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FISHERIES**

**CalCOFI genomic advances:  
*Development and implementation of cutting  
edge genetic tools to improve ecosystem  
assessment in the CCE***

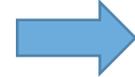
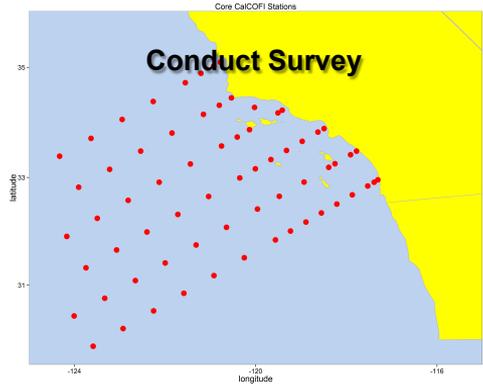
Andrew R. Thompson  
SWFSC, FRF, Ichthyoplankton Ecology

CCLME-2.2 April 19, 2016

**TOR question addressed:**

4. What is the status of oceanographic, habitat, climate and ecological data required to fulfill ecosystem-related science needs? Has the center developed strategies to obtain and manage such data?

# CalCOFI Ichthyoplankton and Ecosystem Analysis



Time-series of abundances of > 700 larval fish species and oceanographic conditions from 1951-present

## Goal

Use modern genetic tools to augment or improve ichthyoplankton sampling to benefit Integrated Ecosystem Assessment



## Outline

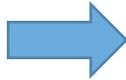
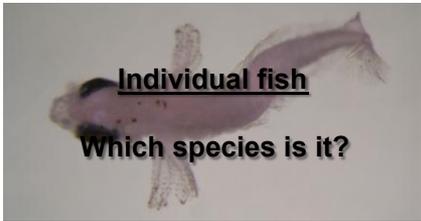
CalCOFI ichthyoplankton genetics and ecosystem analysis:  
Past, Present and Future:

- I. Past: Sanger sequencing to identify larval rockfishes
- II. Present: Metabarcoding to bulk-identify plankton samples
- III. Future: environmental DNA (eDNA)

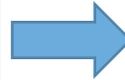


I. Sanger sequencing to identify larval rockfishes

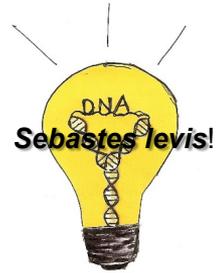
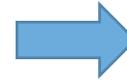
## What is Sanger sequencing? Sequence one individual at a time



Genetics  
Tissue extraction  
PCR  
Sequencing reaction



Match larval  
sequence to  
reference sequence



IEA Value

Increase taxonomic resolution of ichthyoplankton samples



I. Sanger sequencing to identify larval rockfishes



## Example: Rockfish Assemblages in southern California

- So. CA: world's center of rockfish species richness (~ 56 species)
  - Fill important ecological roles from forage to apex predators
  - Economically important for recreational and commercial fishing
    - Most large species overfished
      - NMFS declared several stocks as officially overfished
- NMFS implemented rockfish conservation areas (RCAs) in southern CA in 2001
  - No good time-series for rockfishes in southern CA

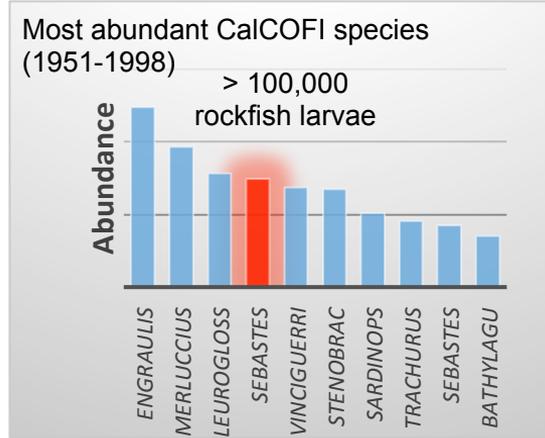
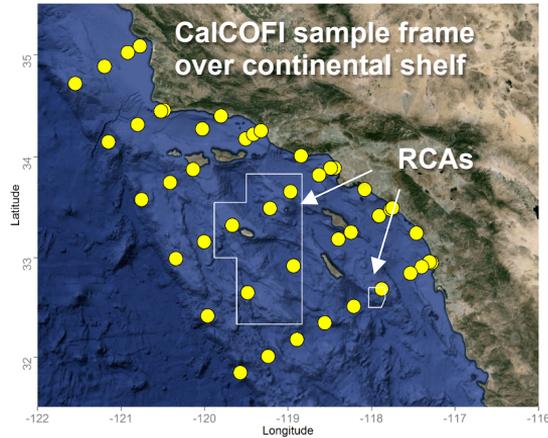
### Goal

Elucidate distribution and abundance dynamics of the rockfish assemblage in southern CA from 1998 to present

## I. Sanger sequencing to identify larval rockfishes

### Background

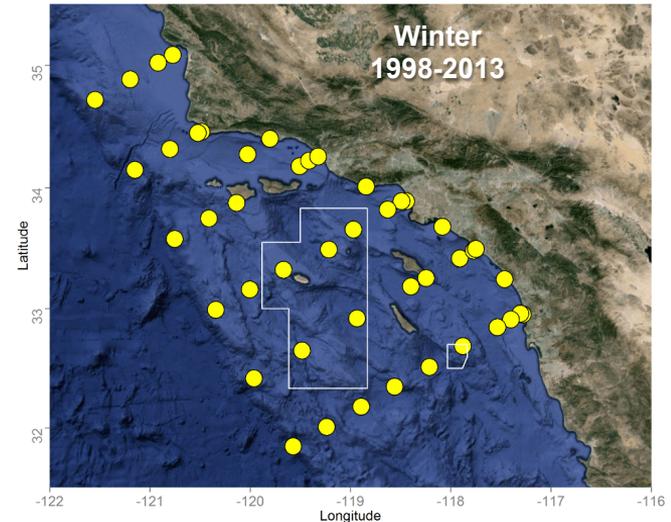
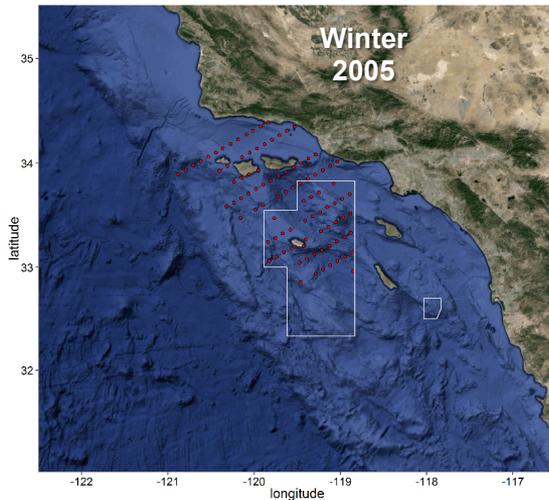
- CalCOFI has collected rockfish larvae since 1951
  - Rockfish larvae are very common
  - Can't ID to species based on morphology



## I. Sanger sequencing to identify larval rockfishes

### Background

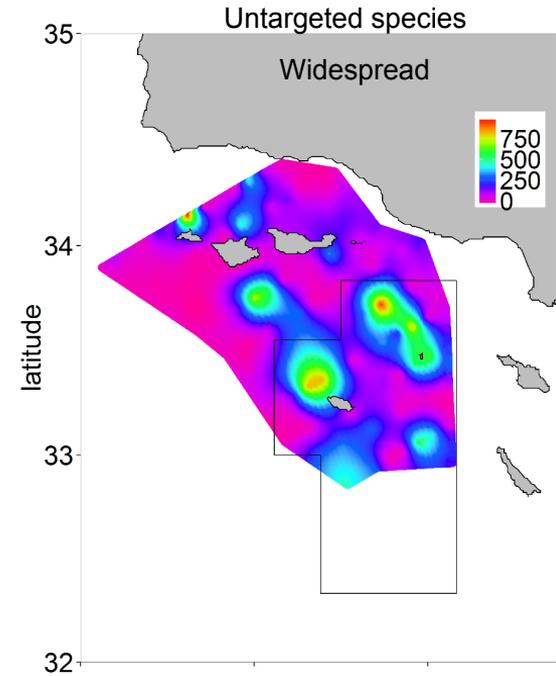
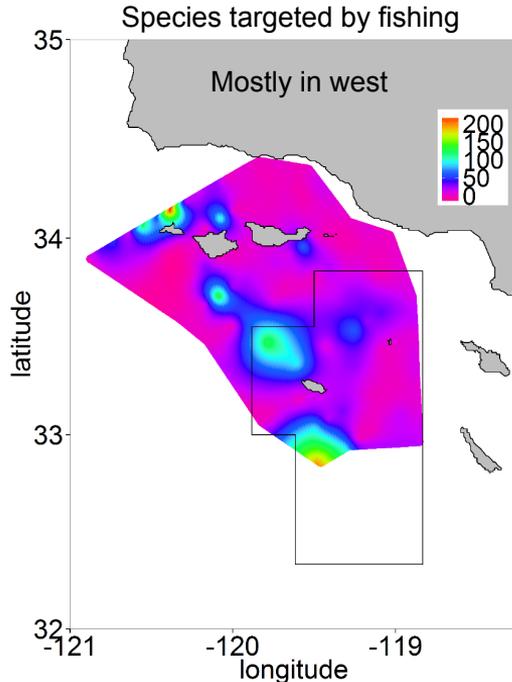
- CalCOFI samples have been preserved in ethanol since 1998
- We genetically identified ~3500 larvae from a 2005 fine-scale cruise
  - ~6000 larvae from winter CalCOFI samples
  - Funded by NMFS's FATE Program to improve IEA



I. Sanger sequencing to identify larval rockfishes

2005 Results

- Identified 36 species
- Abundance of untargeted larvae >> targeted larvae
- Different distributions for targeted vs. untargeted species



I. Sanger sequencing to identify larval rockfishes

1998 - 2013 Results

- Identified 39 species
- Abundances increased for 50% of targeted species and 47% of untargeted species
  - Targeted species consistently in the west
  - Targeted species more prevalent in latter years



## II. Metabarcoding to bulk-identify plankton samples

### What is metabarcoding?

Genetic identification of multiple species from a sample



Genetics  
Bulk tissue extraction  
ddPCR  
High throughput sequencing



Identification  
Match larval sequences to reference sequences



*Sebastes levis*  
*Leuroglossus stilbius*  
*Stenobranchius leucopsarus*  
*Sardinops sagax*  
*Engraulis mordax*  
*Triphoturus mexicanus*  
*Sebastes jordani*

### Goal

Develop and evaluate metabarcoding methods to bulk-identify all fish species from plankton samples

### IEA Value

Increase efficiency of sample identification  
Increase spatial and temporal sampling

## II. Metabarcoding to bulk-identify plankton samples

### Current Goal

Develop and validate ichthyoplankton metabarcoding methods



Determine:

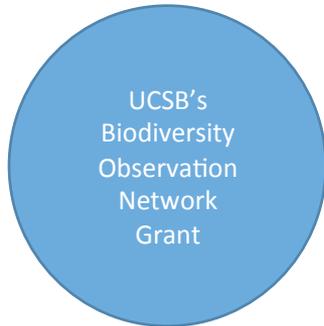
- whether metabarcoding matches morphological id results
  - the probability of detection for common to rare species
- if metabarcoding can provide reliable information on rank/abundance

II. Metabarcoding to bulk-identify plankton samples

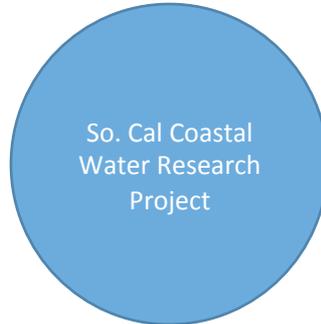
Broad network of collaborators:

Common Goal

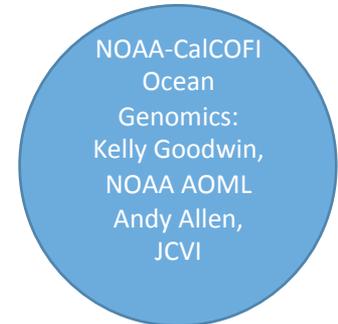
Implement advanced genetic techniques to better sample multiple ecosystem elements in So. Cal



Purpose: Develop a comprehensive ecosystem monitoring program (viruses to whales).  
Focus = SB Channel



Purpose: Develop ecosystem monitoring program for water agencies.  
Focus = coastal so. Cal  
Hired a postdoc, **Dovi Kacev**, who is leading ichthyoplankton metabarcoding project



Purpose: Metagenomics of microbial communities & ichthyoplankton  
Focus = CalCOFI

### III. 'omics - eDNA and metagenomics

## What is eDNA?

DNA taken from a water sample that contains sloughed tissue or DNA



Collect water directly from ocean



Genetics  
Bulk tissue extraction  
Metatranscriptomics  
and high throughput sequencing



Identification  
Match larval sequences to reference sequences



*Sebastes levis*  
*Leuroglossus stilbius*  
*Stenobranchius leucopsarus*  
*Sardinops sagax*  
*Engraulis mordax*  
*Triphoturus mexicanus*  
*Sebastes jordani*



## Goal

Develop and evaluate eDNA methods to bulk-identify all fish species from water samples and effluent from preserved samples

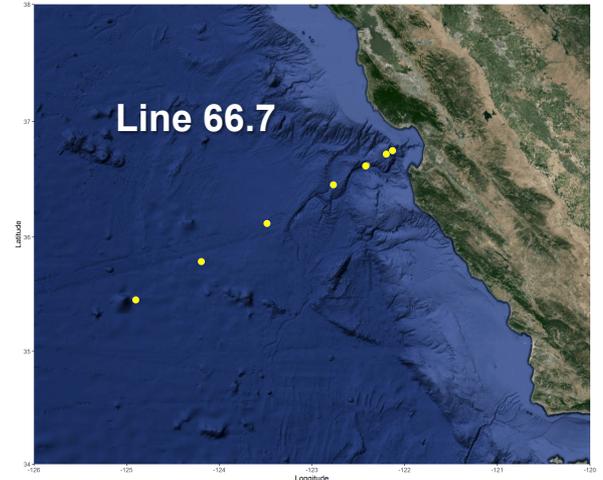
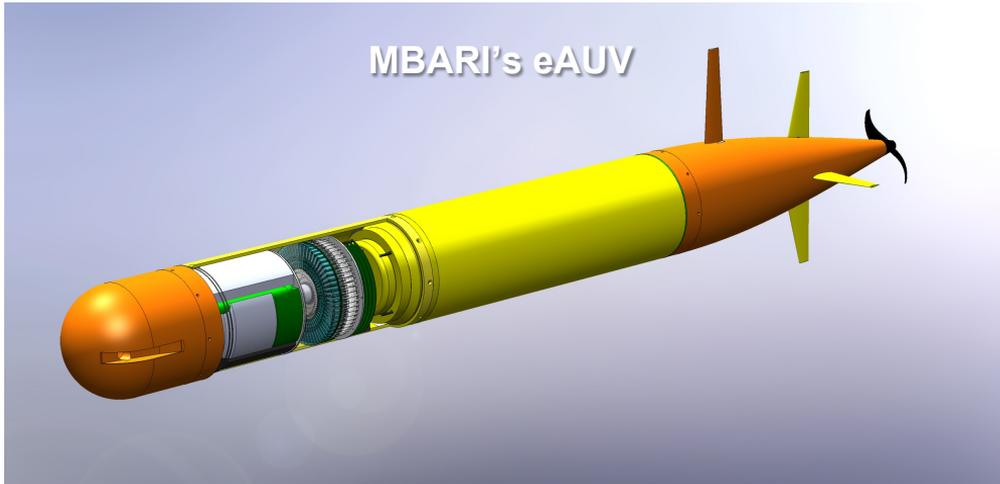
## IEA Value

Increase efficiency of sample identification  
Increase spatial and temporal sampling

### III. 'omics - eDNA and metagenomics

#### Planned Projects

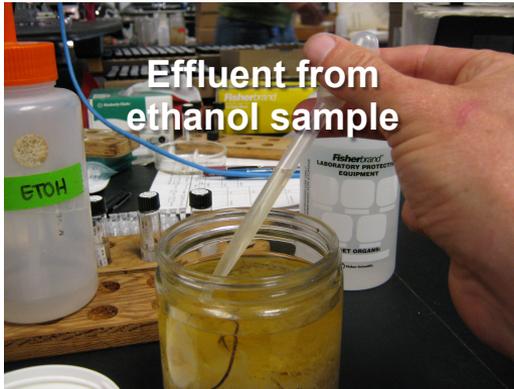
- Collect water and plankton samples from CalCOFI line 66.7 in Spring 2017.
  - Use an eAUV to filter water
- Evaluate both fish and microbes (i.e., archae, bacteria, phytoplankton).
- In conjunction with the Monterey Bay Aquarium Research Institute (MBARI),
  - Funded by OAR (Kelly Goodwin PI)



### III. 'omics - eDNA and metagenomics

## Planned Projects

- Collect effluent from existing ethanol-preserved CalCOFI samples
- Process non-destructively backlogged samples from spring, summer and fall (1998-present)



### Strengths

- Genetics can increase taxonomic resolution, spatial & temporal sampling to provide better indices for IEA
  - Example: Developed a rockfish time-series in so CA
  - Complement existing monitoring to better sample the ocean ecosystem

### Challenges

- Need to maintain funding to test and implement these cutting-edge tools
  - Need to stay on top of new developments in the field
- Need to thoroughly test new methods before they are used in policy decisions

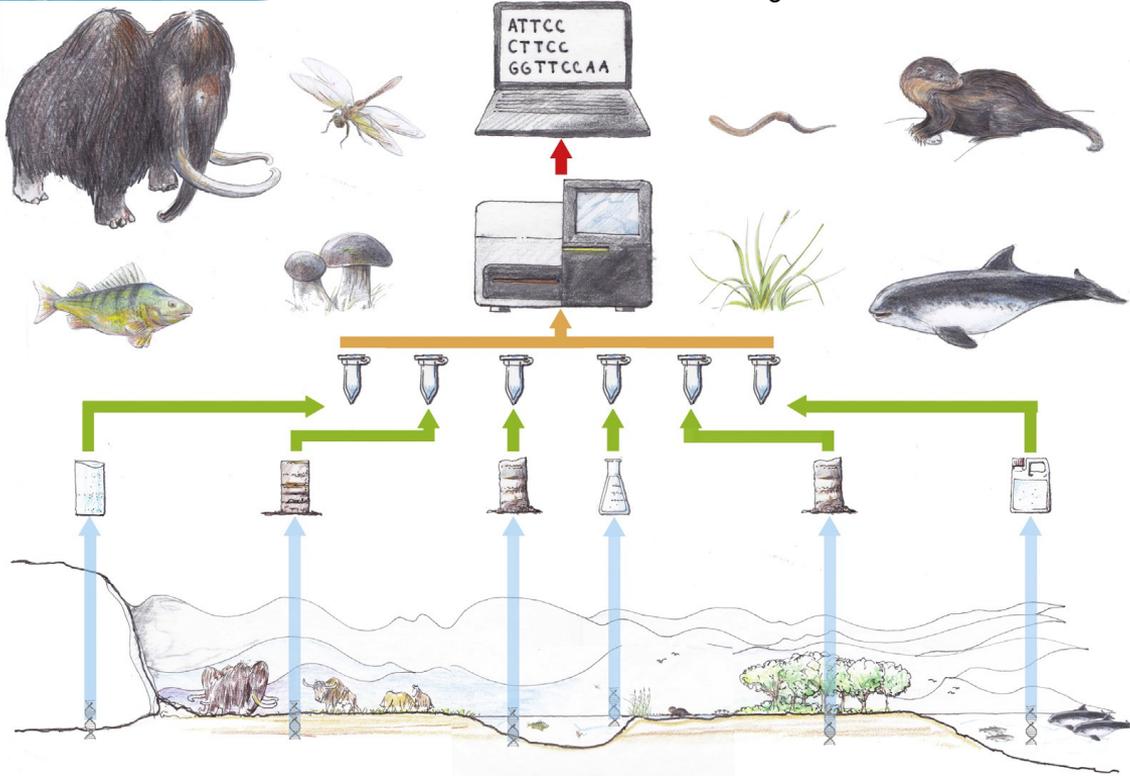
### Strategies

- Continue working with IEA modelers to best tailor modern genetics technologies to pertinent ecosystem questions
- Continue finding and working with partners to build a comprehensive assessment network in southern CA
- Don't jump ahead; do research to vet methods prior to integration into surveys

Thomsen & Willerslev 2015 Biological Conservation 183: 4-18



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Genetics holds the potential to integrate monitoring across ecosystems and temporal scales  
We hope to develop and use these tools to produce the best possible ecosystem monitoring program