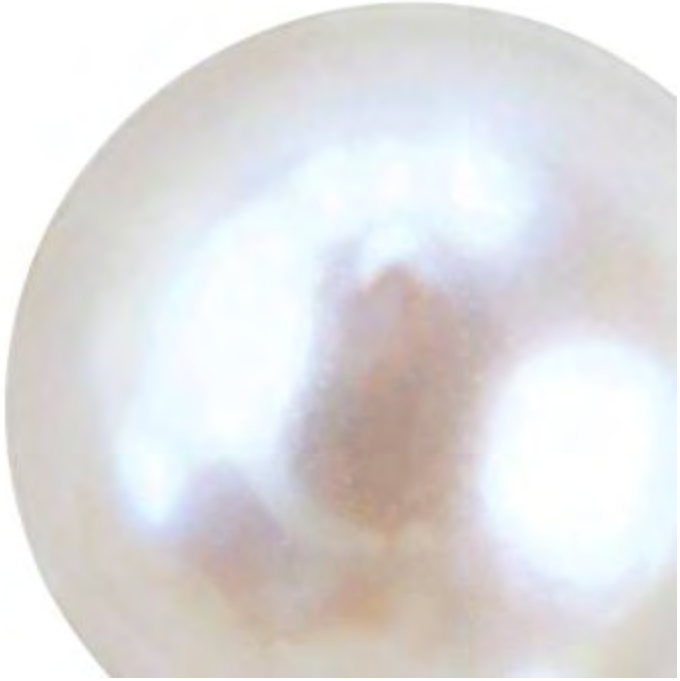
PML | Plymouth Marine Laboratory

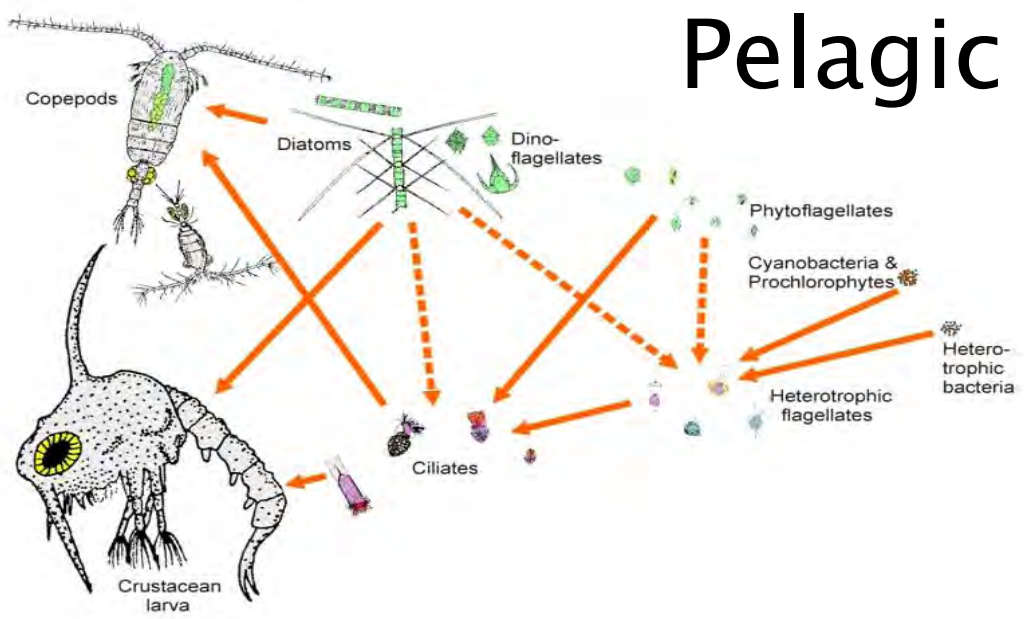
Marine Matters

Meat and two veg? Determining feeding selectivity of bivalve larvae in the Western English Channel with traditional and molecular techniques.



Pennie Lindeque, Elaine Fileman,
Claudia Halsband-Lenk, Helen Parry.

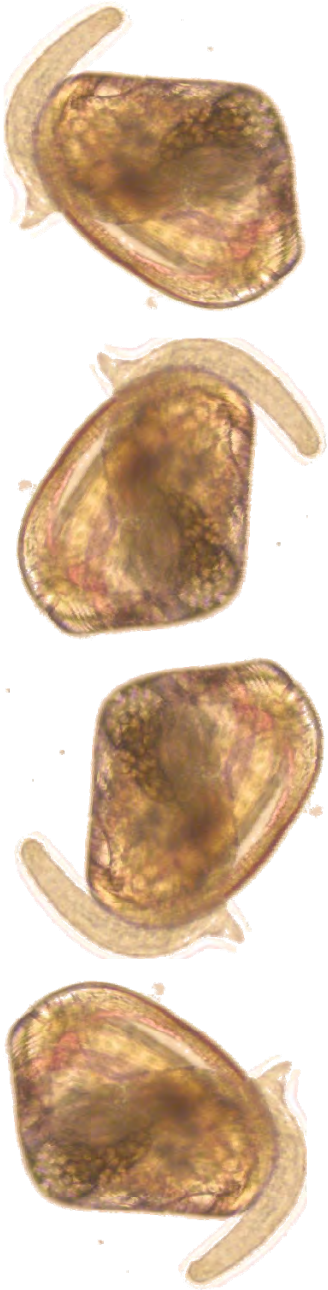
Pelagic



Meroplankton

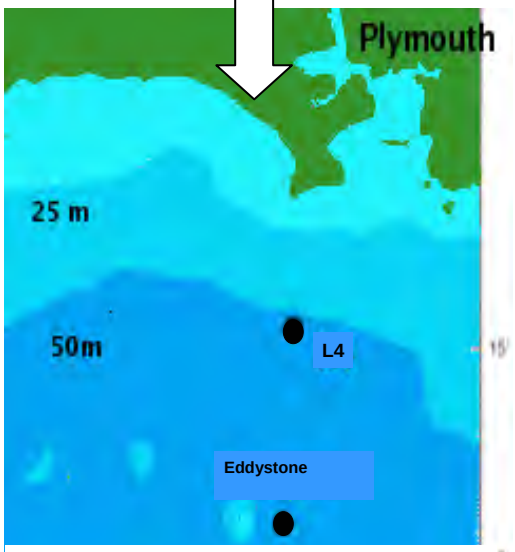
– an under-researched component

Benthos



Bivalve larvae

- Bivalves produce large numbers of pelagic larvae
- These larvae are an important dispersal mechanism for bivalves
- Compete with other plankton grazers
- Source of prey for fish larvae and other organisms
- Need to gain sufficient energy and nutrients to metamorphose and develop so they can recruit to the seafloor



Study Site - Station L4
50.258ºN, 04.2178ºW

Aim

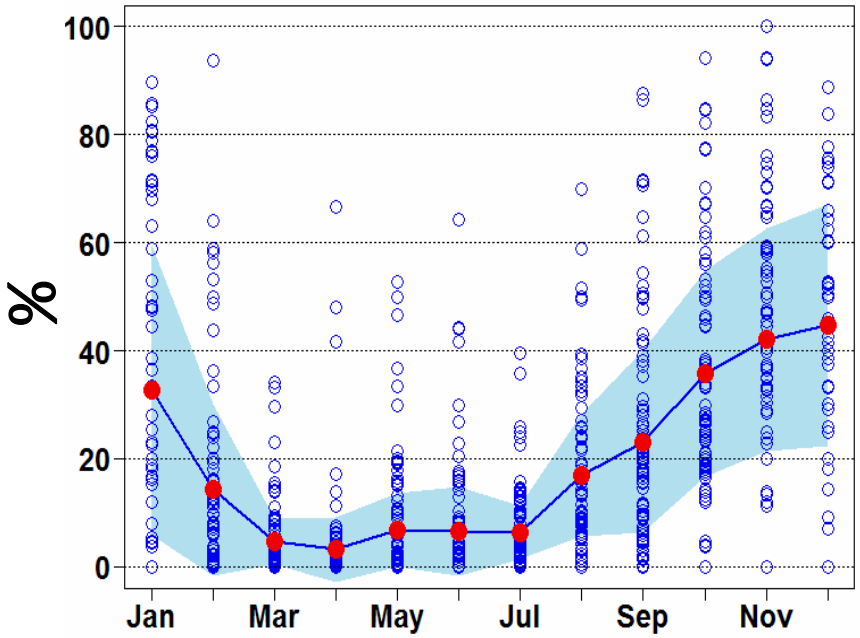
Quantify impact of bivalves in pelagic foodweb of the Western English Channel over a seasonal cycle

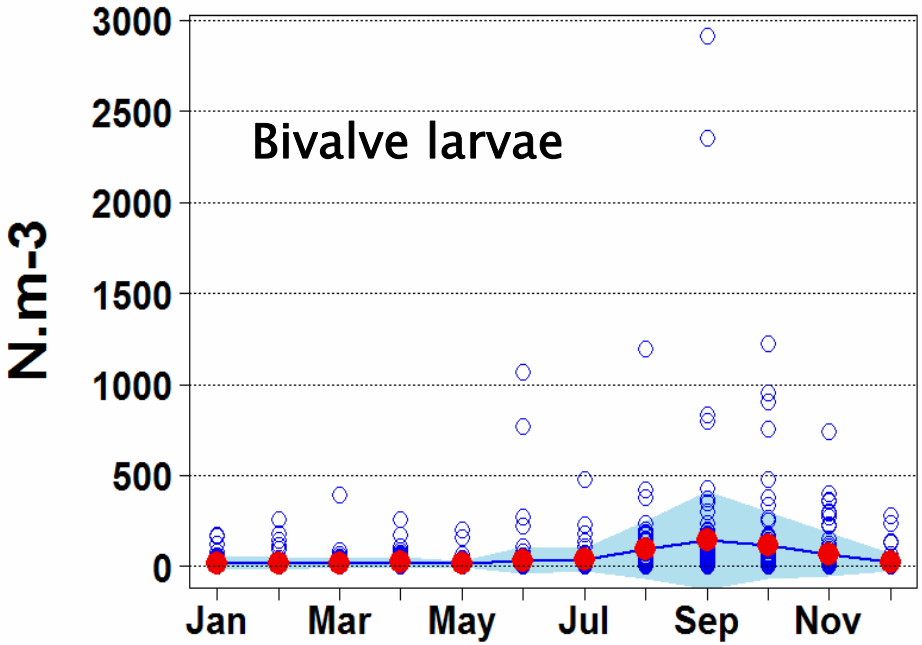
Objectives

- Conduct series of experiments to determine bivalve larval feeding selectivity and rates
  - Complimentary gut content analysis using molecular techniques to compare feeding in the experiments and the field
- Molecular identification of bivalve larvae

Western Channel Observatory (Station L4): 20 yr averages (1988-2008)

Bivalves % total meroplankton





Bivalve larvae feeding experiments

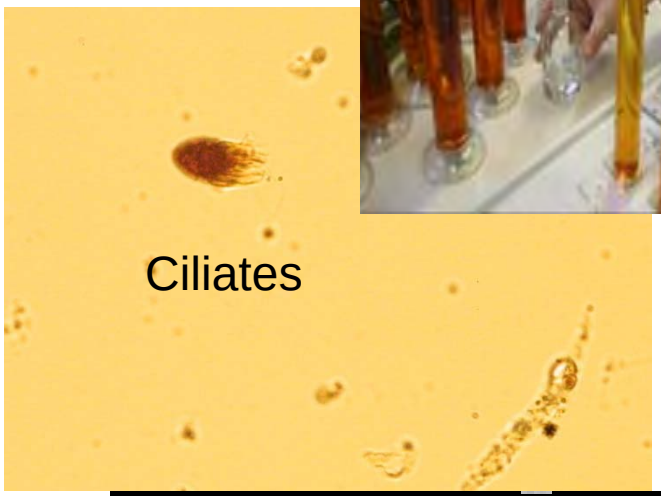
Determine larval feeding rate on the natural mixed plankton assemblage

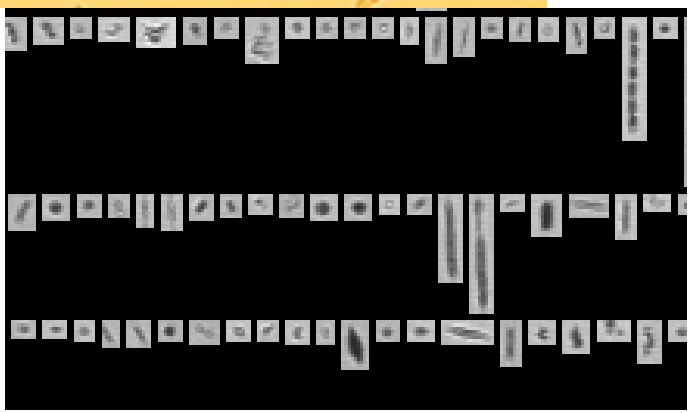
Methods

- Water and live zooplankton collected from L4 (WP2 vertical net)
- ~230 bivalve larvae isolated
- Water screened to remove competitive grazers
- 24 hour incubation of bivalves on plankton wheel at ambient sea temperature
- Feeding rates calculated from changes in food concentration measured at beginning and end of experiment

Plankton Community Analysis

Fluorometry	Chlorophyll a
FlowCAM	all cells 5-50µM
Flow Cytometry	*Synechococcus* picoeukaryotes (0.2-2.0µM) nanoeukaryotes (2-20 µM) (cryptophytes, coccolithophores)
Microscopy	small ciliates <30µM





Grazing Results

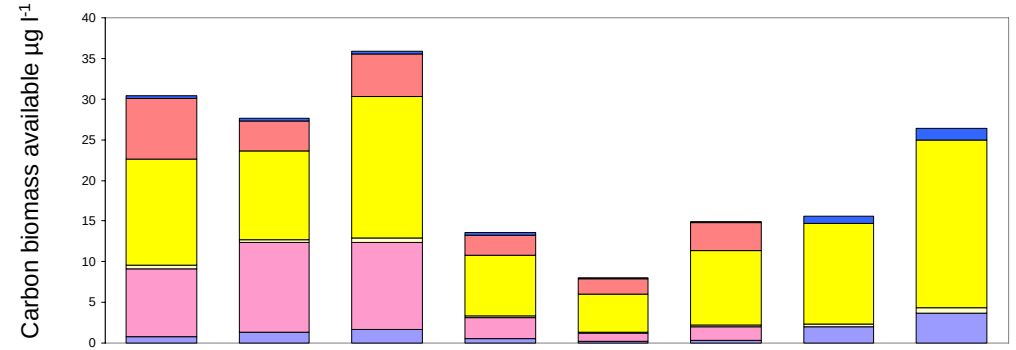
Fluorometry	
Experiment	Chlorophyll
1	**
2	***
3	***
4	***
5	***
6	***
7	***
8	***

FlowCam	
Experiment	5µm <50µm Particles
1	**
2	**
3	**
4	tbd
5	tbd
6	tbd
7	tbd
8	tbd

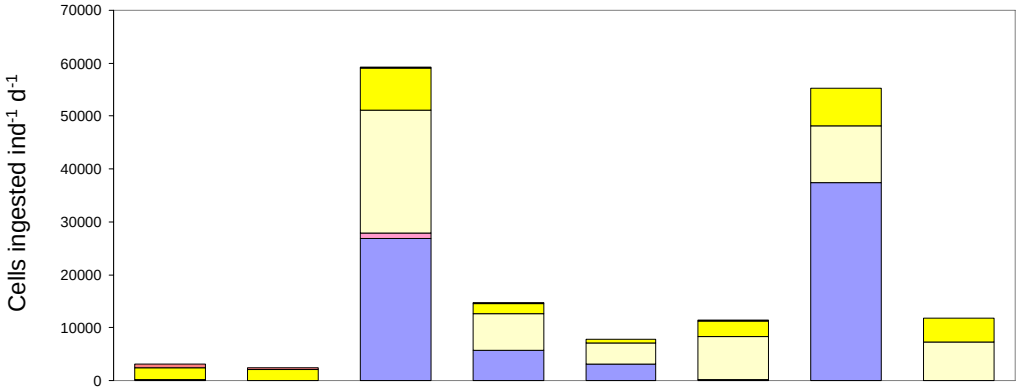
Microscopy	
Experiment	Ciliates
1	*
2	*
3	*
4	***
5	*
6	***
7	
8	*

Flow Cytometry						
Expt	Bacteria LNA	*Synechococcus*	Cryptophytes ~7-10µm	Picoeukaryotes <2µm	Nanoeukaryotes 2-20µm	Coccolithophores ~ 1-20µm
1					***	***
2					**	***
3	**	**	**	**	***	**
4	*	*		*	**	***
5		**		***	***	***
6	**		**	***	***	***
7	na	**	Na	**	***	na
8	na		Na	**	***	na

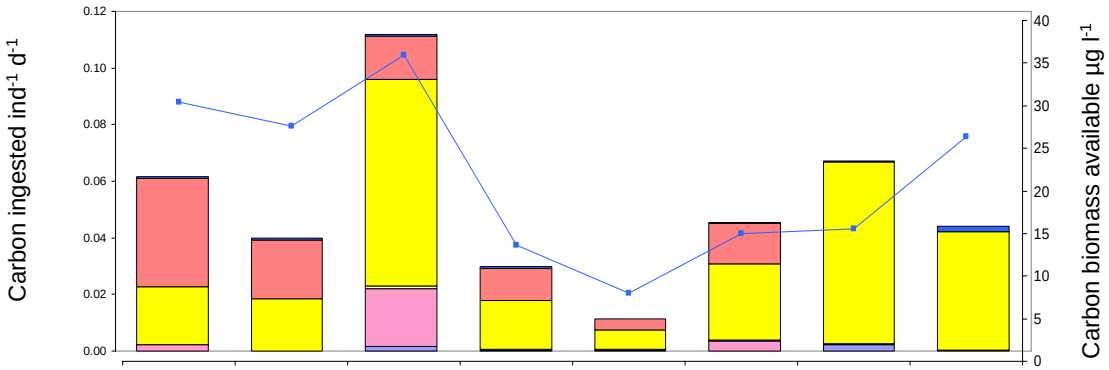
Prey Composition and Feeding Rates



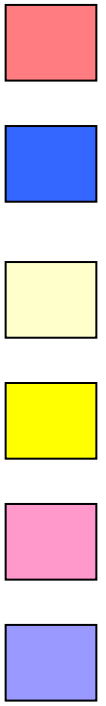
C Biomass available



Cells ingested

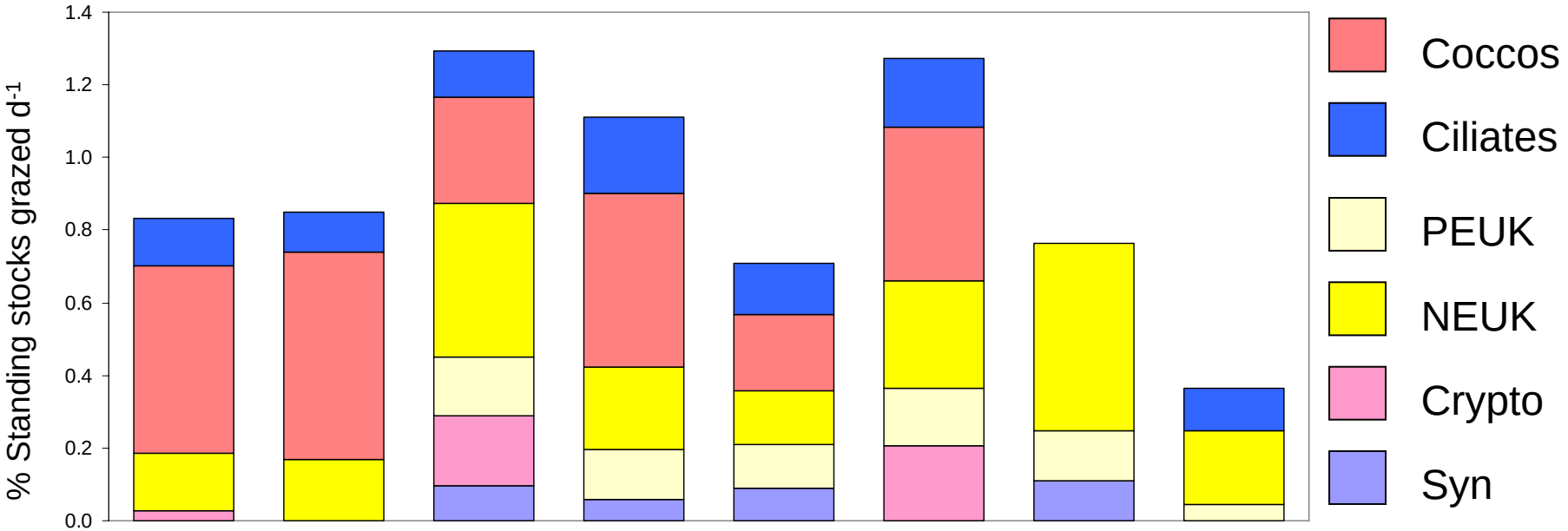


C ingested

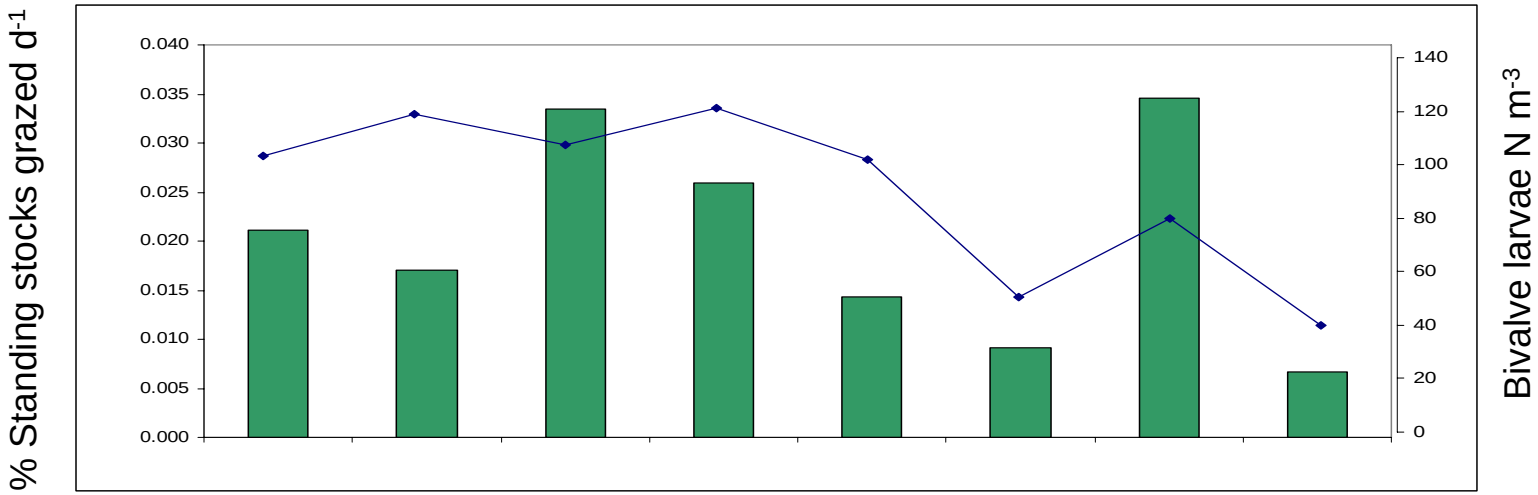


Late Autumn ‘09 **Winter ‘10** **Early Autumn ‘10**

Trophic impact in bottles



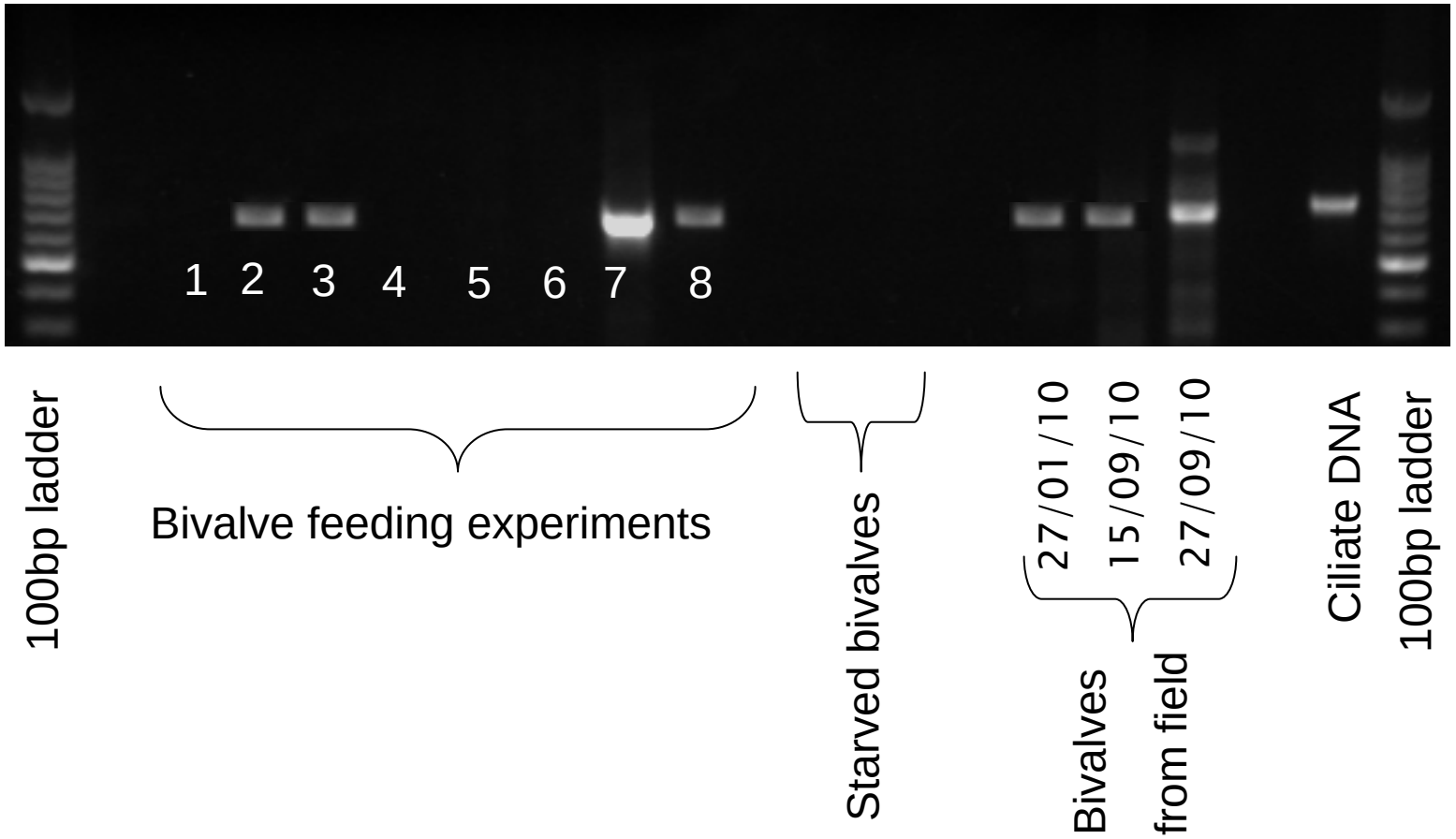
Trophic impact in field



Molecular approach to determine prey selection in meroplankton

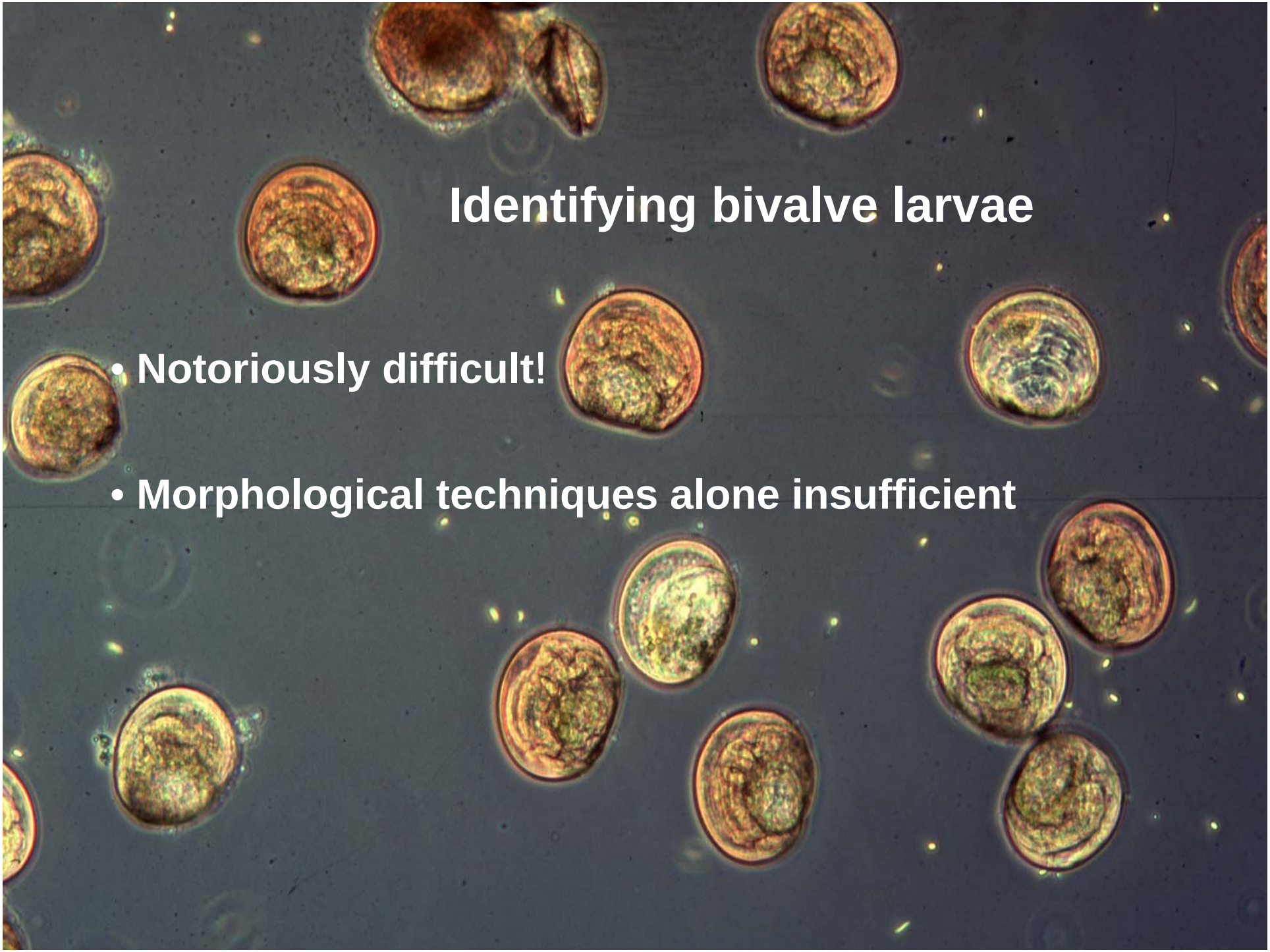
- Results from the feeding expt. can be used to determine what larvae eat.
- Primers can then be designed to the ingested prey
- Can we use these primers to:
  - Detect the prey in the gut of larvae after the feeding expt?
  - Detect the prey in the gut of larvae straight from the field?

Molecular detection of ciliates



•Primers from Dopheide et al (2008) used to amplify a 750bp fragment of 18S rDNA

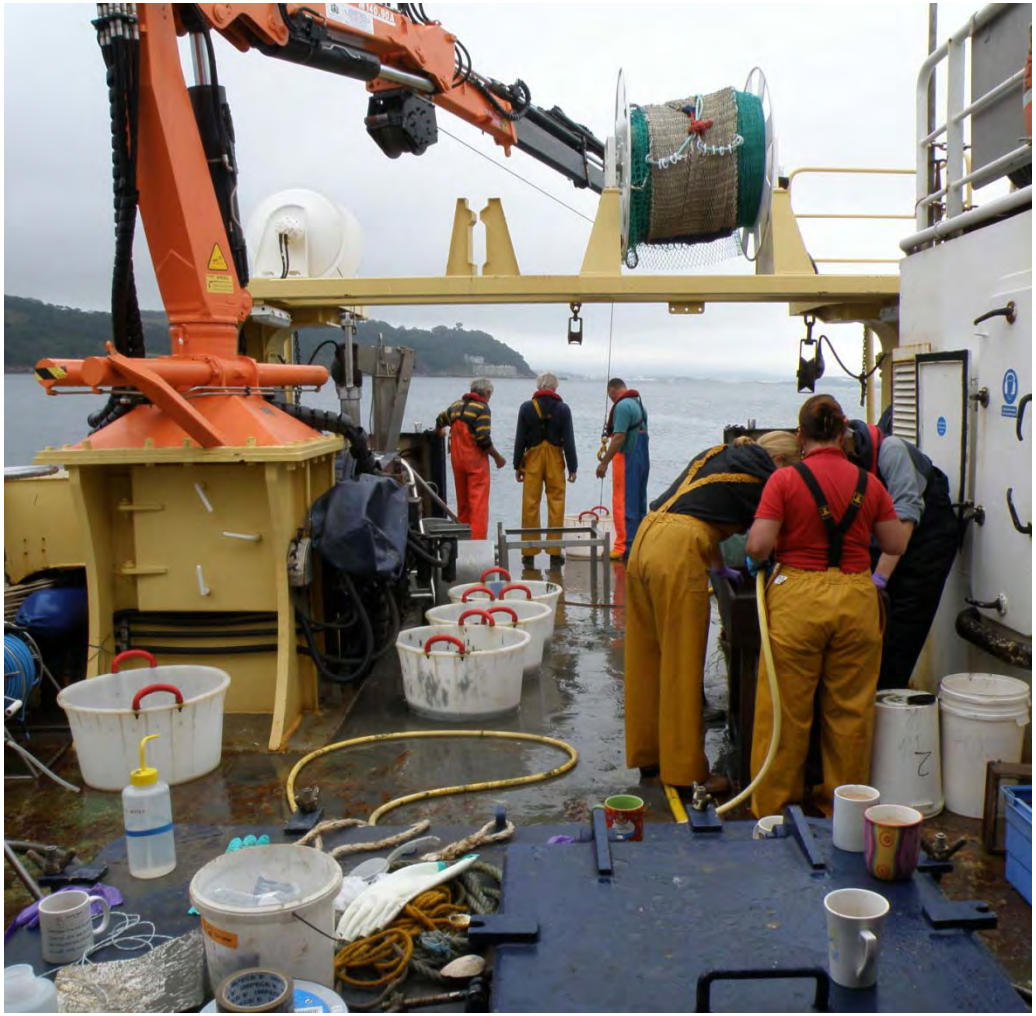
Dopheide et al (2008) Appl Env Microbiol 74 (6) pp1740-1747.



Identifying bivalve larvae

- Notoriously difficult!
- Morphological techniques alone insufficient

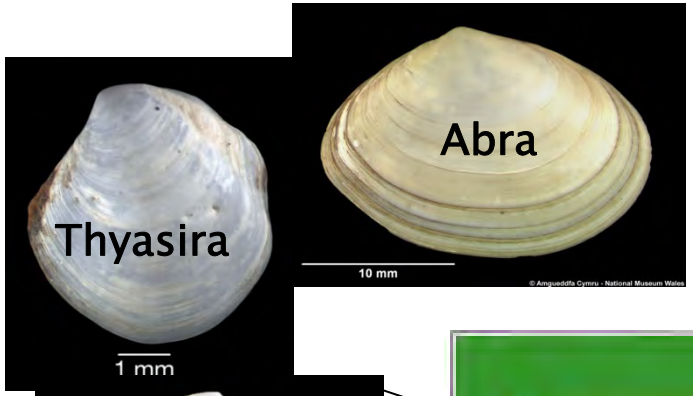
Benthic Survey

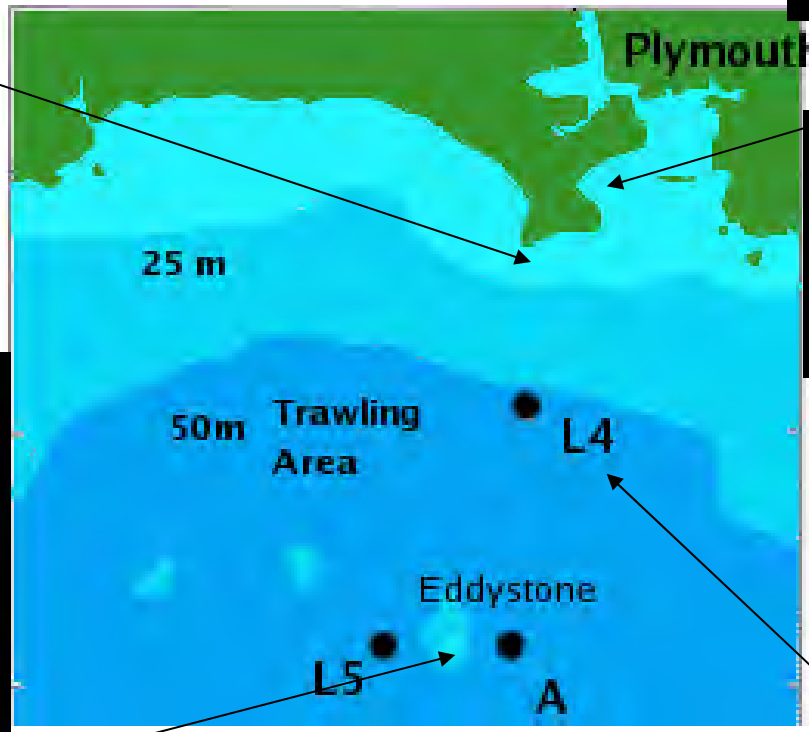


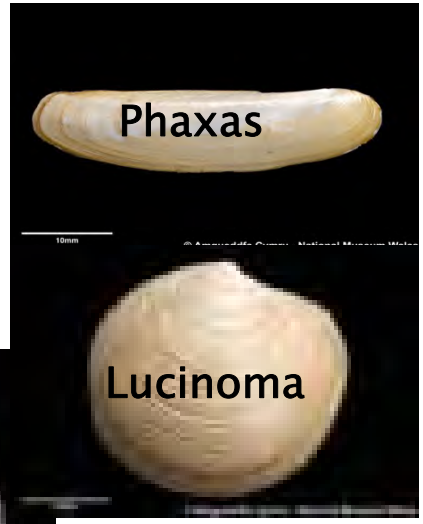
Sites

- Cawsand
- Hilmars Box (L4)
- Rame Mud
- Eddystone

Benthic Survey



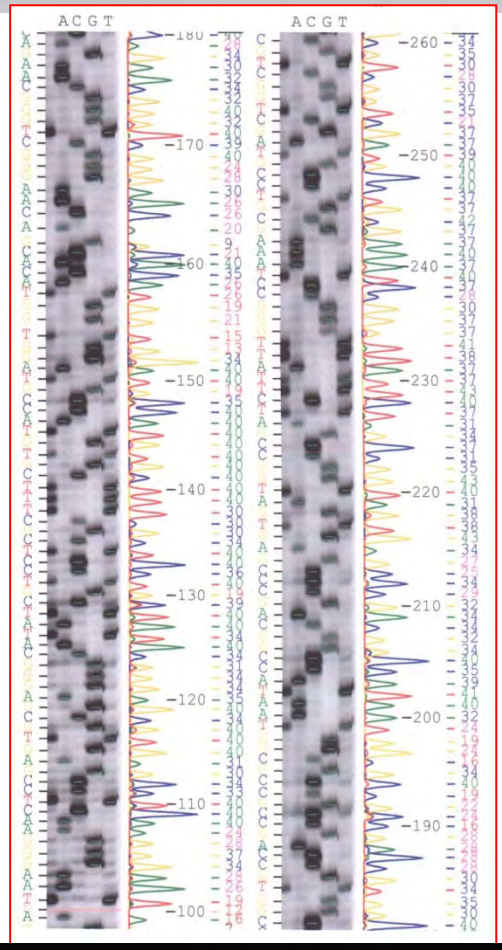


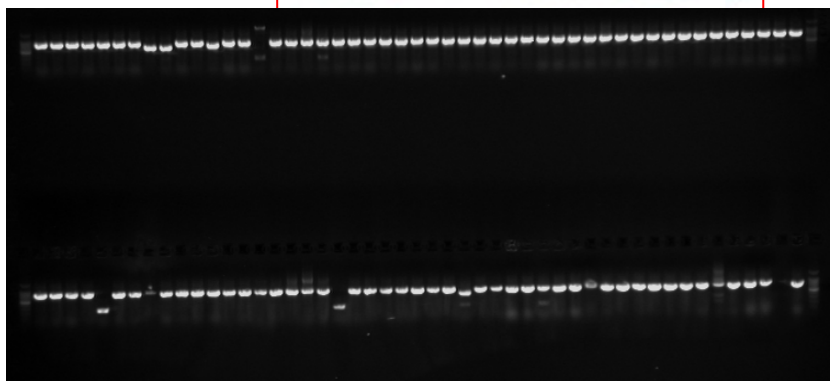


Molecular Identification

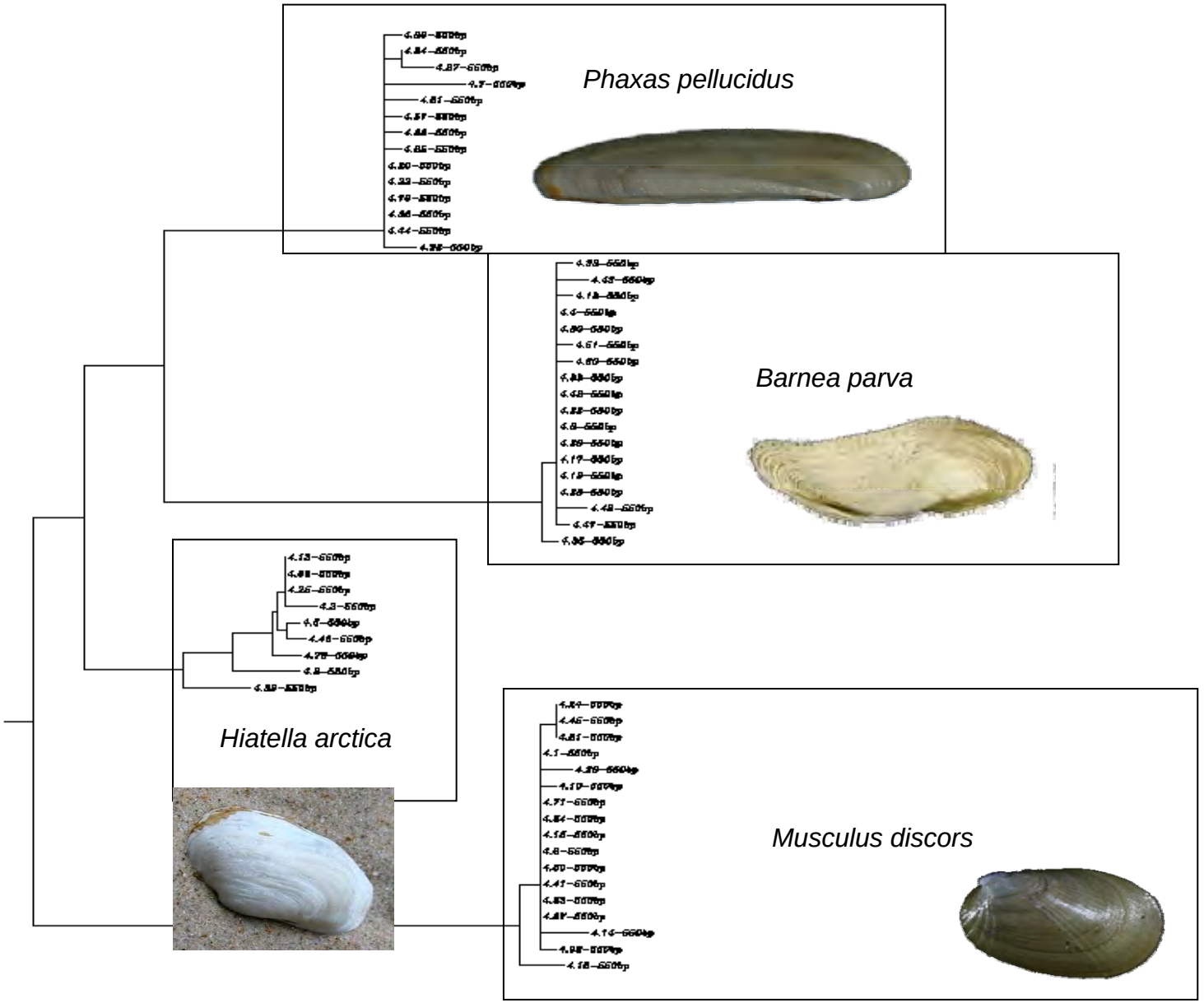
- Total DNA extraction from 200 bivalve larvae
- PCR amplification of a partial region of 18S rDNA gene with universal eukaryotic primers
- Clone library for each cohort of larvae
- >40 colonies sequenced from each clone library
- Sequences assigned to species by comparison with genetic database

  (>98% homology)



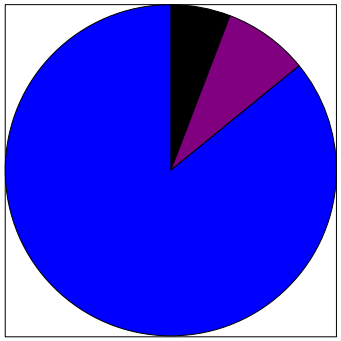


Example Experiment 3

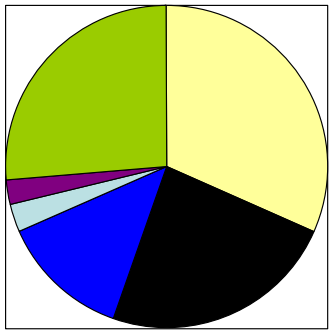


Seasonal comparison of species composition

19.10.09



27.10.09



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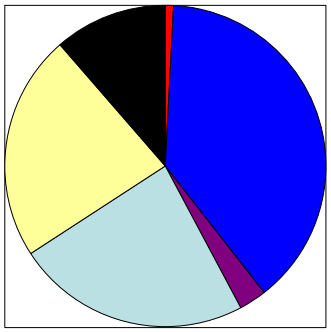

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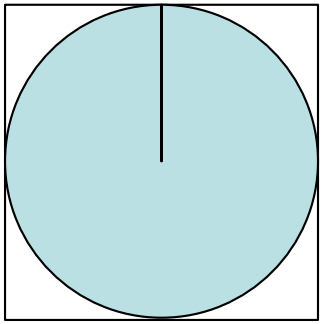

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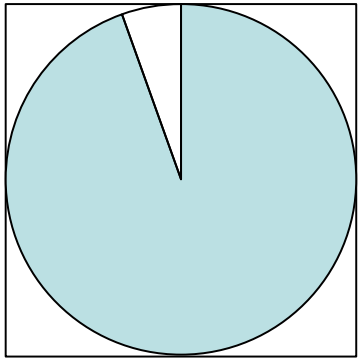

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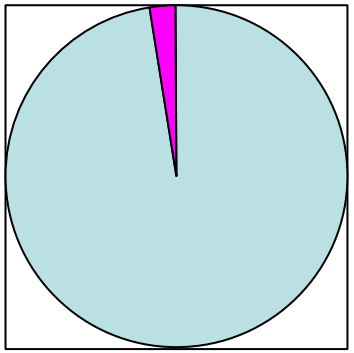

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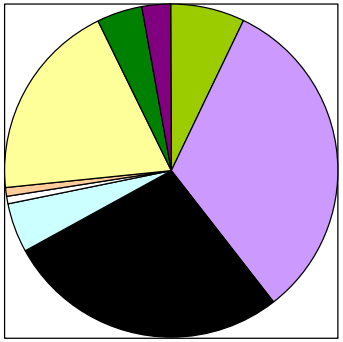

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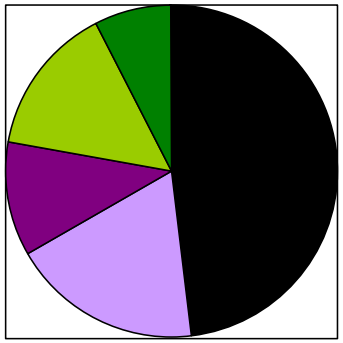
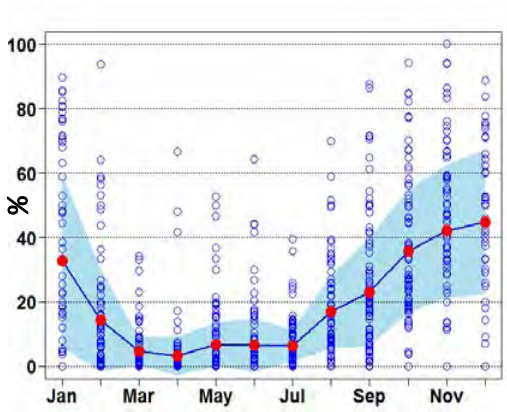


Legend:

Lasaea Musculus* Phaxas* Spisula* Pecten* Mysella*

Barnea Telina Mytilidae Sphenia Hiatella* Kellia

Boring Bivalves



Summary

- Seasonal distribution of bivalve larvae in the plankton
- Abundance can be 50 % total meroplankton
- What the bivalve larvae are
    - Seasonal differences in bivalve diversity
    - Morphologically similar larvae can belong to many different species
- What they are eating: Meat and two veg!
- Grazing rate
- Potential trophic impact
- Prey can be detected in bivalve guts with molecular markers directly from the field

Future Work:

- Prey selection indices
- Predation on bivalve larvae
- Respiration
- Survival and recruitment success

Reverse particle tracking

Sequence prey 18S

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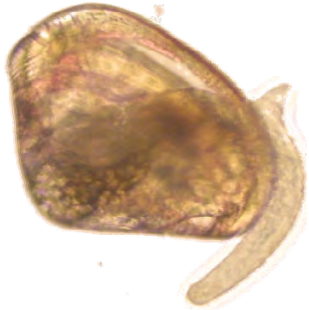

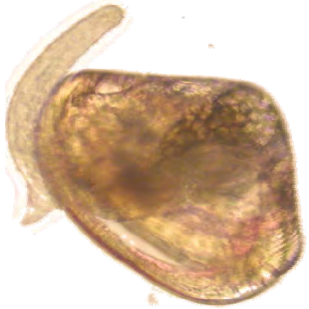

Acknowledgements

•Oceans 2025 Theme 4

• Theme Leader Paul Somerfield

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