Development of Autonomous Sampling Capability for Small Marine Organisms:

Species and stage – specific barnacle larval distributions obtained from AUV sampling and genetic analysis in Buzzards Bay, Massachusetts, USA

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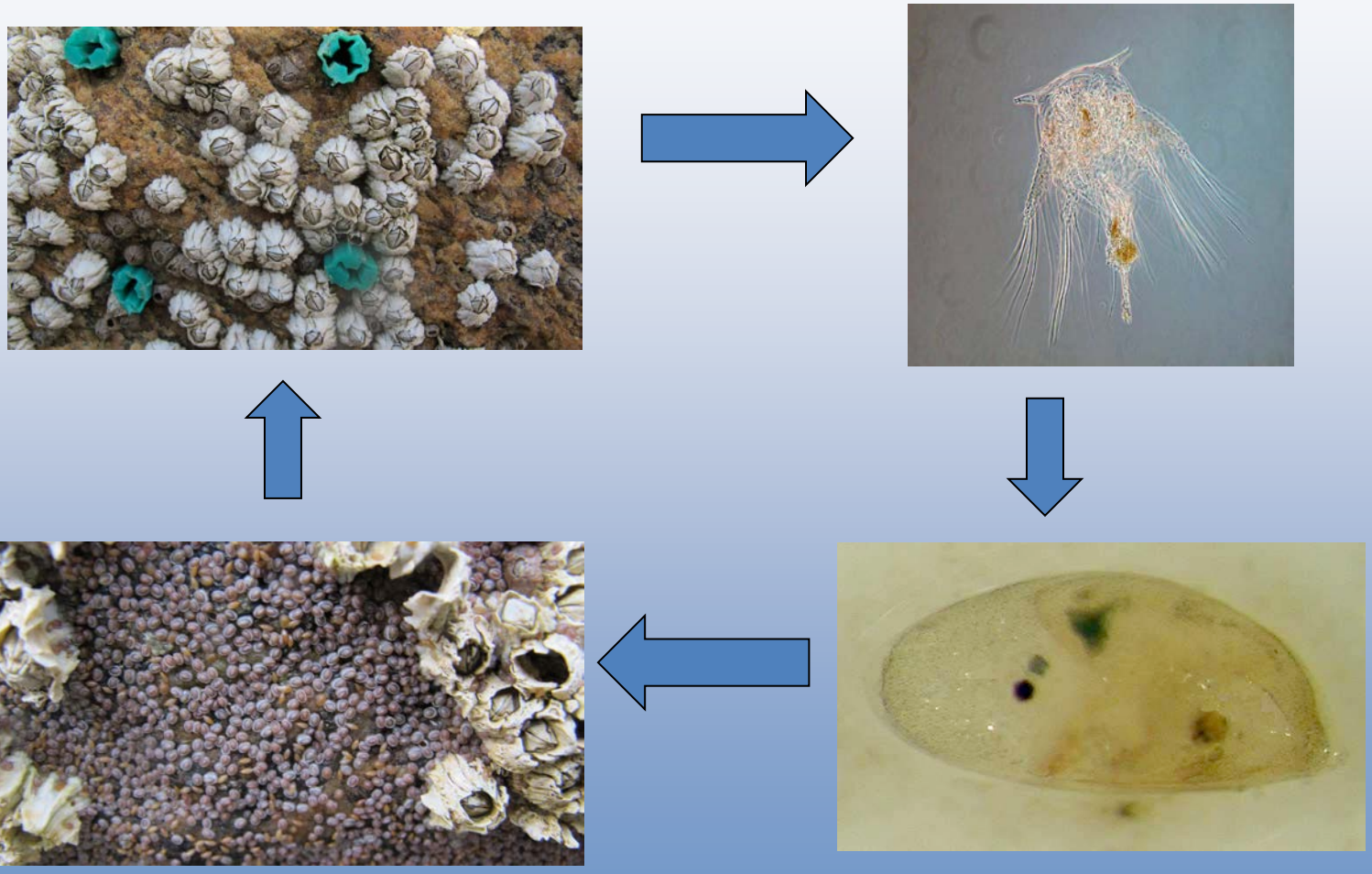
Understanding larval dispersal is critical for benthic animals that are fixed to a substrate or have limited mobility

The rocky intertidal in Buzzards Bay, Massachusetts is dominated by the barnacle *Semibalanus balanoides*

Barnacle life cycle

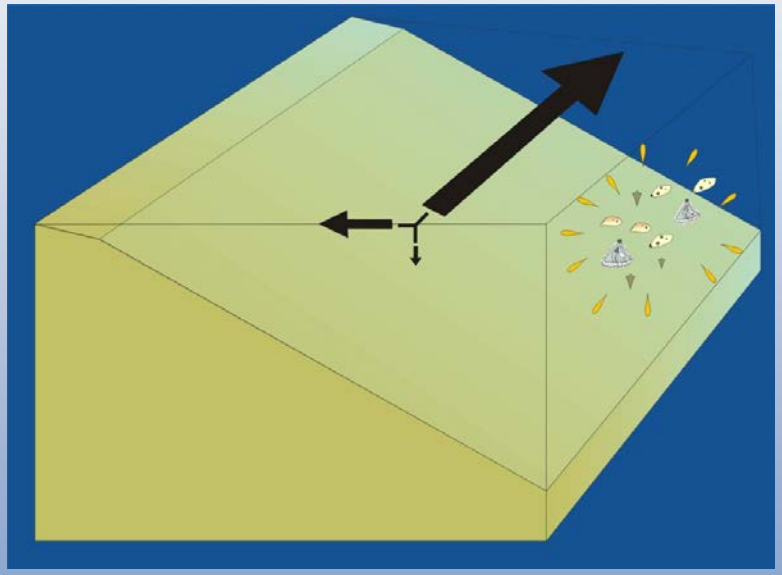
Typical of many invertebrates (e.g., bivalves) – benthic adults and planktonic larvae



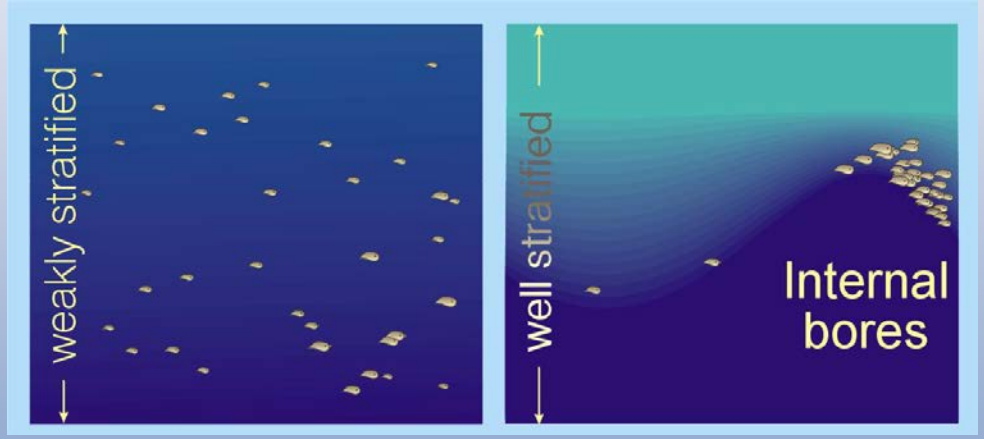
Planktonic larval dispersal may influence adult population connectivity

Which larvae are where, when?

Vertical distribution of larvae impacts dispersal



Larvae are weak swimmers, but may influence where they go by controlling their vertical position



Oceanographic features like internal bores may concentrate larvae in parts of the water column

Characterizing larval distributions is challenging

- Traditional sampling uses pumps and nets (e.g., MOCNESS)
- *Sampling close to the bottom*
- *Obtaining contextual environmental data*
  - fine scale distribution patterns relative to environmental features like fronts
- *Species identification*
  - Behaviors are species-specific, but difficult and time consuming to identify larvae using morphology

The MOCNESS can collect discrete depth samples, but only in open water

New technologies can overcome existing limitations

- Autonomous Underwater Vehicles (AUVs) can sample closer to the bottom and collect environmental data

- Genetic barcoding can expedite accurate species identification

Our goal is to combine robotic and genetic technologies to obtain species-specific vertical distributions of larvae

- We developed an autonomous method ("SUPR-REMUS") for sampling larval vertical distributions

- We deployed SUPR-REMUS in Buzzards Bay when barnacle larvae are thought to be abundant (*Semibalanus balanoides*)

- We barcoded sampled larvae to obtain species-specific patterns

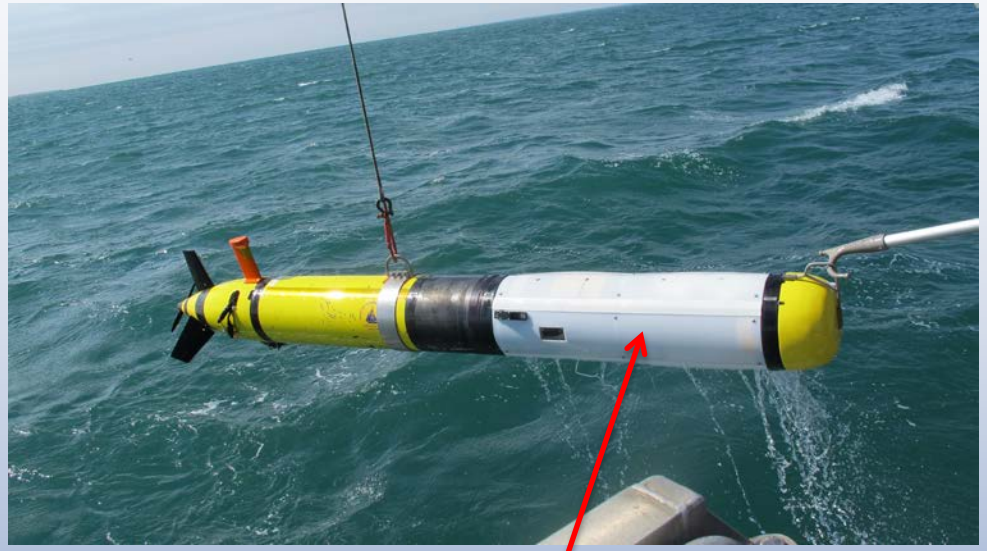
Methods

- A pump ("SUPR") was integrated into the AUV REMUS
- REMUS was equipped with sensors to obtain conductivity, temperature, and pressure
- Two deployments in March 2014
  1st deployment: ~ 9.9 km through relatively shallow water
  2nd deployment: ~11.2 km and more complex, navigating through a relatively deep channel and taking vertically discrete samples
- Barnacle larvae were enumerated, staged (early nauplii, late nauplii, cyprids), and genetically identified

The Suspended Particulate Rosette Sampler (SUPR)

- Large volume *in situ* filtration pump
- Originally developed for deep-sea geochemical and microbial sampling
- With up to 14 filter holders (samples)

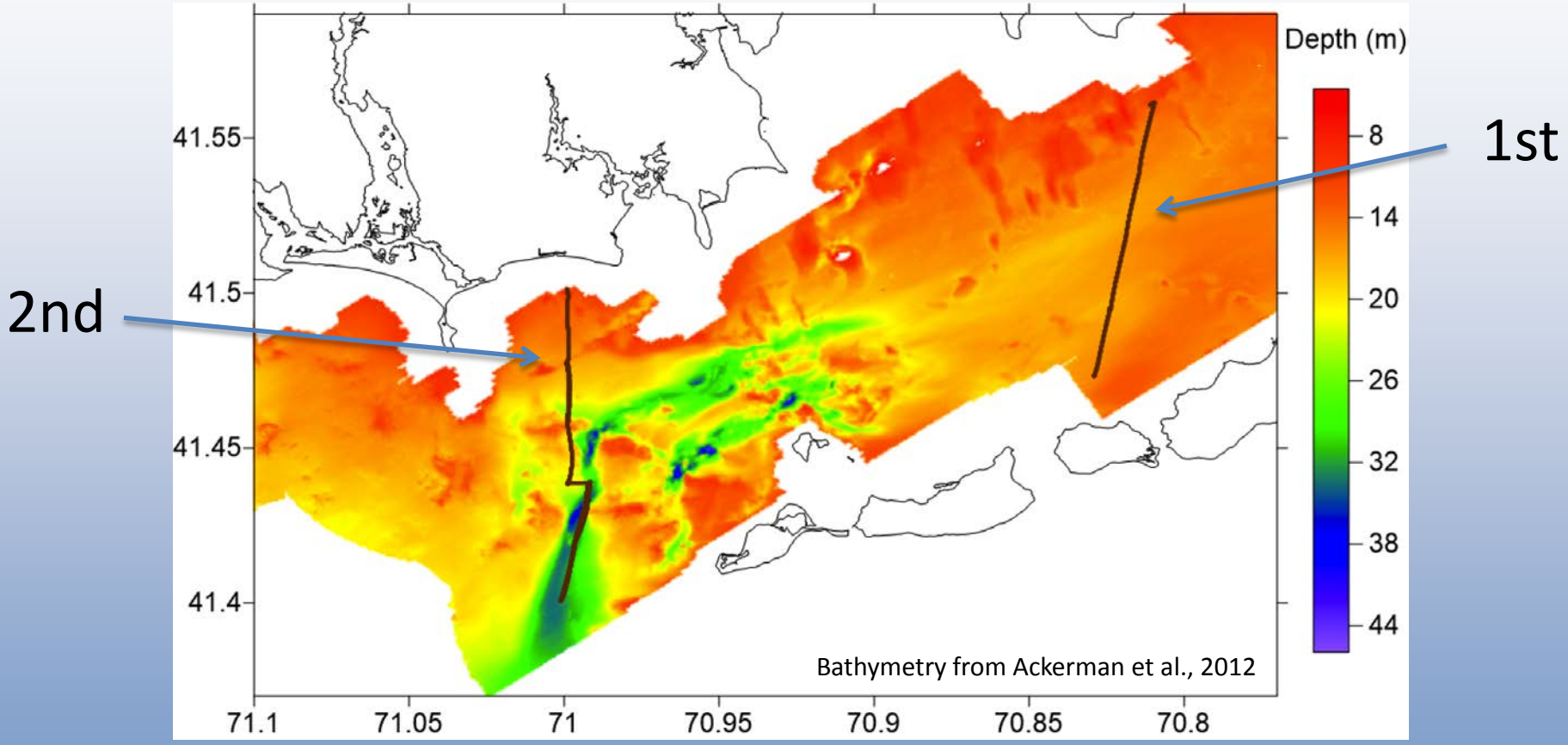
REMUS 600



SUPR mounted in forward payload section

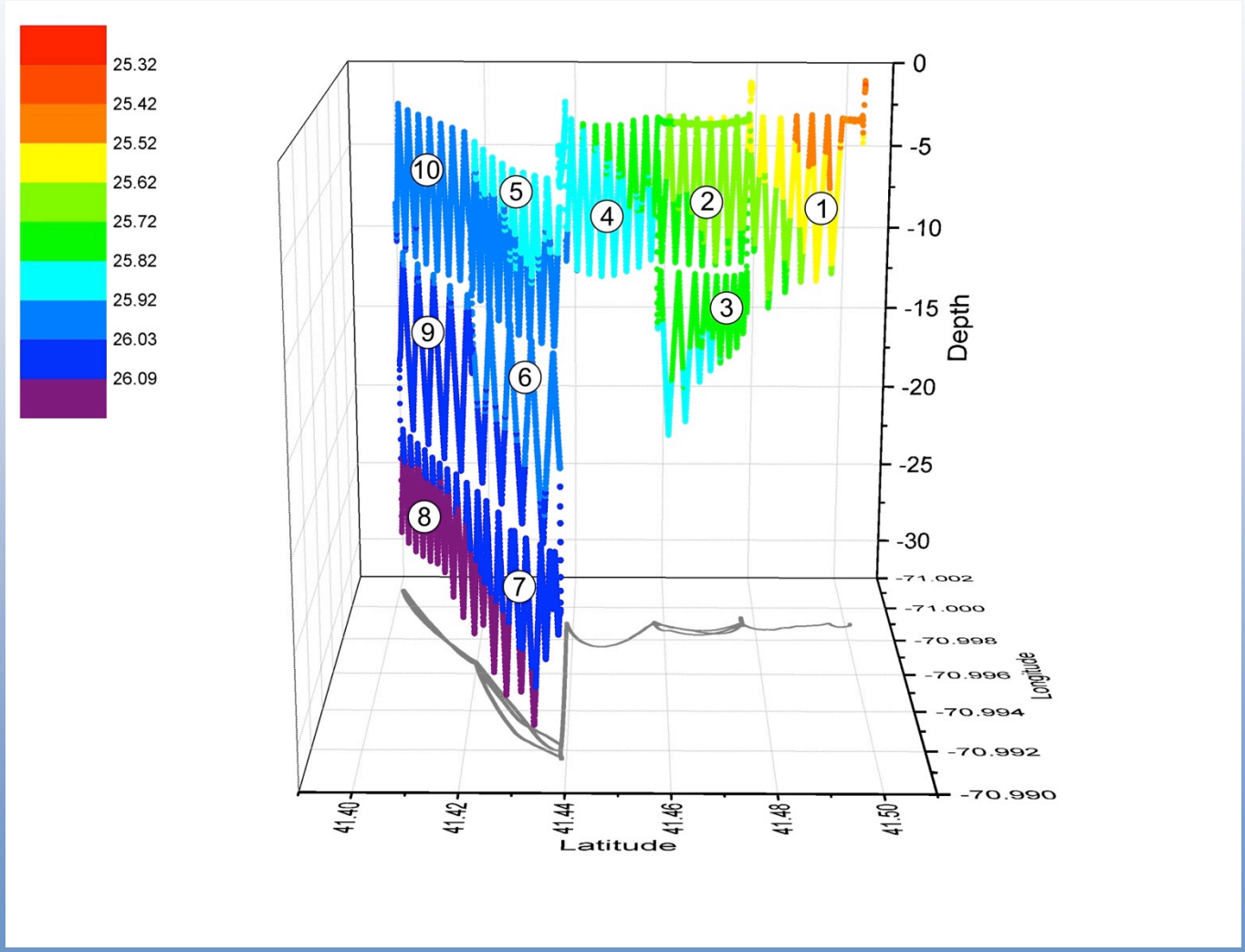
- Operates in waters up to 600 m
- Sensors collect environmental data such as temperature, conductivity, depth, turbidity
- Samples within 1 m of the seafloor

Deployments

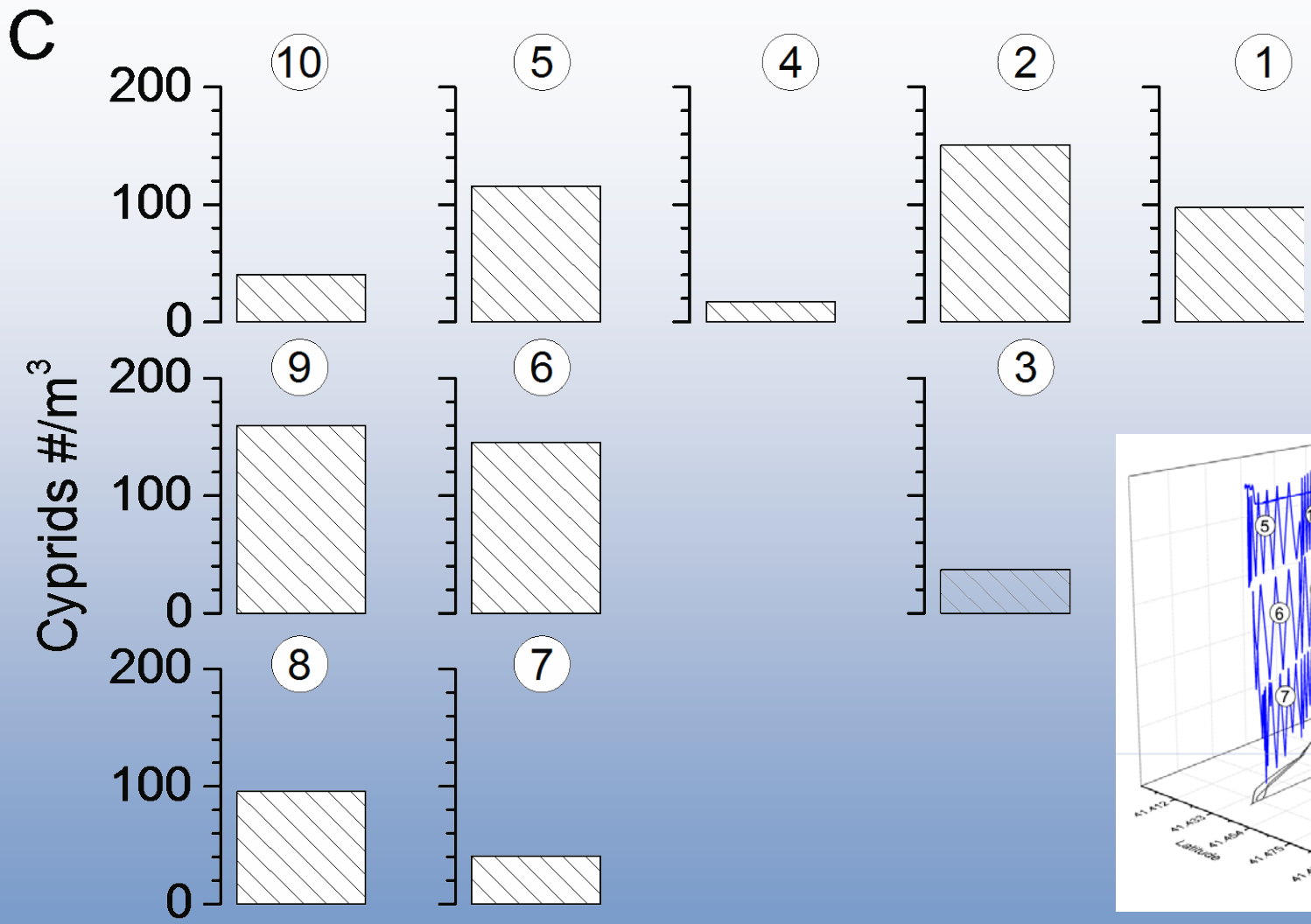


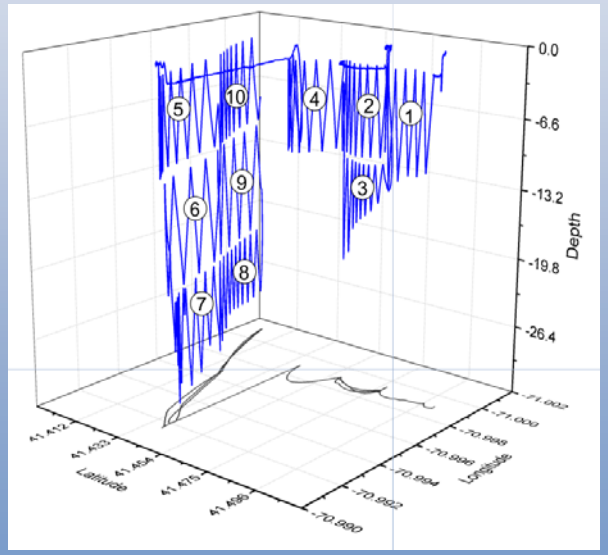
2nd Deployment

Cross-shore gradient in density and vertical stratification

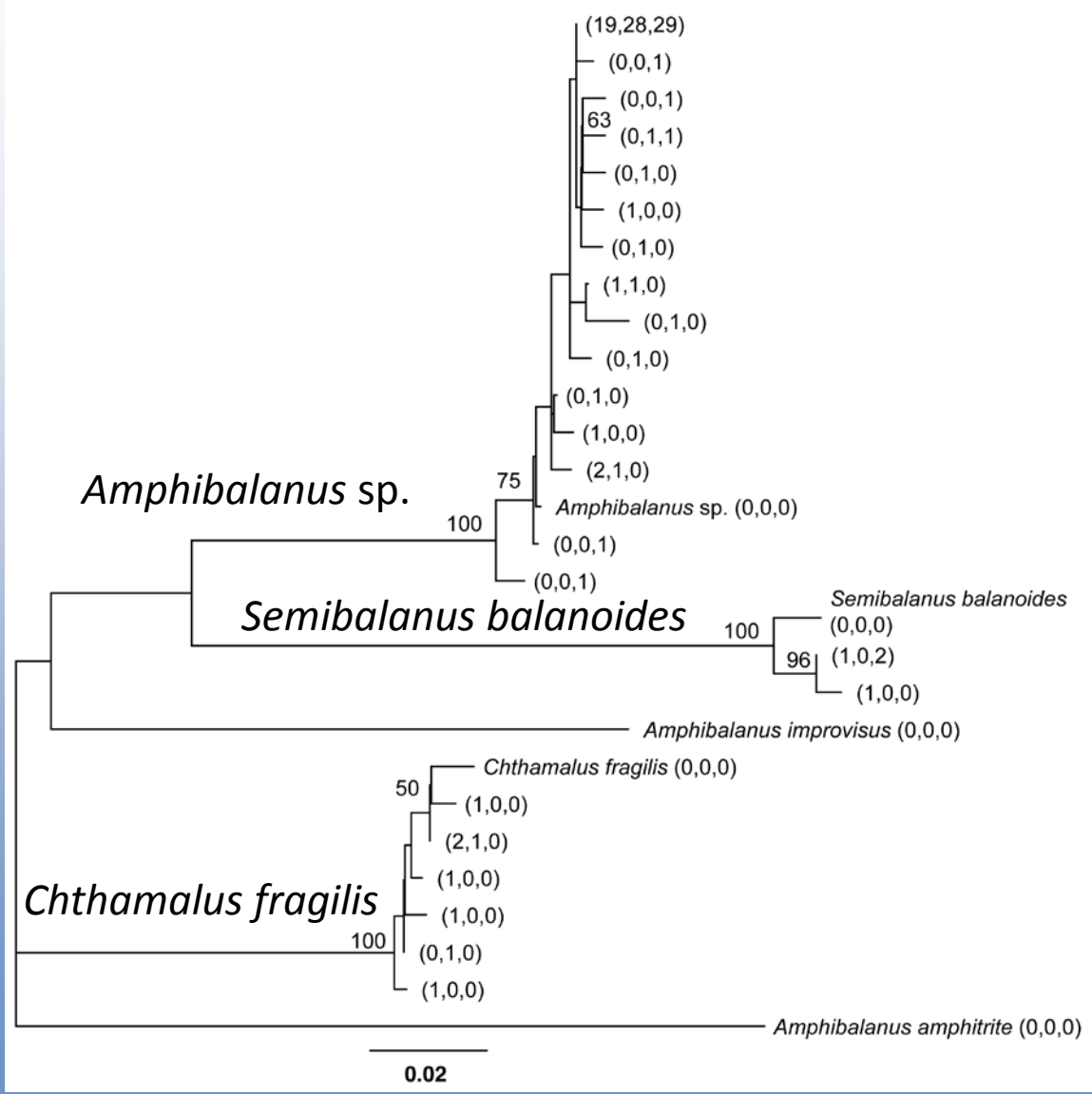


Depth distribution of cyprids (all)



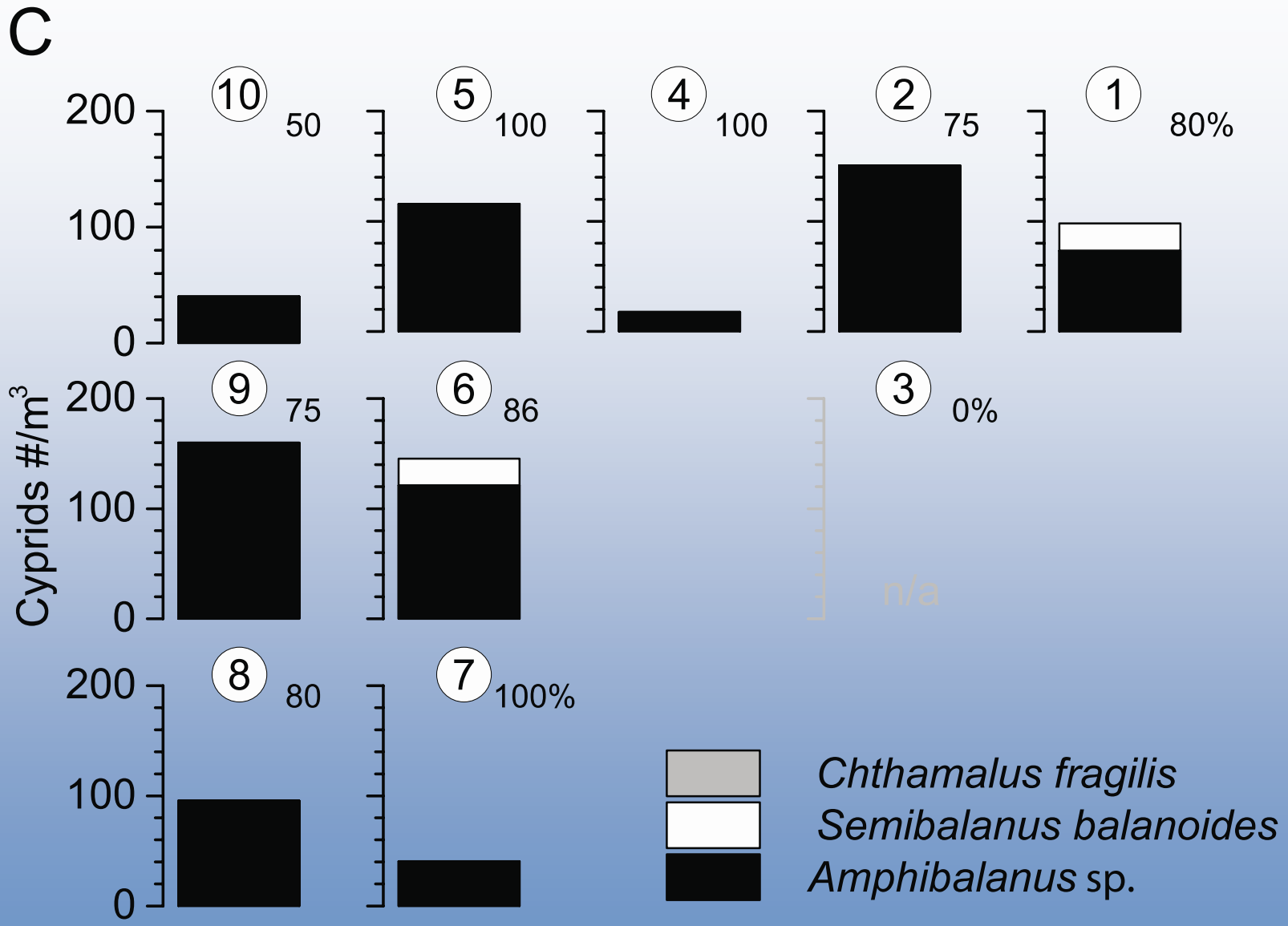


Barcode (mtCOI) results – all larvae, 2nd deployment



- Unexpectedly, 3 barnacle species were found
- Could not match most common larval taxon with a Genbank reference

Species-specific depth distribution of cyprids



Conclusions

- SUPR-REMUS is a useful new method for characterizing vertical distribution of barnacle larvae
- Need more data to corroborate distribution patterns
- Barcoding revealed unexpected larval species composition

Future Directions

- Increase flow rate to increase sample sizes
- Incorporate adaptive sampling (in response to environmental thresholds)
- Build a comprehensive reference barcode database
- Develop community barcoding methods (metabarcoding) using next generation sequencing (NGS) technology to apply to bulk environmental samples
- Planned for 2016 – use SURPR-REMUS to sample scallop and bivalve larvae (sea scallops, ocean quahogs, and surf clams) and couple with NGS metabarcode analysis

Thank you!

Kaitlynn Tradd, Mark Dennett, Andrew Girard, Gwynneth Packard, Ken Houtler