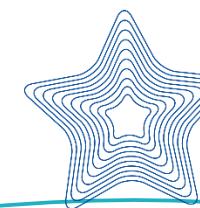




(RECOBI)

PERFORMANCE of BIVALVES

Pruebas previas al diseño experimental (junio 2023)



Centro de Investigación Mariña
Universida de Vigo



GOBIERNO
DE ESPAÑA

MINISTERIO
DE CIENCIA
E INNOVACIÓN



Plan de
Recuperación,
Transformación
y Resiliencia



Experimento de temperatura

5.1 & 5.3. Salinity and temperatura experiments in mesocosms (july 2023)

-Performance curves for recruits and juveniles: constant temperatura without tides, 2 days.

4 Treatments:

18 °C

21 °C

24 °C

27 °C

4 Species:

Fina

Babosa

Japónica

Berberecho

2 Sizes:

2 – 5 mm

10 – 12 mm

3 pilot experiments to answer:

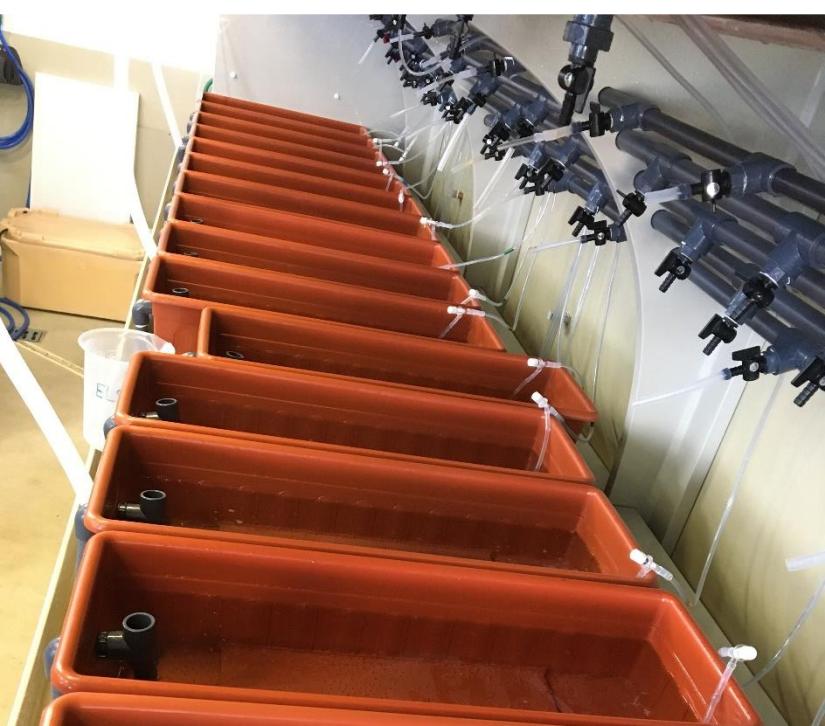
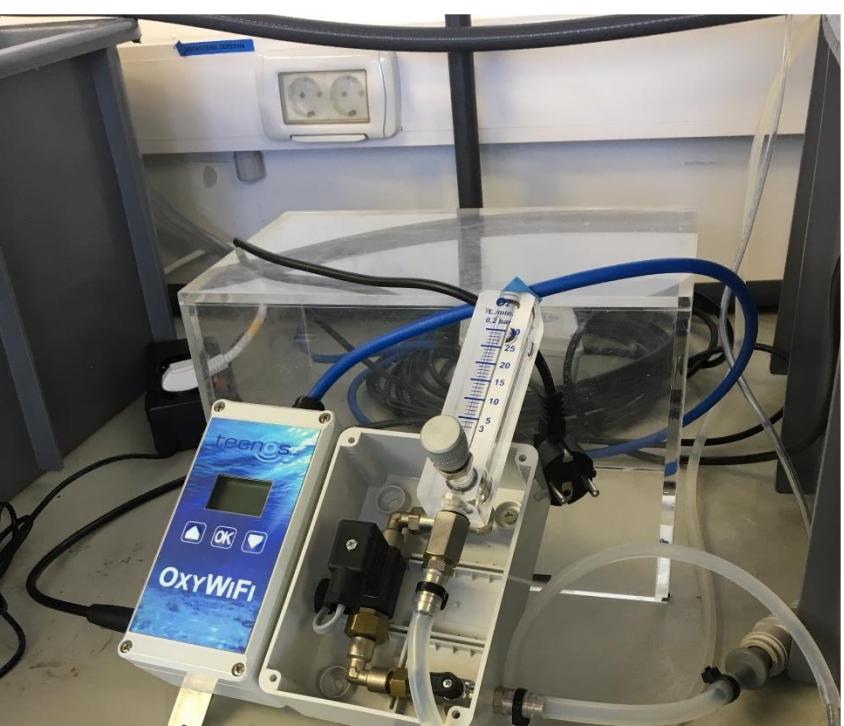
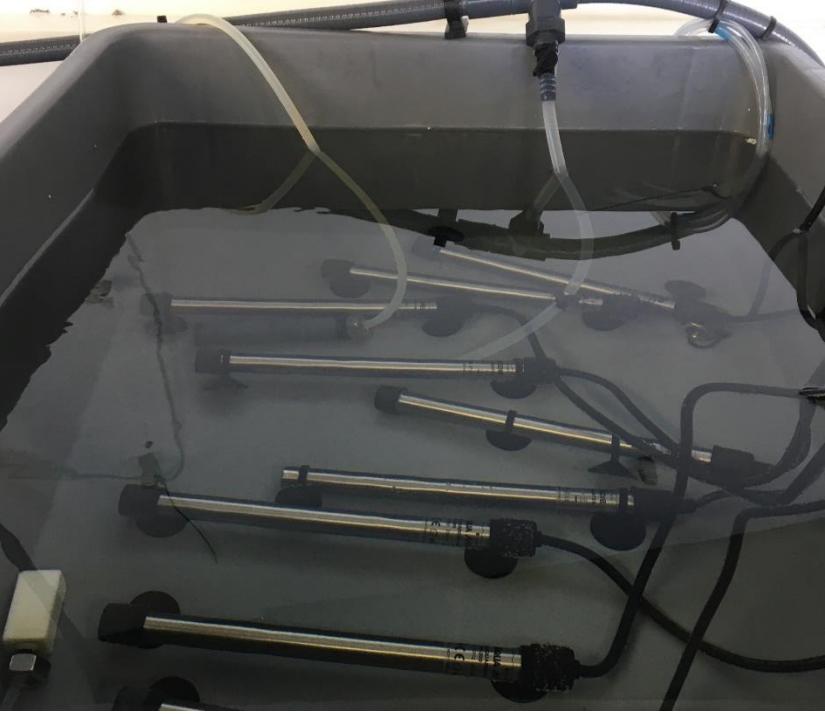


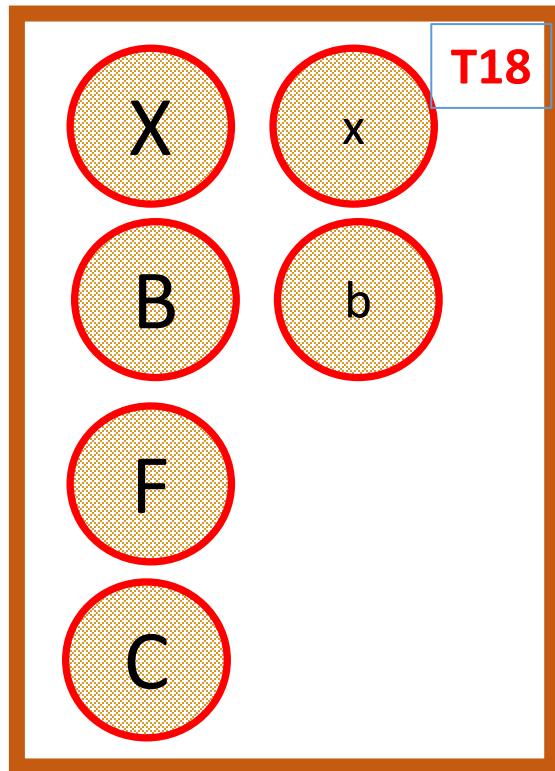
1. What is the the experimental unit? ¿1 individual? ¿3? ¿5?
2. Are there significant differences in [O₂] at different temperatures?
3. What volumen do we need to register ammonium signal? ¿20, 40, 60 u 80 ml?

Objectives

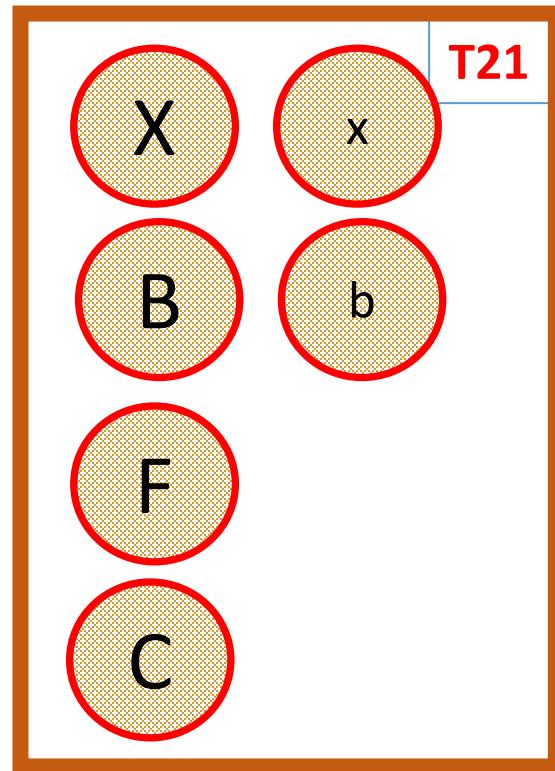
Objective 5: Mesocosm experiments to analyze the effect of the changes determined in Objectives 1, 2, 3 and 4.

- Task 5.1: Preliminary mesocosm experiments based on bibliographic data to analyze the effect of extreme events on mortality, scope-for-growth and reproduction.
- Task 5.2: Reproducing future extreme sediment alterations in mesocosm experiments to analyze their effect on mortality.
- Task 5.3: Reproducing future extreme low salinity and high temperature conditions in mesocosm experiments to analyze their effect on mortality, scope-for-growth and reproduction.
- Task 5.4: Post processing experimental data.

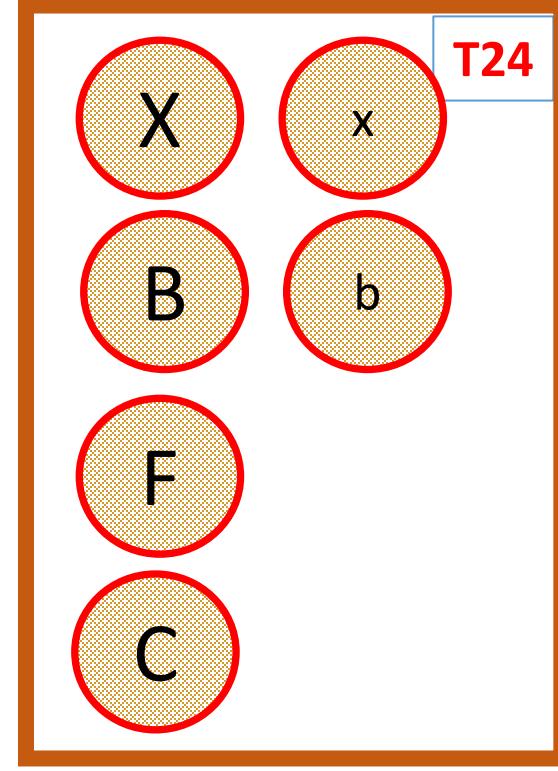




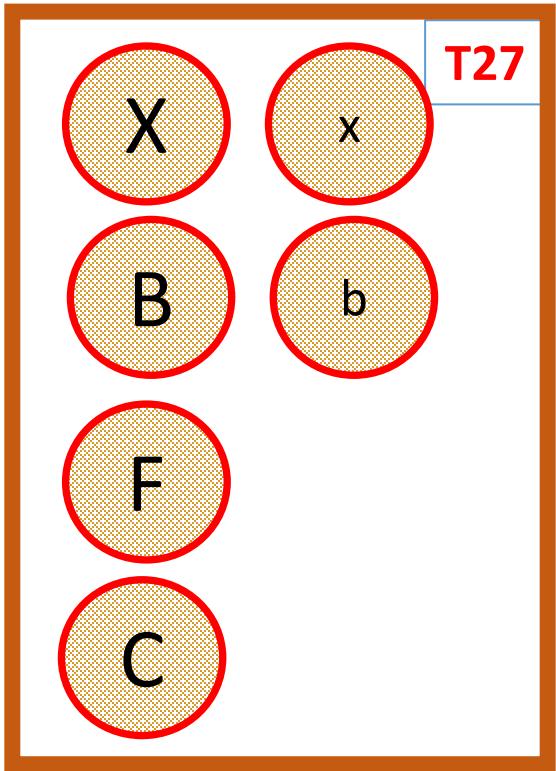
x 12



x 12



x 12



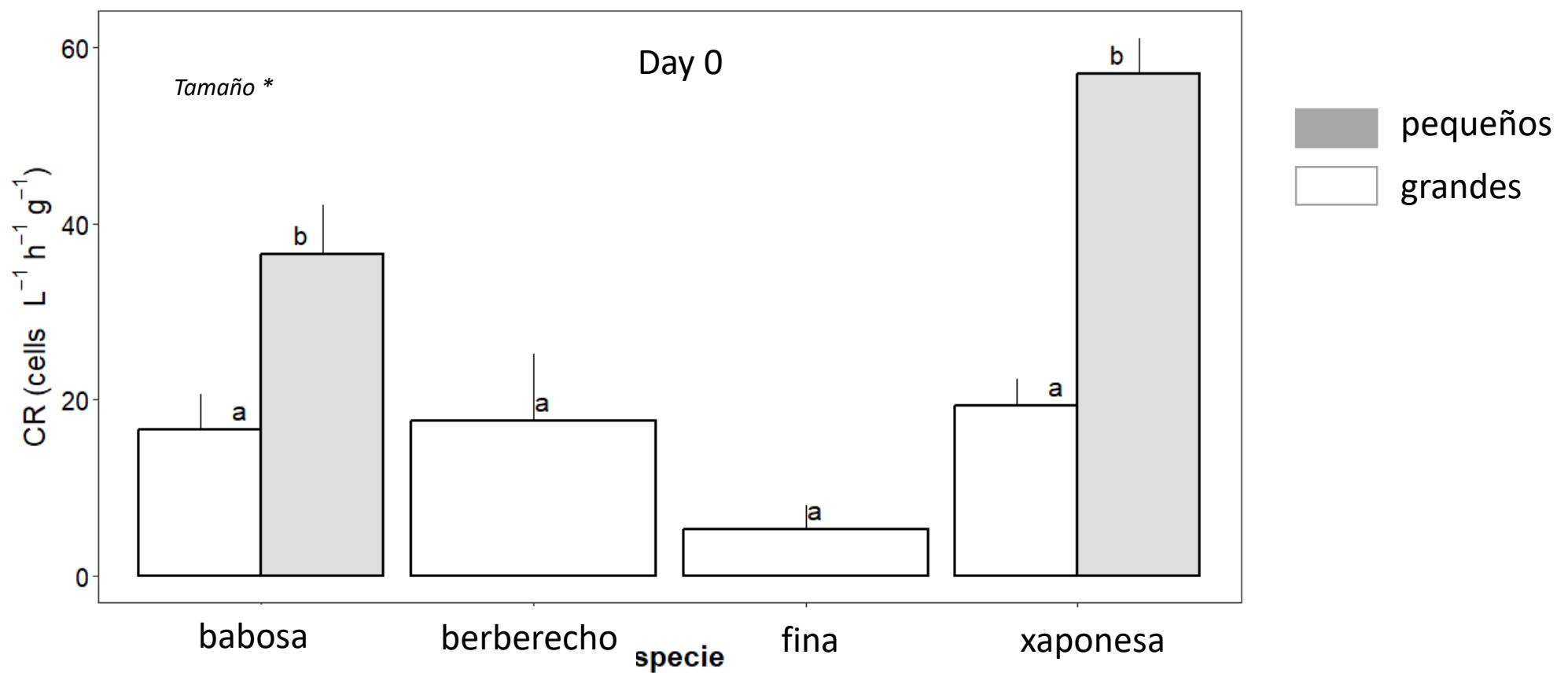
x 12

Cada individuo en un vasito y cada vasito en una maceta



- *Preliminary results*

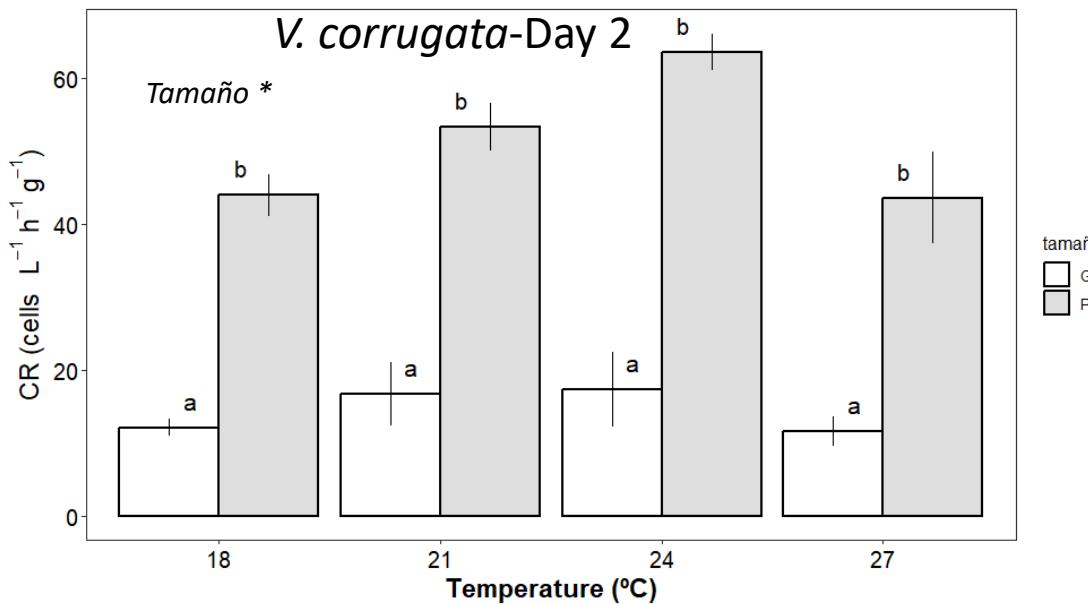
Feeding



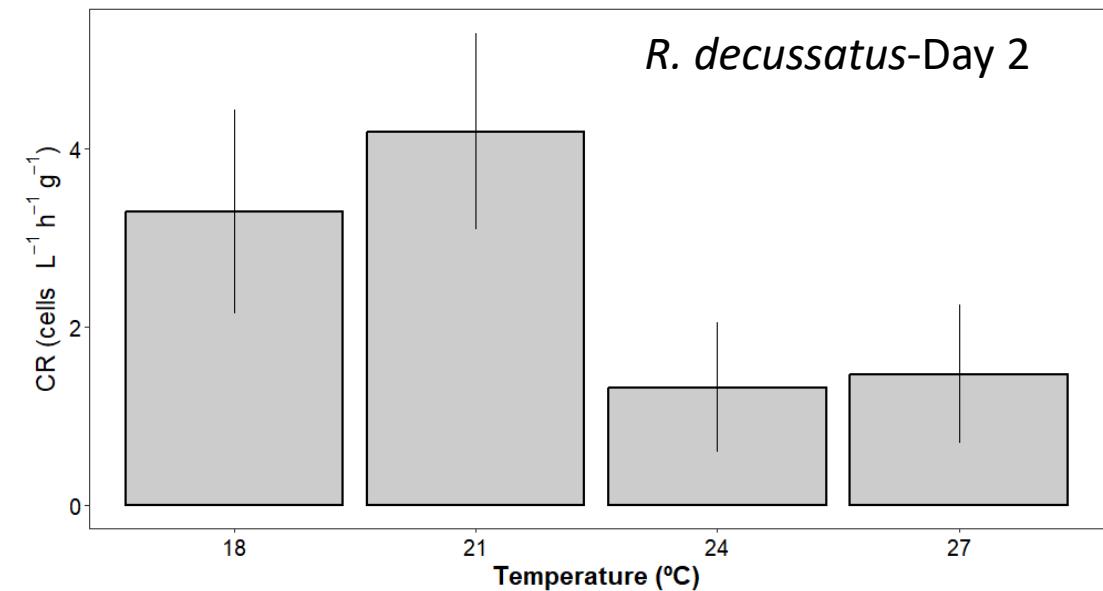
Fina es la que menos come

Feeding

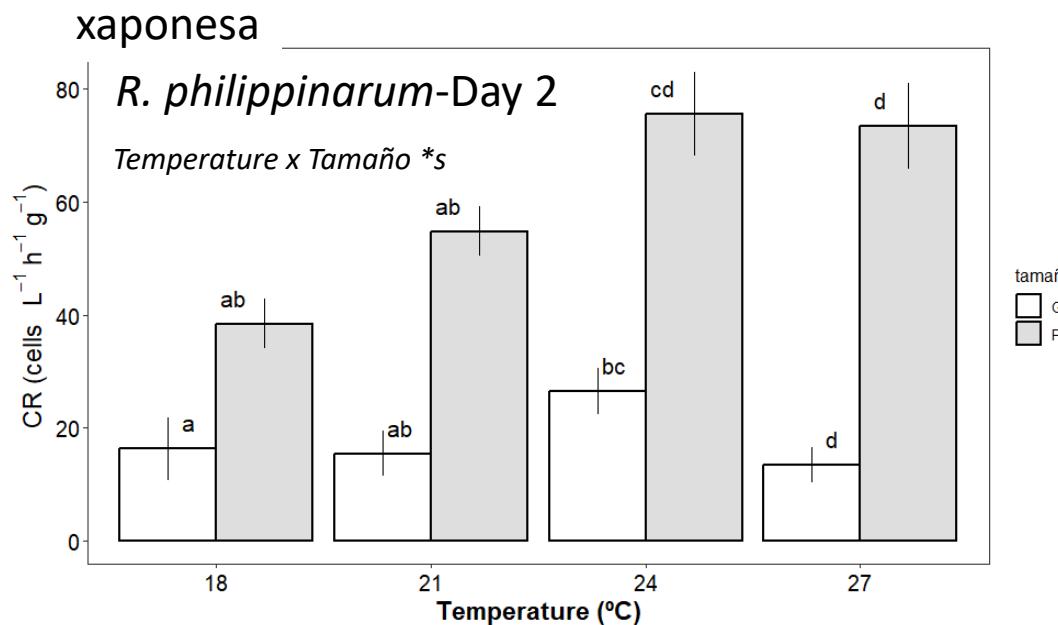
babosa



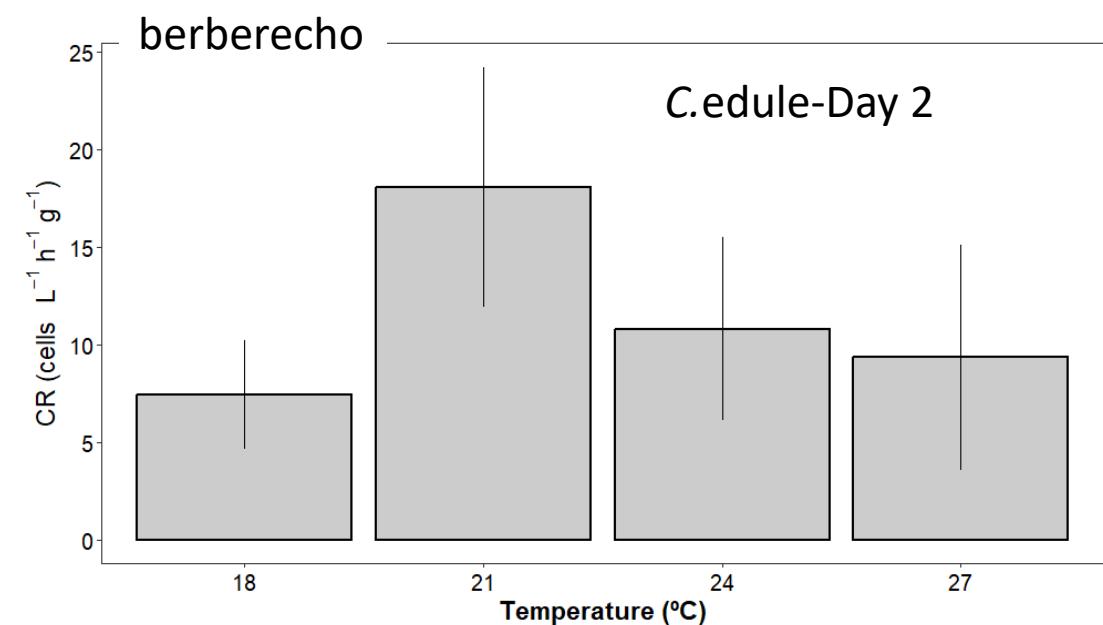
fina



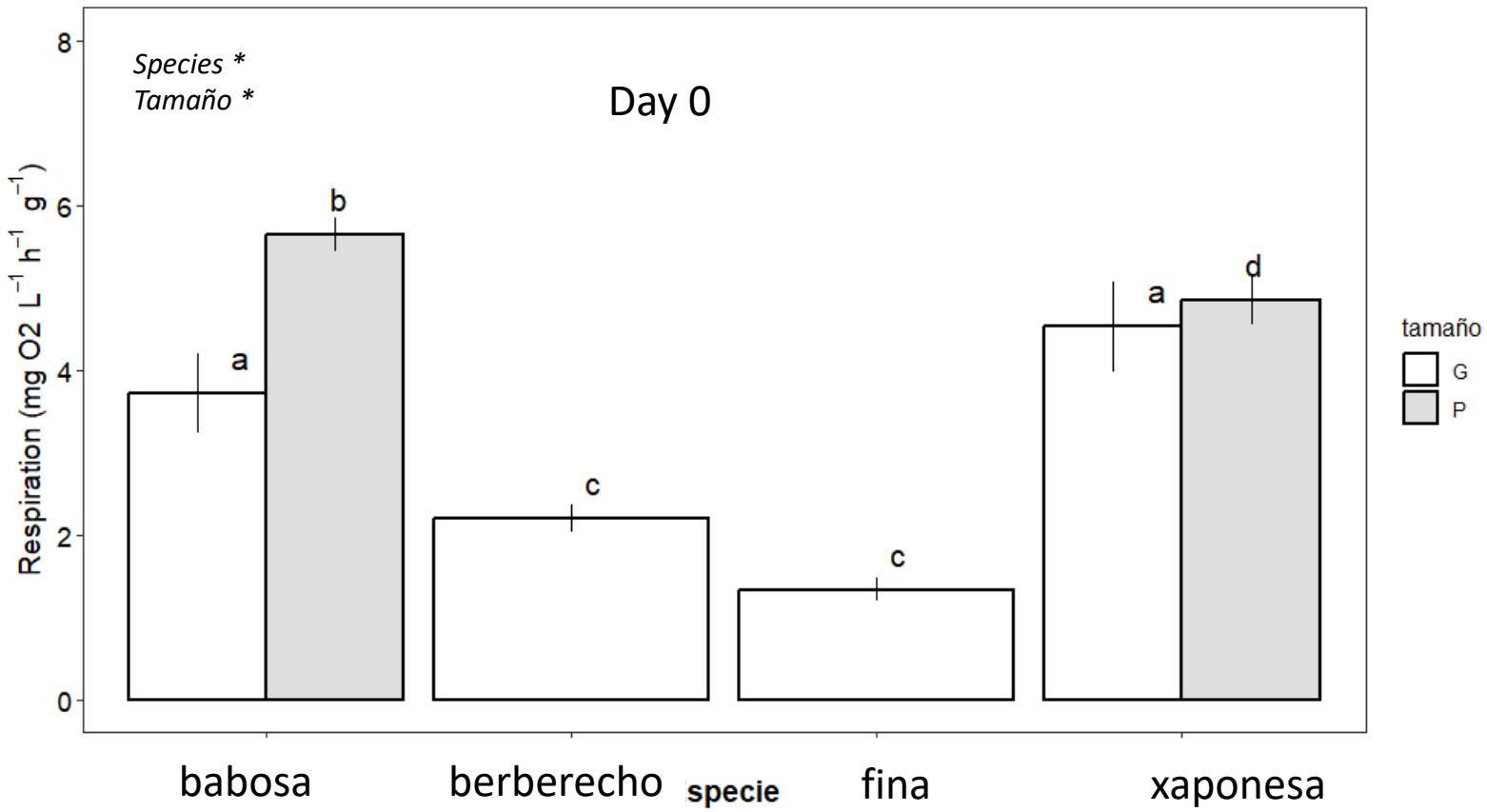
xaponesa



berberecho

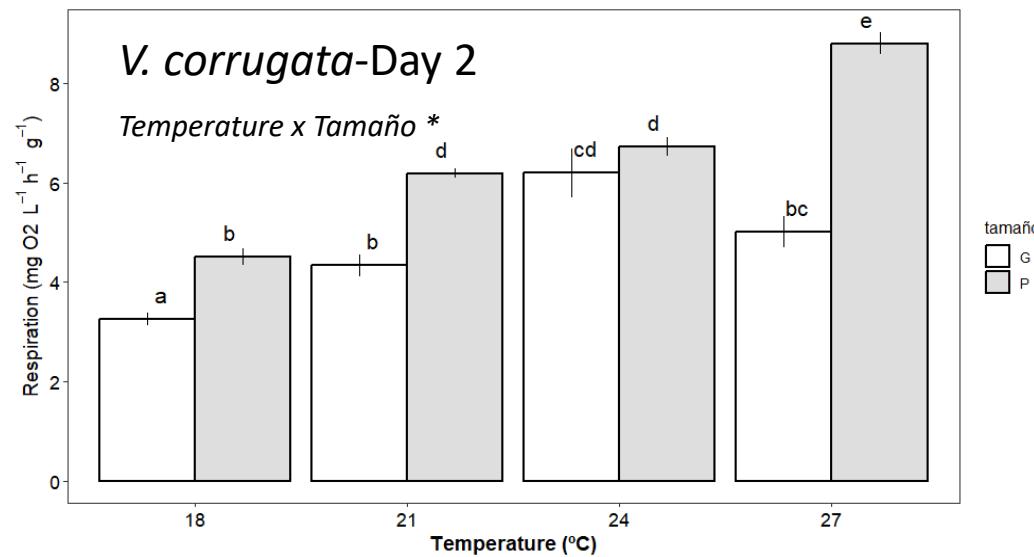


Respiration

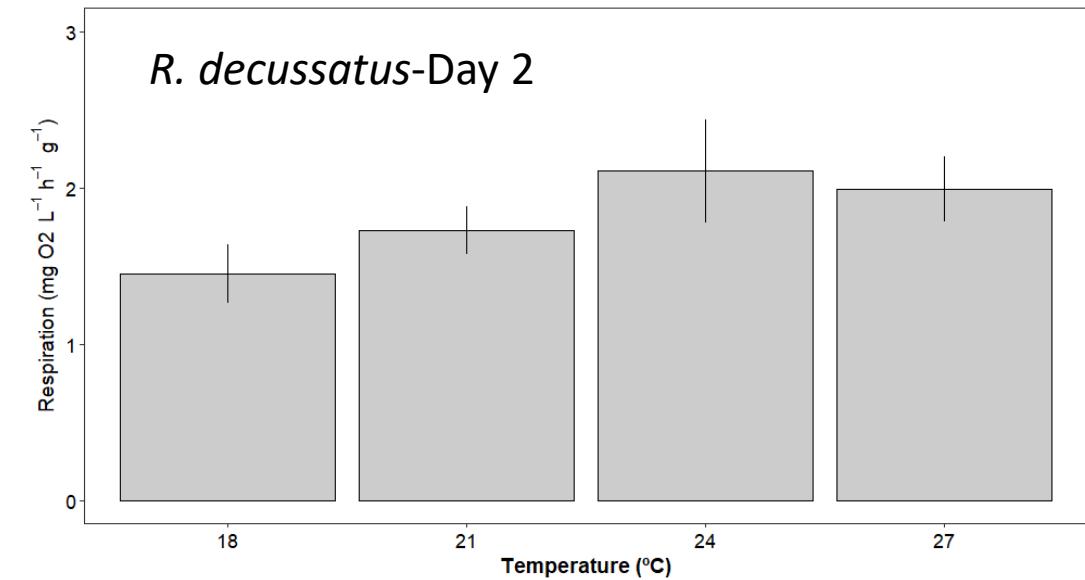


Respiration

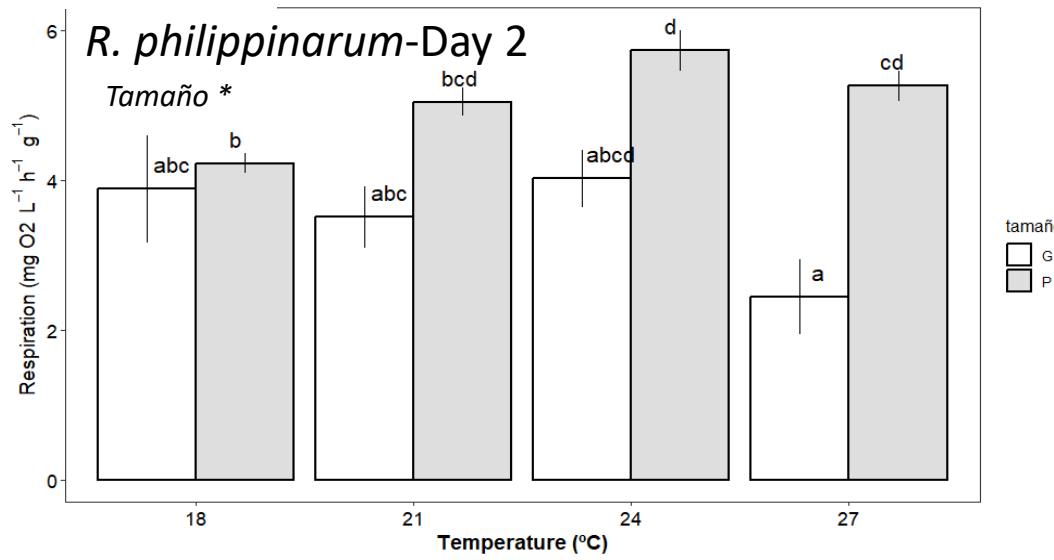
babosa



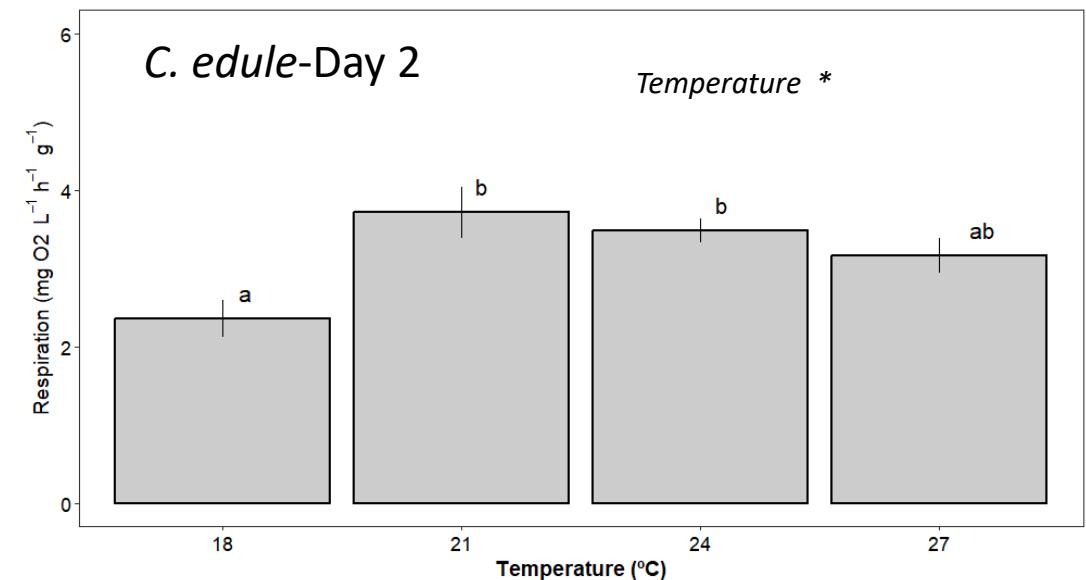
fina



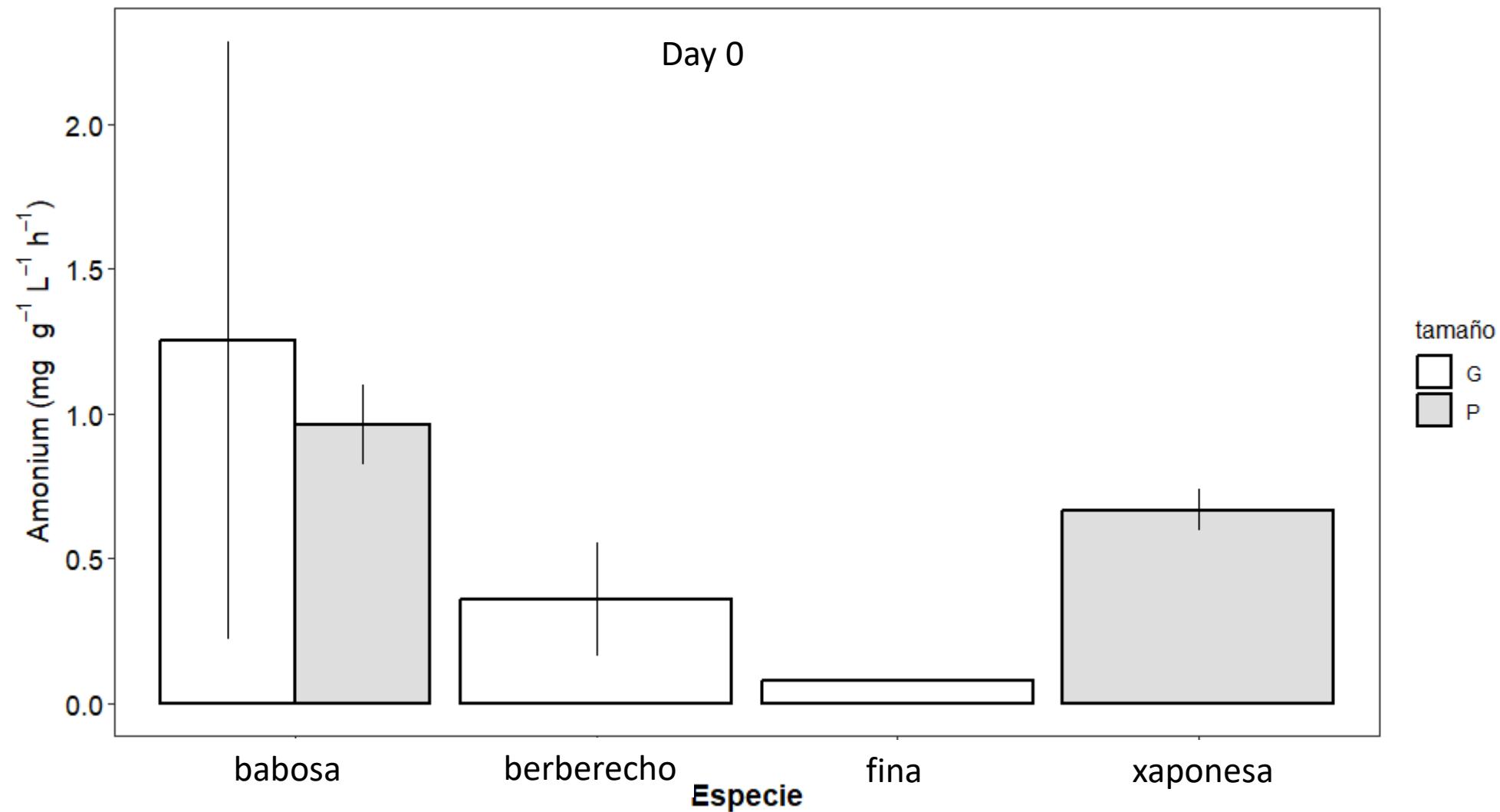
xaponesa



berberecho

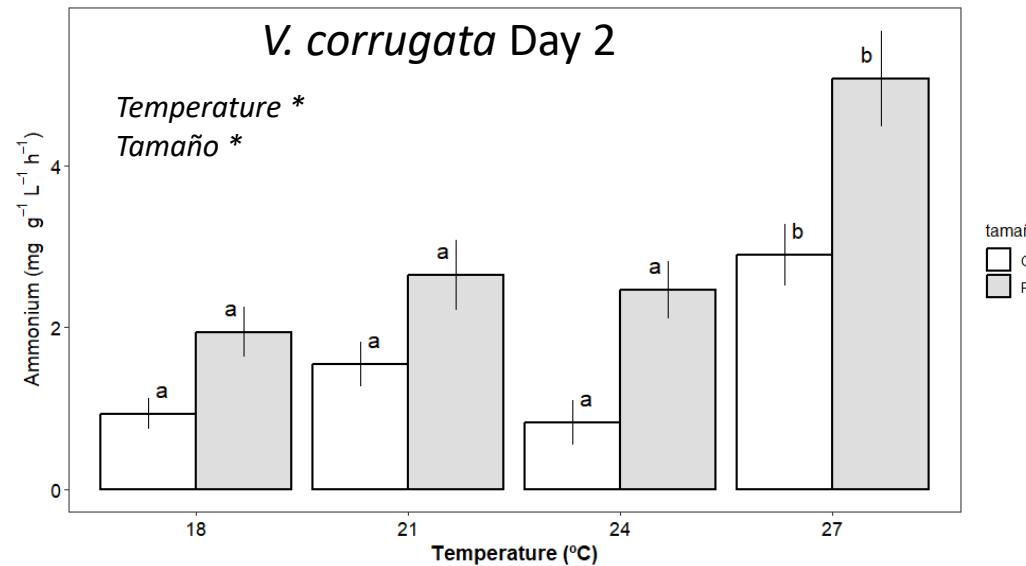


Excretion



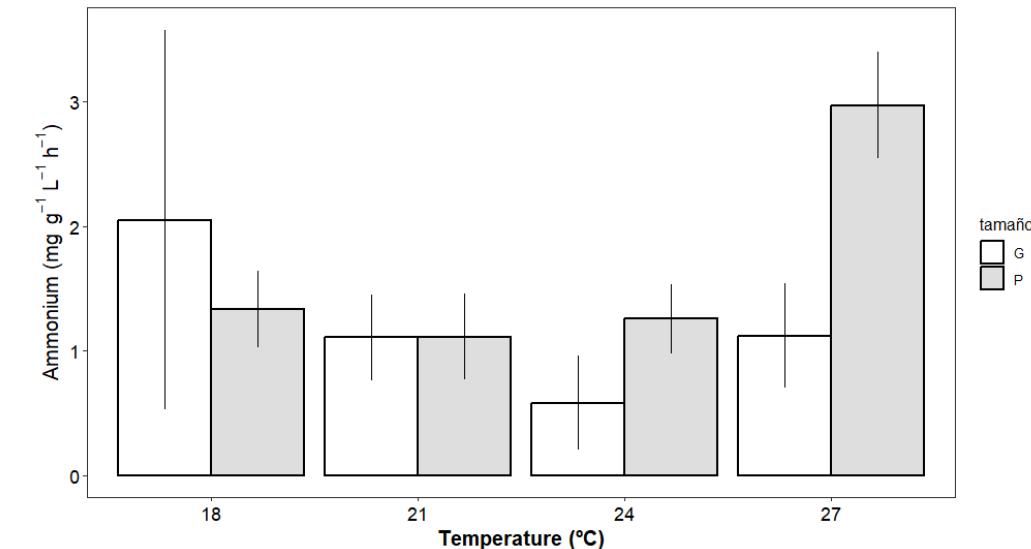
Excretion

babosa

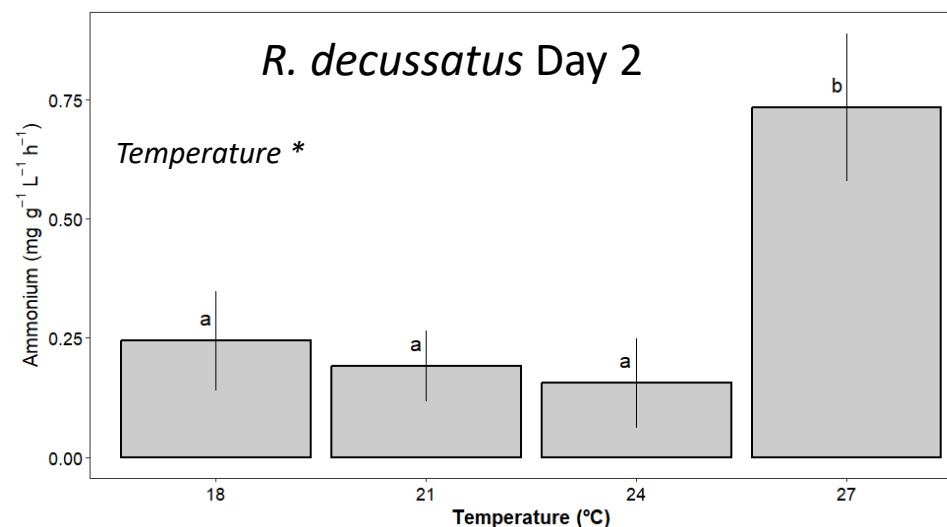


R. philippinarum Day 2

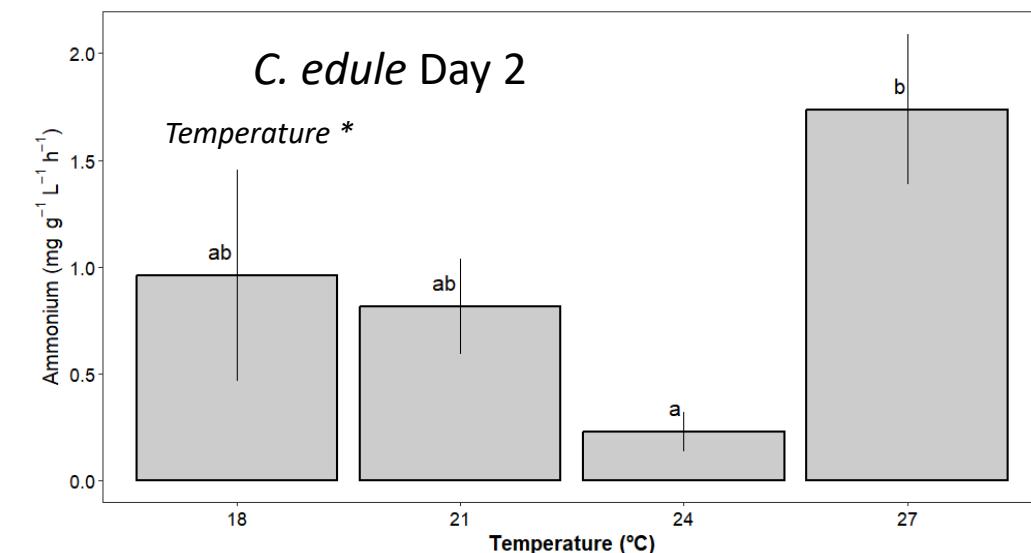
xaponesa



fina



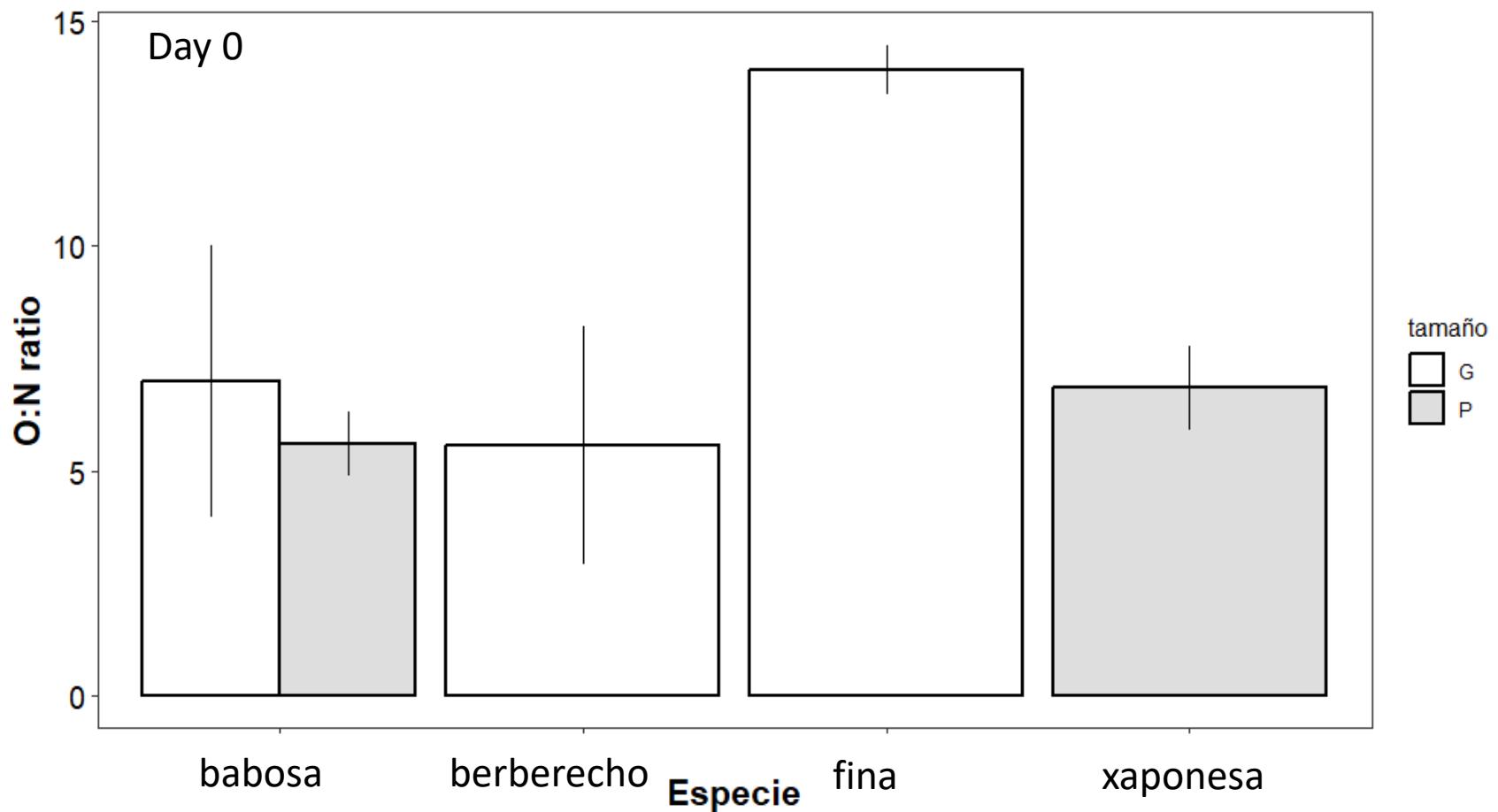
berberecho



O:N ratio

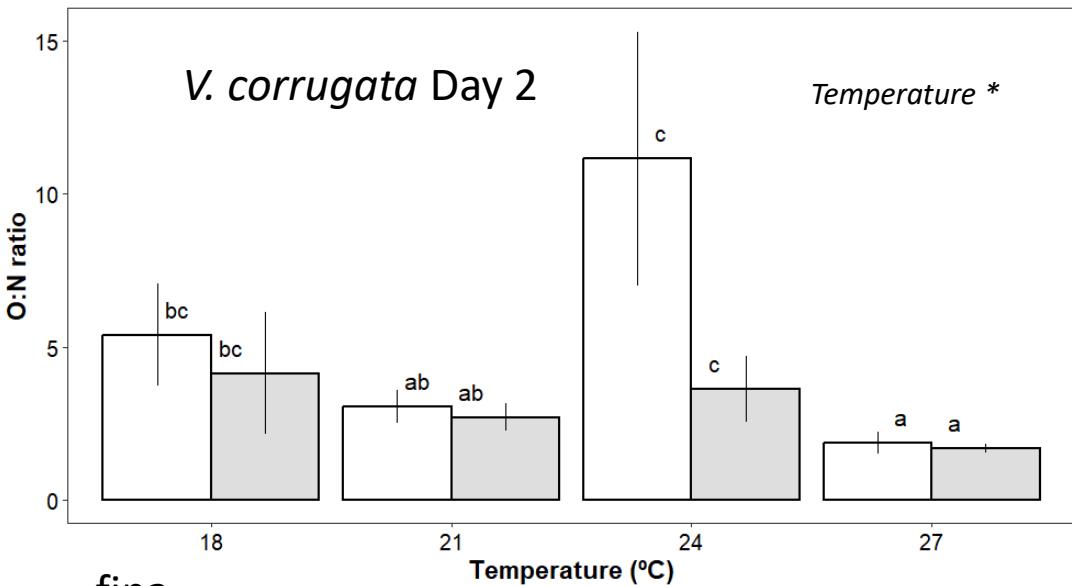
Balanced catabolism would oscillate between 50 and 60 while values below 30–20 are indicative of stress and pure protein catabolism will reach values between 3 and 13

Peteiro et al. 2018: berberechos 9-10 mm valores por debajo de 20 y muchos por debajo de 10 en la respuesta aguda que sería la equivalente a la nuestra. A salinidad 25 está sobre 10 y a salinidad 30 es algo inferior a 20.

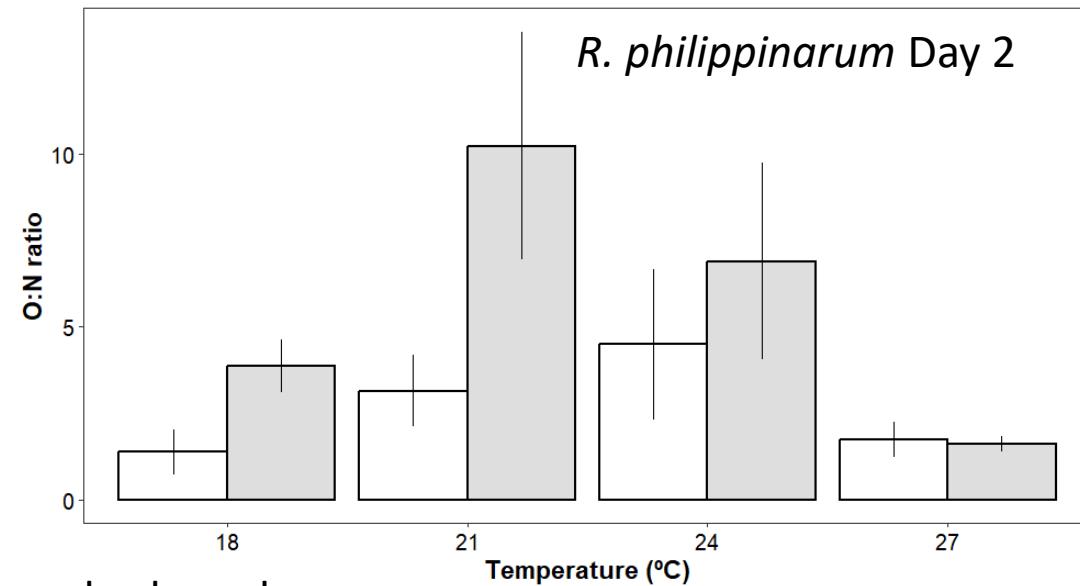


O:N ratio

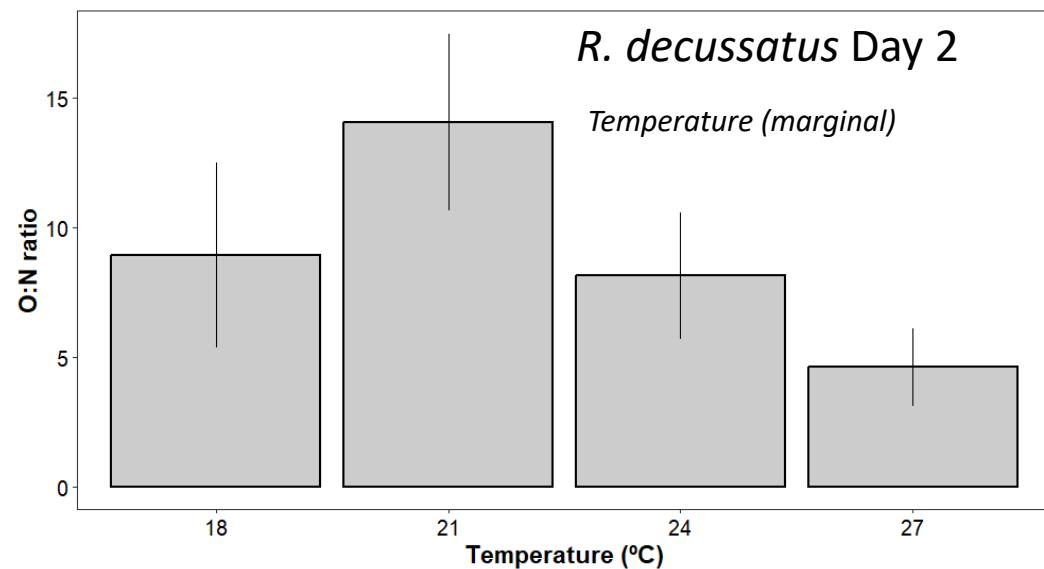
babosa



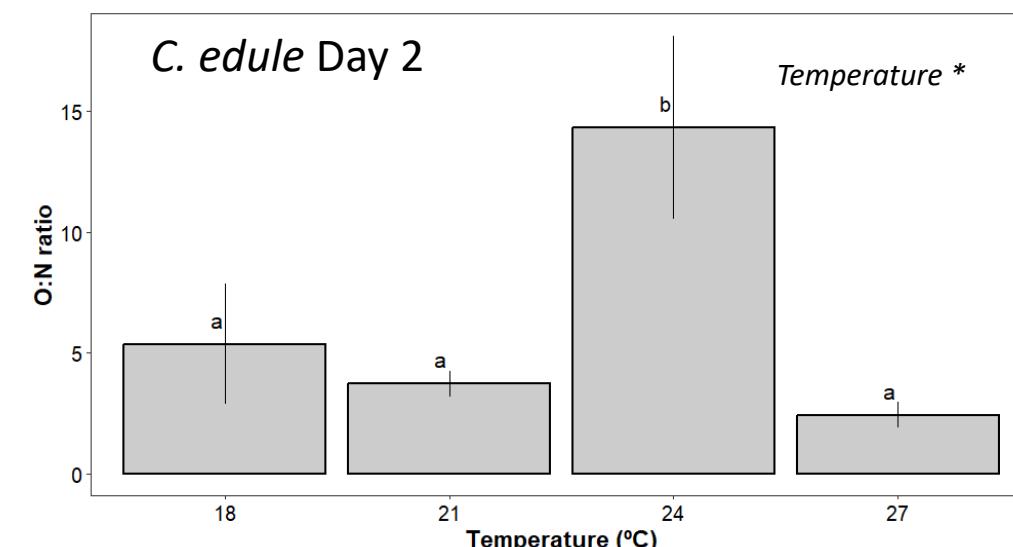
xaponesa



fina



berberecho



Salinity experiment

5.1. Experimentos salinidad (February 2024 & May-June 2024)

-Performance curves for recruitis and juveniles: constant salinity without tides, 4 days

Especies (x4)

Fina
Xapo
Babosa
Berberecho X

Tamaño
<10 mm

Salinidad (x5)

6
9
12
15

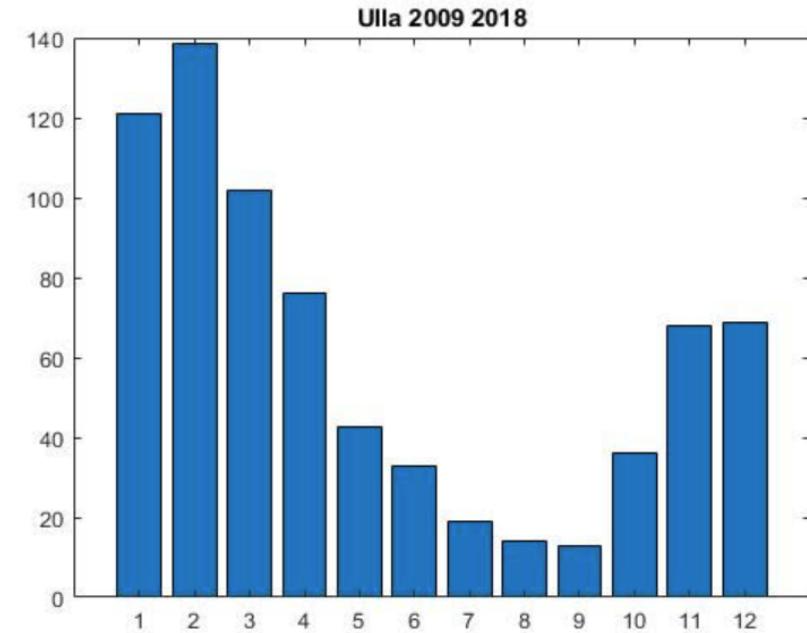
35 (control)

x 10 réplicas

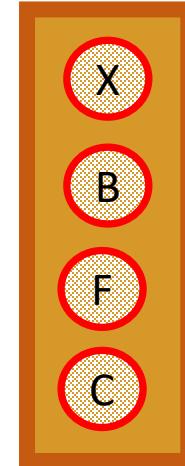
Exposición
4 días
Medidas D0, D4

Variables Respuesta

- ✓ Respiración (30 minutos)
- ✓ Alimentación (Citometría de flujo)
- ✓ Amonio 4h en 100 ml (ECIMAT)
- ✓ Volumen
- ✓ Peso seco



- Respuesta en 4 días
- Sin mareas; a temperatura ambiente
- Aireación suave para evitar evaporación
- Tanques de cabecera (uno por tratamiento) y poca renovación para que se consuma un tanque de cabecera en 24h.
- Cada 24h preparar nuevos tanques de cabecera
- 30 individuos en cada vaso
- Poner malla superior en cada vaso para evitar que salgan de los vasos



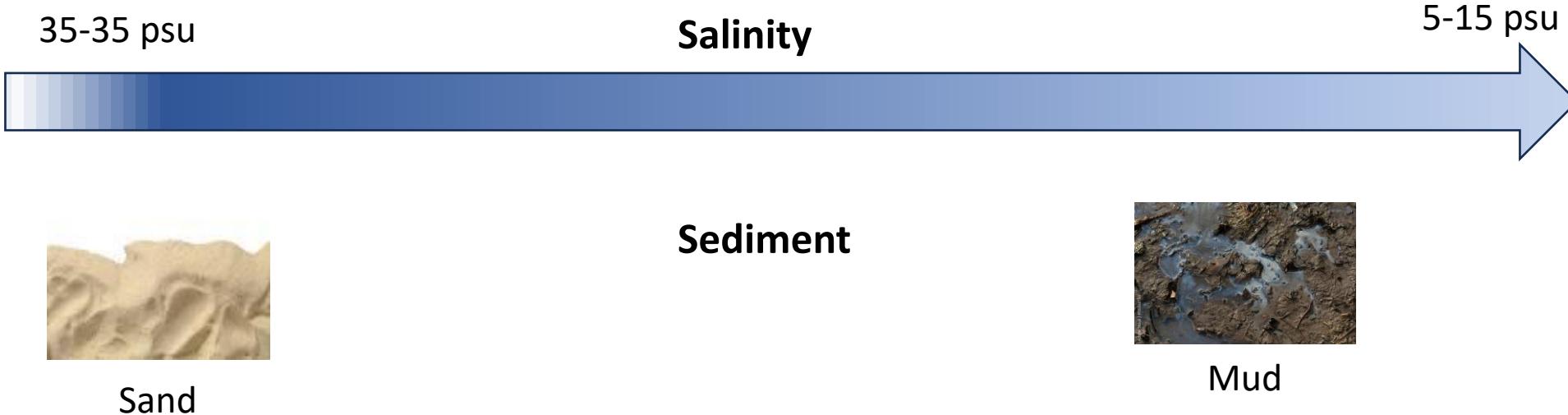
x 10 para cada
salinidad



Objective 4

Task 4.2 :

1. Effects of salinity on physiology of clams in two different sediments
2. The effects of clams on sediment differ under different salinity conditions



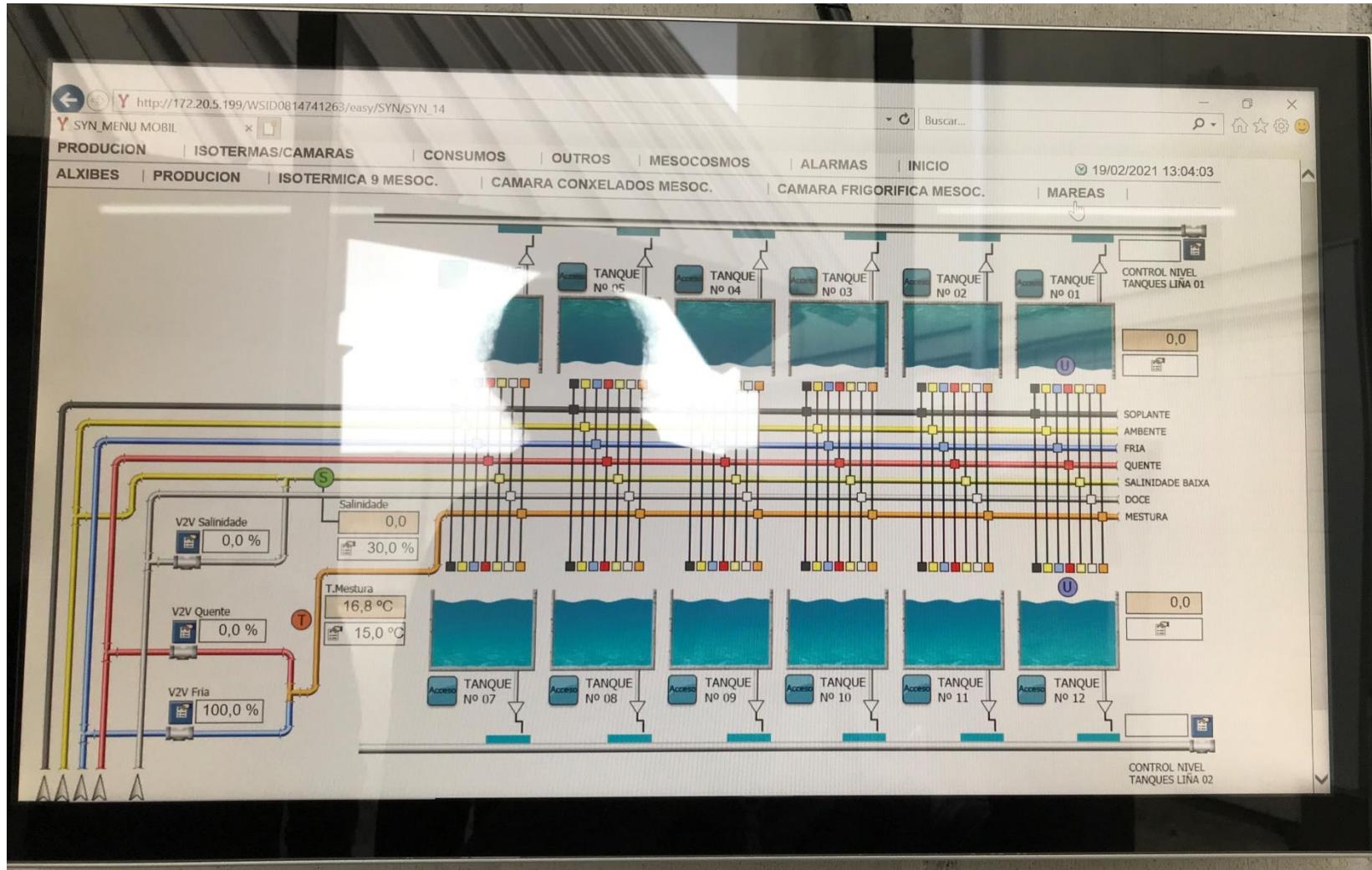
We think this species (*Venerupis corrugata*) is the most interesting to test salinity effects



Small-sized adults 20-25 mm, depending on availability

Mesocosms

RECOBI



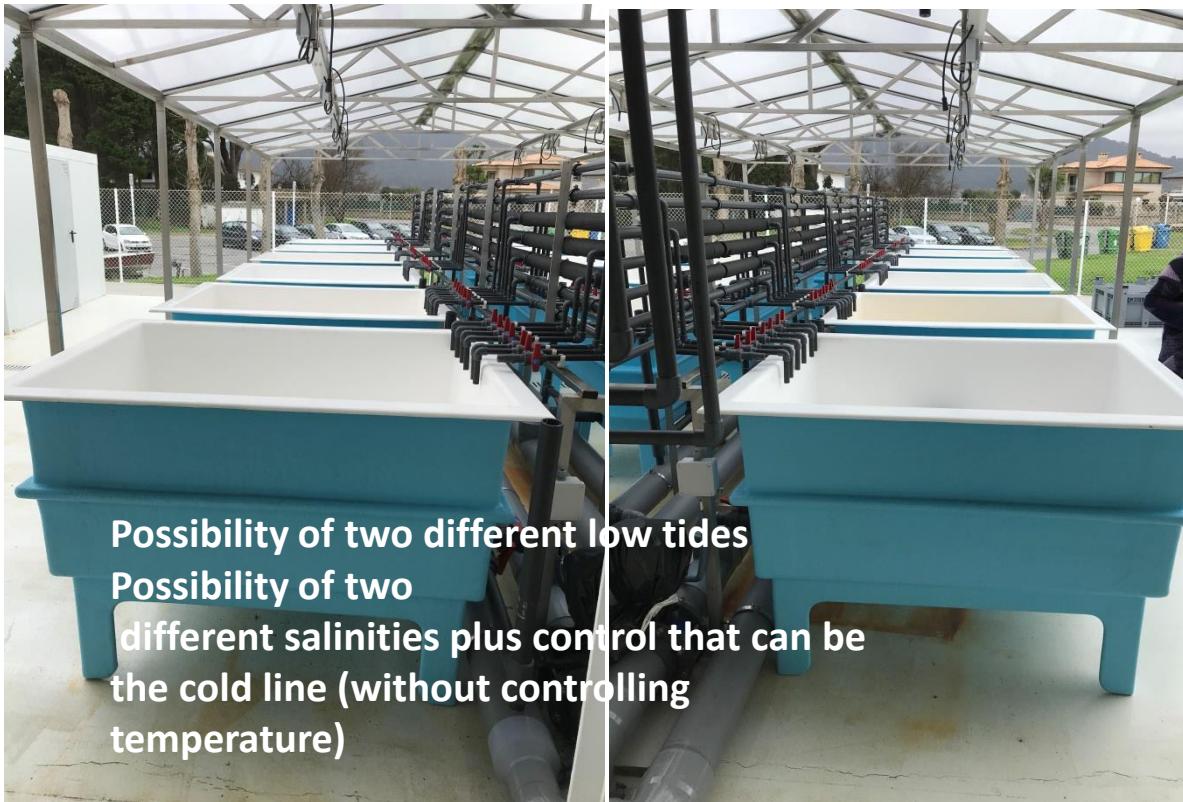
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la Unión Europea
NextGenerationEU

TR Plan de Recuperación,
Transformación y Resiliencia

12 tanks in each 18 black baskets ($0,25 \text{ m}^2$)



25x25x15 cm
 0.0625 m^2

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la Unión Europea
NextGenerationEU

PR
Plan de Recuperación,
Transformación y Resiliencia

Experimental design

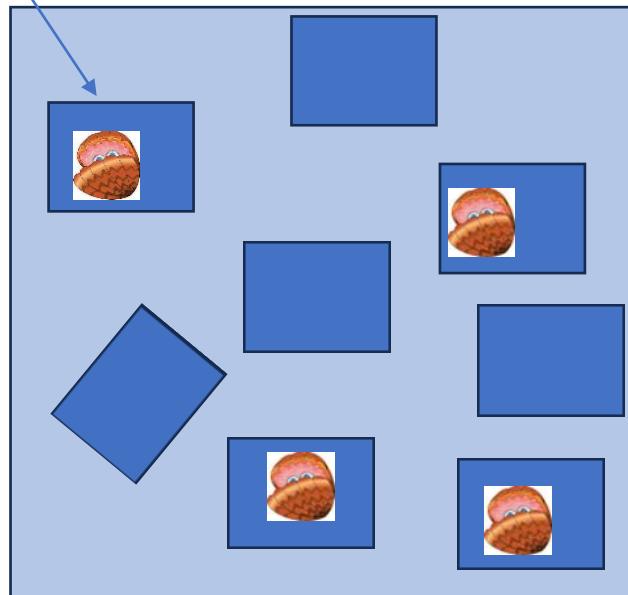
Salinity and Sediment are two orthogonal fixed factors: 35-35 sand, 35-35 mud, 5-15 sand, 5-15 mud. Each combination of combination of treatments replicated in 3 random Tanks (third factor), with 4 randomly positioned replicates (basket) of each treatment with and without clams (fourth factor). Keeping sediment type separated in different experimental big tanks

25x25x15 cm
0.0625 m²



Eg: 3 Big tanks per condition Salinity-Sediment: Salinity (35-35)-Sediment (sand)

n= 4



Keeping densities similar to field:

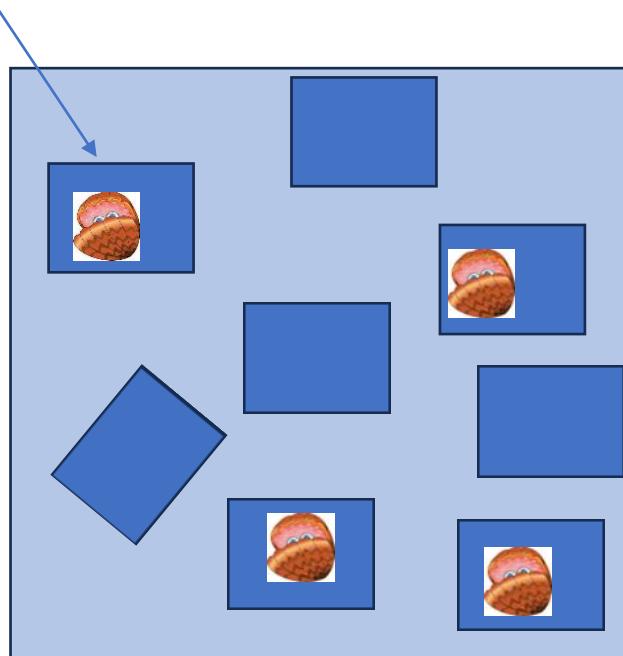
225 ind/m² (big adults): 14 individuals/basket, maybe a little bit more as they will not be big adults.... 18 individuals (6 of each species)

One of the most vulnerable to salinity changes is *V. corrugata*, we think it is better to focus on one species.

Experimental design- Sediment variables (pH, OM, redox..)

To be defined by Geo team

Eg: 3 Big tanks per condition Salinity-Sediment: Salinity (35-35)-Sediment (sand)



If data of sediment are analysed by ANOVA (MO, redox, etc.)

| | df | F-versus |
|-----------------------------------|----|-----------------------------------|
| Salinity | 1 | Tank (Salinity *Sediment) |
| Sediment | 1 | Tank (Salinity *Sediment) |
| Tank (Salinity *Sediment) | 8 | Residual |
| Clam | 1 | Clam x Tank (Salinity * Sediment) |
| Salinity x Sediment | 1 | Tank (Salinity *Sediment) |
| Salinity x Clam | 1 | Clam x Tank (Salinity * Sediment) |
| Sediment x Clam | 1 | Clam x Tank (Salinity * Sediment) |
| Clam x Tank (Salinity * Sediment) | 8 | Residual |
| Salinity *Sediment*Clam | 1 | Clam x Tank (Salinity * Sediment) |
| Residual | 72 | |

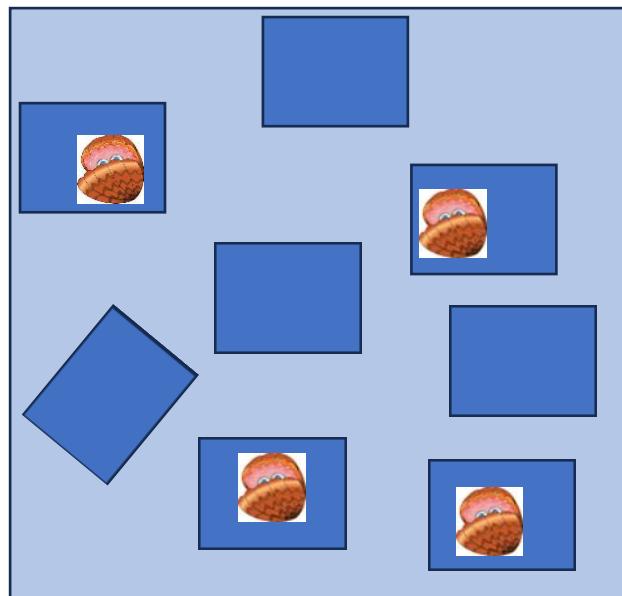
n= 4

Experimental design- Physiological variables (clams)

Eg: 3 Big tanks per condition Salinity-Sediment: Salinity (35-35)-Sediment (sand)

If data of clams are analysed by ANOVA (feeding, respiration...)

4 baskets each



| | df | F-versus |
|------------------------------------|-----|------------------------------------|
| Salinity | 1 | Tank (Salinity x Sediment) |
| Sediment | 1 | Tank (Salinity x Sediment) |
| Tank (Salinity x Sediment) | 8 | Basket(Salinity x Sediment x Tank) |
| Basket(Salinity x Sediment x Tank) | 36 | Residual |
| Salinity x Sediment | 1 | Tank (Salinity x Sediment) |
| Residual | 144 | |

Animals to measure for physiological ~ 4