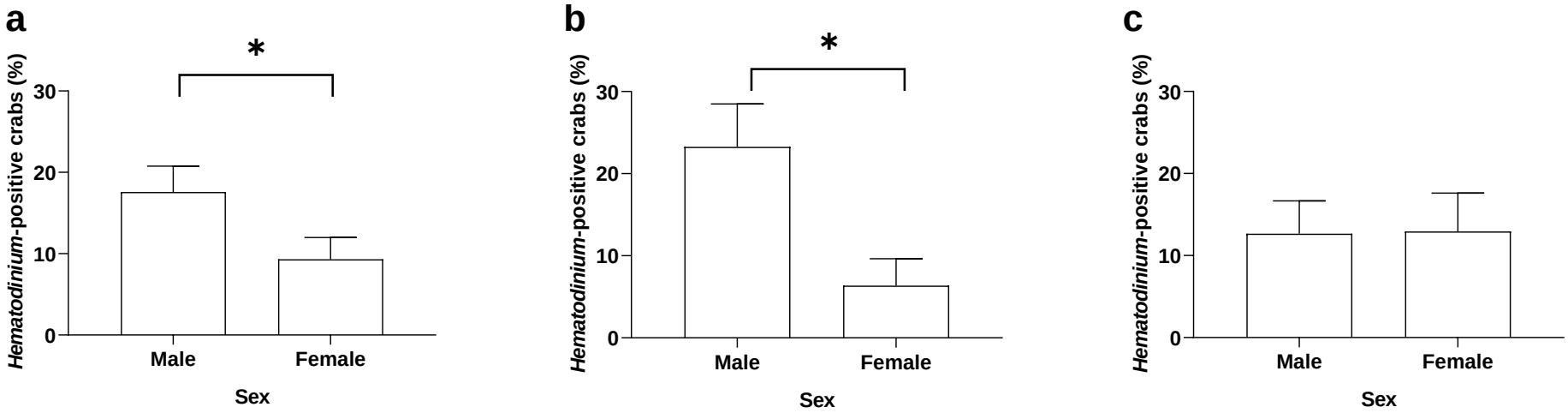
Total
13.6% infected

Dock
14.4% infected

Pier
12.8% infected



Sex
Males nearly twice as likely to become infected overall.

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Results - PCR

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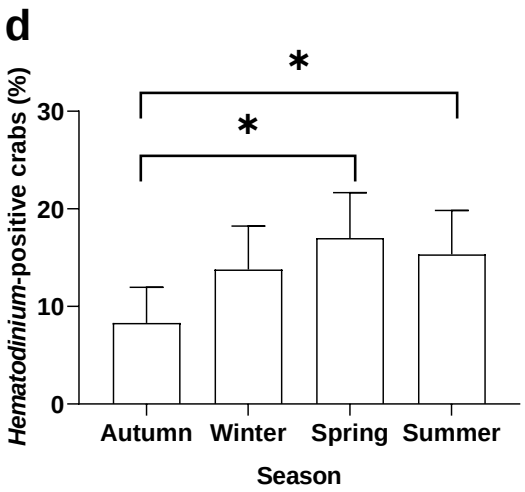
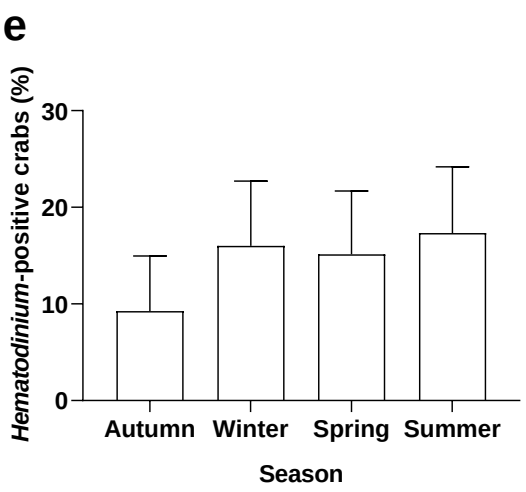
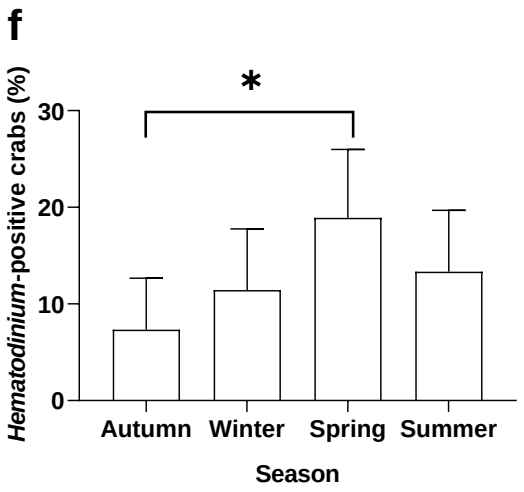
Total



Dock



Pier



Season

Crabs in spring and summer more likely to be infected.

Davies et al. (2019) *Parasites & Vectors*
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Results - PCR

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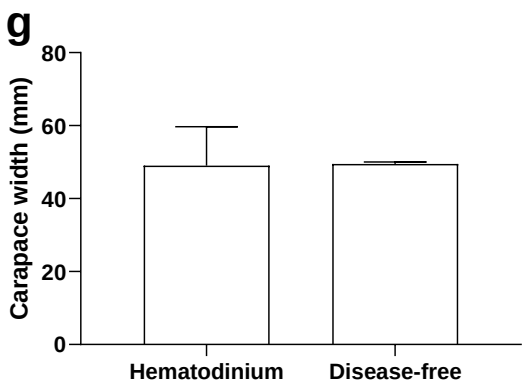
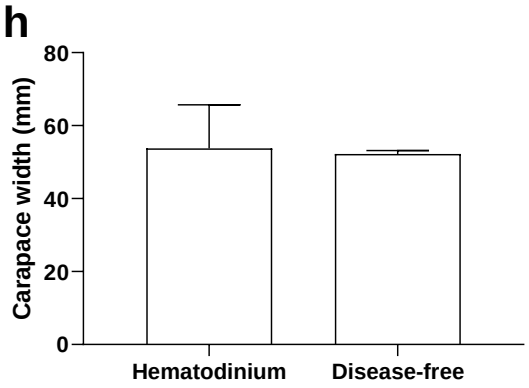
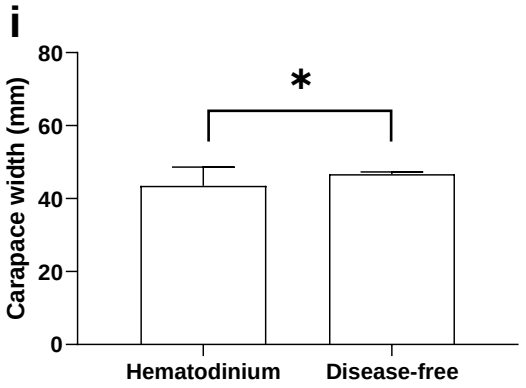
Total



Dock



Pier



Size

Smaller crabs more likely to be infected, in **Pier** location only.

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Results

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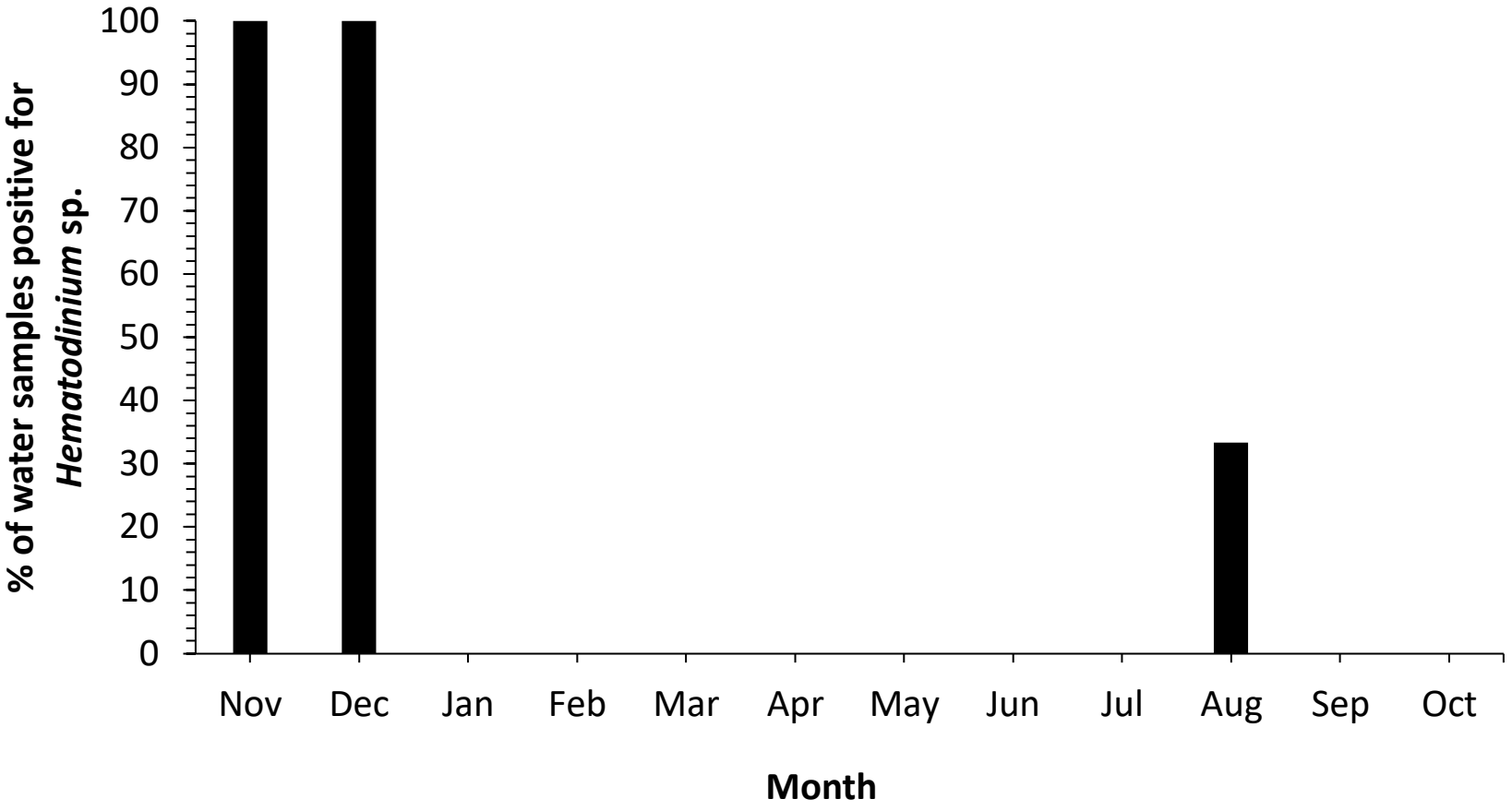
Water eDNA

Hematodinium sp. ONLY found in the **Pier**, not the Docks.



Davies et al. (2019) Parasites & Vectors
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Life Cycle

Possible *Hematodinium* sp. life cycle?

Spring

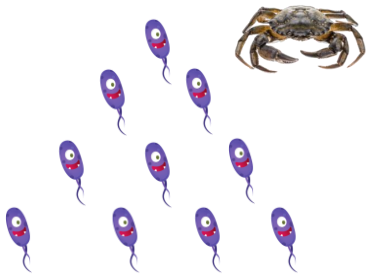
Summer

Autumn

Winter

Peak **prevalence** of *Hematodinium* sp. in crabs

- Multiplication of *Hematodinium* sp. in crab tissues



Highest **severity** of *Hematodinium* sp. infection in gills, hepatopancreas and haemolymph of crabs 

Severly infected, moribund crabs release infective stage dinospores into water Infection of new crabs *Hematodinium* sp. found in **eDNA** from water column

Davies et al. (2019) Parasites & Vectors doi: 0.1186/s13071-019-3727-x

Co-infections

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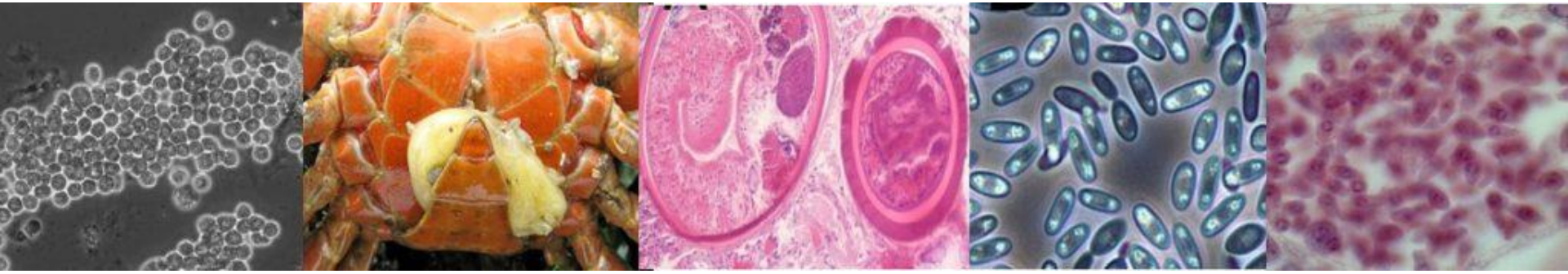
Pathogen	*C. maenas*		Seawater eDNA	
	Dock	Pier	Dock	Pier
Haplosporidia	✗	✓	✓	✓
Microsporidia	✓	✗	✓	✗
Mikrocytids	✗	✗	✓	✓
Paramyxids	✗	✗	✗	✓
Vibrio spp.	✓	✓	✓	✓
Fungal species	✗	✓	✓	✓
Trematode parasites	✗	✓	NA	NA
Sacculina sp.	✓	✗	NA	NA

Davies et al. (2021) *Preprint. BioRxiv* DOI: 10.1101/2021.05.26.445743



Co-infections

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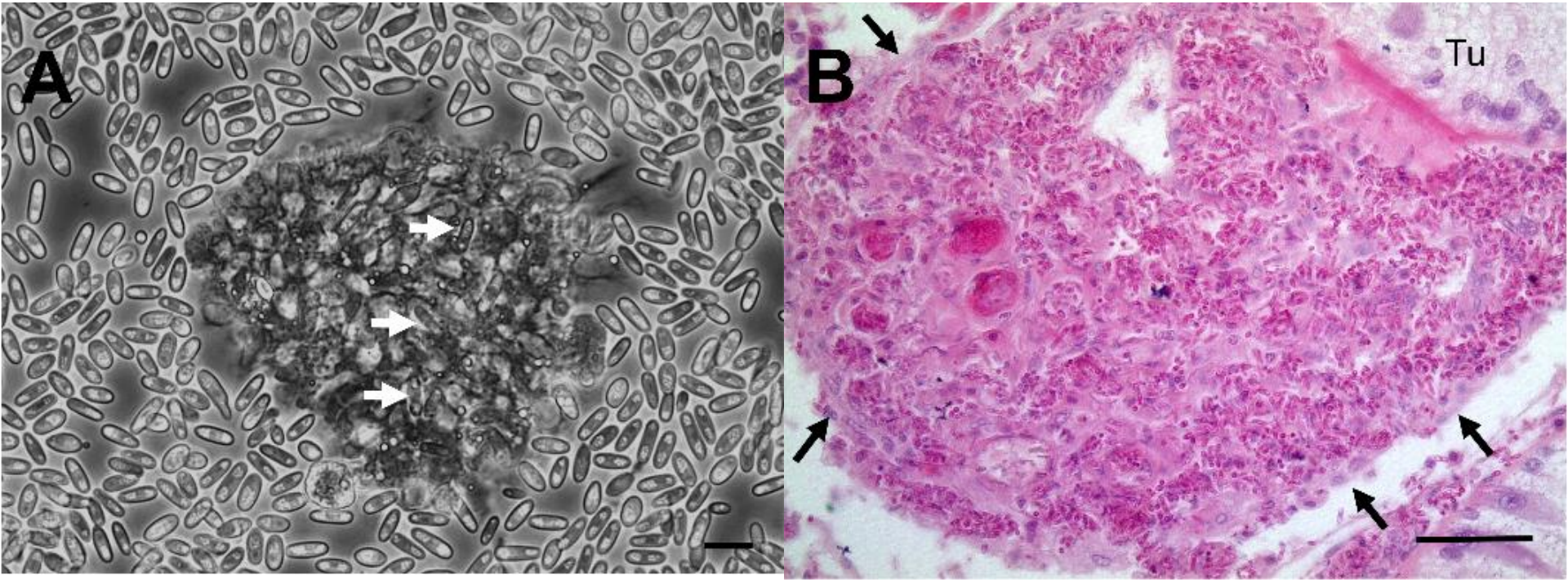
Fungi, or a novel mycosis?



Davies et al. (2020) *Pathogens* doi:10.3390/pathogens9060462

Fungi

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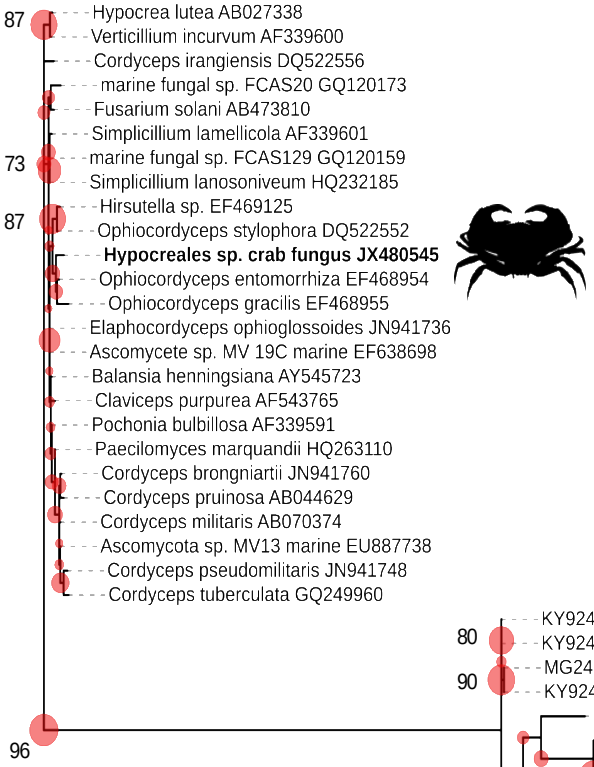
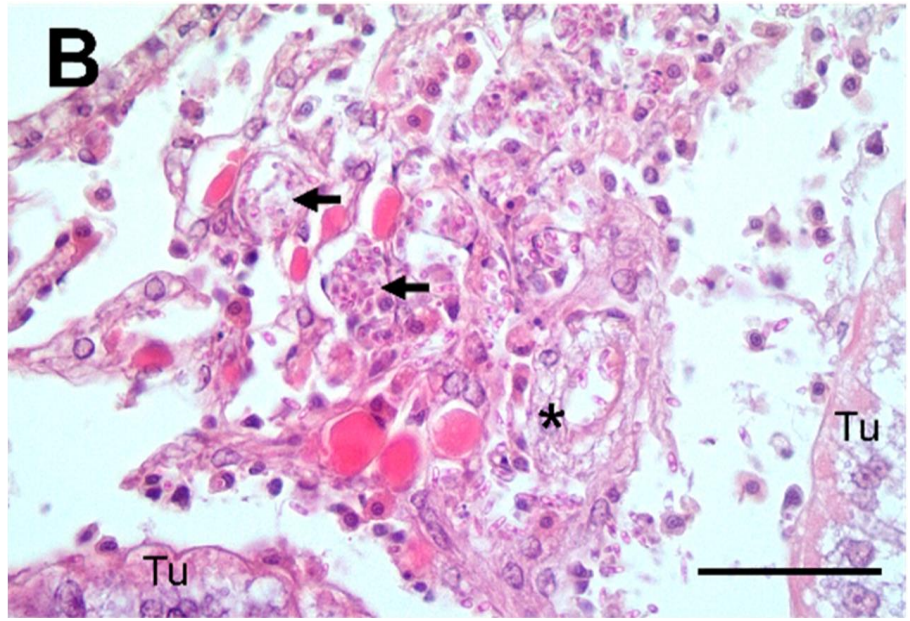
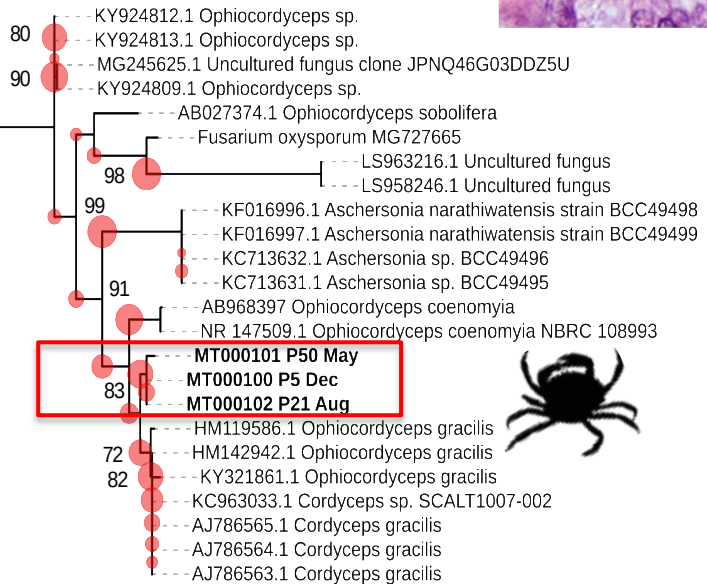
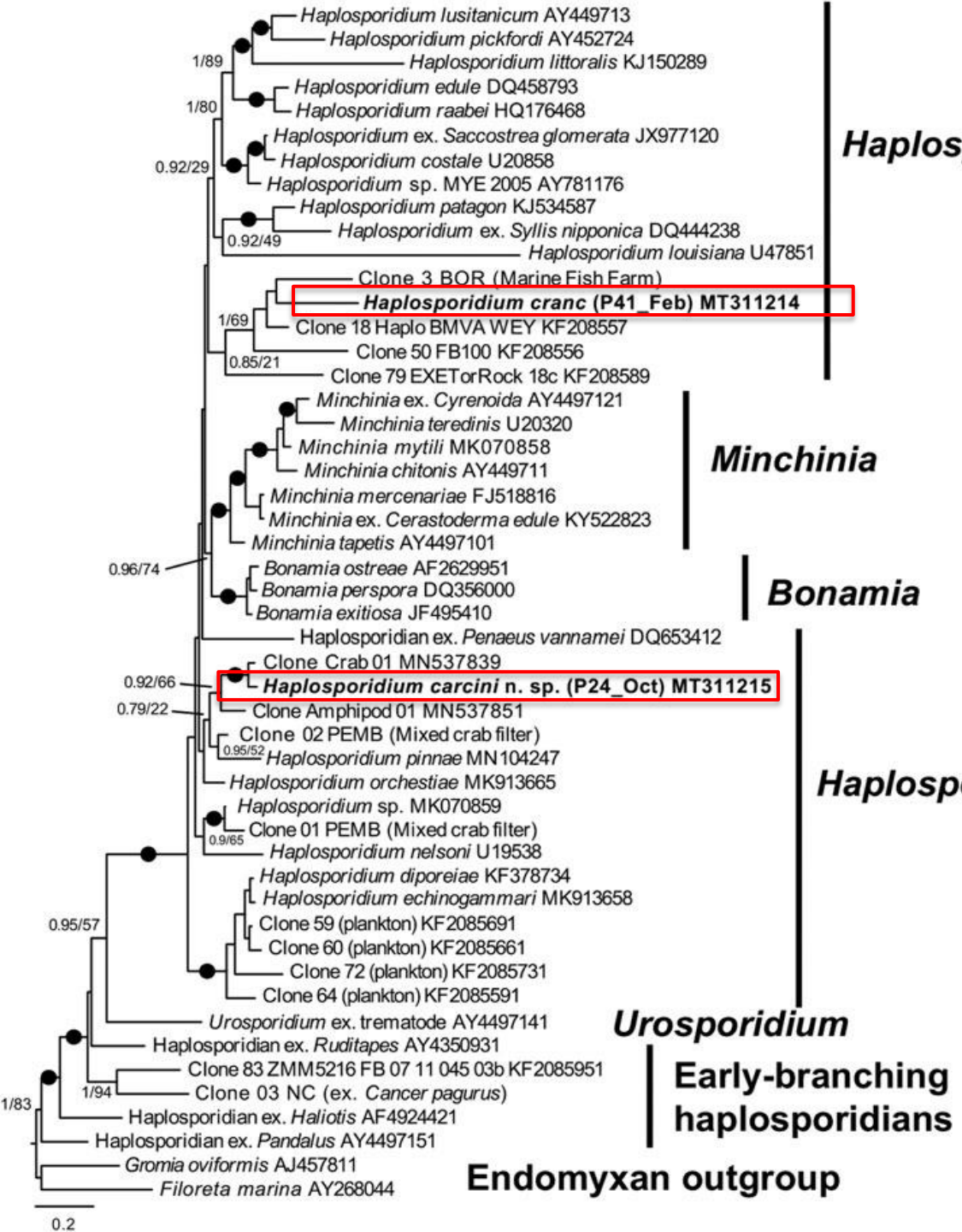






Tree scale: 0.1

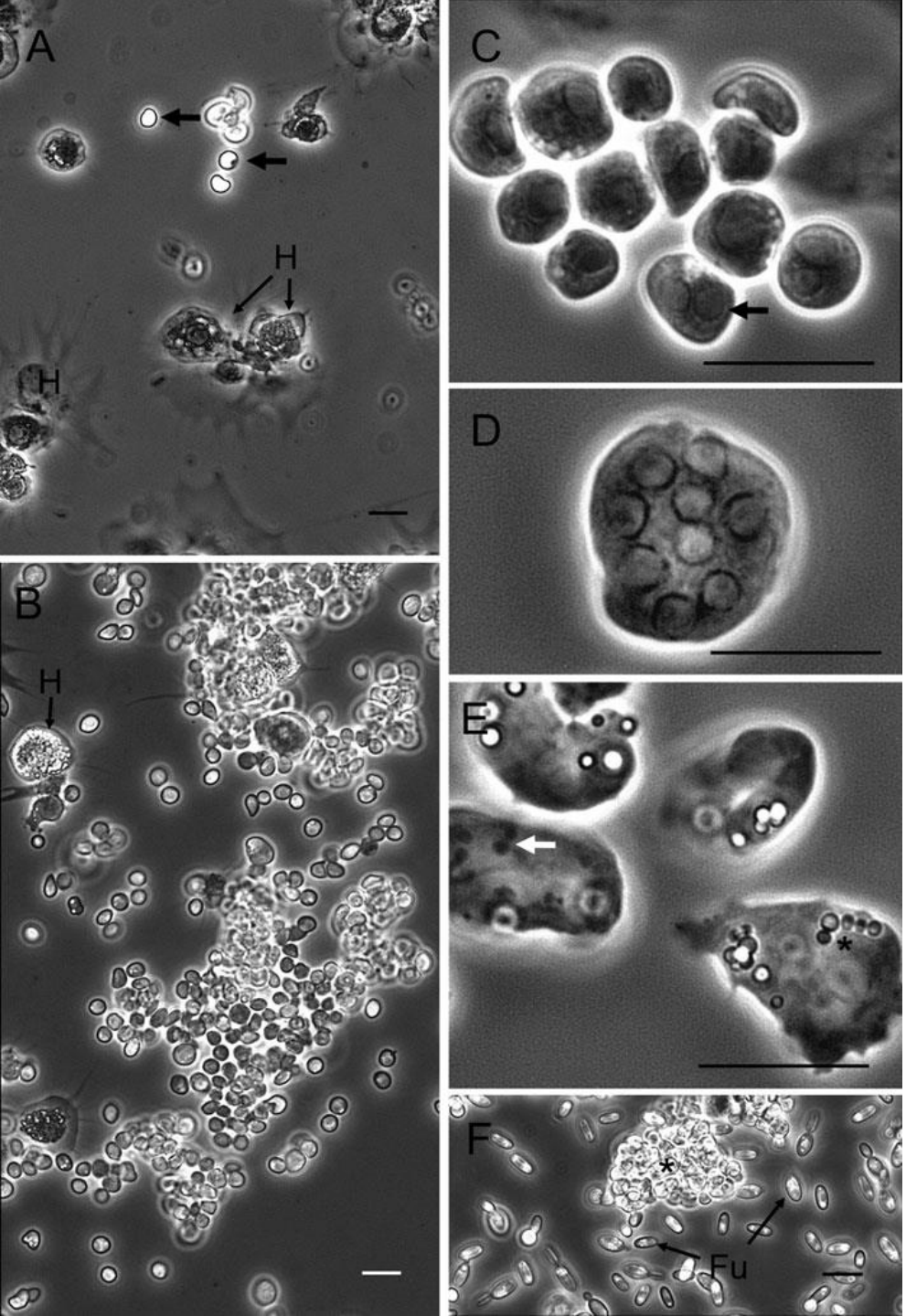
Davies et al. (2020) *Pathogens*
doi:10.3390/pathogens9060462



Co-infections

Haplosporidia, two new species!

Davies et al. (2020) *Parasitology*
doi: 10.1017/S0031182020000980



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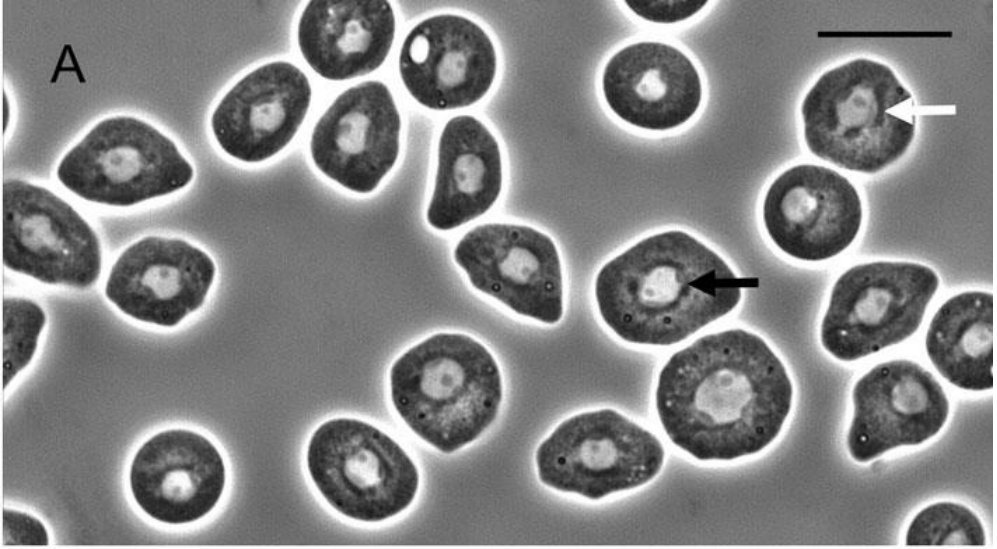
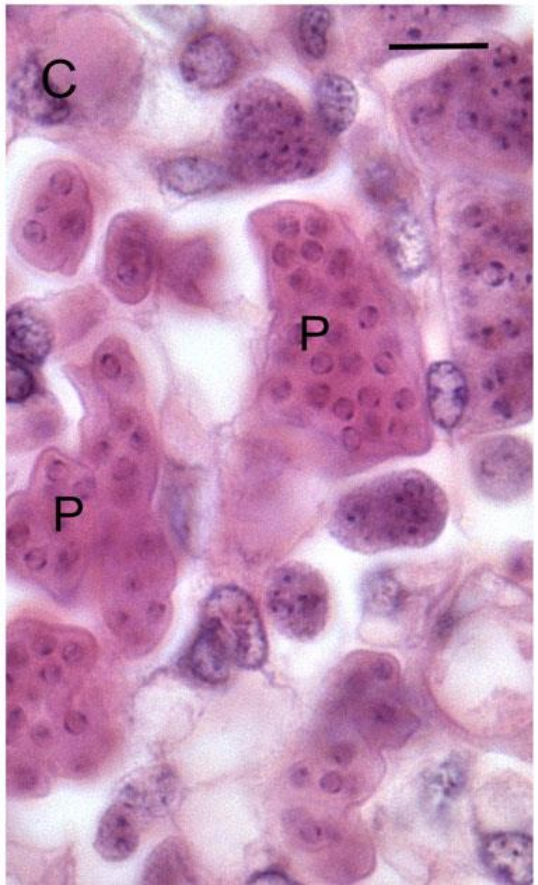
Haplosporidium carcini

(A). Apparent low-grade infection with adherent haemocytes (H) and non-adherent, refractile haplosporidia (unlabelled arrows).
(B). Higher severity infection with numerous uninucleate parasites and smaller number of haemocytes (H).
(C). Uni- and bi-nucleate (arrow) forms of parasite.
(D). Multinucleated plasmodium.
(E). Spread cells of unknown origin with cytoplasmic refractile bodies (*) and bacteria-like structures (arrow).
(F). Coinfection of H. carcini (*) and an unidentified yeast-like fungus (Fu). Scale bars = 10 µM.

Davies et al. (2020) *Parasitology*
doi: 10.1017/S0031182020000980





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Haplosporidium cranc

(A). Mononucleated haplosporidians in the haemolymph of an infected crab. Note unusual chromatin arrangement (unlabelled arrows).
(B). Uninucleate haplosporidians in the terminal vessel of a gill lamella. Note prominent chromatin blocks in these cells (unlabelled arrow).
(C). Multinucleated plasmodial forms (P) of this parasite in the interstitial space of the hepatopancreas. Scale bars = 10 µM.

Davies et al. (2020) *Parasitology*
doi: 10.1017/S0031182020000980

Co-infections

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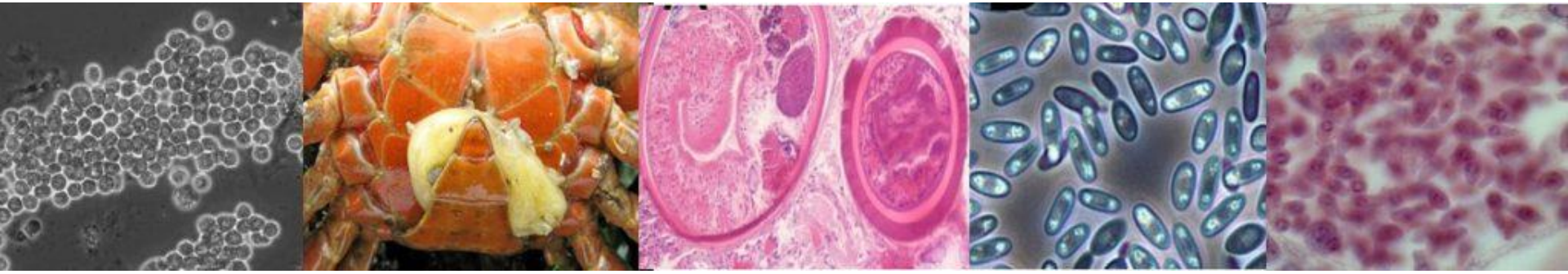
Pathogen	*C. maenas*		Seawater eDNA	
	Dock	Pier	Dock	Pier
Haplosporidia	✗	✓	✓	✓
Microsporidia	✓	✗	✓	✗
Mikrocytids	✗	✗	✓	✓
Paramyxids	✗	✗	✗	✓
Vibrio spp.	✓	✓	✓	✓
Fungal species	✗	✓	✓	✓
Trematode parasites	✗	✓	NA	NA
Sacculina sp.	✓	✗	NA	NA

Davies et al. (2021) *Preprint. BioRxiv* DOI: 10.1101/2021.05.26.445743



Co-infections

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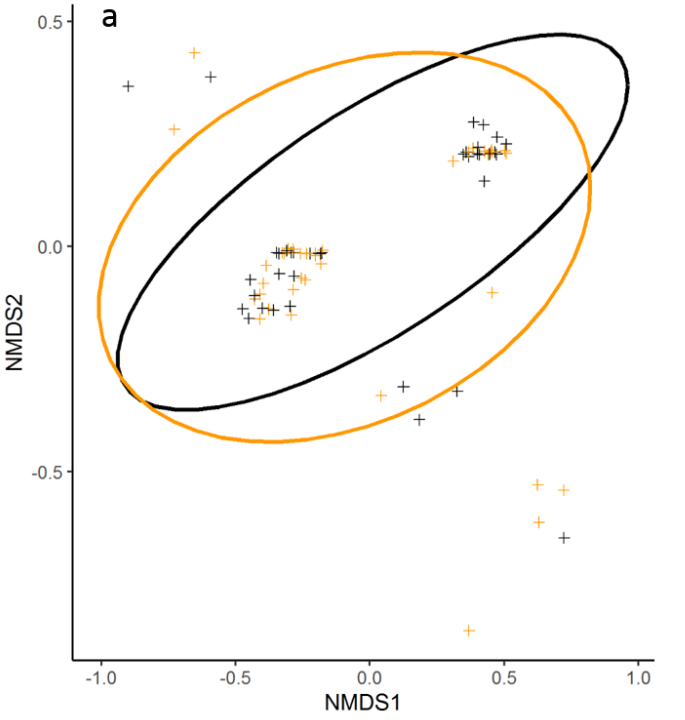

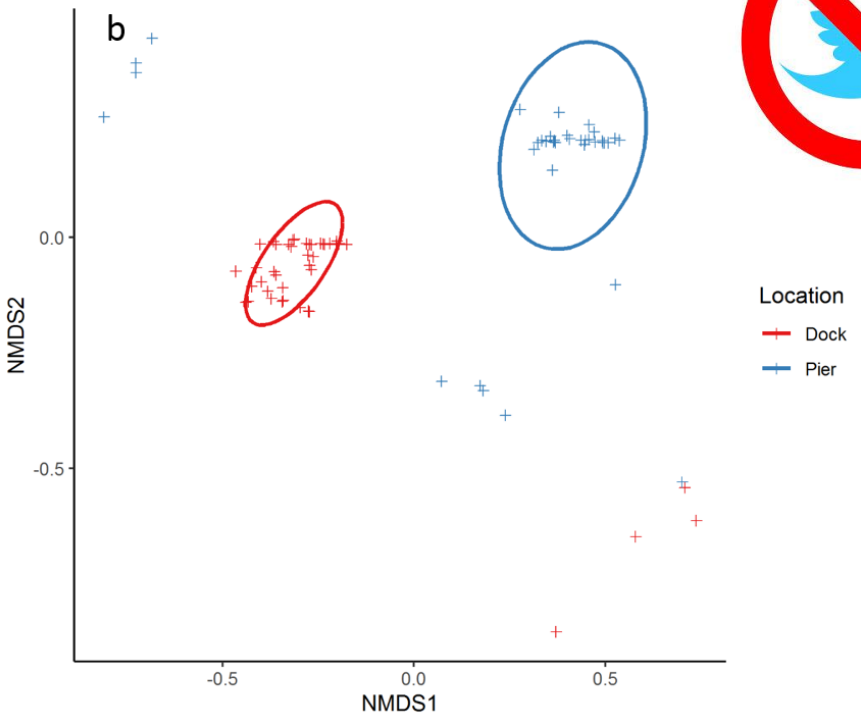

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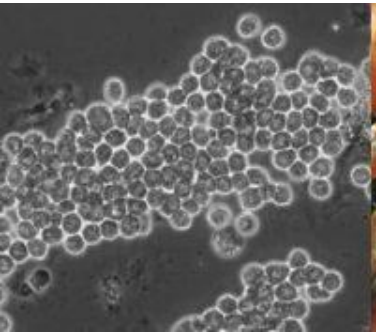

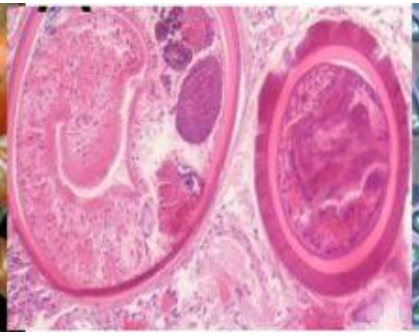

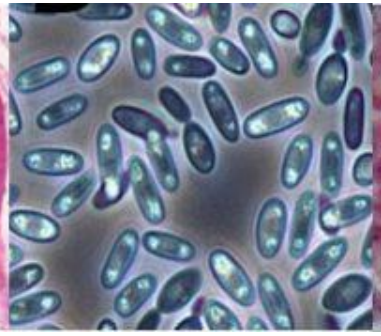

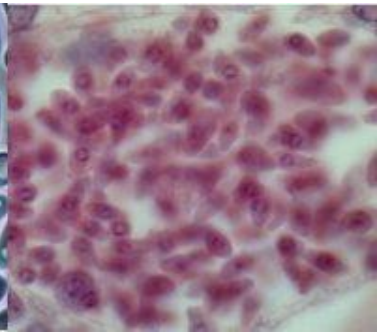

Conclusions

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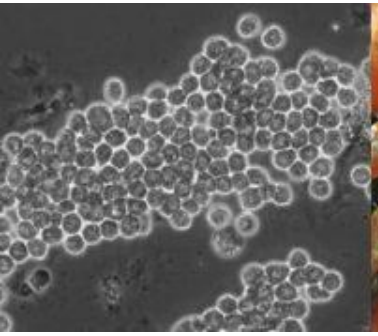
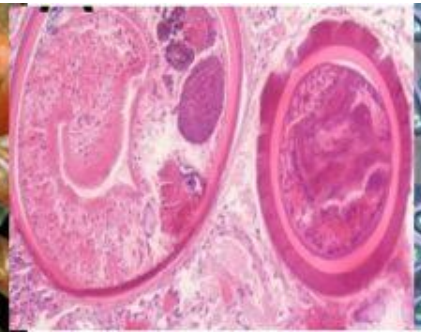
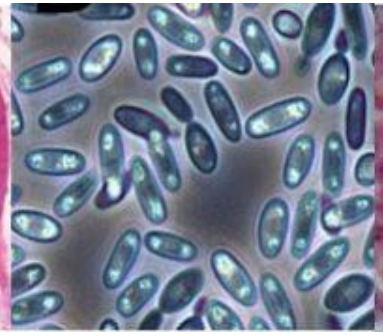
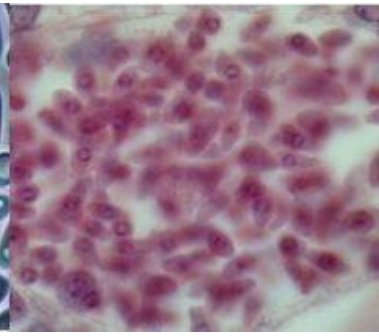
- A systematic approach is always best
  - Using multiple detection methods
- eDNA can reveal difference stages of life cycles which may not be present in the host
- Location/environment may play a role in co-infection and disease dynamics
  - As opposed to only the host ecology



Thank you

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Colleagues and students:

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- Frederico Batista, David Bass, Kelly Bateman and Stephen Feist at **Cefas**.
- Georgia Ward at the **Natural History Museum**.

- **Davies et al.** (*preprint*) Environment, rather than *Hematodinium* parasitization, determines collateral disease contraction in a crustacean host. *BioRxiv* DOI: 10.1101/2021.05.26.445743
- **Davies et al.** (2020) Diagnosis and prevalence of two new species of haplosporidians infecting shore crabs *Carcinus maenas*: *Haplosporidium carcini* n. sp., and *H. cranc* n. sp. *Parasitology* **147:**1229–1237 DOI: 10.1017/S0031182020000980
- **Davies et al.** (2020) Mycosis is a Disease State Encountered Rarely in Shore Crabs, *Carcinus maenas*. *MDPI Pathogens* **9**(6):462 DOI: 10.3390/pathogens9060462
- **Davies et al.** (2019) Spatial and temporal disease dynamics of the parasite *Hematodinium* sp. in shore crabs, *Carcinus maenas*. Parasites & Vectors **12**:472 DOI: 0.1186/s13071-019-3727-x

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