Brief overview

Biological Rationale

Resources & Timeline

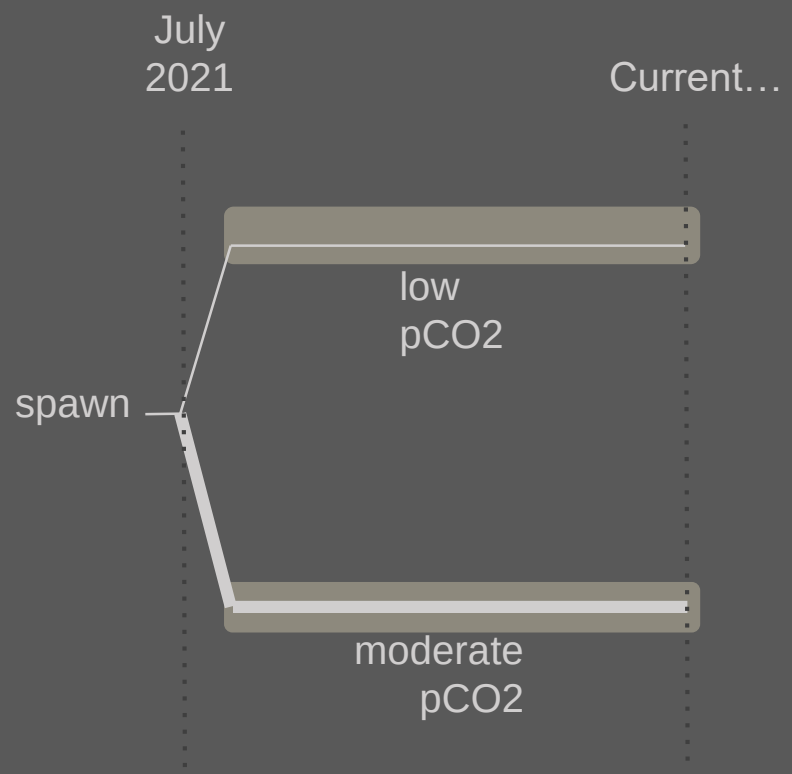
by Sam Gurr

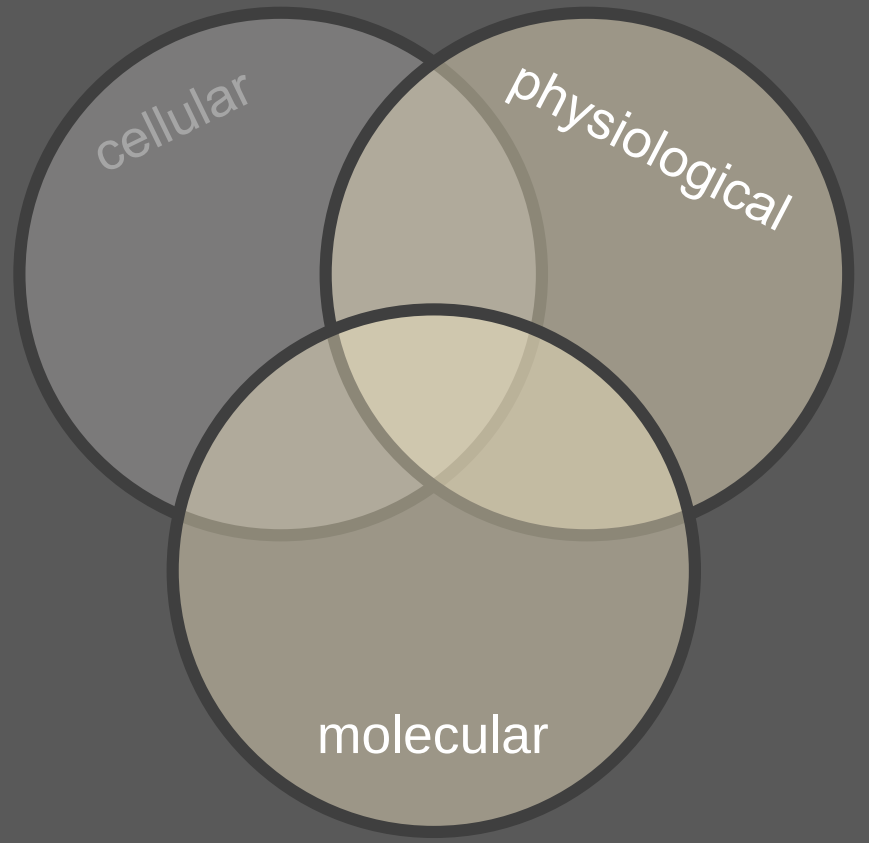
Brief overview

Biological Rationale

Resources & Timeline

Our ongoing scallop experiment…



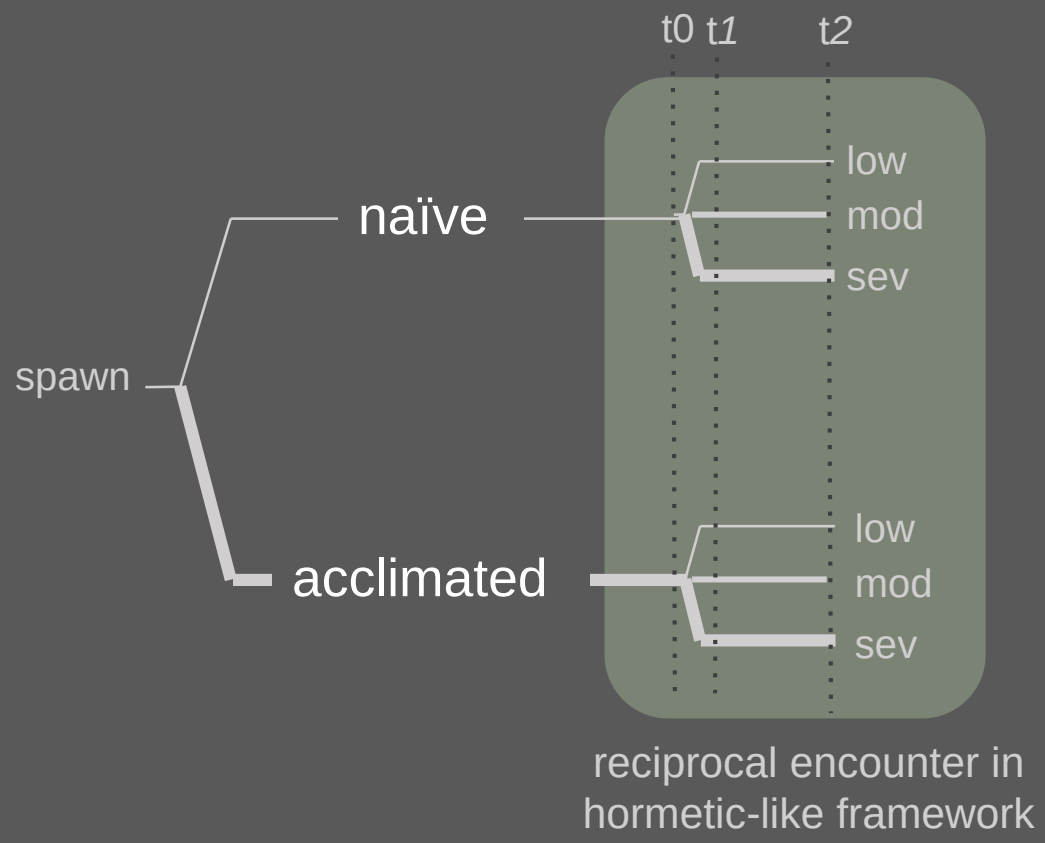


Suite of physiological measurements
- growth/survivorship
- respiration rate
- clearance rate
- biodepositon
- excretion

genetic variation
- allele frequency
- putatively adaptive loci

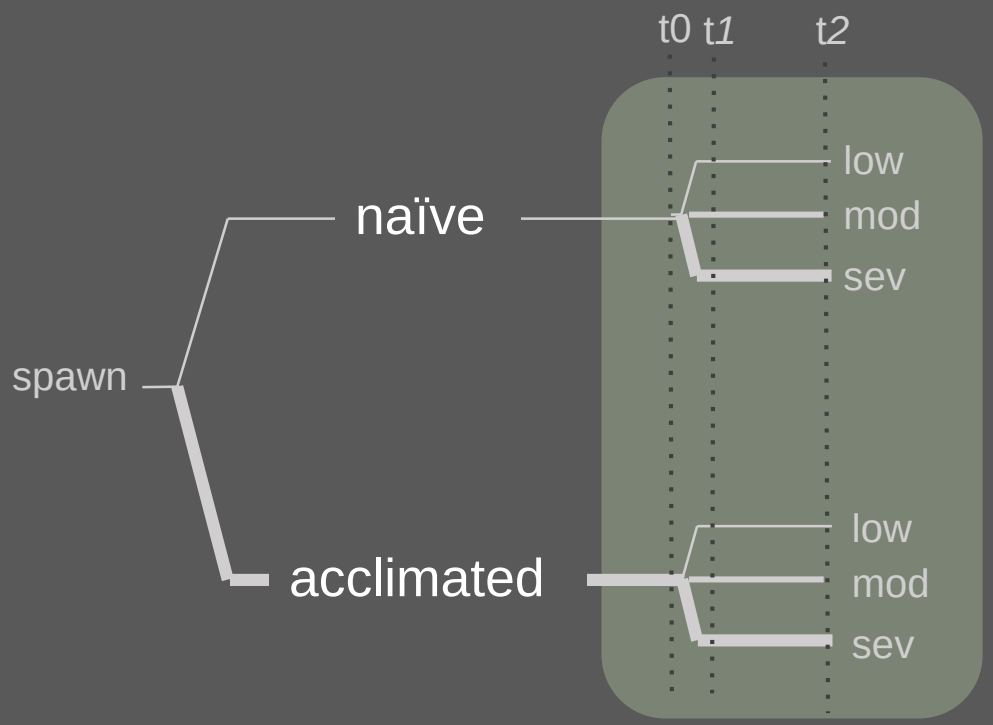
gene expression
- enriched functions/pathways
- differentially-expressed genes

Understand cellular mechanisms of OA response

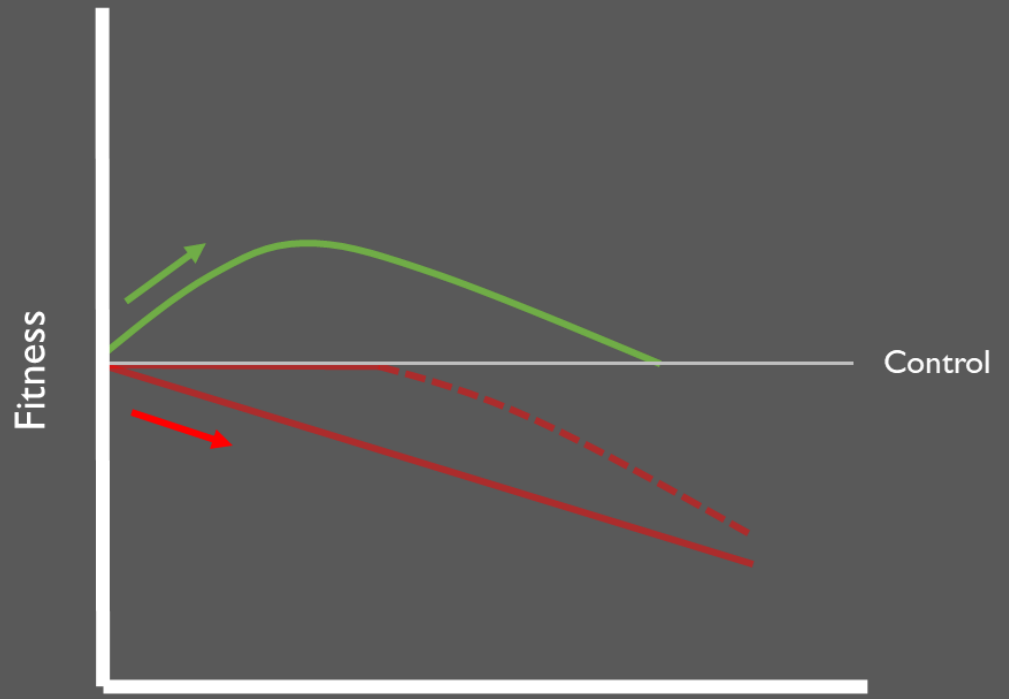


reciprocal encounter in
hormetic-like framework

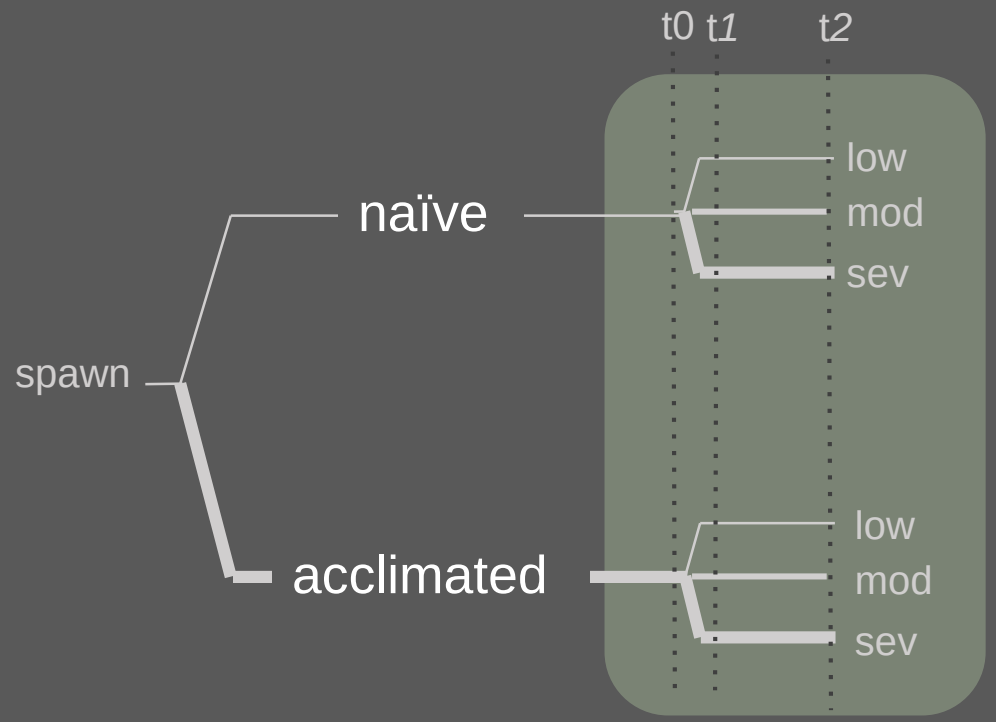
Understand cellular mechanisms of OA response



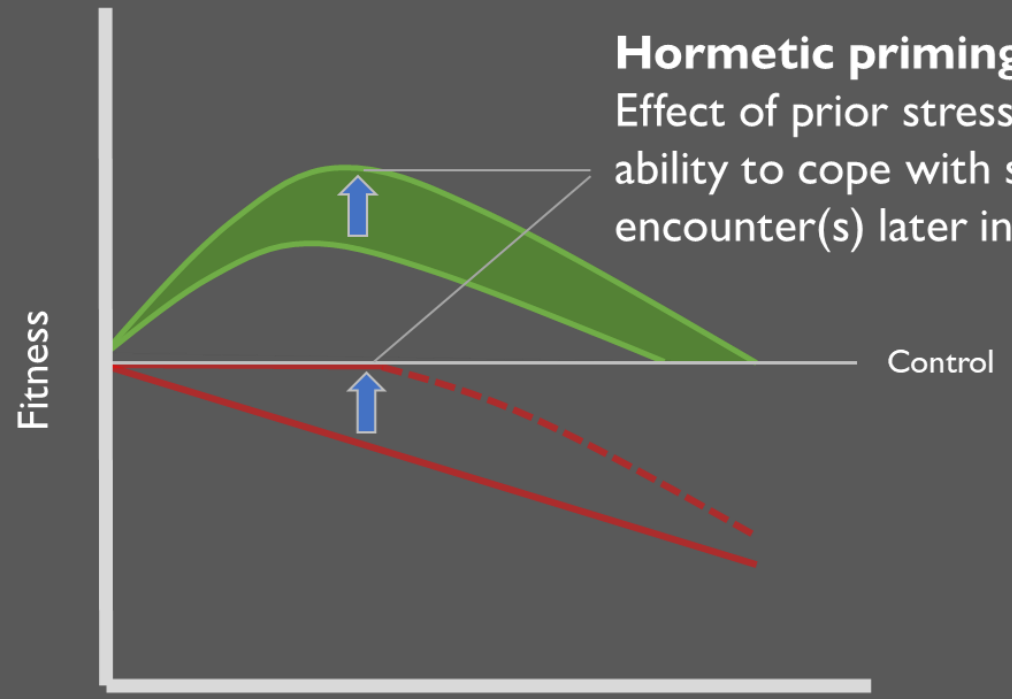
reciprocal encounter in hormetic-like framework



Understand cellular mechanisms of OA response

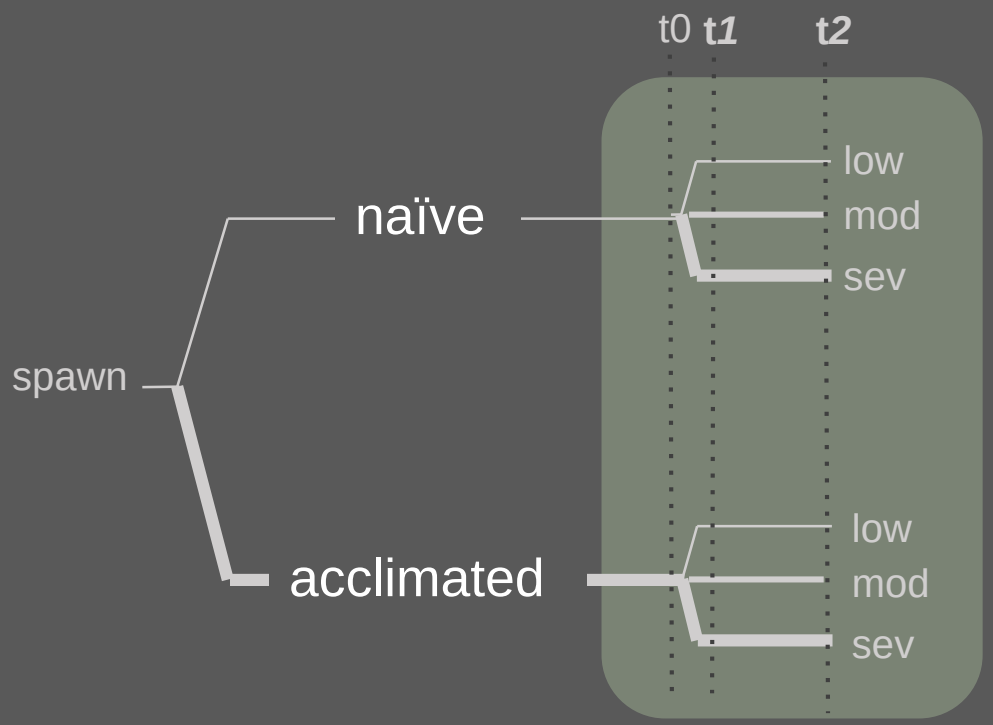


reciprocal encounter in hormetic-like framework



Hormetic priming =
Effect of prior stress exposure on ability to cope with subsequent encounter(s) later in life

Understand cellular mechanisms of OA response



hemolymph extraction

measured same day

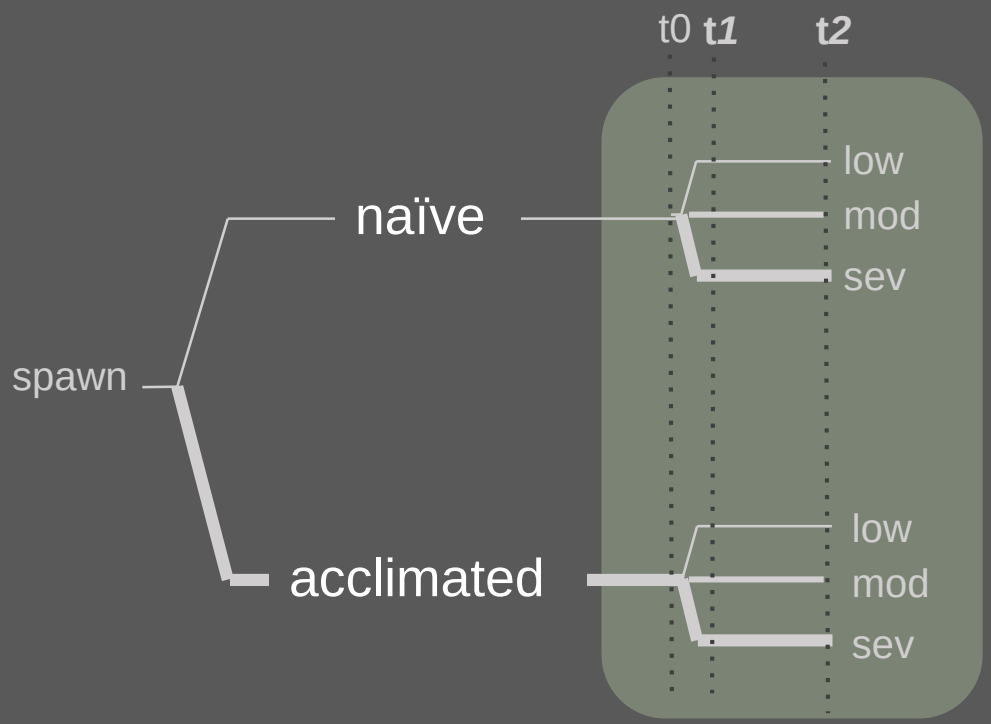
- mitochondrial & hemocyte status

findings incentivize later investigations…

tissue preservation

- qPCR for *a priori* genes of interest
- colorimetric assays

Understand cellular mechanisms of OA response



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Environmental sensor

Mitochondrial dysfunction & alternative pathways

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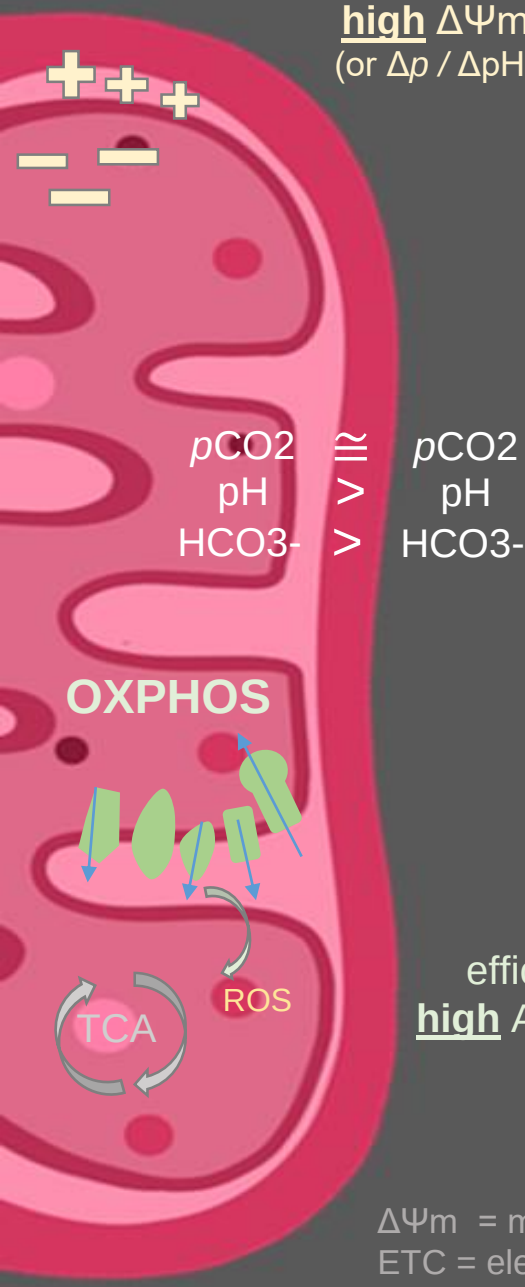
Mitochondria under normal/optimal conditions



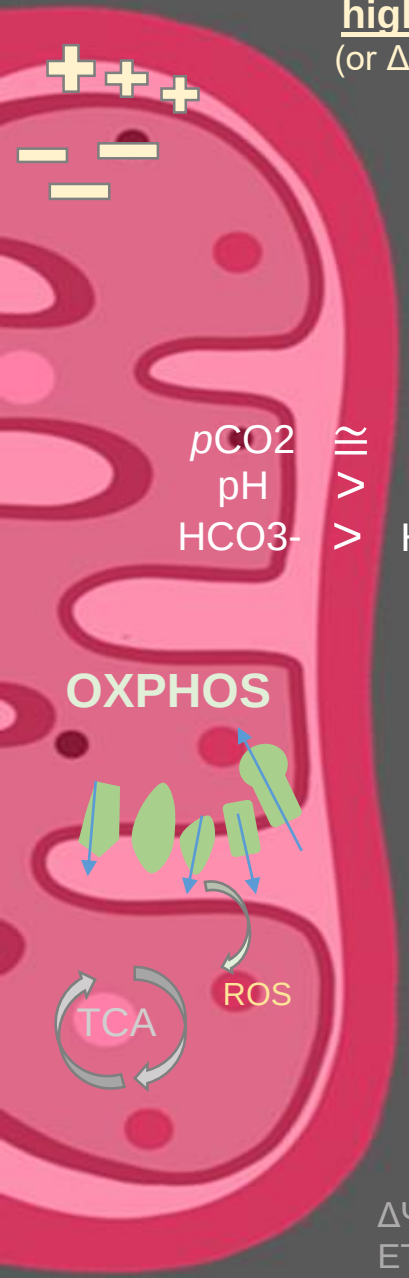
efficient ETC /
high ATP synthesis

‘Power house’ of the cell

- electropositive gradient drives ATP synthesis
  (+) intermembrane space vs (-) inner matrix
- main source of metabolically-produced CO2
  - high HCO3- in mitochondria acts as a buffer, inner mitochondrial pH typically > cytosol

ΔΨm = mitochondrial membrane potential
ETC = electron transport chain
OXPHOS = oxidative phosphorylation

Mitochondria under normal/optimal conditions



efficient ETC /
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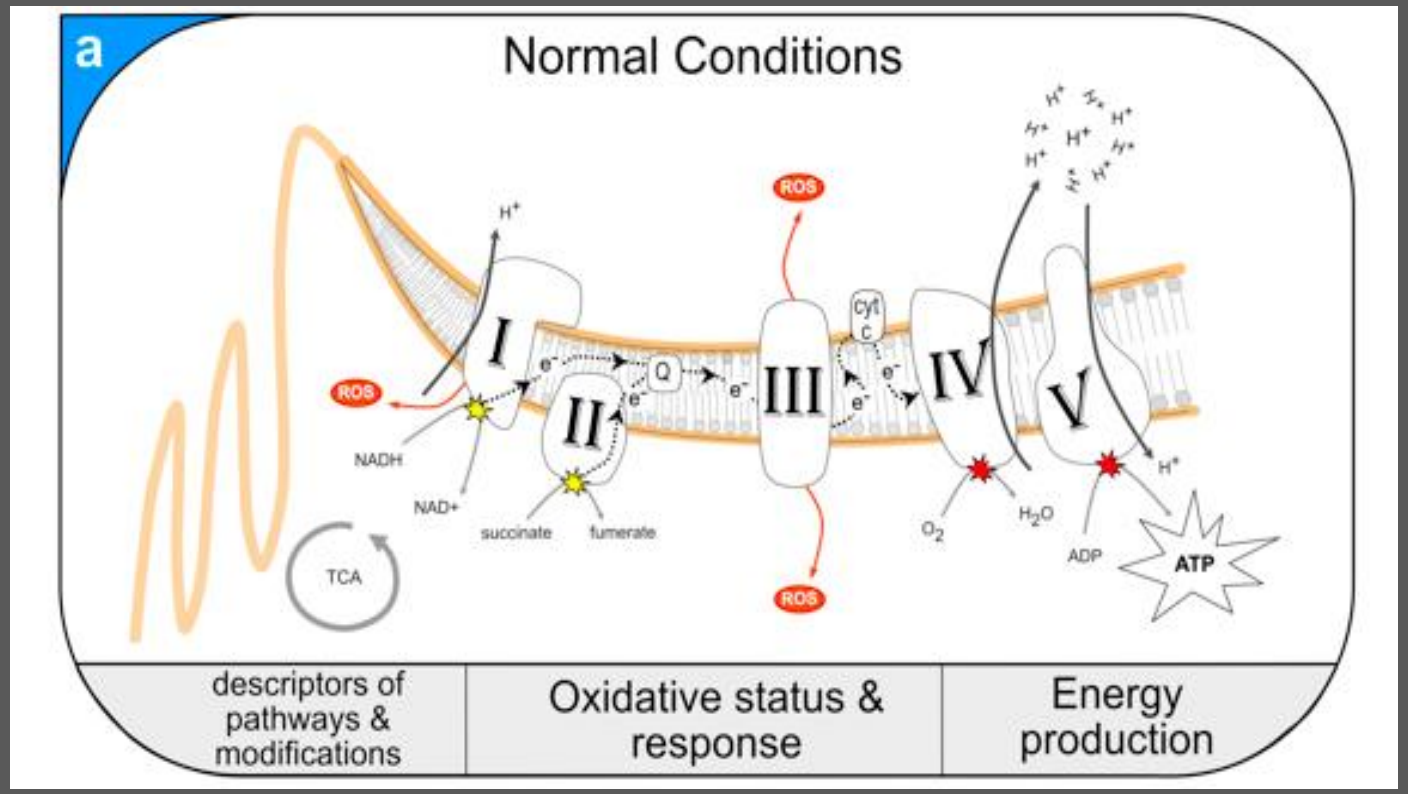

ΔΨm = mitochondrial membrane potential
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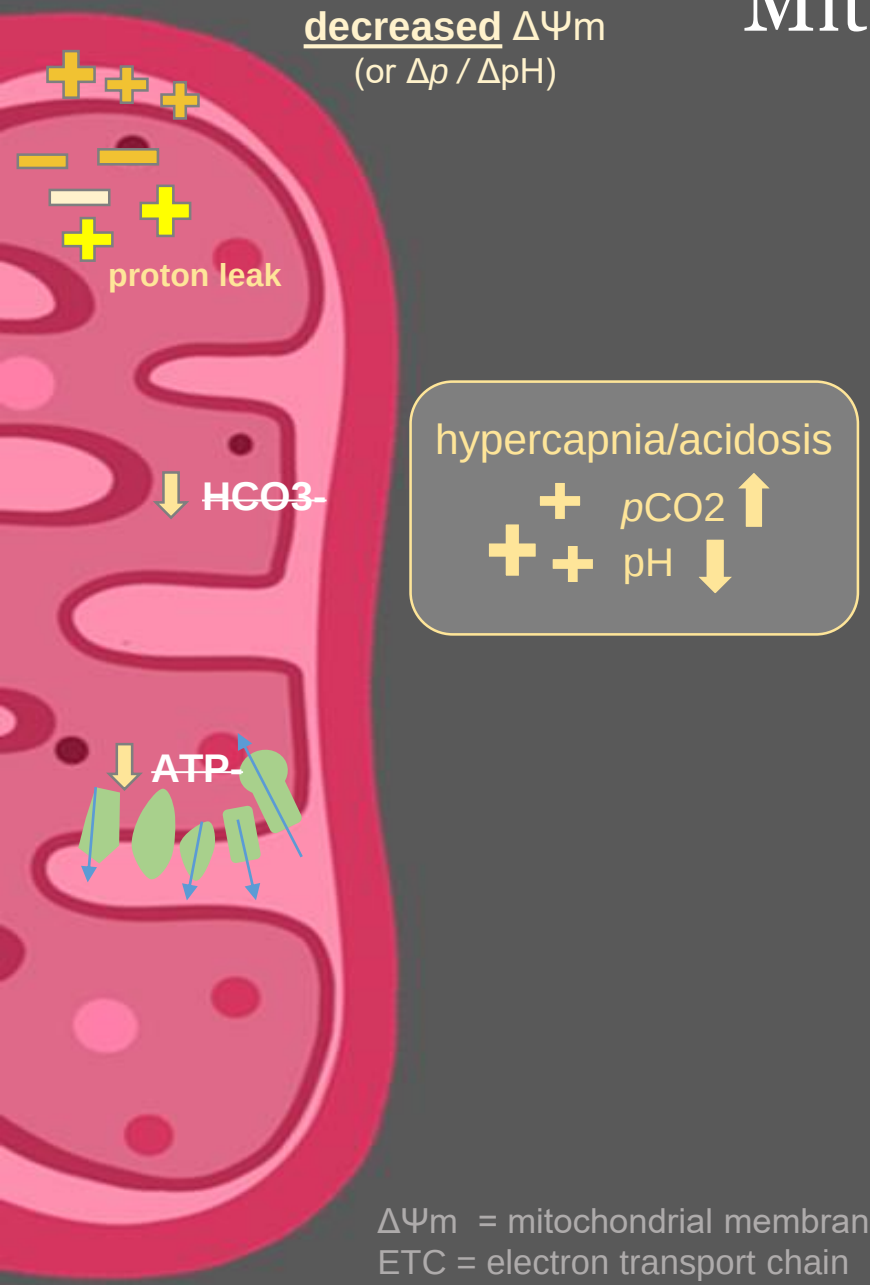
Mitochondrial dysfunction under OA conditions



hypercapnia/acidosis

*p*CO2 ↑

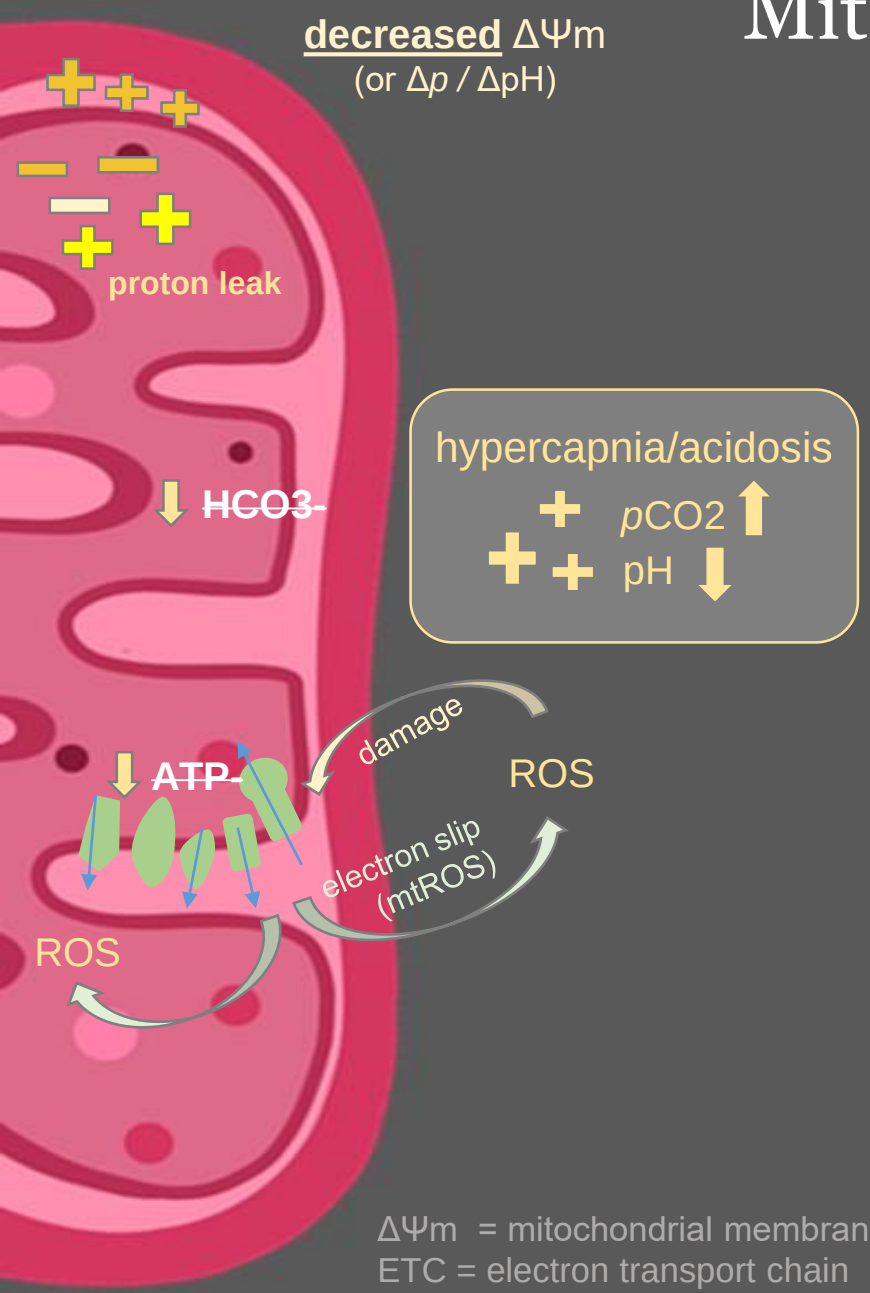
pH ↓

Several routes to decrease ATP

- overloads bicarbonate buffer in matrix
  - proton leak
  - lower membrane potential (ΔΨm)
  - decreased efficiency of OXPHOS

ΔΨm = mitochondrial membrane potential
ETC = electron transport chain
OXPHOS = oxidative phosphorylation

Mitochondrial dysfunction under OA conditions



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- increased production of free radicals
  - damage to mitochondrial membrane proteins
  - electron slip, superoxide byproduct (O2-)

Mitochondrial dysfunction under OA conditions

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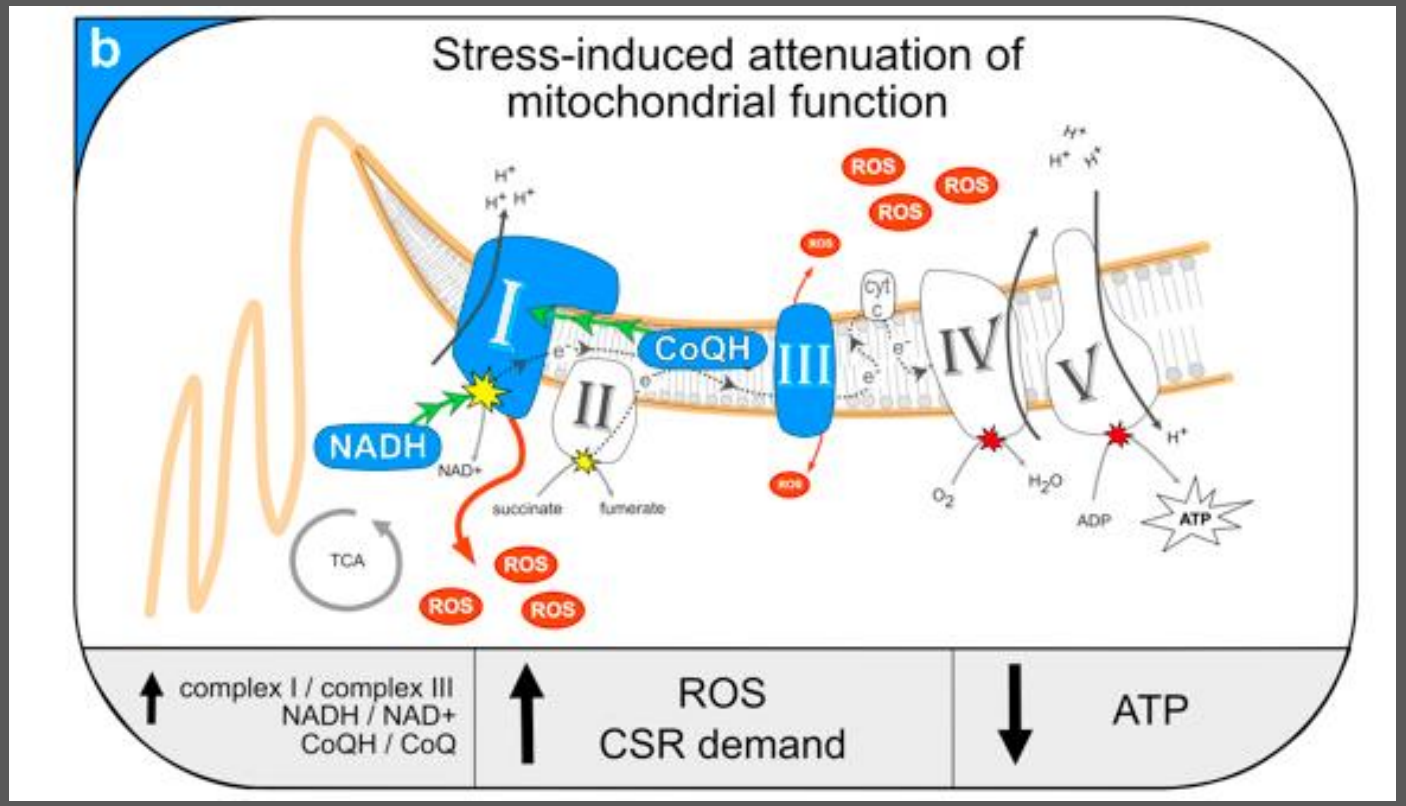
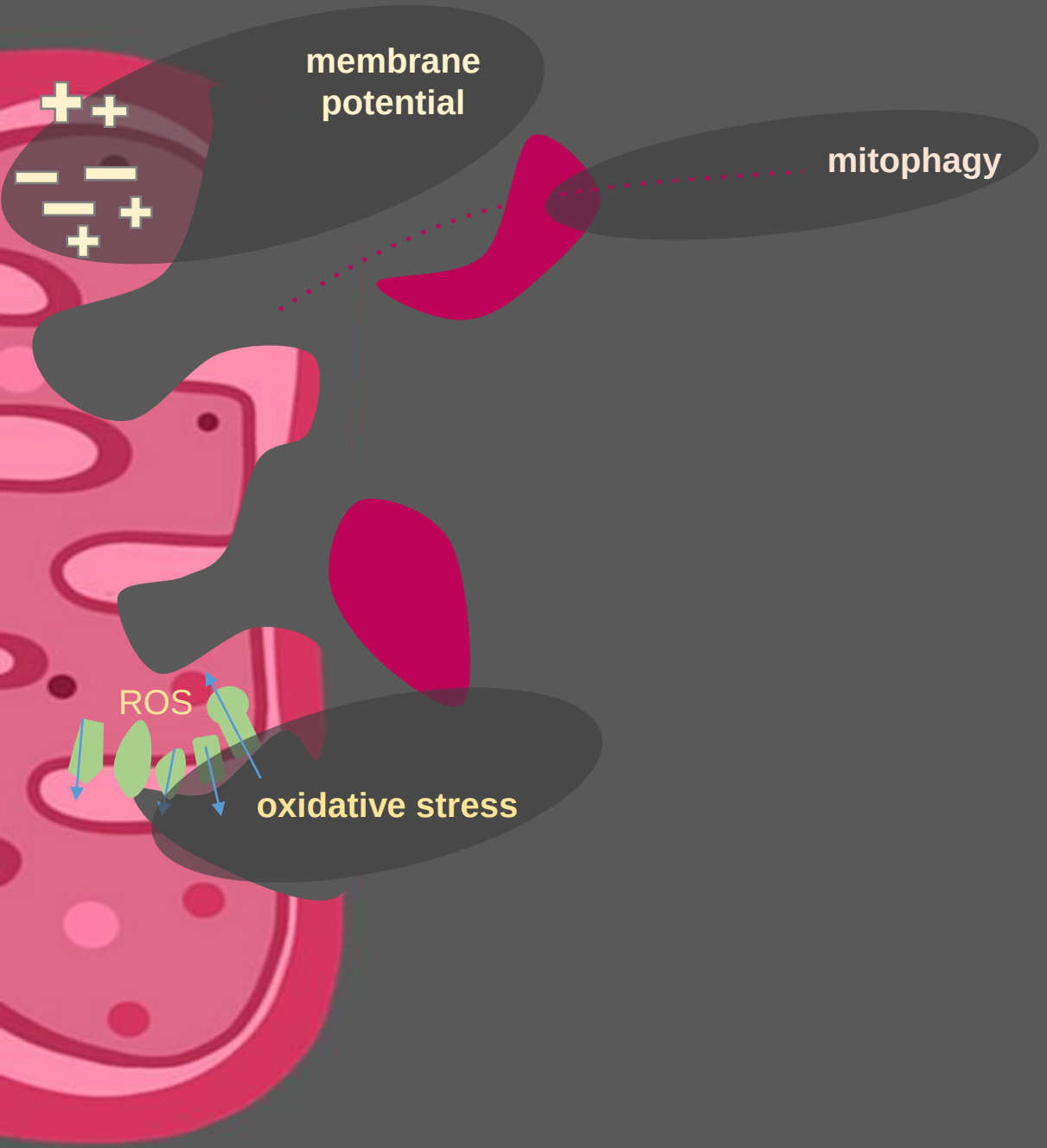
  - decreased efficiency of OXPHOS
- increased production of free radicals
  - damage to mitochondrial membrane proteins
  - electron slip, superoxide byproduct (O2-)
- mitochondrial recycling and potential cell death
  - dysfunction signals for mitophagy, can be precursor to apoptosis

Mitochondrial dysfunction under OA conditions





…targets for flow cytometry!

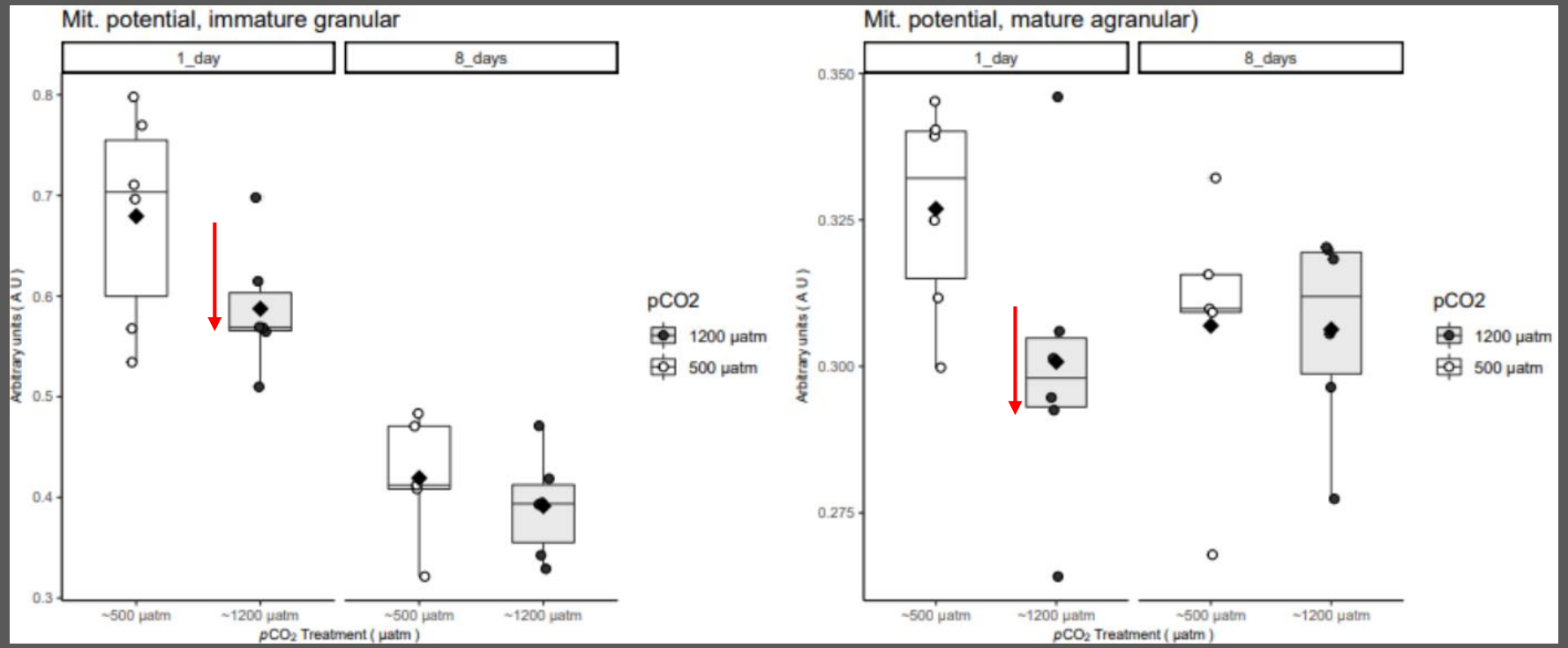
mitochondrial **membrane potential** (ΔΨm)
- probe = JC-10

mitochondrial production of **free radicals**
- probe = DCHF-DA

mitophagy & lysosomal content
- probe = MitoTracker (Green or Deep Red)
- probe = LysoTracker Red DND-99

hemocyte status / **mortality** / cytomorphology
- probe = syber green and propidium iodide

preliminary results in adult oysters…



significant decrease in membrane potential for immature granular and mature agranular cells, ‘recovery’ after 8 days of exposure

- hypothesized effect persisted albeit a low sample size!
- animals were also starved throughout this trial, confounding timepoint comparison

NOAA DFO grant

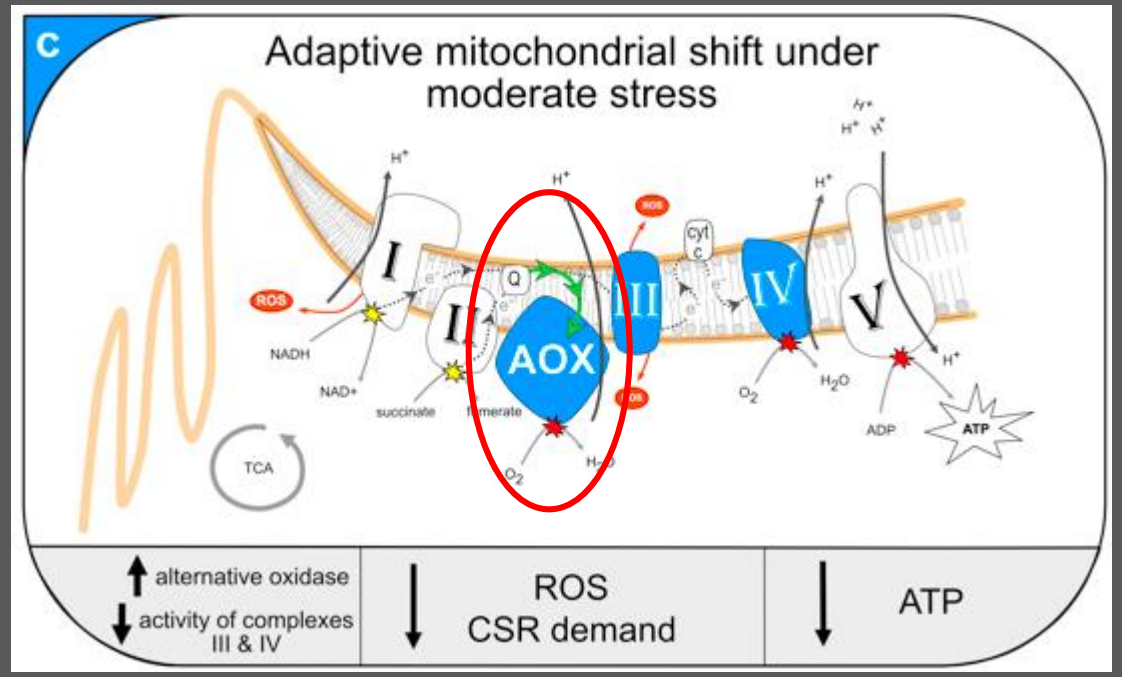
Colorimetric assays

- NAD/NADH ratio
  - marker of reverse electron transport (dysfunction)
  - optimum level mitochondrial respiration

- Lipid peroxidation
  - a damage signal necessary to infer oxidative stress (coupled with DCFH-DA)

a priori genes of interest (qPCR or TagSeq)

- mitochondrial complexes I and III
  - major components that change activity/concentration under dysfunction
- uncoupling proteins
  - regulate the proton gradient and cell resp.
- alternative oxidase (AOX, view figure →)
  - of growing interest as an adaptive response
- mitophagy pathway
  - FOXO, pink-1 parkin, etc.
- carbonic anhydrase etc.
  - acid-base regulatory proteins



Future directions (with preserved tissues!)

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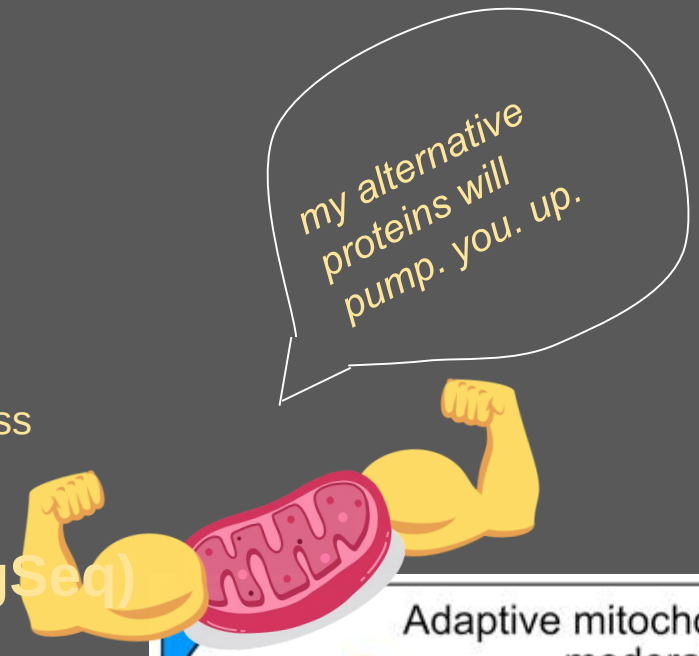
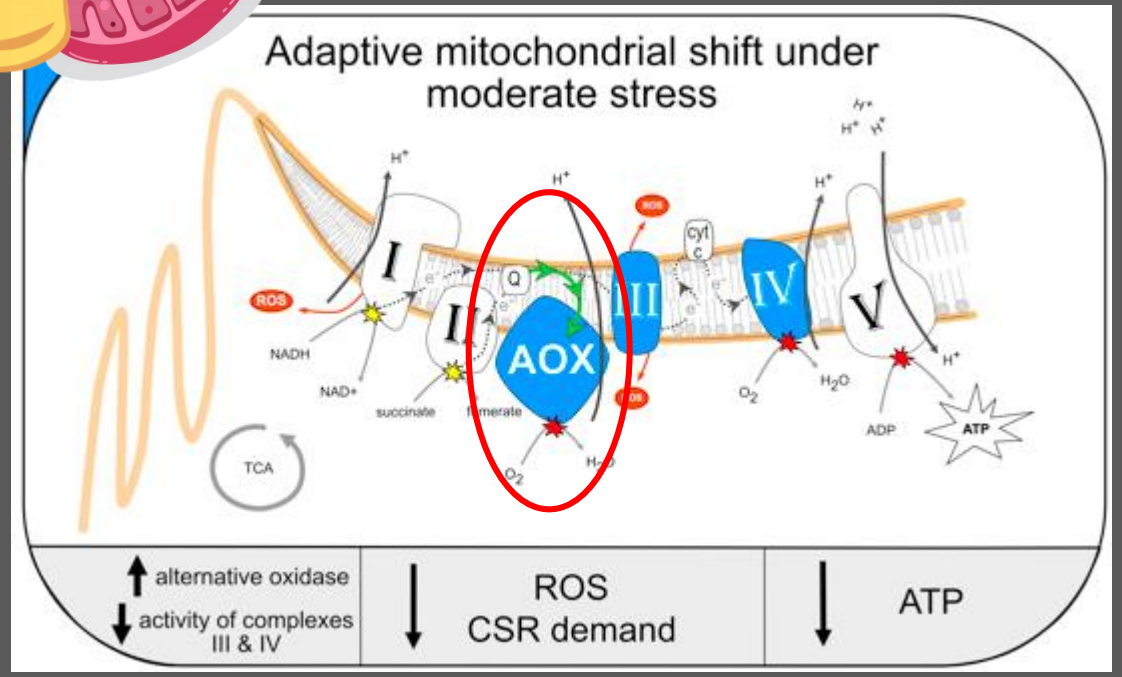
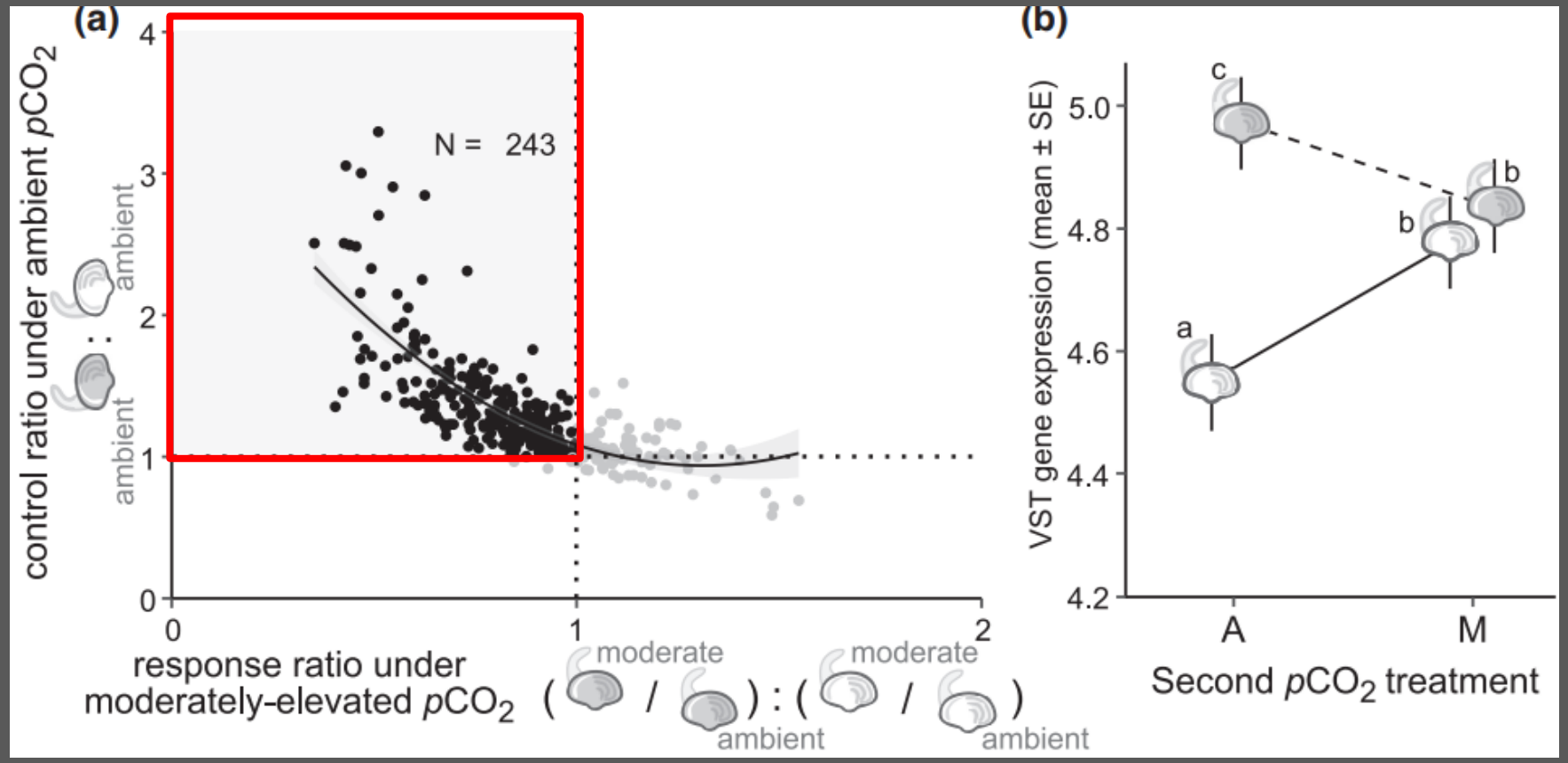




Future directions (with preserved tissues!)

constitutive gene frontloading of our acclimatized cohort



Gurr et al. 2022

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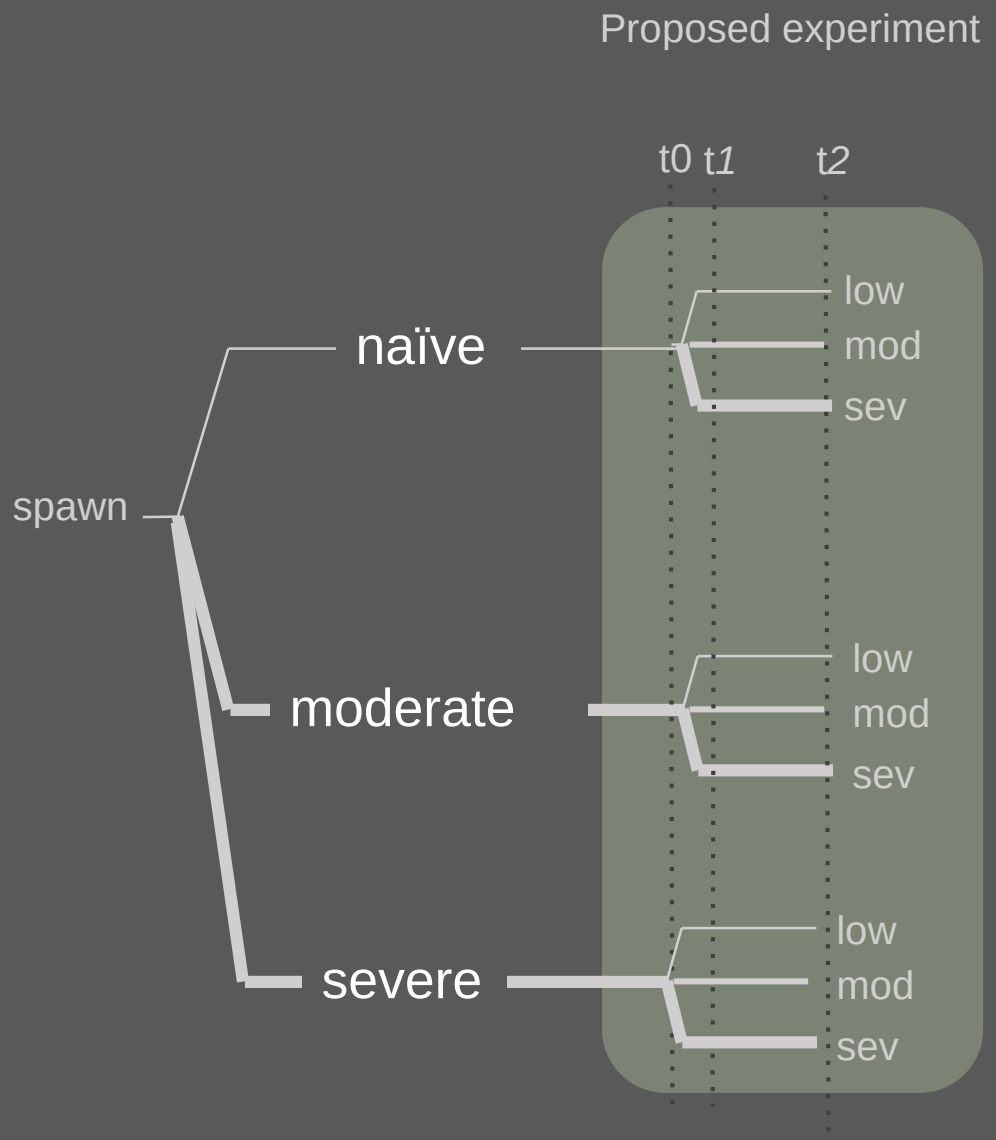


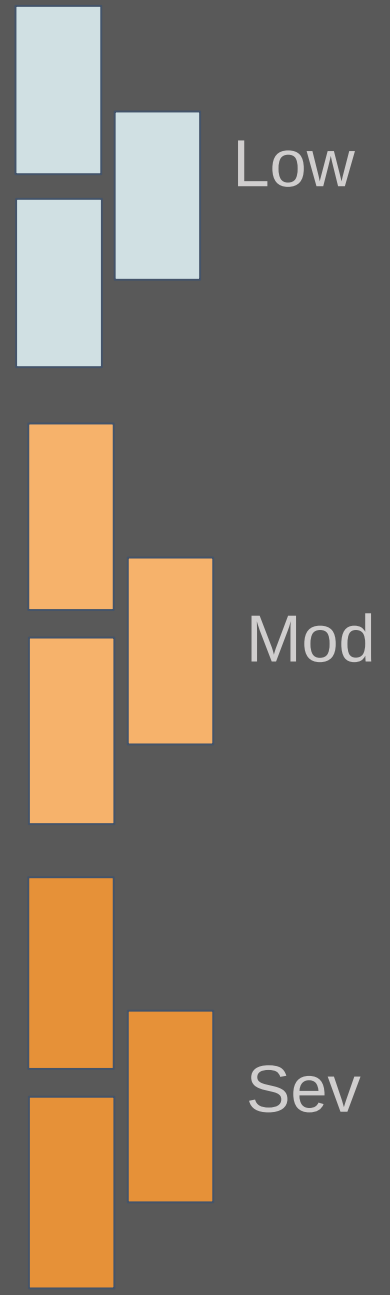
About treatments

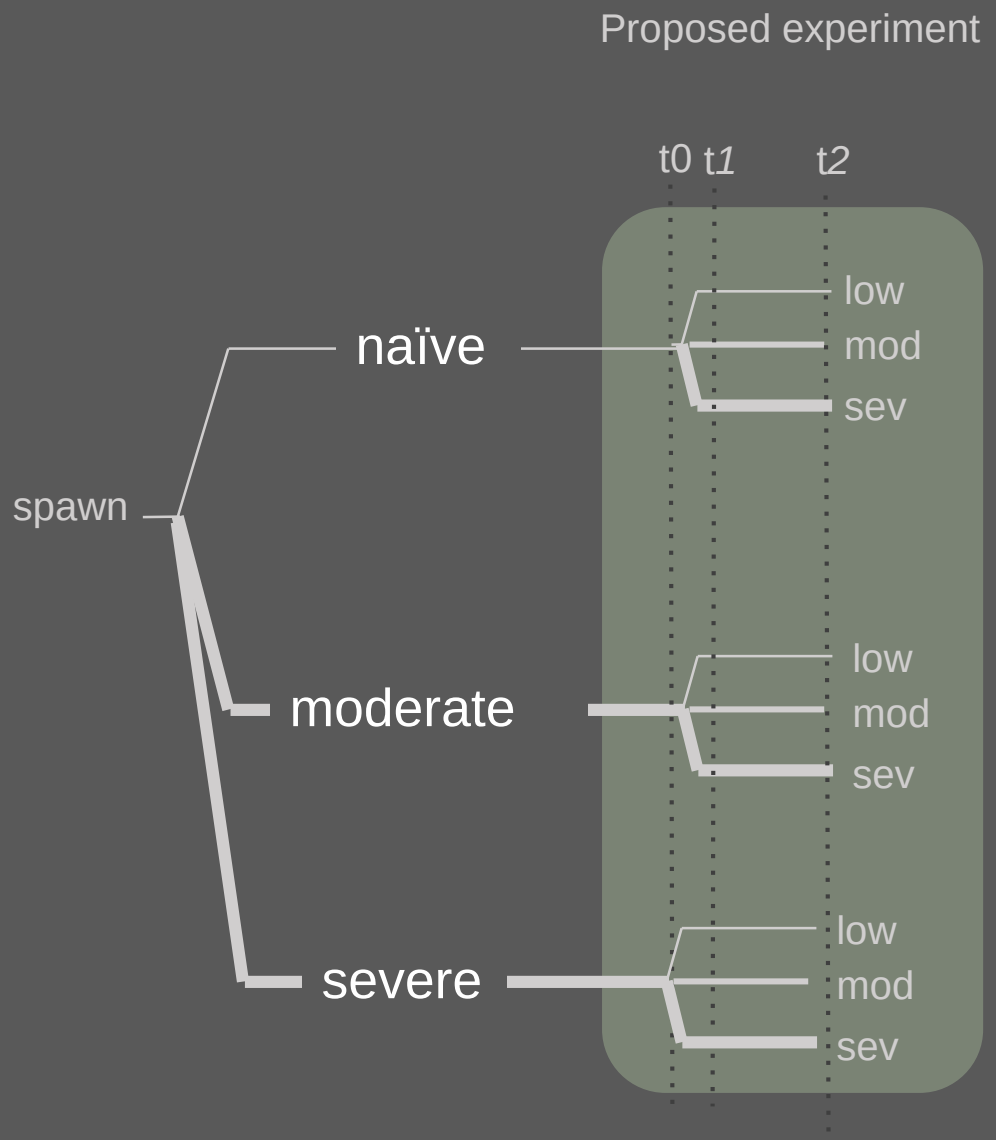
$pCO2_{HISTORY}$: $N = 3$
$pCO2_{EXPOSURE}$: $N = 3$

$pCO2_{HISTORY}$ x $pCO2_{EXPOSURE}$: $N = 9$

tank replication: $N = 3$

total tanks: $N = 9$





Number of F2s

Low pCO2

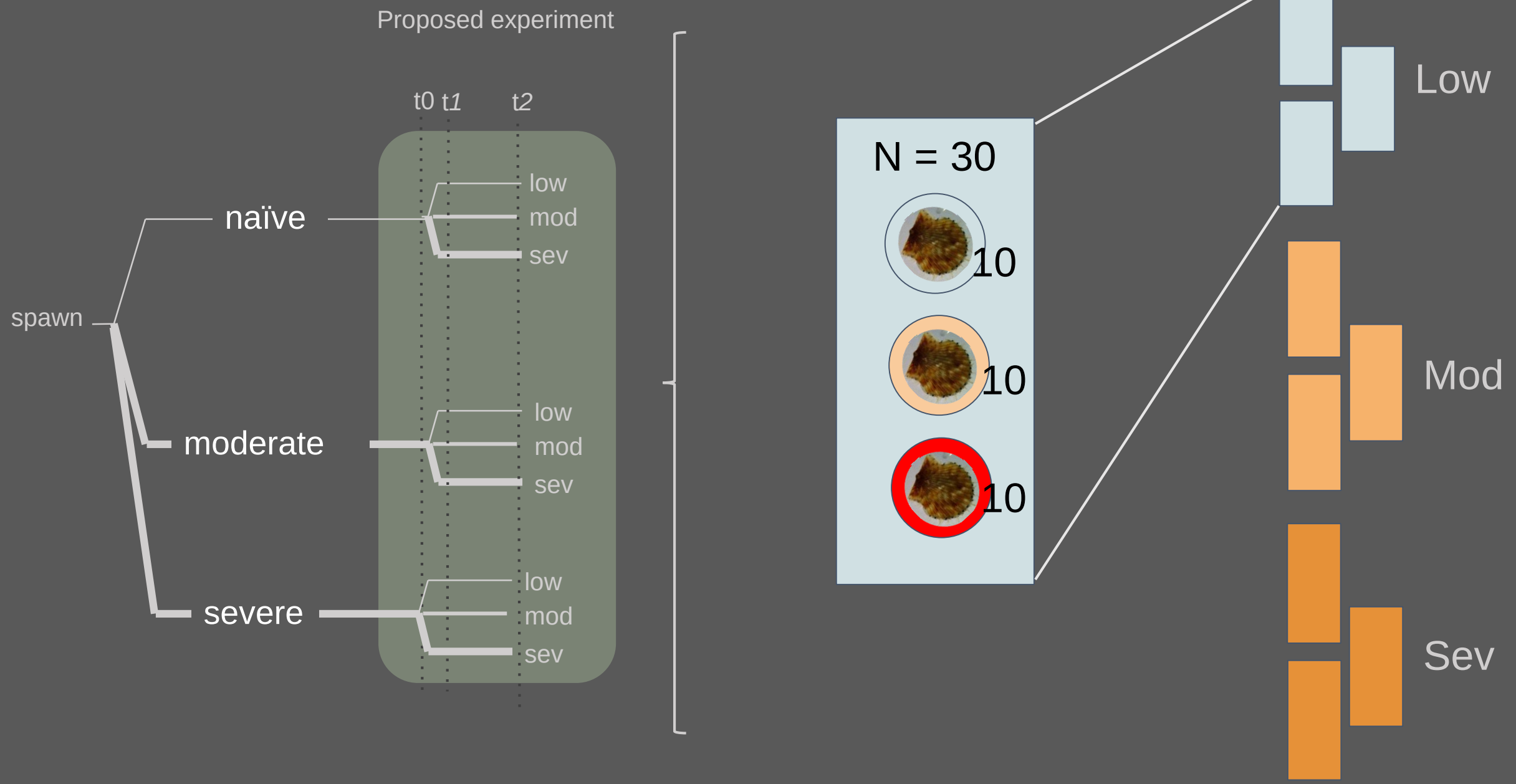
= 30

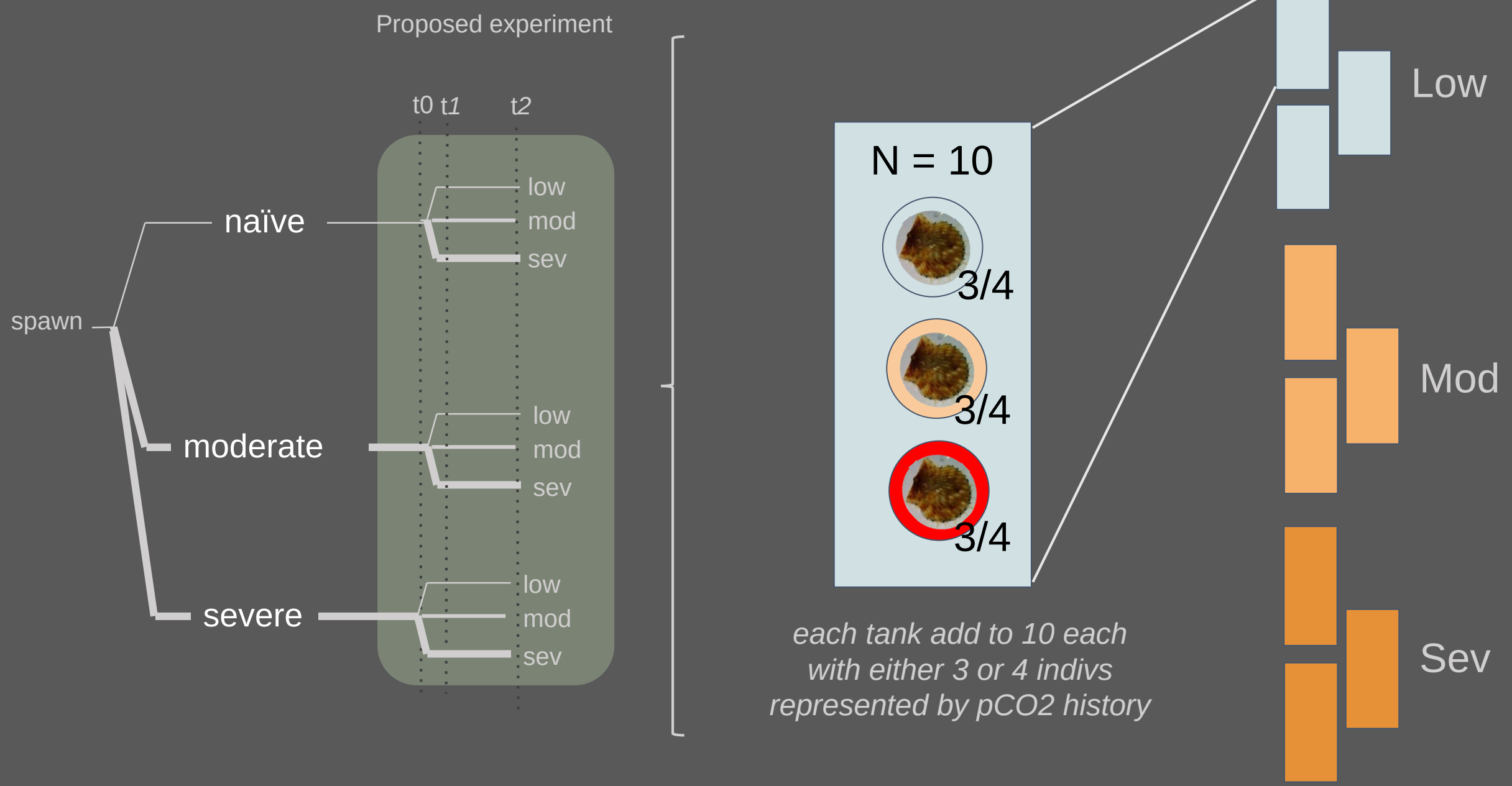
‘Moderate’ pCO2

= 30

‘Severe’ pCO2

= 30


Proposed experiment
t0
t1
t2
spawn
naïve
moderate
severe
low
mod
sev
low
mod
sev
low
mod
sev
N = 30
10
10
10
Low
Mod
Sev


Proposed experiment
t0 t1 t2
naïve
spawn
moderate
severe
low
mod
sev
low
mod
sev
low
mod
sev
N = 10
3/4
3/4
3/4
each tank add to 10 each
with either 3 or 4 indivs
represented by pCO2 history
Low
Mod
Sev

Timepoint 1: **April 4th**

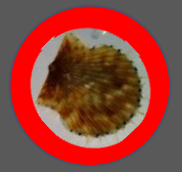
x Low x Mod x Sev

5 per treatment; total = 15

x Low x Mod x Sev

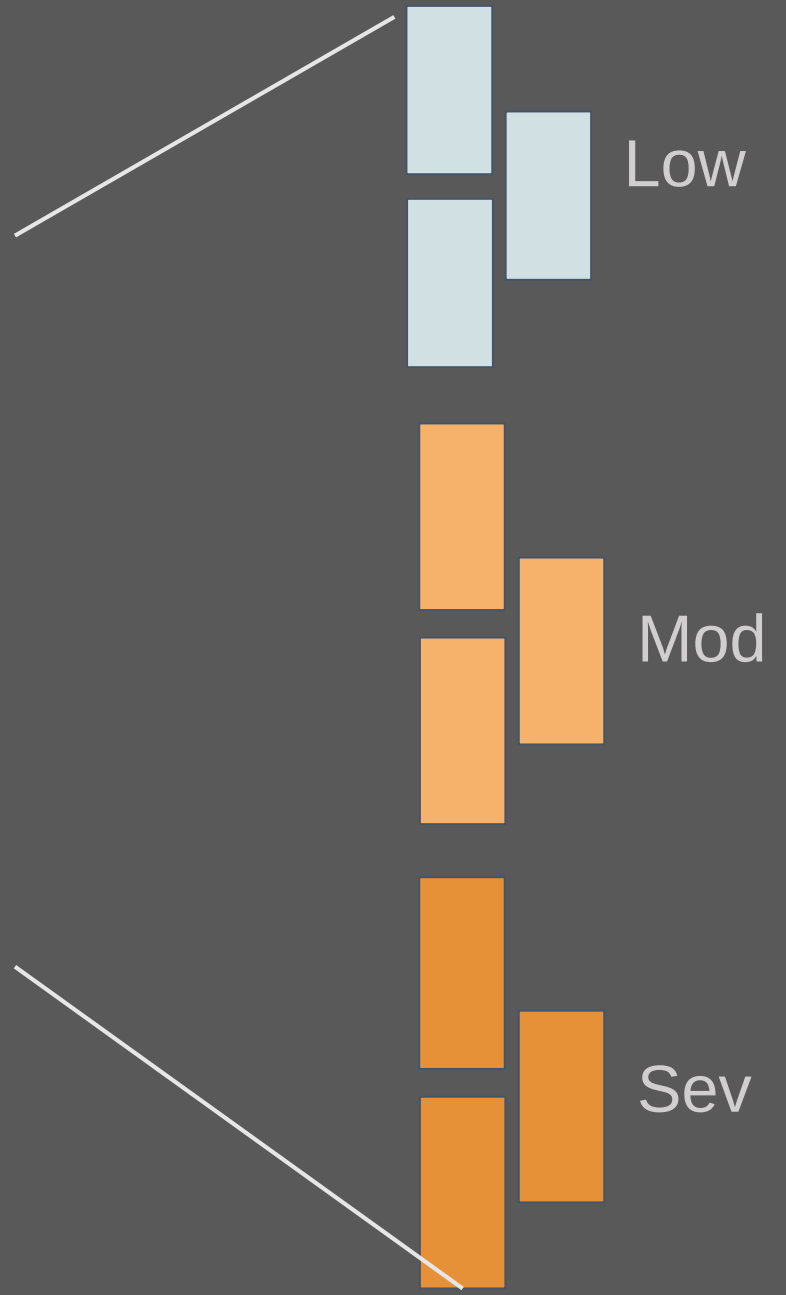
5 per treatment; total = 15

x Low x Mod x Sev



5 per treatment; total = 15

N = 45

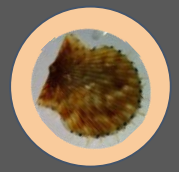


Timepoint 2: **April 18th**

x Low x Mod x Sev

5 per treatment; total = 15

x Low x Mod x Sev

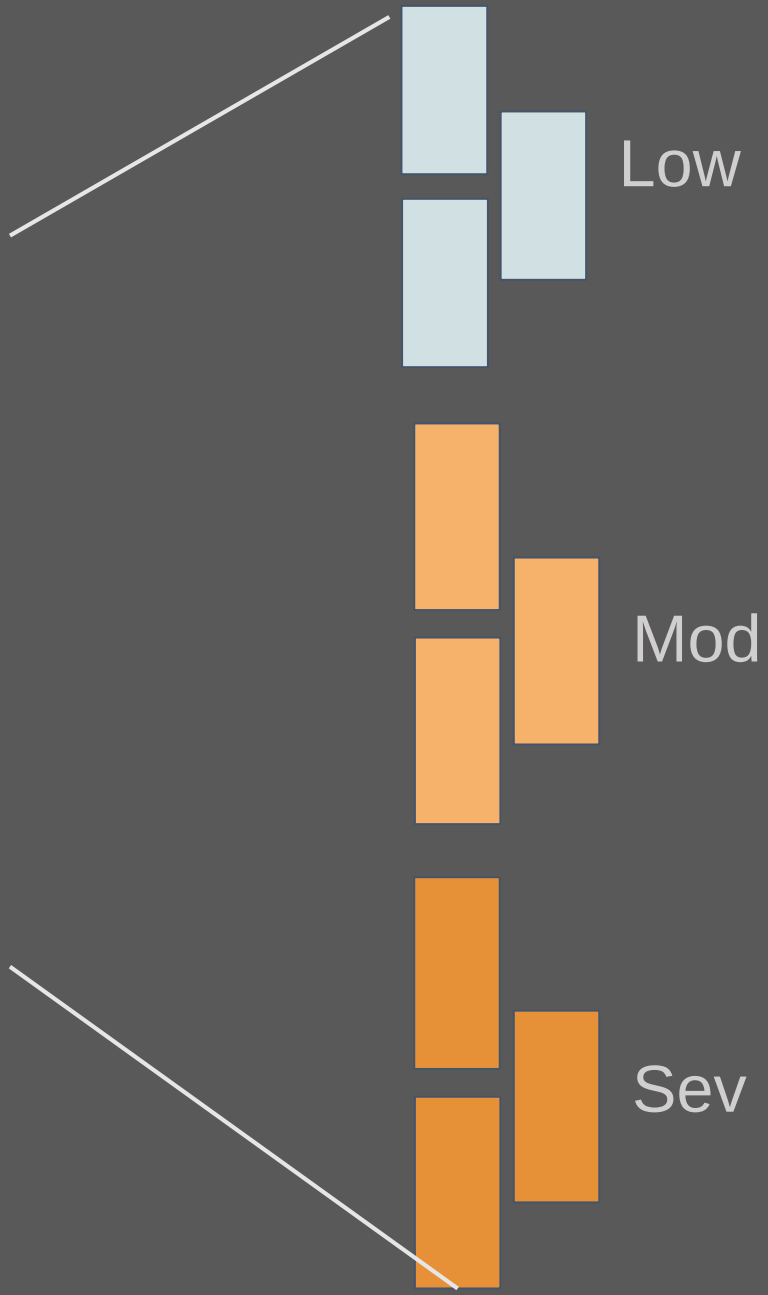


5 per treatment; total = 15

x Low x Mod x Sev

5 per treatment; total = 15

N = 45.. again!



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5 per treatment; total = 15

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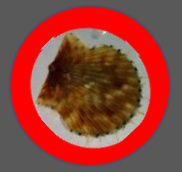

5 per treatment; total = 15

45 animals at each time point (90 total)

same day:
hemolymph

future analysis:
gill tissue x2 per individual!

Hemolymph probes

- **SYBR green** = live cells
- **JC-10 + CCCP** = mitochondrial membrane potential with and without depolarizing (control)
- **MitoSox Green** = mitochondrial free radicals

same day:
hemolymph

45 animals at each time point (90 total)

45 animals at each time point (90 total)

Future analysis of gill tissue!

Gene frontloading

- TagSeq (N=64 samples max)

Colorimetric assays *with tissue homogenate*

- NAD/NADH Quantification
- Lipid peroxidation (MDA)
- ADP/ATP
- corrected for Total protein

future analysis:
gill x2