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# *Echoes of Color: Hyperspectral Imaging of Coral Reef Fishes Through the Fixation Process*

SICB 2025

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Blue-barred parrotfish



<https://www.pinterest.com/pin/383720830731867328/>



<https://www.pinterest.com/pin/3588874676139658/>  
<https://inspiraboardsp.blogspot.com/2011/08/saltwater-fish.html>



2,740 results

267 results

Why the big gap?

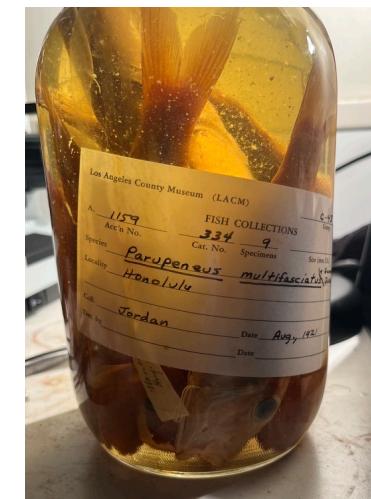
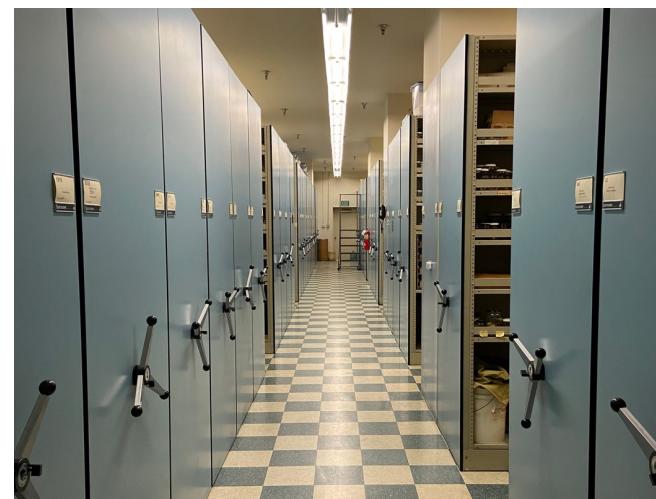


Drawers of birds and Smithsonian scientist Roxie Laybourne at the Smithsonian National Museum of Natural History  
<https://i.insider.com/638a7310b4290800185d0c43?width=1300&format=jpeg&auto=webp>



↑ Ichthyology collection at Scripps Institute of Oceanography

← ↓ Ichthyology collection at the Los Angeles County Museum of Natural History





# It's clear color fades

Achilles tang  
*Acanthurus achilles*



Picasso triggerfish  
*Rhinecanthus aculeatus*



Moorish idol  
*Zanclus cornutus*



# But is all color information lost?

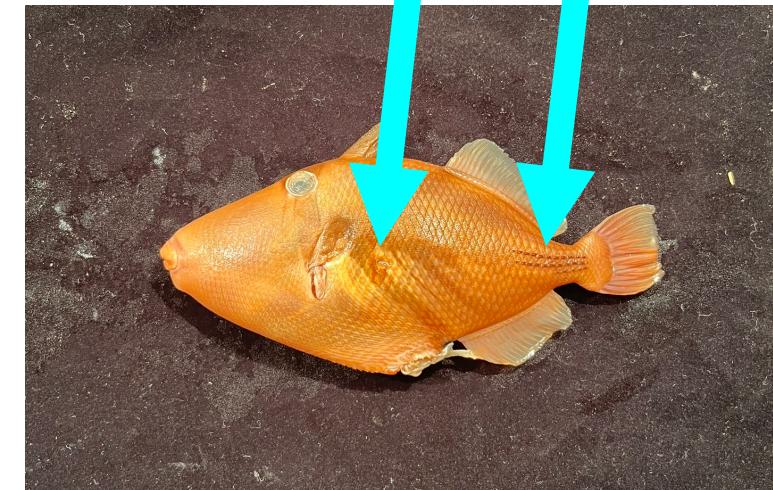
Achilles tang  
*Acanthurus achilles*



Picasso triggerfish  
*Rhinecanthus aculeatus*



Moorish idol  
*Zanclus cornutus*



# Driving Research Questions

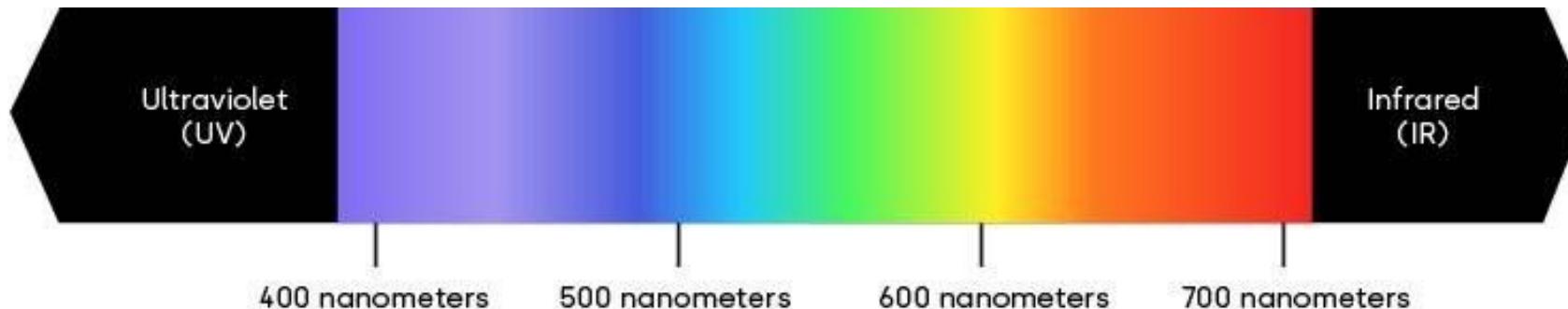
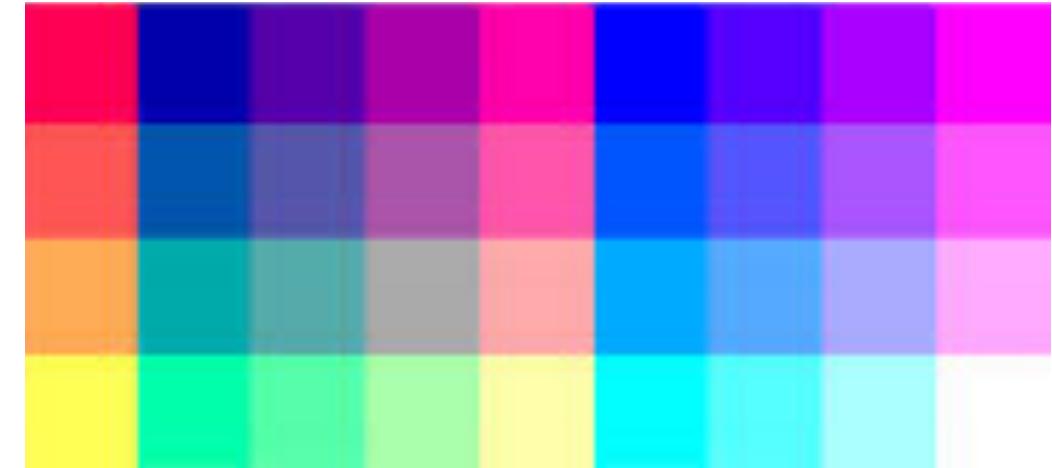
1. Is there any signature of original coloration in museum specimens?
2. How does formalin fixation affect the retention of color in fish specimens?

So how do we do this?

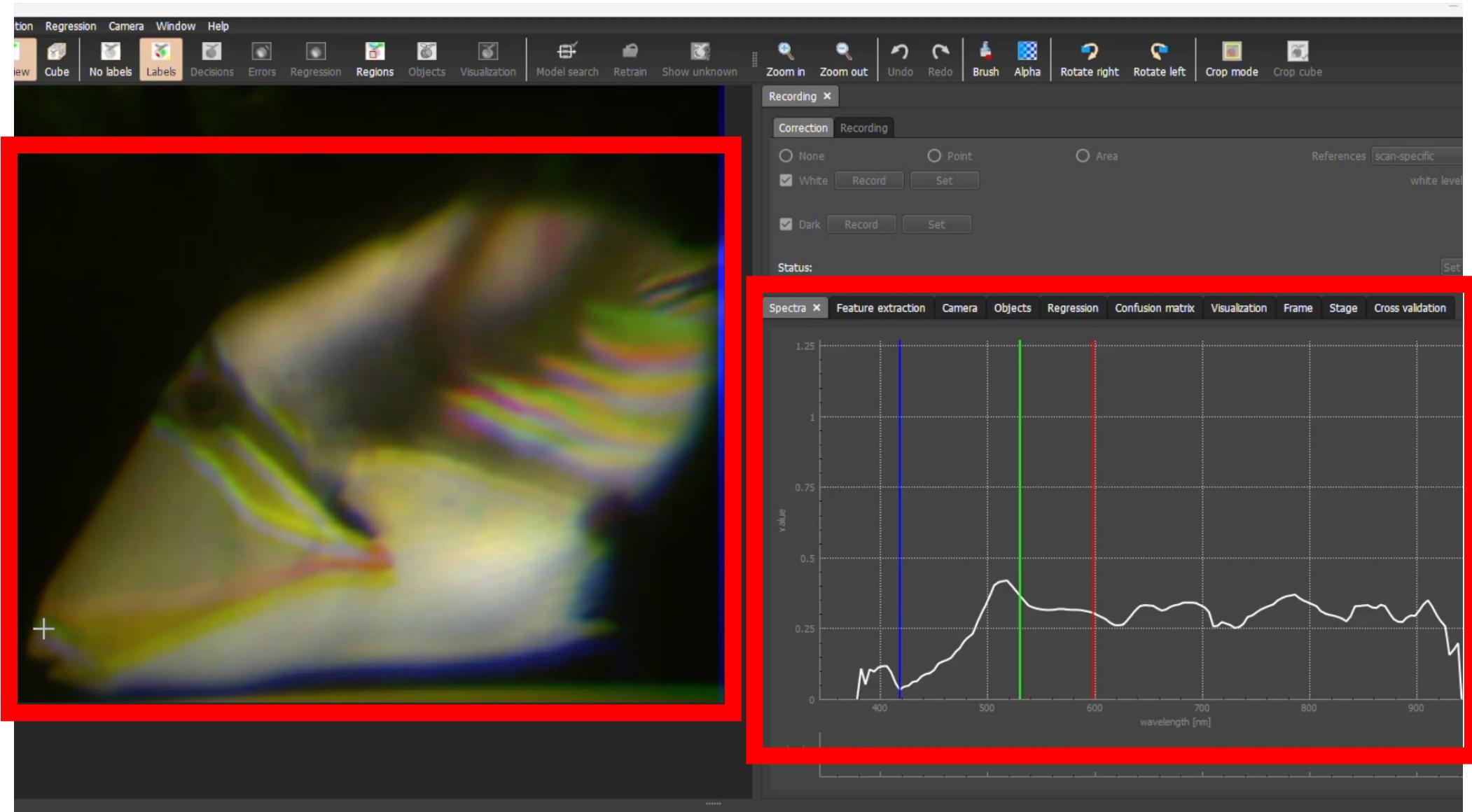
**HYPERSPECTRAL IMAGING**

# ULTRIS X50

- Hyperspectral camera

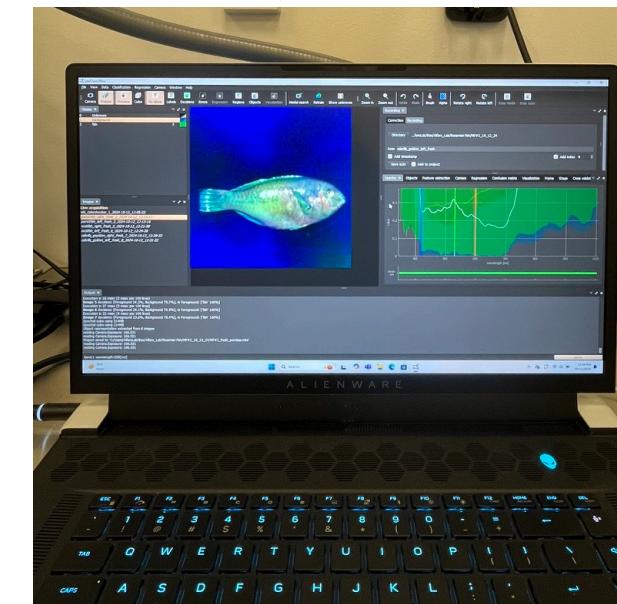
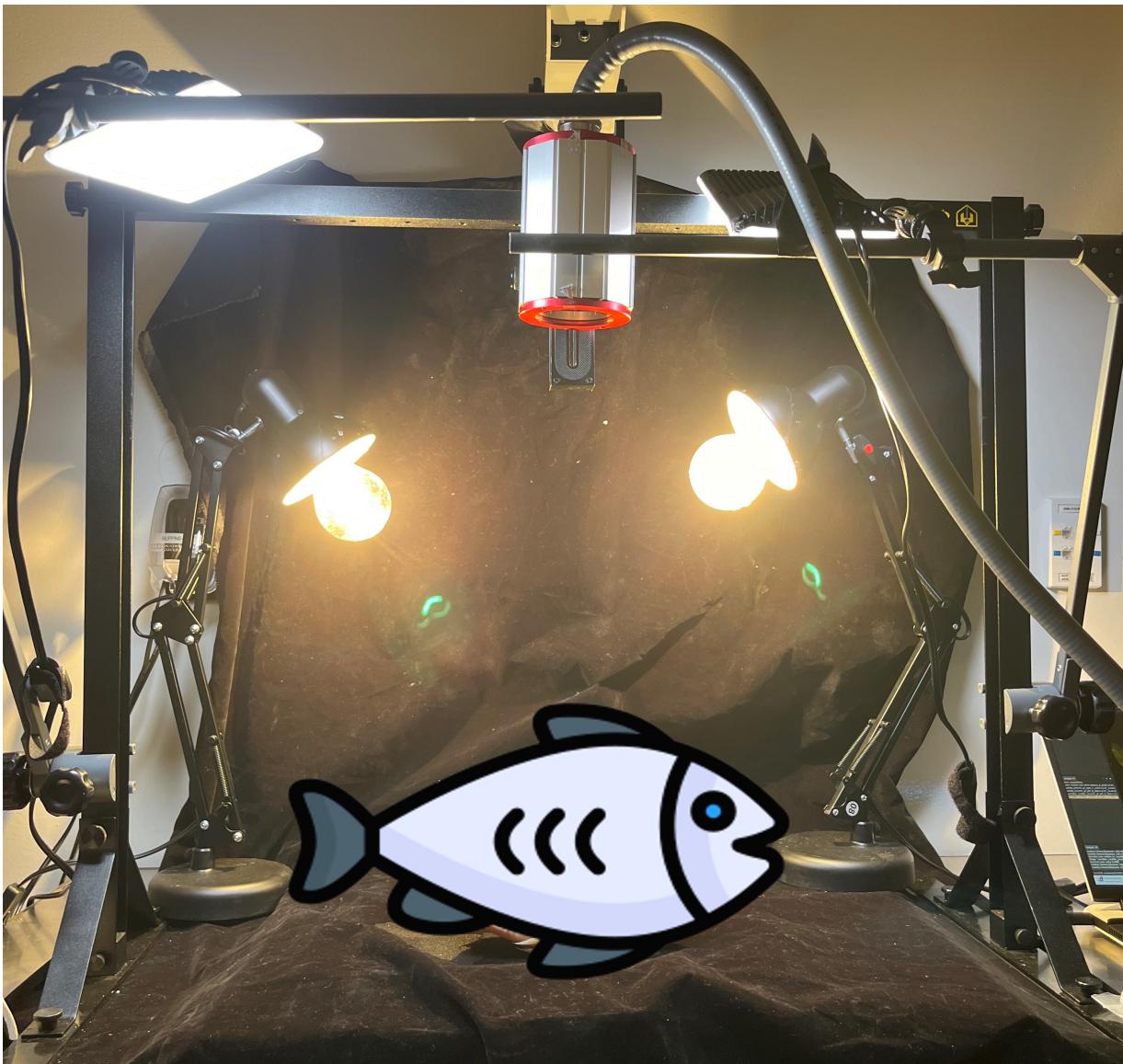


# PerClass in action...



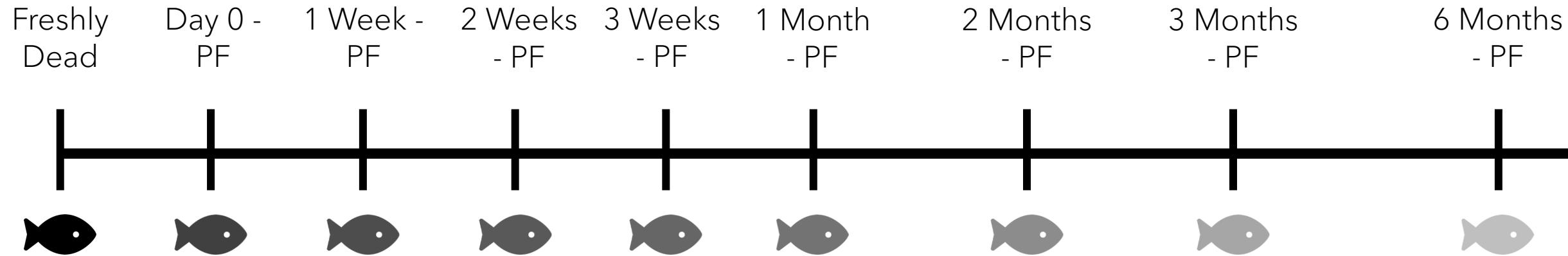
# HSI Camera Setup

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Disclaimer: different fish specimens featured in each photo.

# How does reflectance change throughout the fixation process?

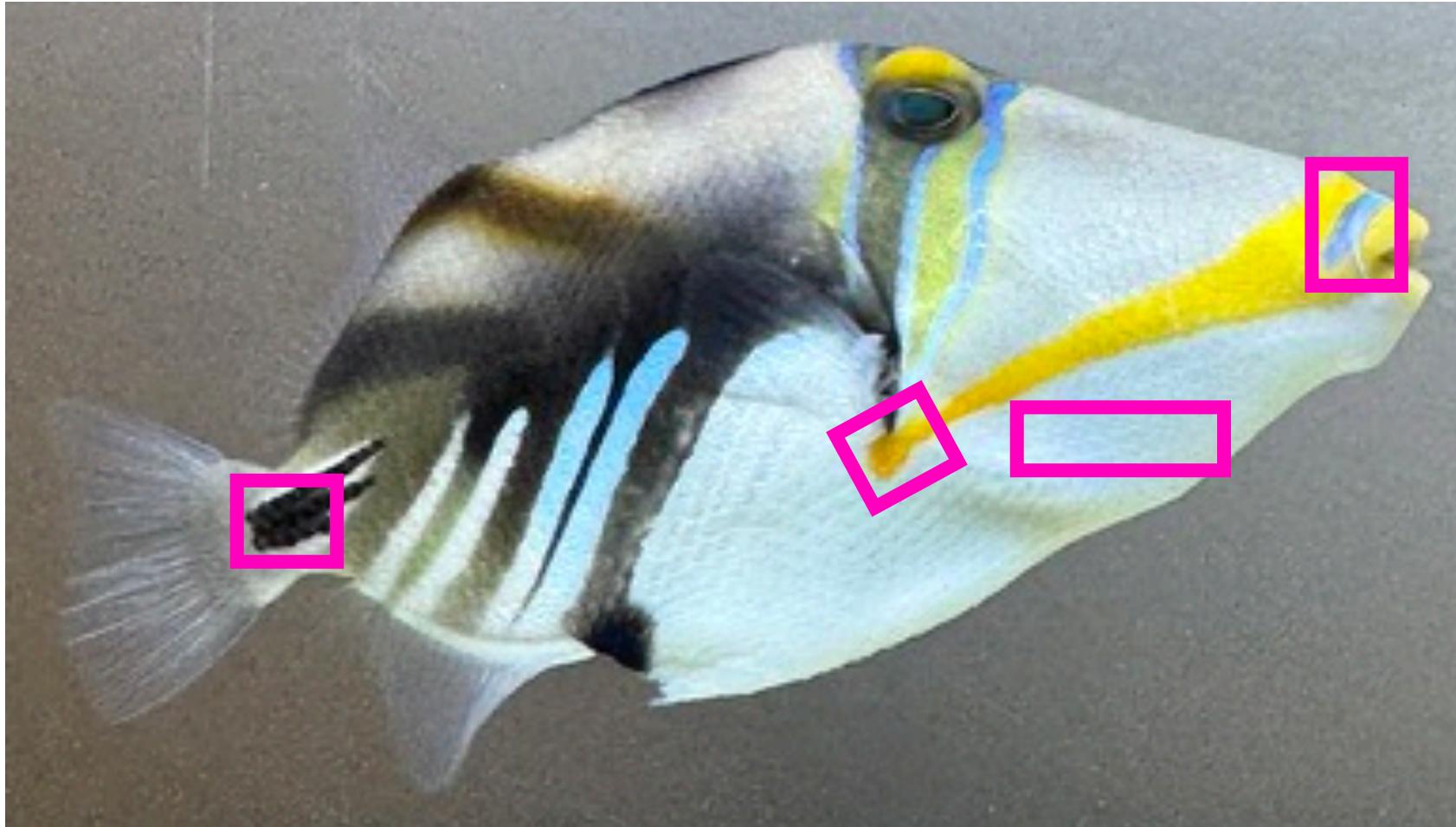


... up until 1 year from specimen acquisition

# Picasso Triggerfish

*Rhinecanthus aculeatus*

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- Blue
- Orange/yellow
- White
- Black

# Comparing Patch Reflectance



black



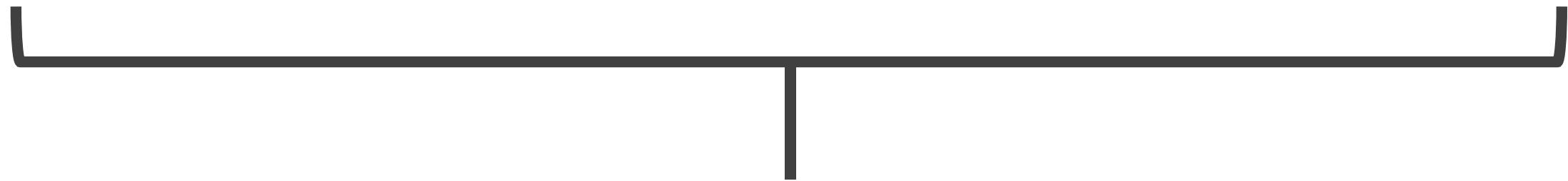
blue



orange



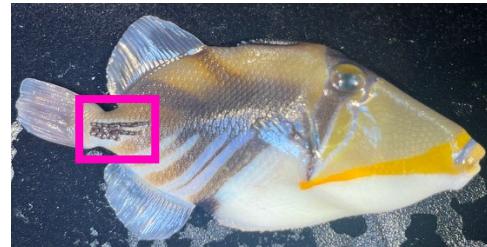
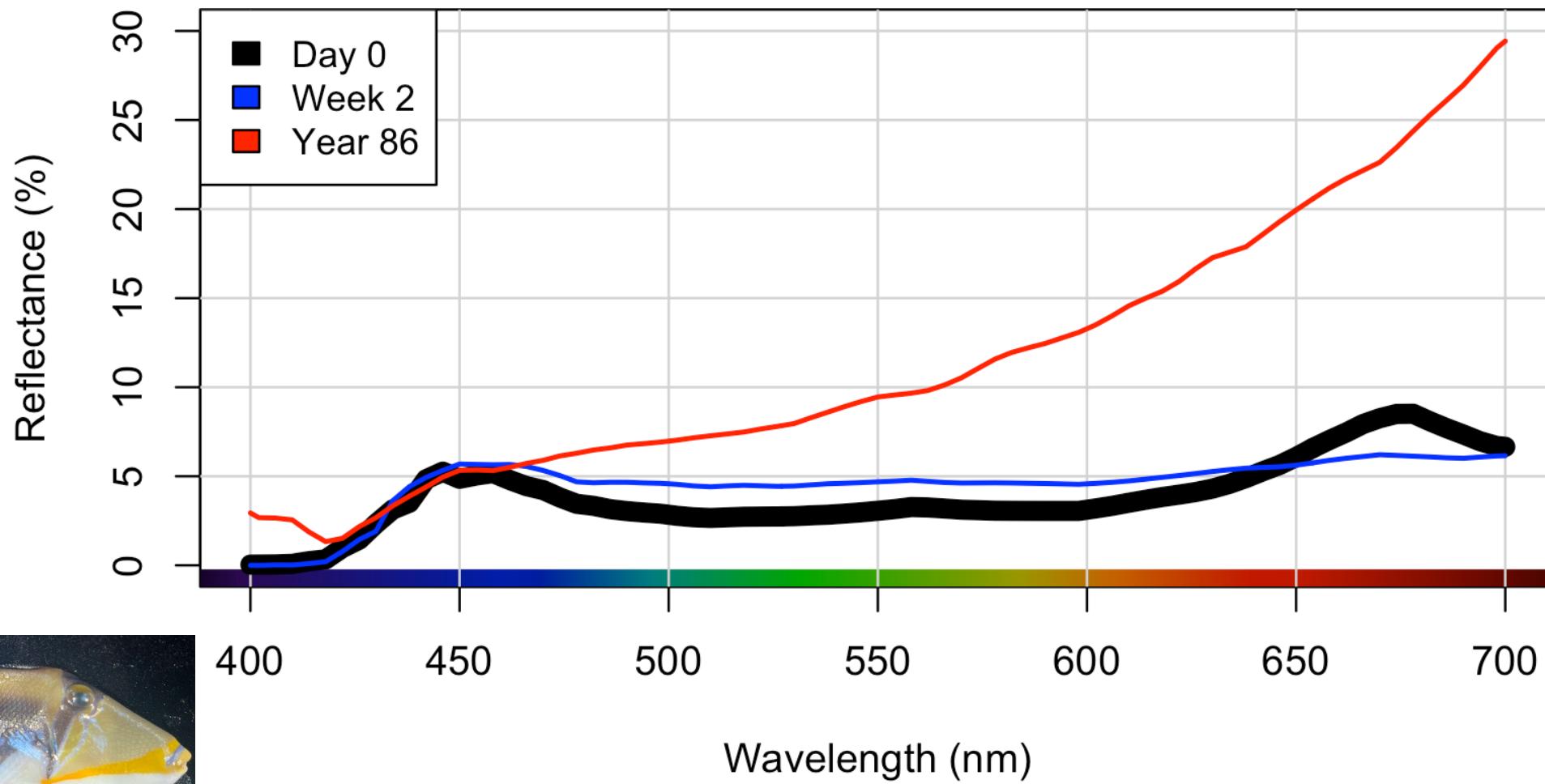
white



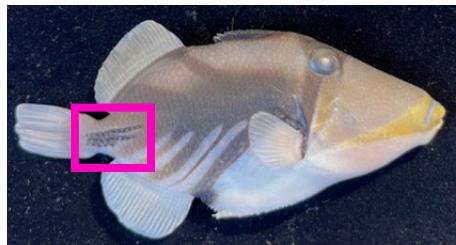
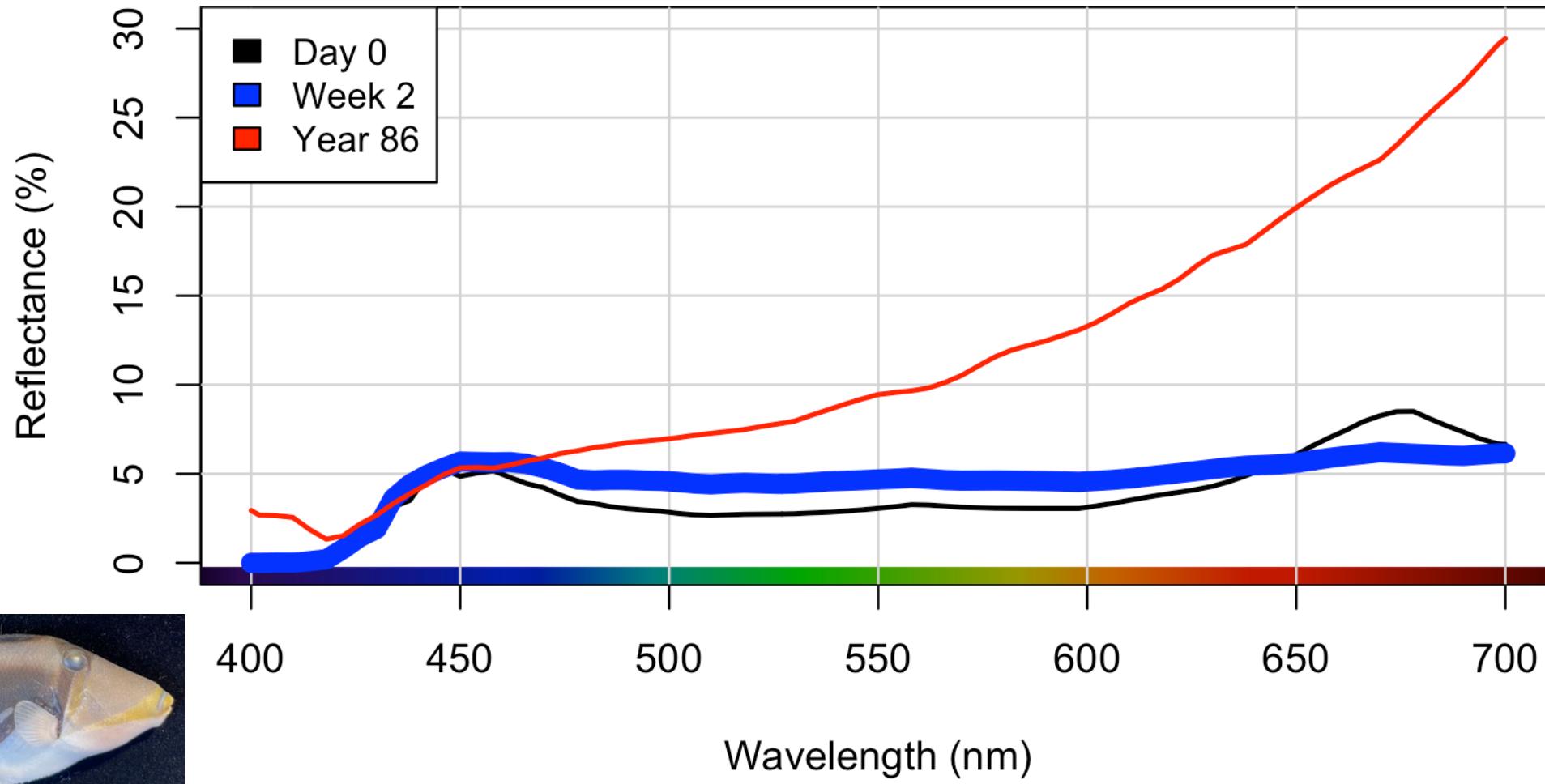
# Black Patch Reflectance



## Black Patch

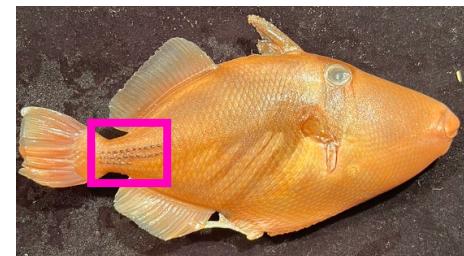
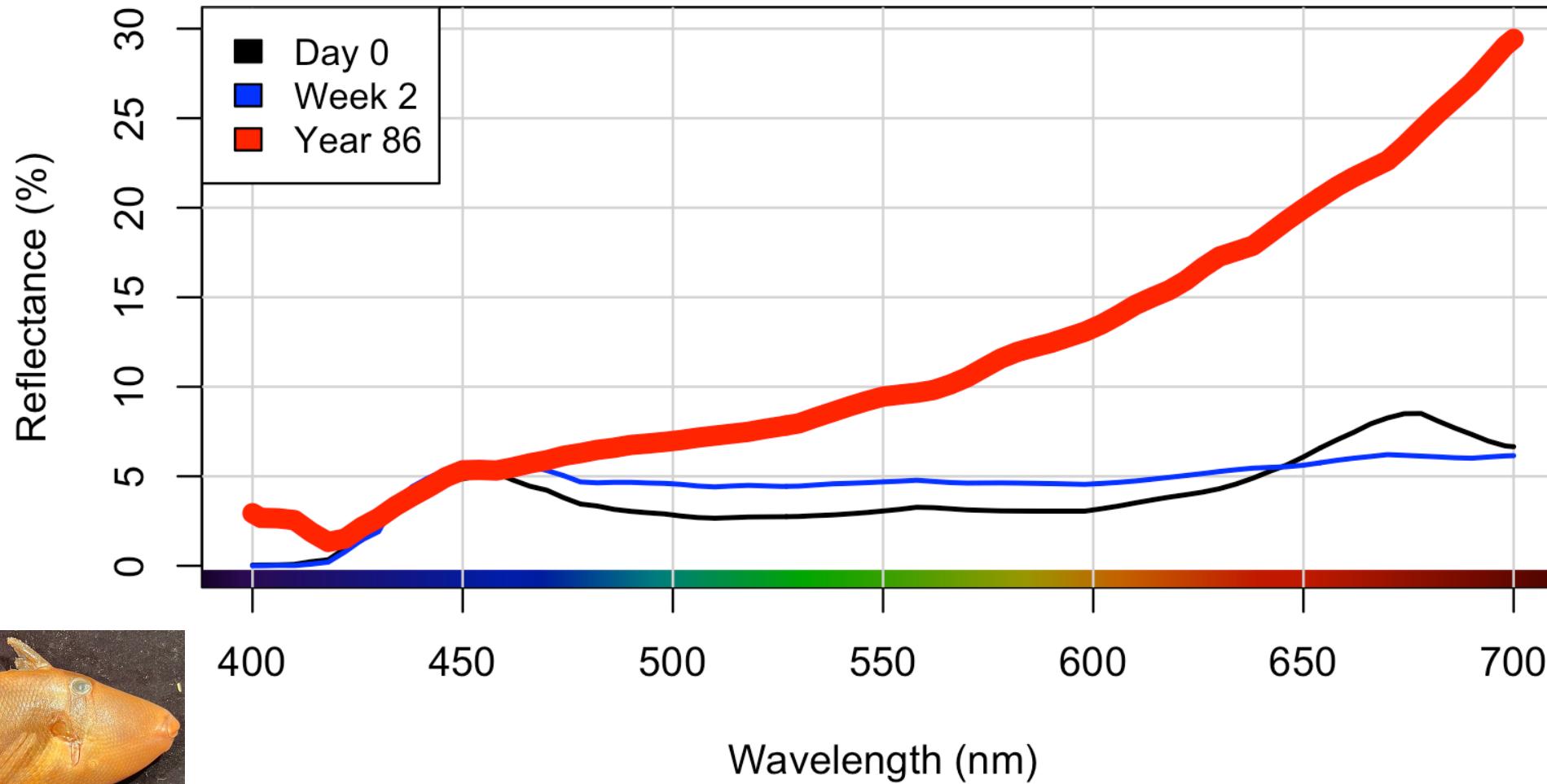


## Black Patch



Black patches have reflectance at higher wavelengths

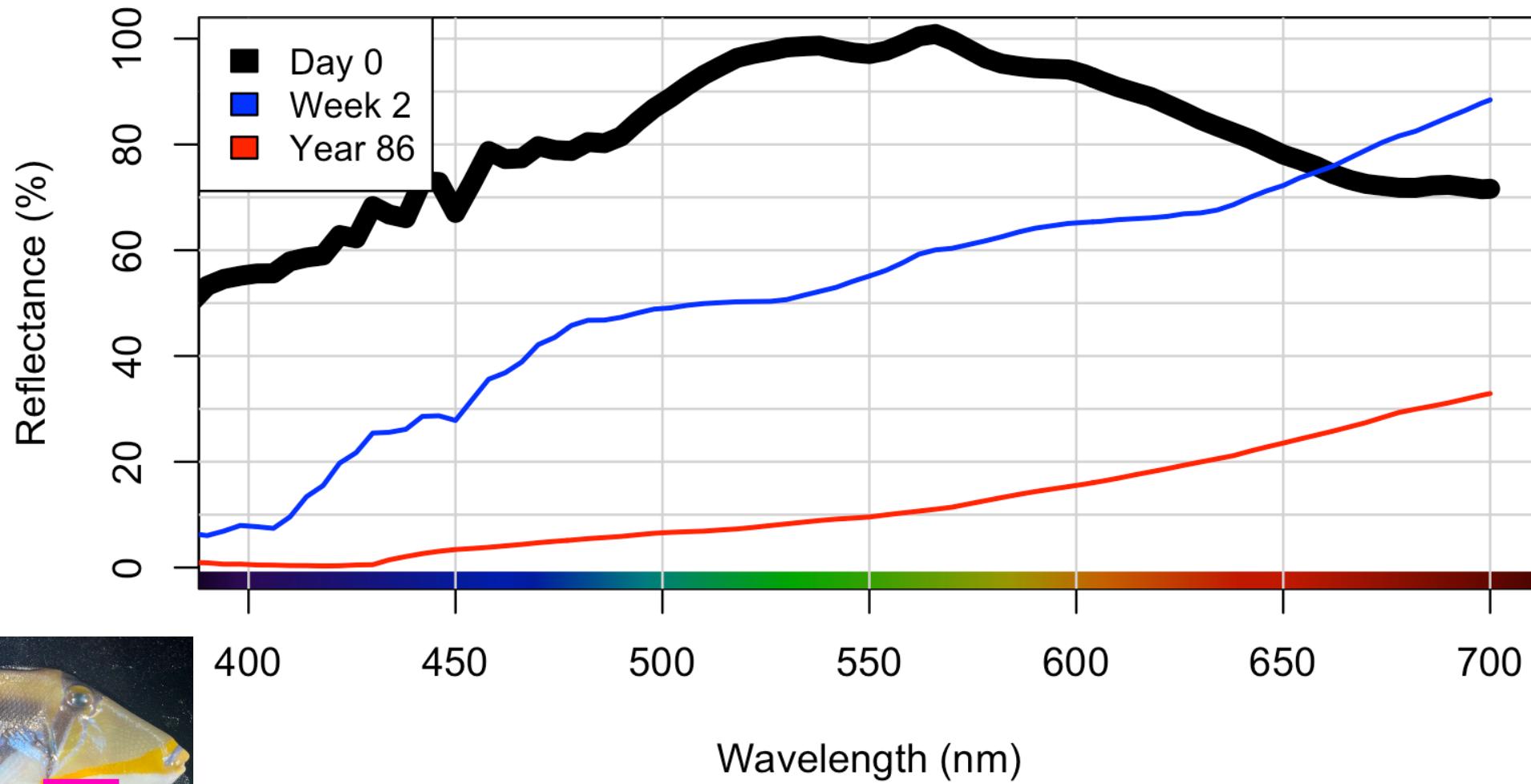
### Black Patch



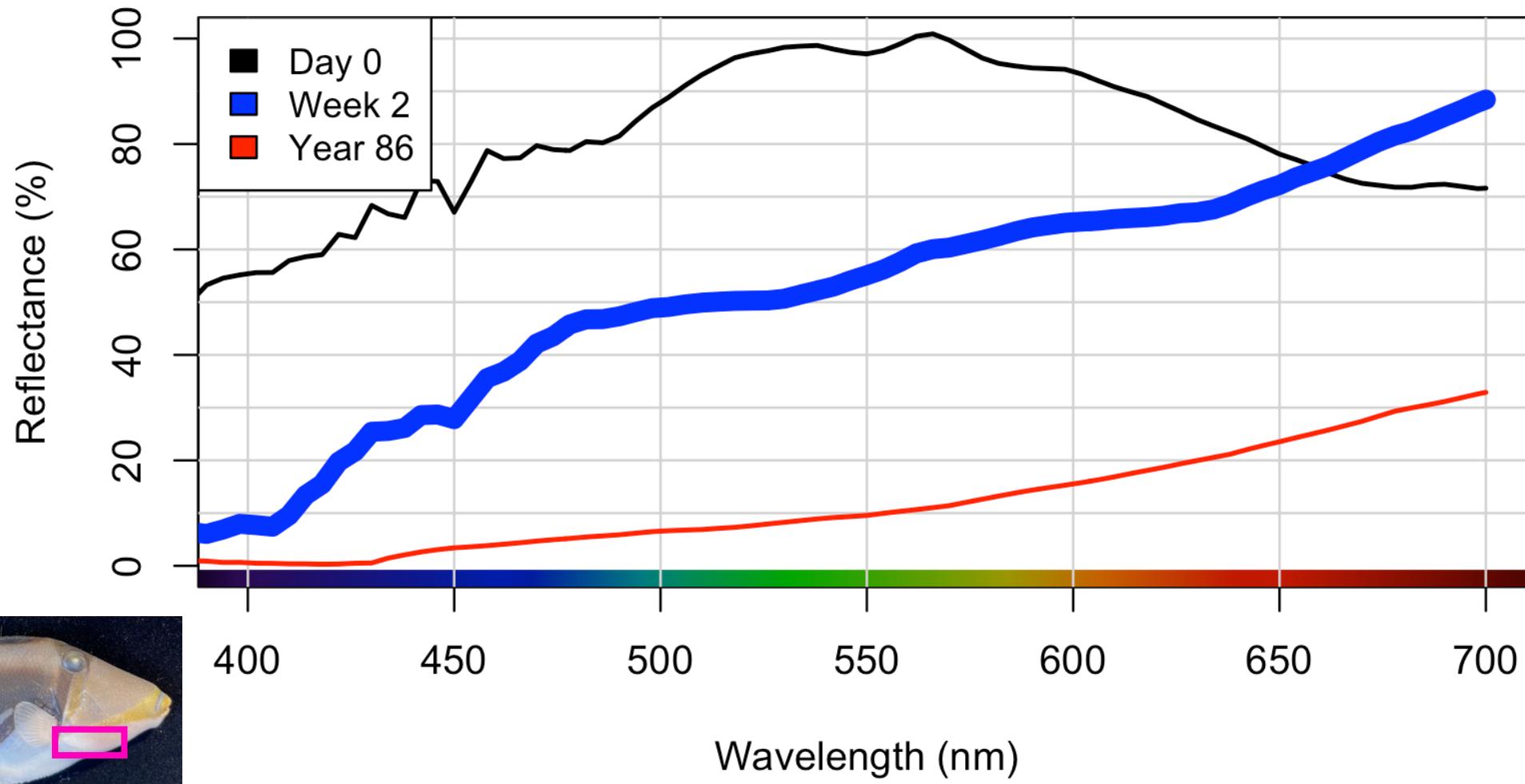
# White Patch Reflectance



## White Patch

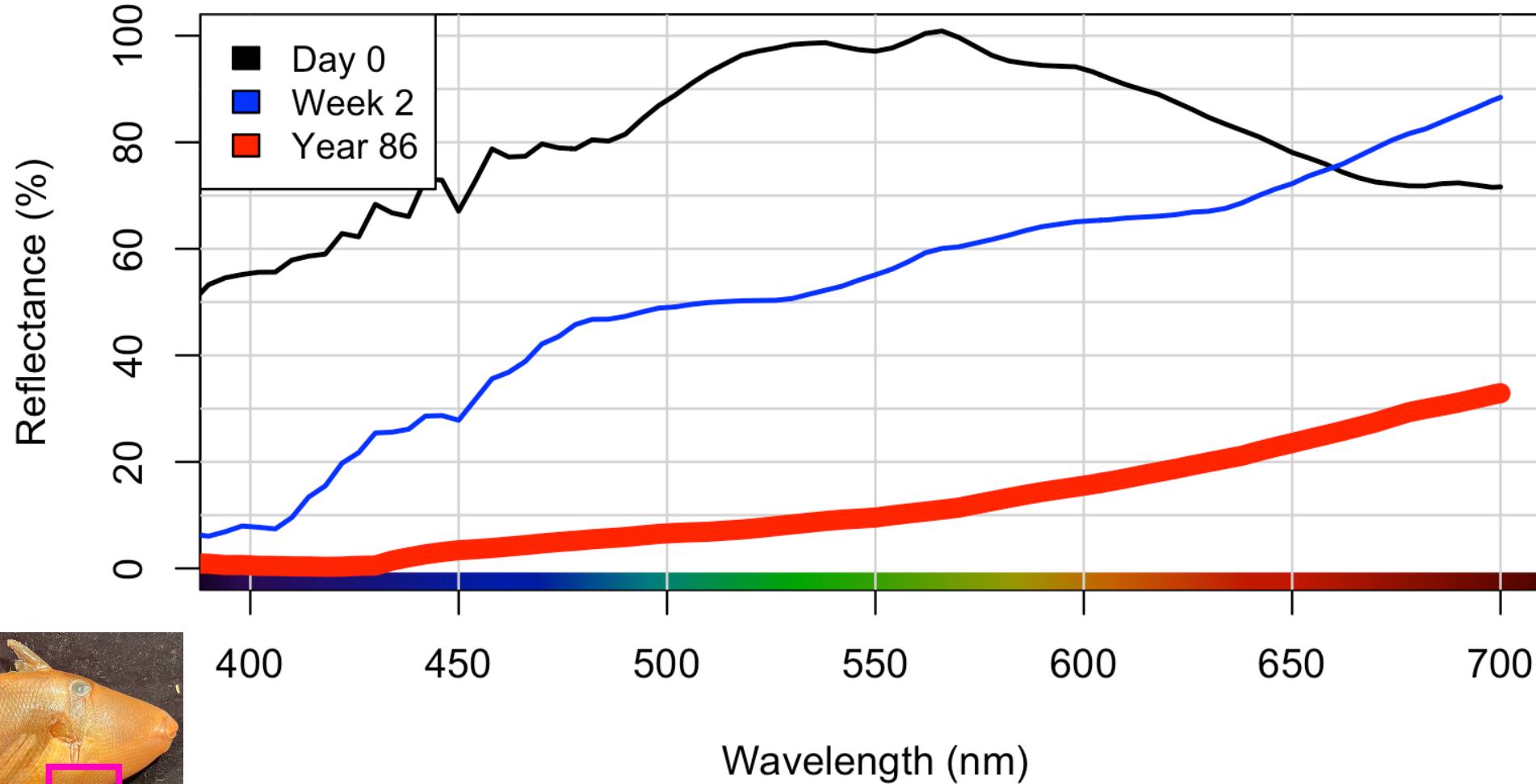


## White Patch



Overall, reflectance is dropping for white patches

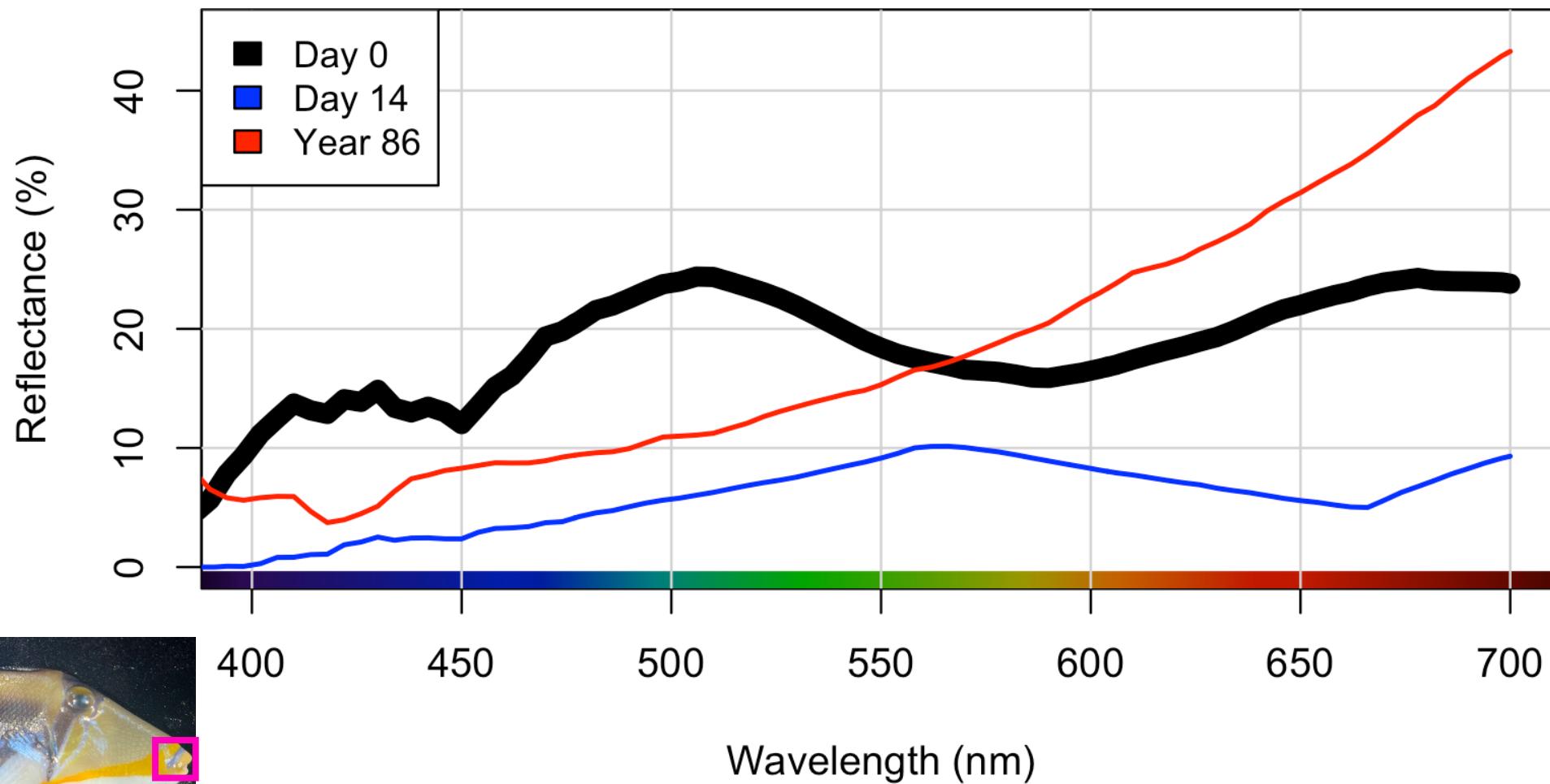
### White Patch



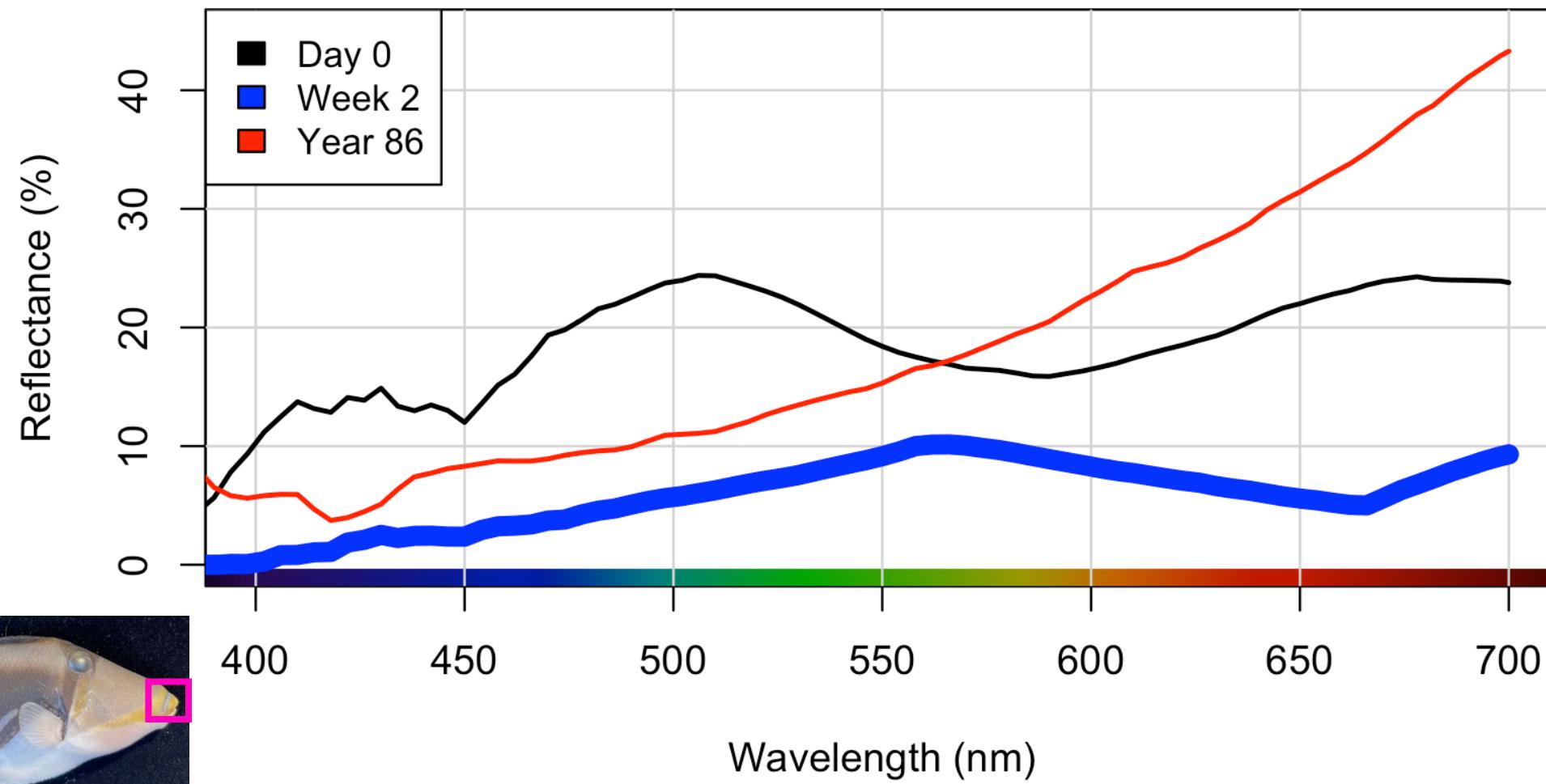
# Blue Patch Reflectance



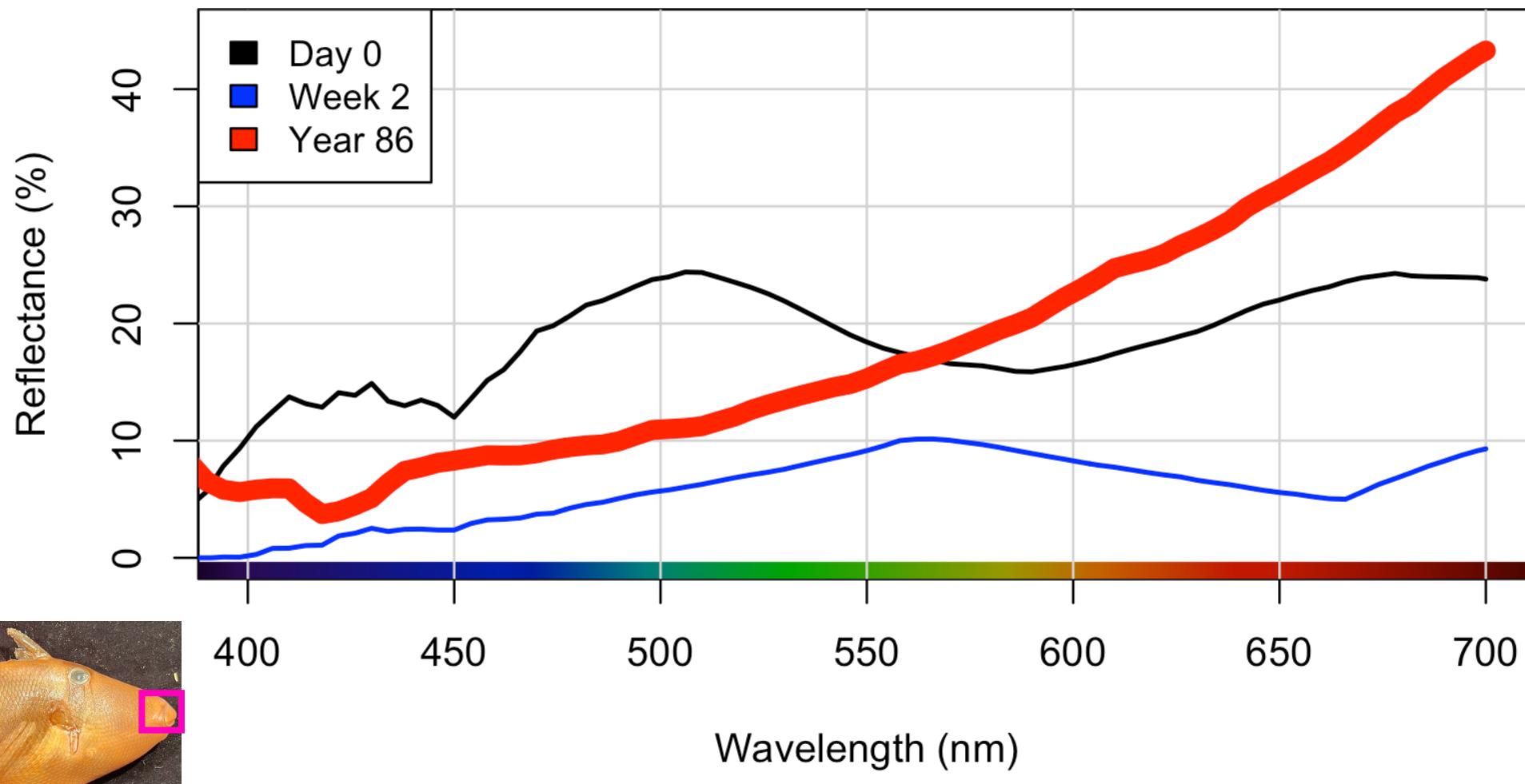
## Blue Patch



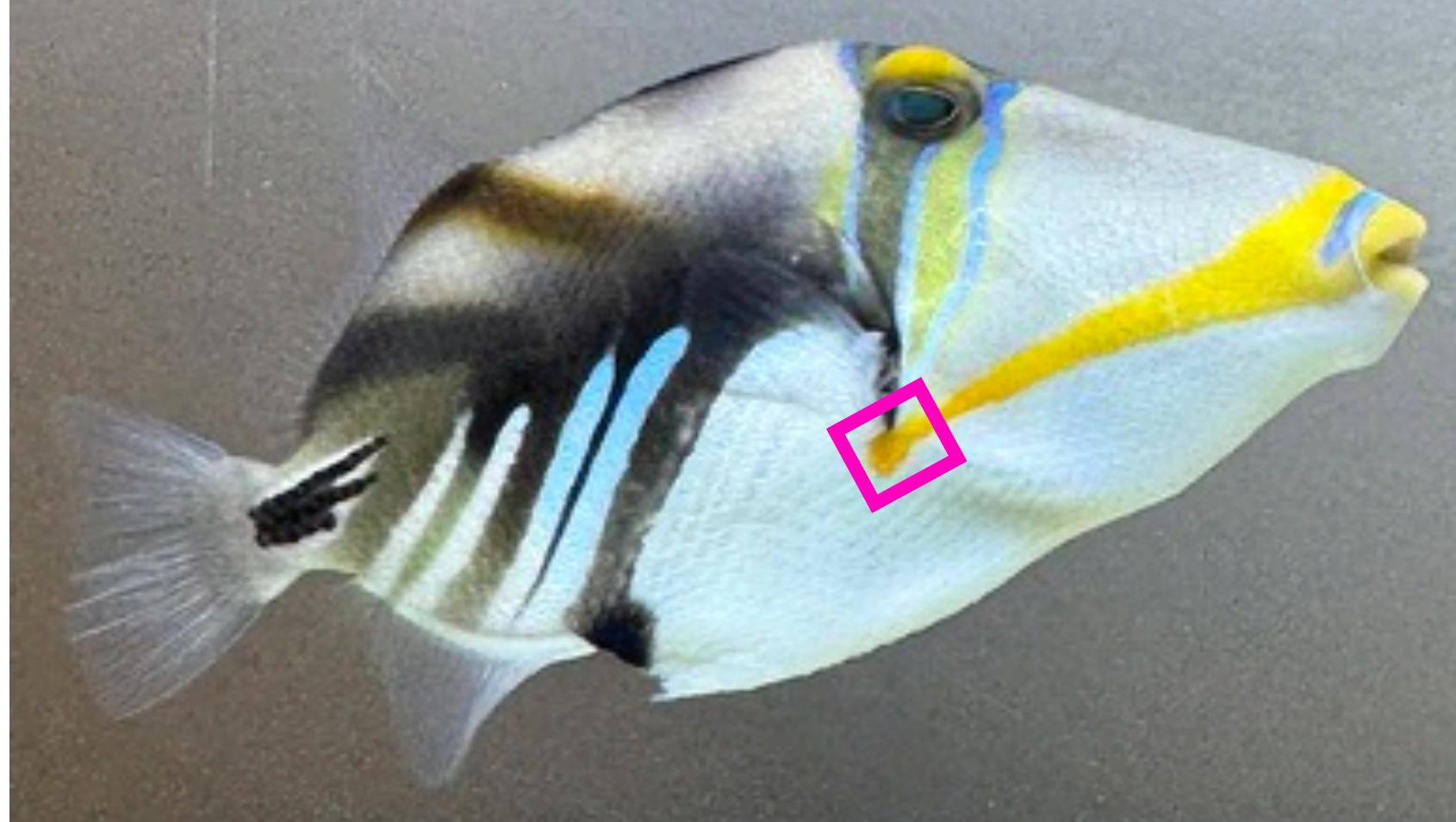
## Blue Patch



## Blue Patch



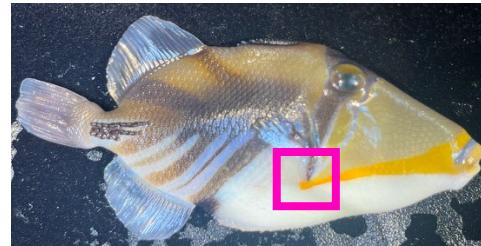
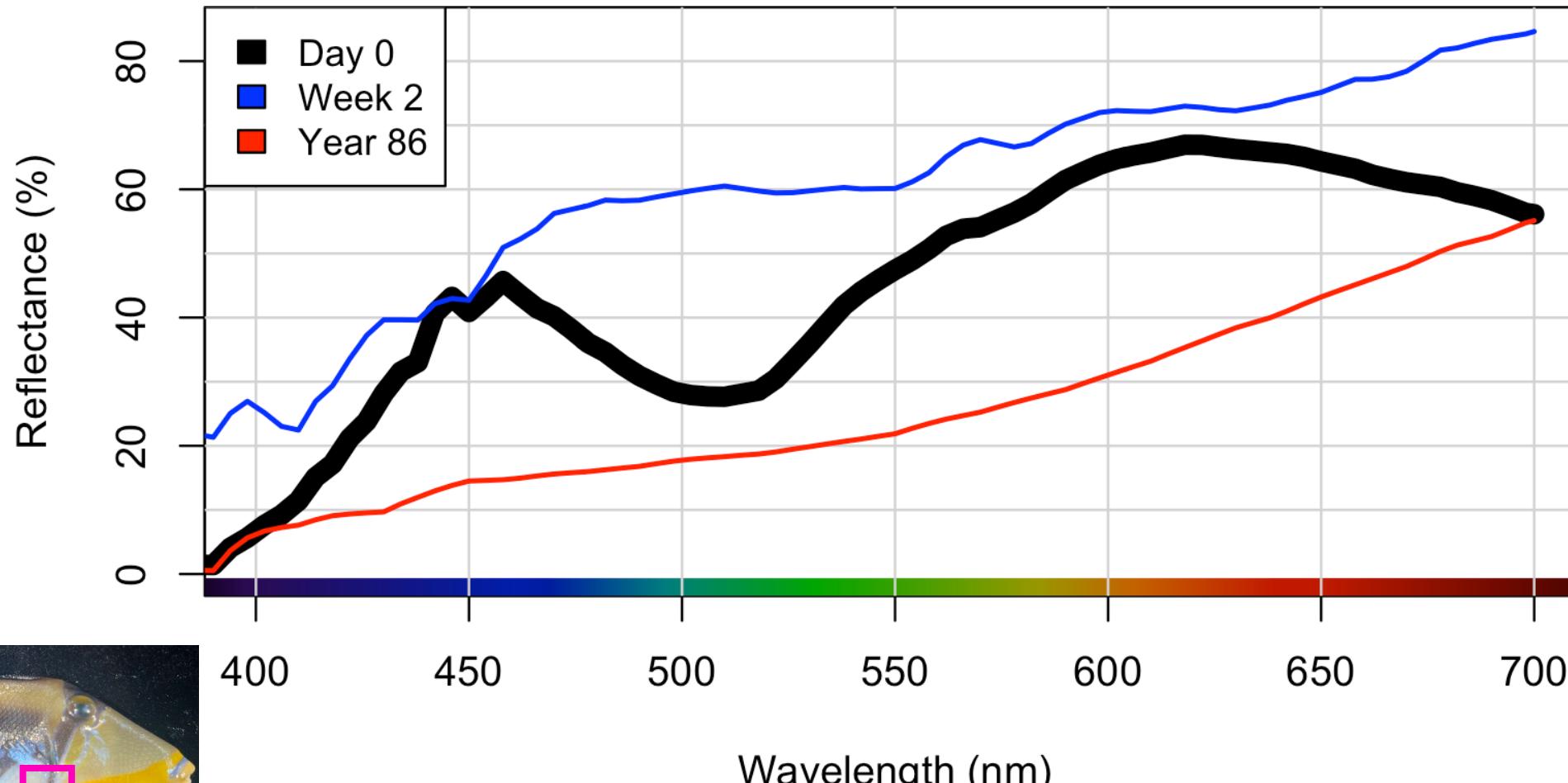
# Yellow/Orange Patch Reflectance



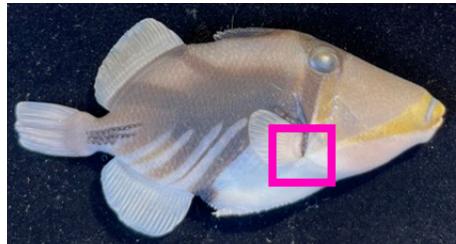
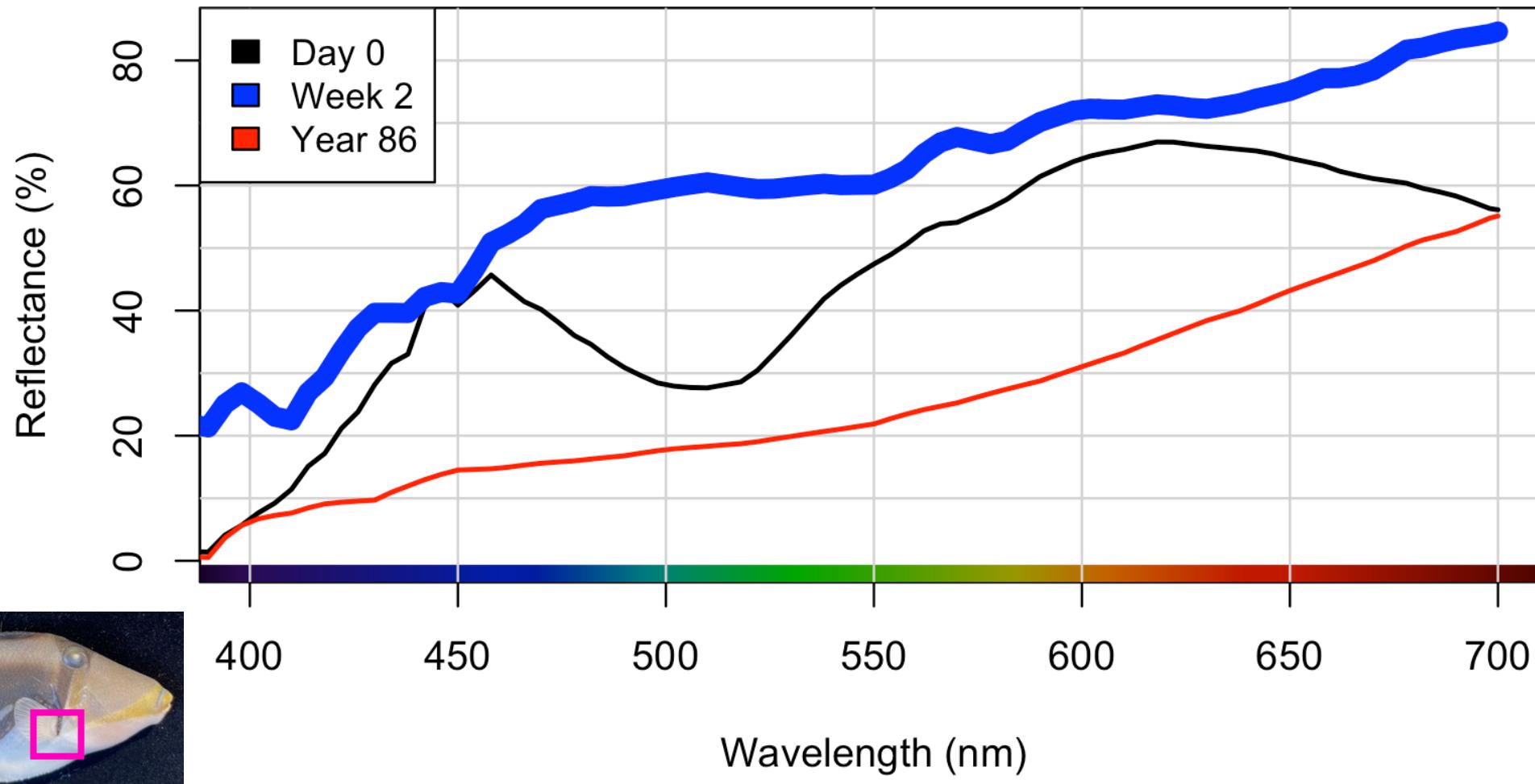
# Yellow/orange patches have higher reflectance %

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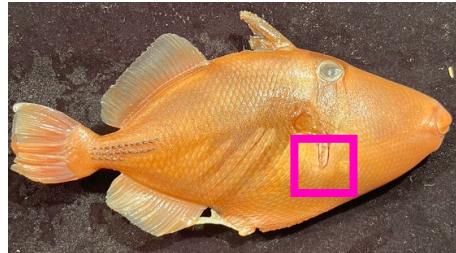
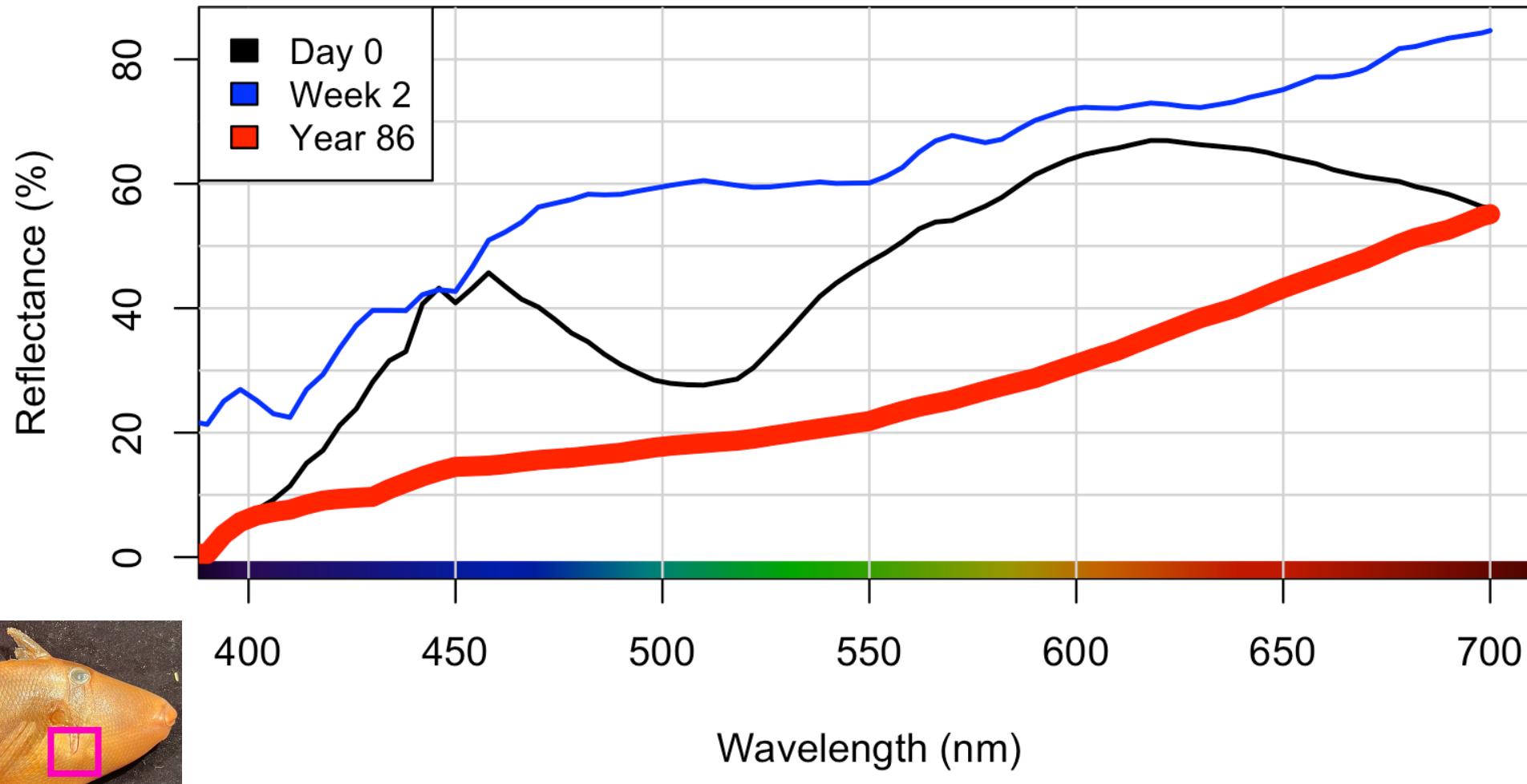
## Orange Patch



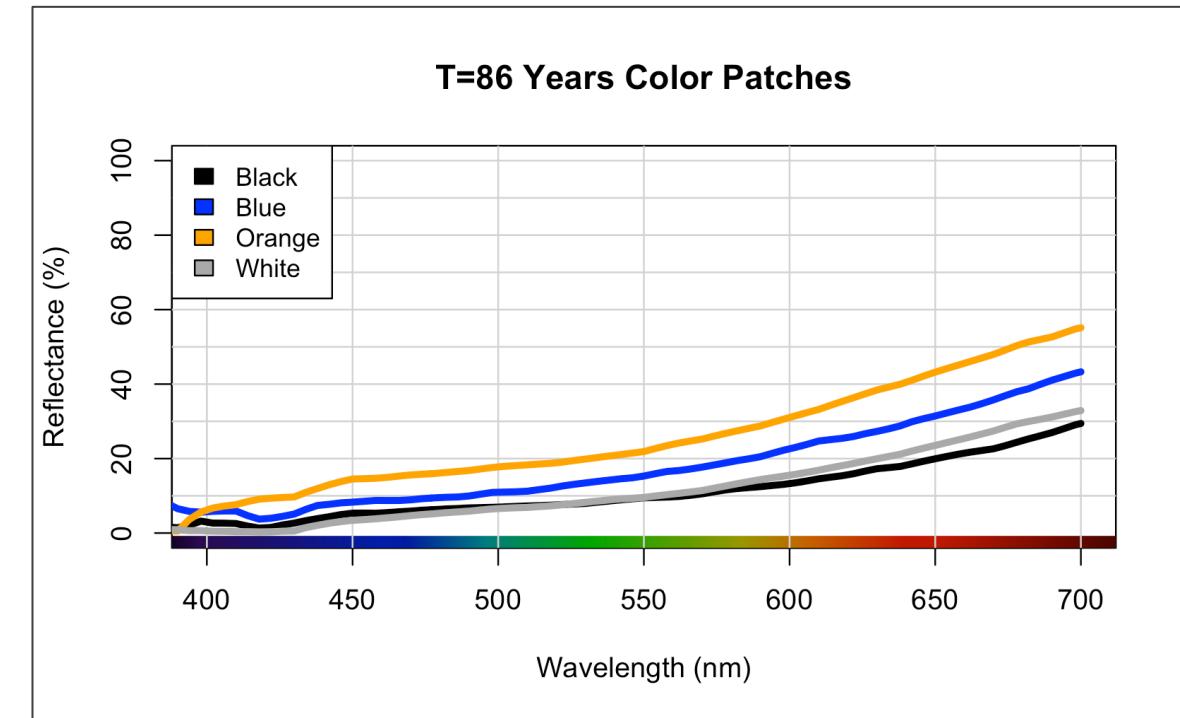
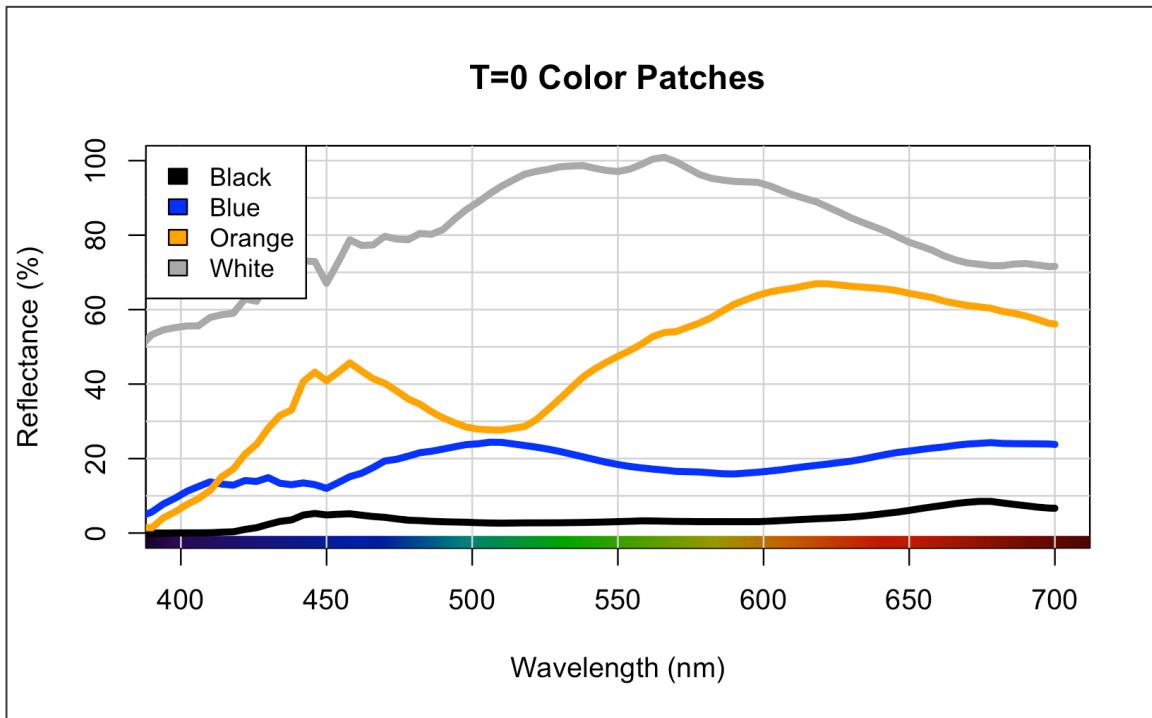
## Orange Patch



## Orange Patch



# In progress...



2. How does formalin fixation affect the retention of color in fish specimens?

# Fish have six types of pigment cells

Five are widespread:

- Melanophores (black)
- Xanthophores (yellow)
- Erythrophores (red)
- Iridophores (iridescent, blue, silver or gold)
- Leucophores (dull, whitish)

One rare pigment:

- Cyanophores (true blue)  
(Bagnara & Matsumoto, 2006; Kelsh, 2004)

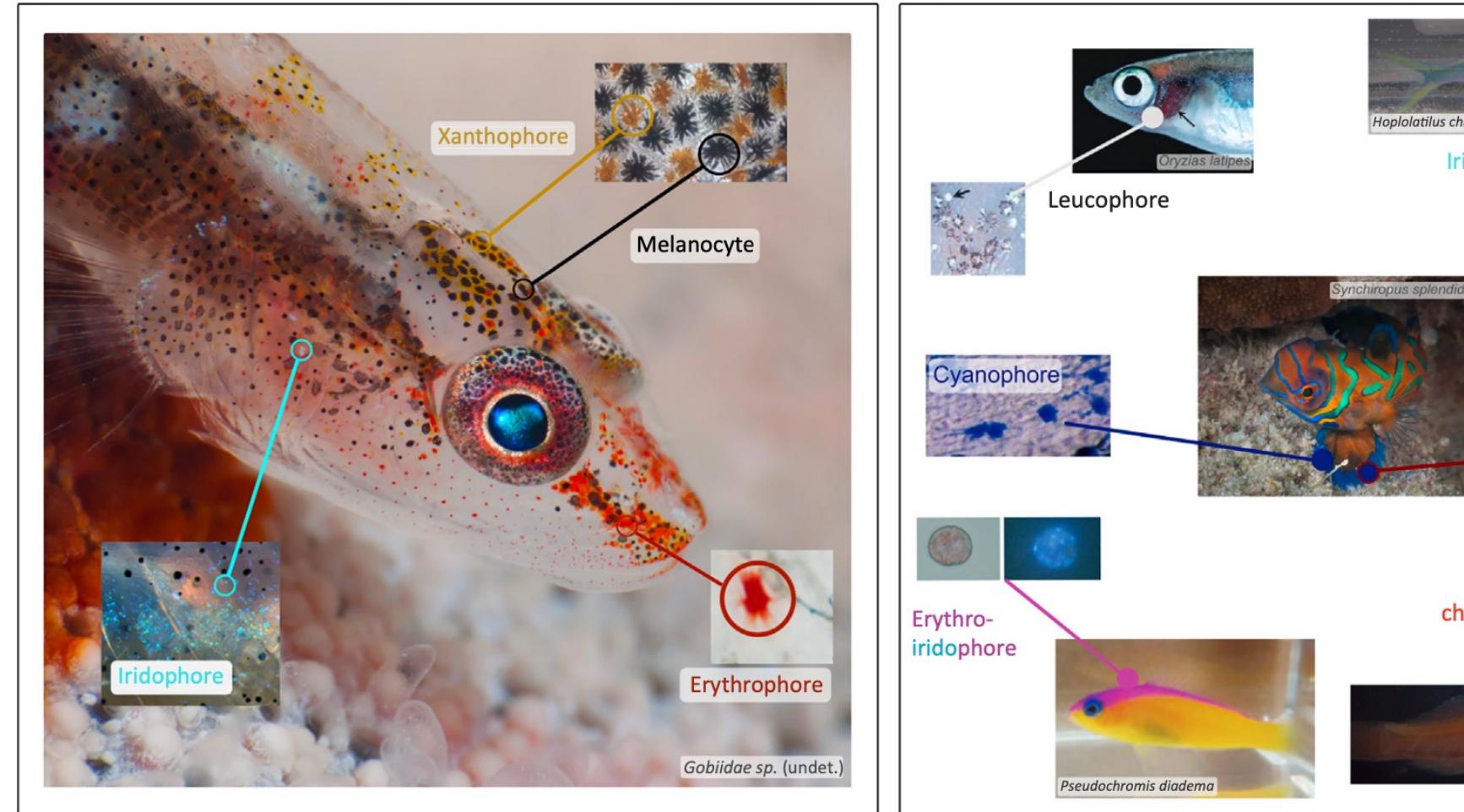
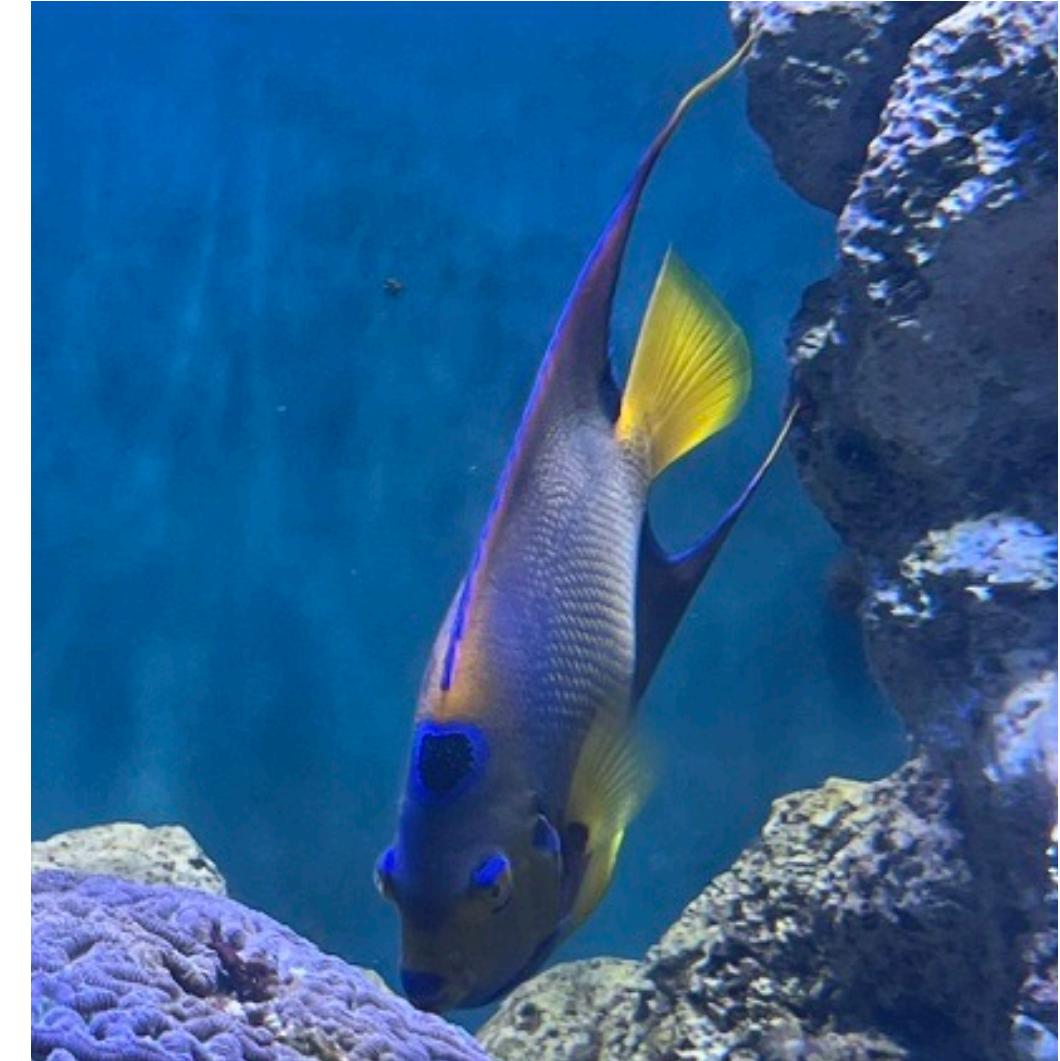


Figure 1. Magic Traits in Magic Fish (Salis et al., 2019)

# Fish also have structural colors



Queen angelfish @ California Academy of Sciences



# What is happening during fixation?



Hirox HRX-01 3-D Digital Microscope

Palenose parrotfish  
*Scarus psittacus*



<https://www.inaturalist.org/observations/22063927>  
Observation © Tom Barnes



T=0



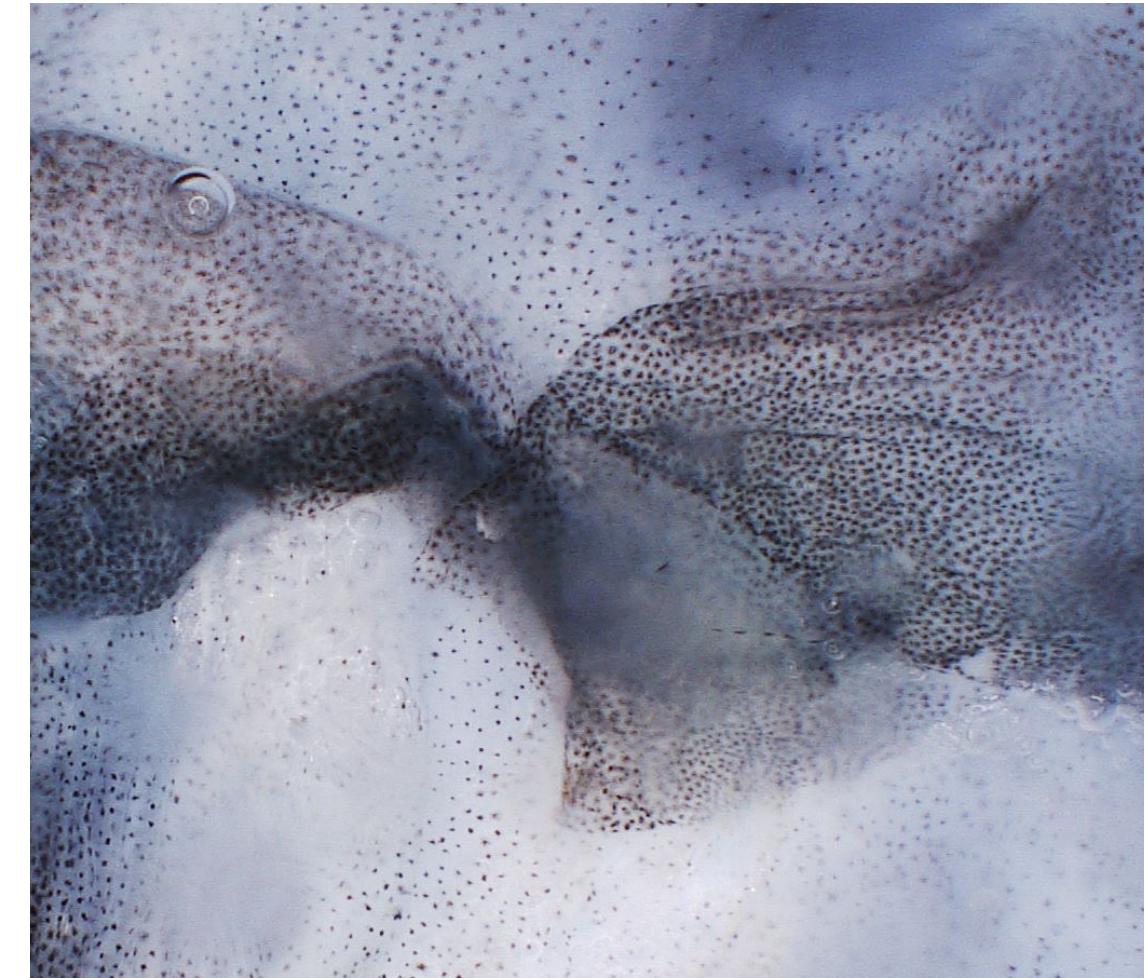
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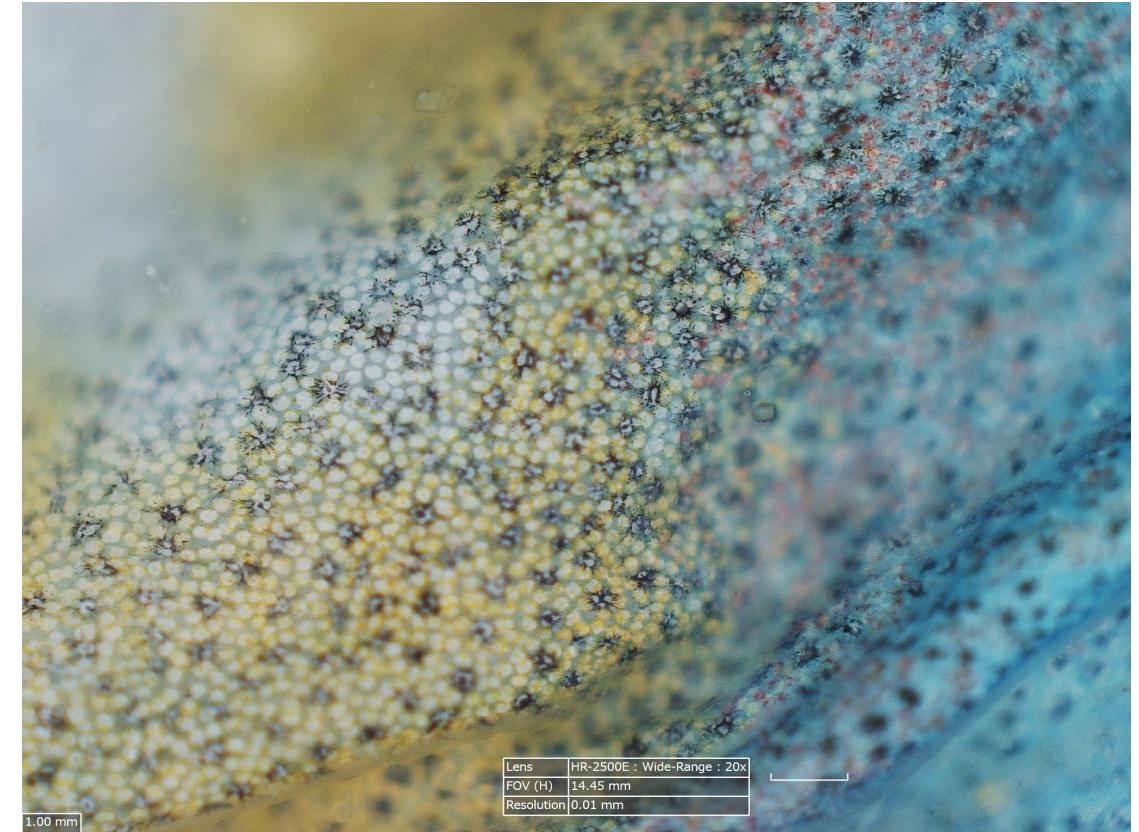


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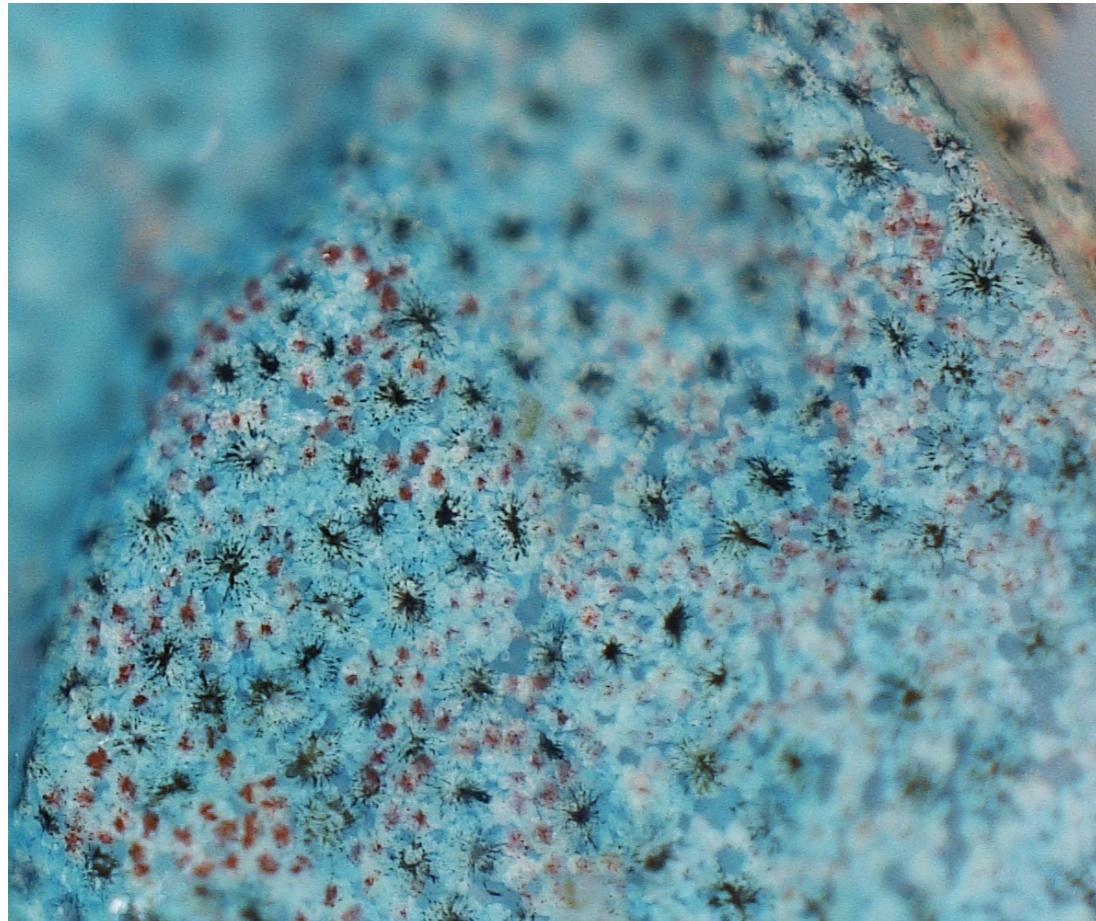
T=14 hours





What pigment cell types do you see?

Color patches have more than one type of pigment cell type present

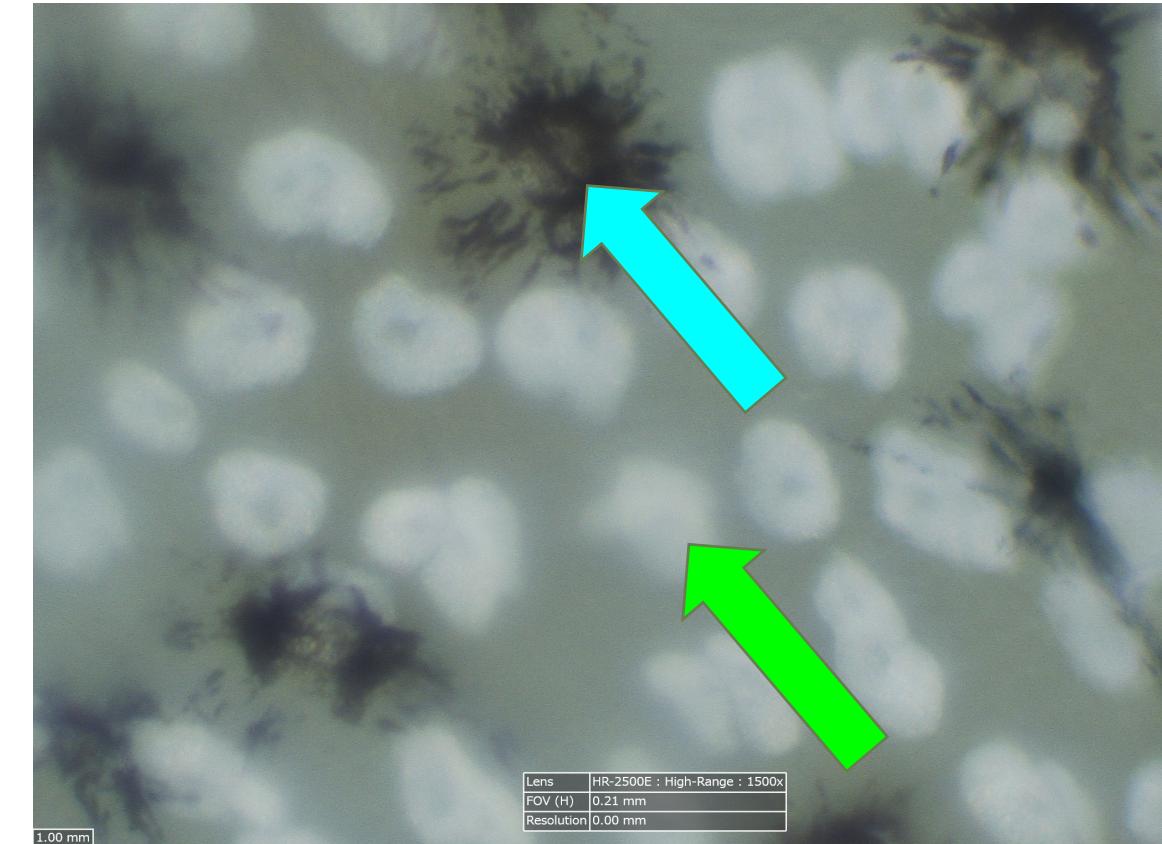
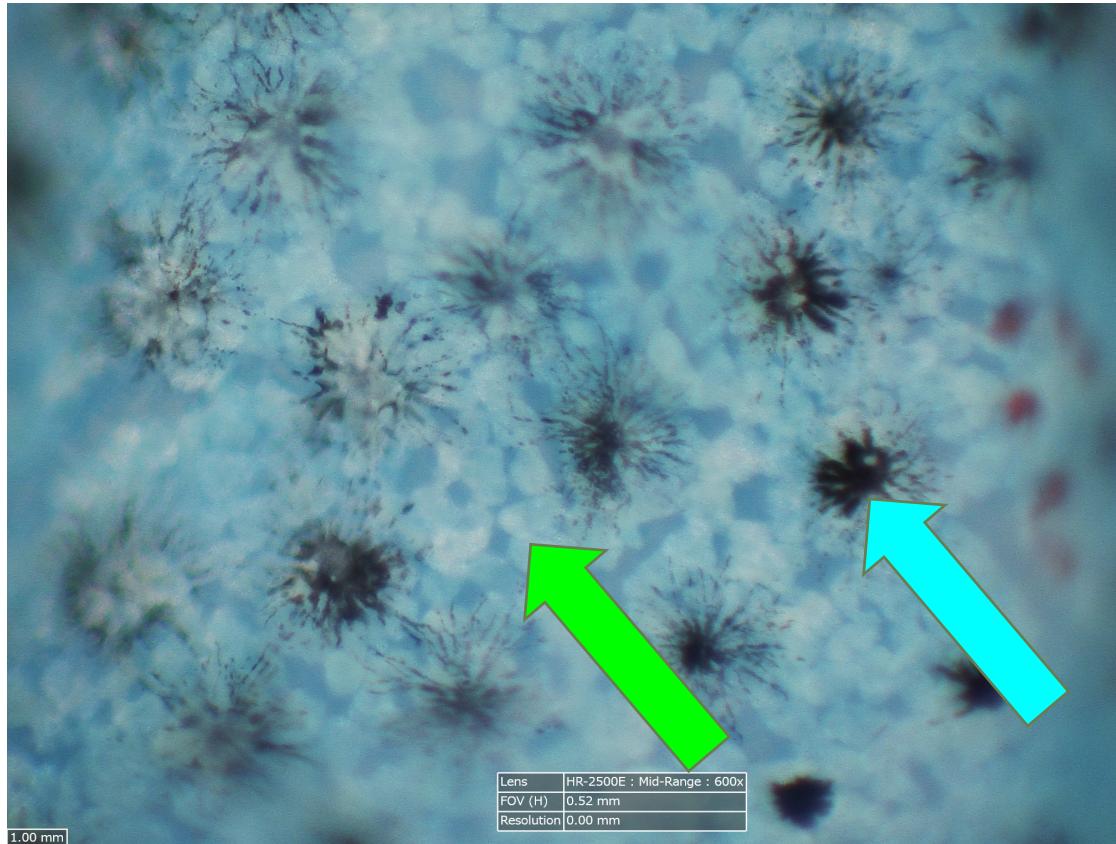


- Melanophores
- Iridophores
- Erythrophores



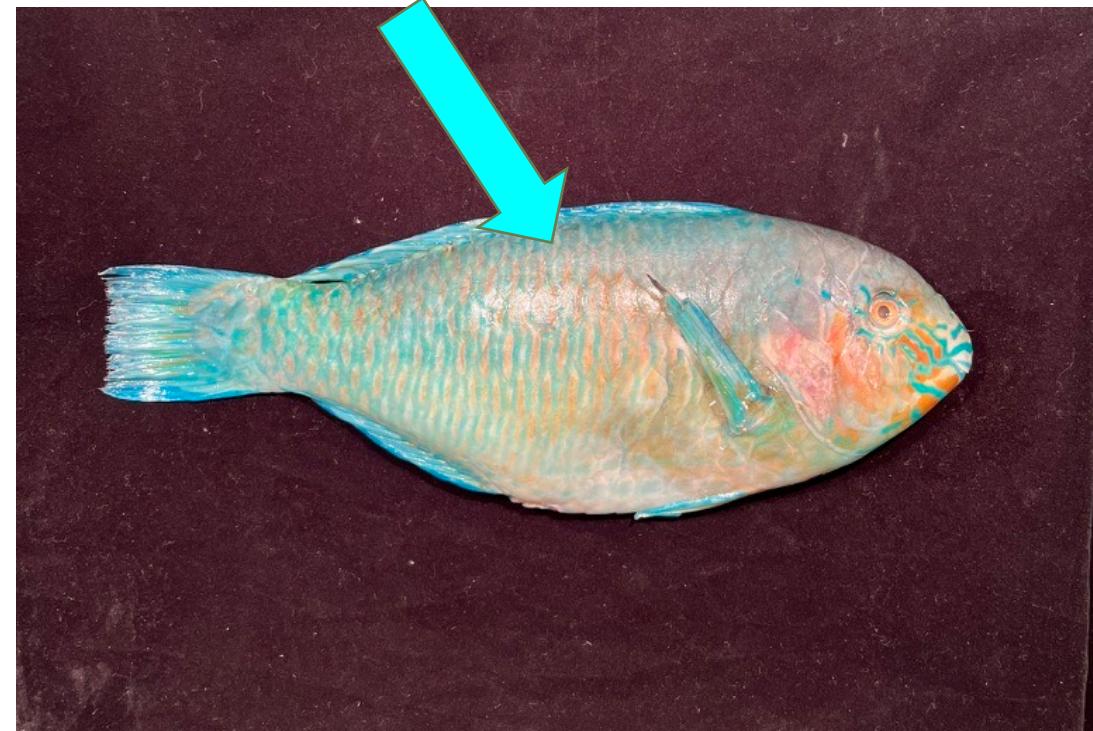
- Xanthophores
- Melanophores
- Iridophores
- Erythrophores

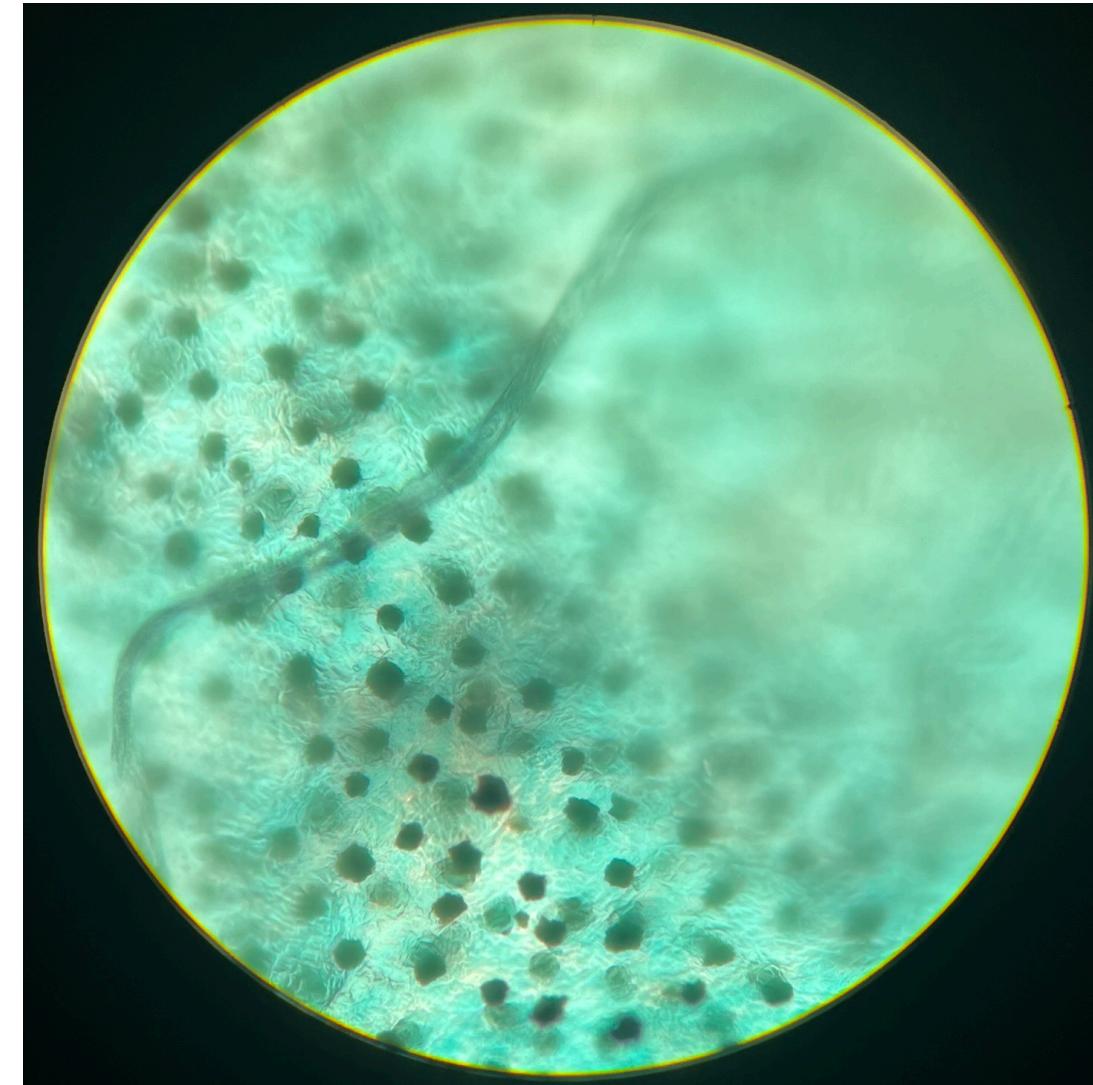
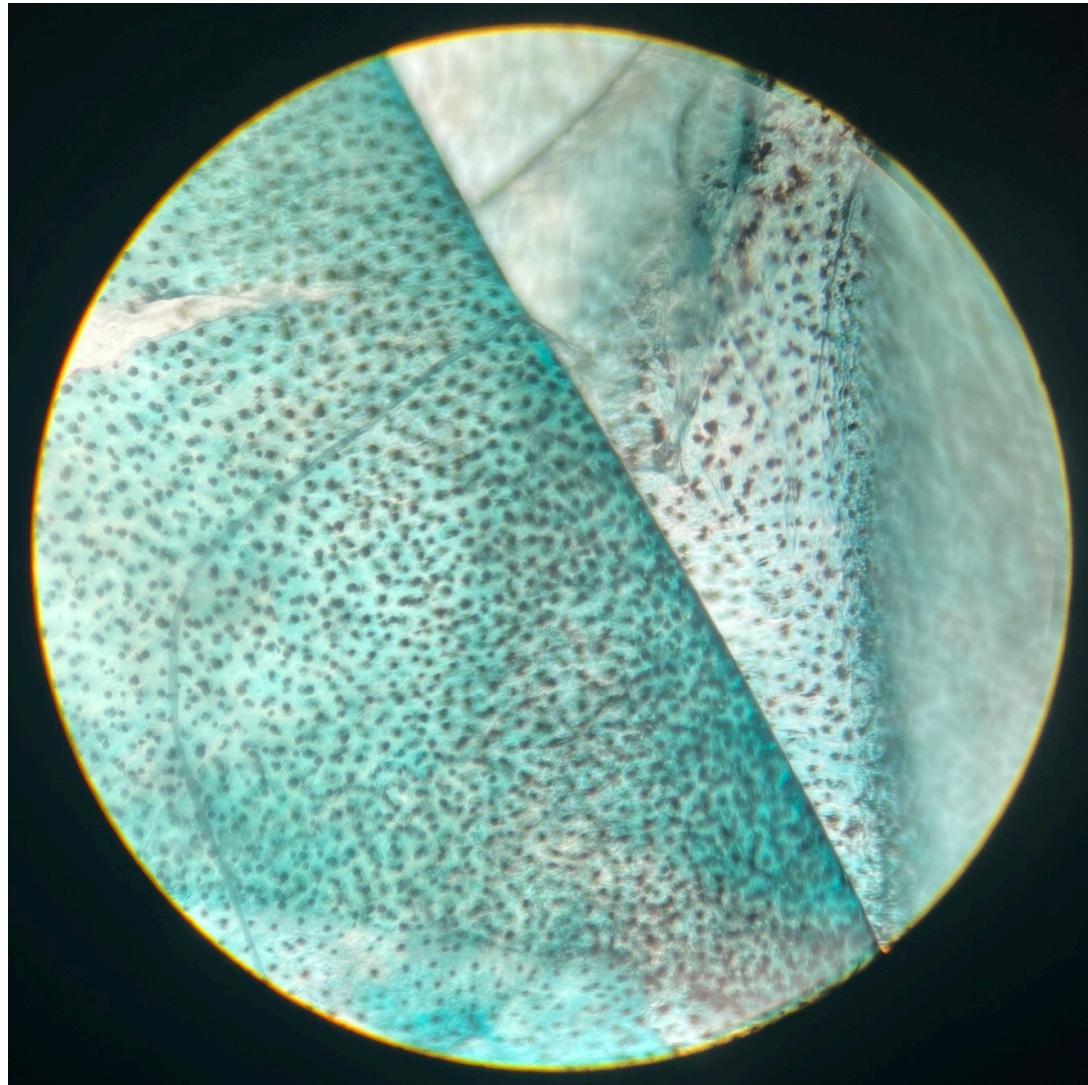
Pigment cells and structural colors combined make the colors we see in fish.



# Blue barred parrotfish

*Scarus ghobban*





# Conclusions

1. Is there any signature of original coloration in museum specimens?
  - Yes!?!??
  - Black & white color patches become more reflective and there's a shift towards warmer wavelengths
  - Blue & Orange - unclear what's going on
    - Refine data collection protocol to account for error
  - Nevertheless, reflectance spectra still differed between colors = hope for using museum specimens 

# Conclusions

2. How does formalin fixation affect the retention of color in fish specimens.

- Pigment is leeched out during this process
- Melanophores are consistently present over time
- But Leucophores, Erythrophores, and Xanthophores are the first to fade away
- Cannot answer this question in detail **-YET!**

# Next Steps

- HIS protocol w/ a more controlled environment
- Compare color patches in preserved specimens across multiple decades
  - Trial data acquired from rockfish and parrotfish
- Observe changes at microscopic level
  - Research abroad at OIST
  - Access to their resources and different fish species
  - Structural vs pigment cells
- Compare organization of pigment cells within skin layers
  - Histology slides

# Acknowledgments

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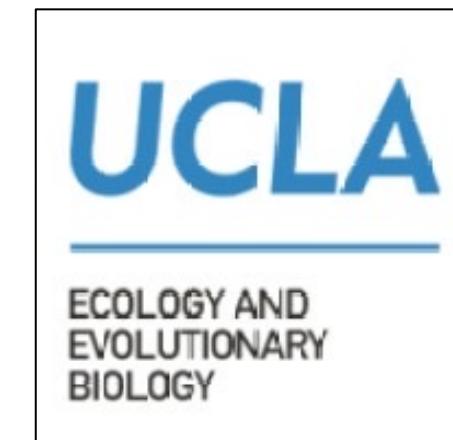
Los Angeles County Museum of Natural History

University of California Los Angeles

SICB Charlotte Mangum Student Support Program

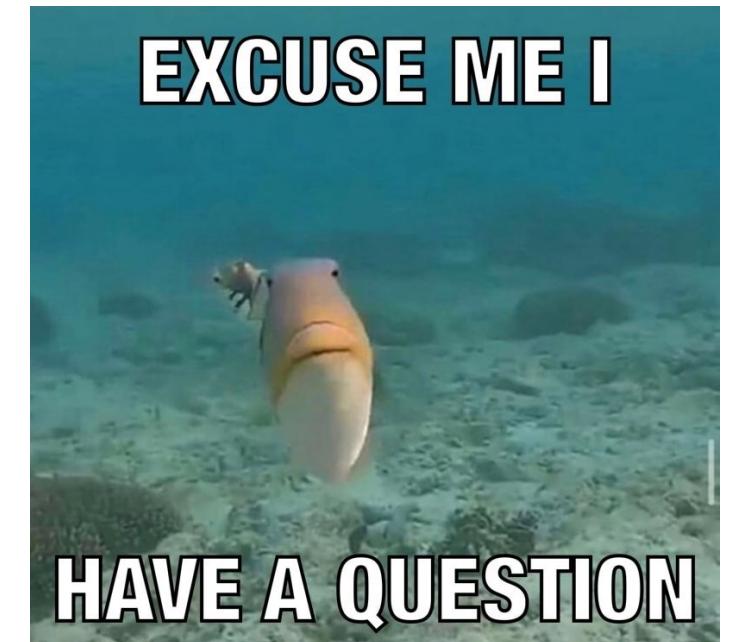
UCLA EEB Conference Grant for Continuing Students

Mom, Dad, and Friends



Thank you!  
Questions?

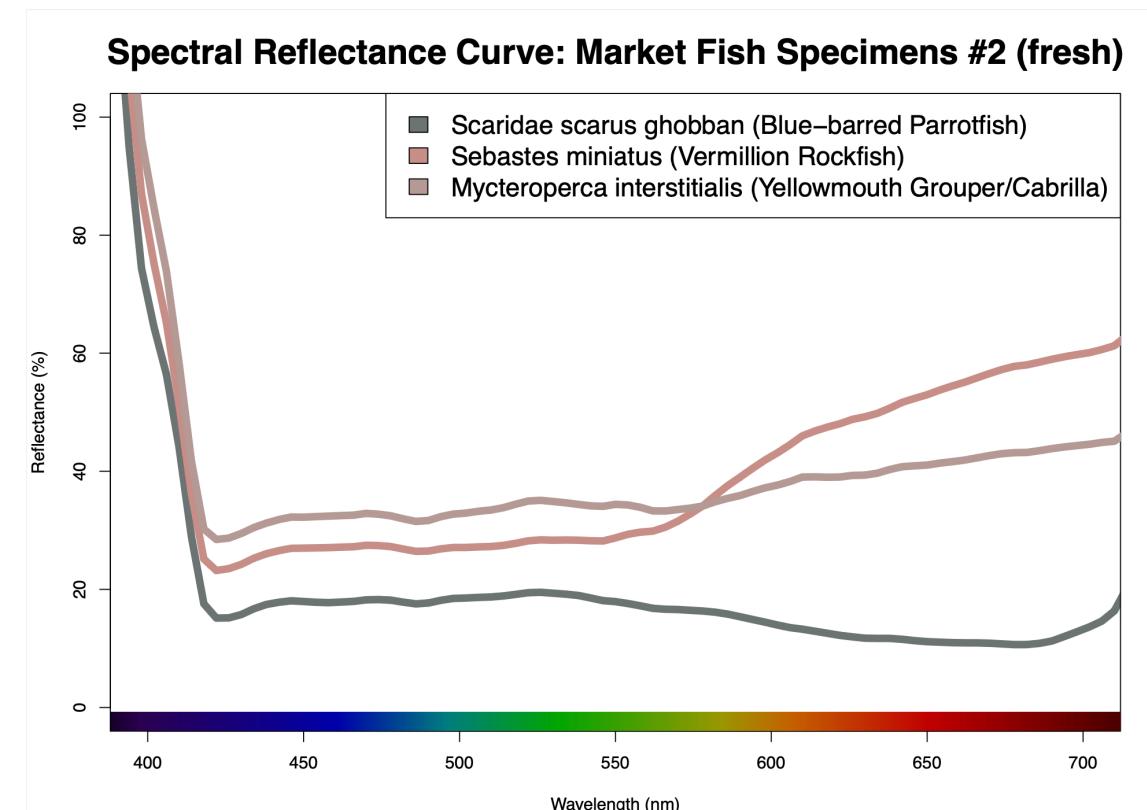
Contact info:  
[rosamariorduna@ucla.edu](mailto:rosamariorduna@ucla.edu)



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- Focus on *scarus ghobban* (have pic of fresh guy on all following slides)
- choose 2-3 different color patches
- Choose 1 patch (blue?) for demonstration
- Show reflectance of patch from fresh day to other time points
- This what it look like after 60 years
- Do this for other color patches
- Put 3 color group graphs condensed into their own graph
- Next slide: when I look at what patches show 1 month in versus 60 years in, there IS/IS NOT color information
- Do same thing w/ patches for MORE fish
- Clear color change, but can I tell red from green from blue, etc????? Show reflectance spectra



- PART TWOOOOOOO
  - Triggerfish
  - Compare to marshall
  - Its colorful, which patches can I tell apart between live specimen and fixed
- 
- PART THREE
  - What is actually happening w/ fixation!!
  - Structural color versus pigment color
  - Show timelapse of color preservation fading
- 
- Conclusion TBD...