CA18221 (WG2)

Feasibility of ecological mesocosms to environmental risk assessment of pesticides on reptiles. A pilot test with wall lizards

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CONTENT

1. Aim of the study
2. Description of the work carried during the STSM
3. Data management
4. Preliminary results
5. Improvements
6. Conclusions
7. Issues to taking in consideration

CA18221

PEsticide RIsk AssessMent for Amphibians and Reptiles (PERIAMAR)

WG2 objectives

1. **WG2O.1**: Characterise amphibian and reptilian taxa depending on their risk of being affected by pesticides (task 2.1)
2. **WG2O.2**: Characterise direct (tasks 2.2, 2.3) and indirect (task 2.4) risks posed by pesticides on amphibian and reptilian populations in nature, as a pillar to identify refinement options within the higher tier assessment and effective risk mitigation measures.

Previous backround from the last STSM (CA18221, WG2): Preliminary analysis of the literature on mesocosm and other manipulative experiments with the reptile and amphibian species.
Conclusion: Studies strongly biased on amphibians, while reptile species are neglected.

Specis tested: *Podarcis bocagei*

- Can occupy agroenvironments and, hence exposed to agrochemicals.

Aim of this STSM

- Covering this gap (few studies of reptile species regarding mesocosms and other manipulative experiments).

How?

- By conducting a pilot study with wall lizards (*Podarcis bocagei*) and establishing all the improvements necessary to evaluate the impacts of pesticide on this species in the future using the mesocosm environment.

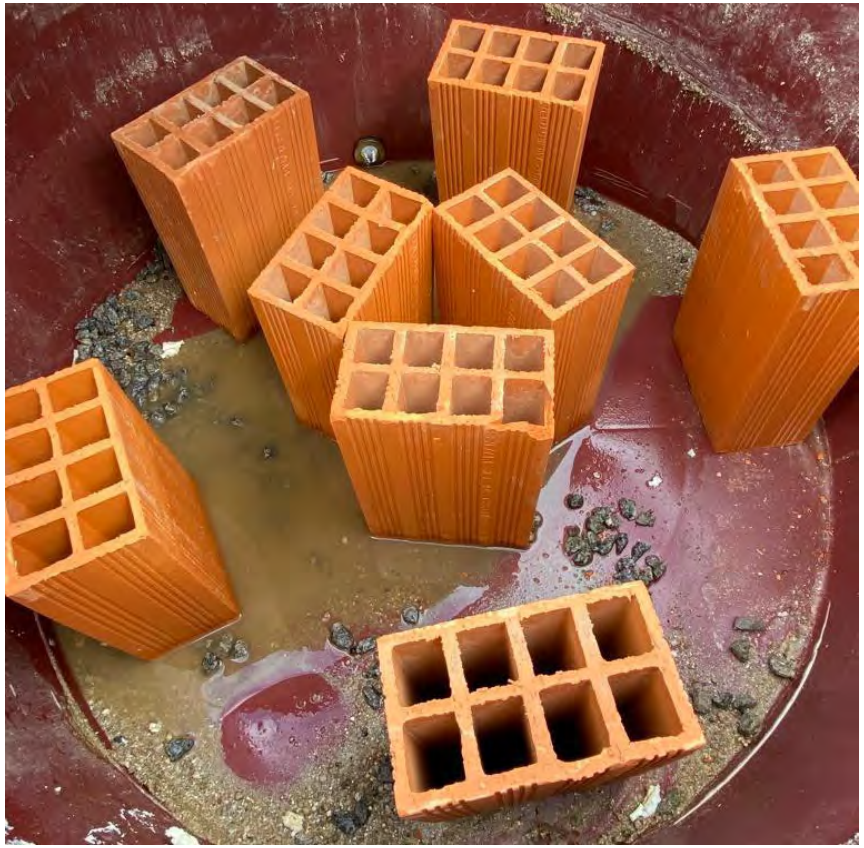
Mesocosm environments

Podarcis bocagei - Male

2. Description of the work carried during the STSM

a) Mesocosm environments

Mesocosm surface

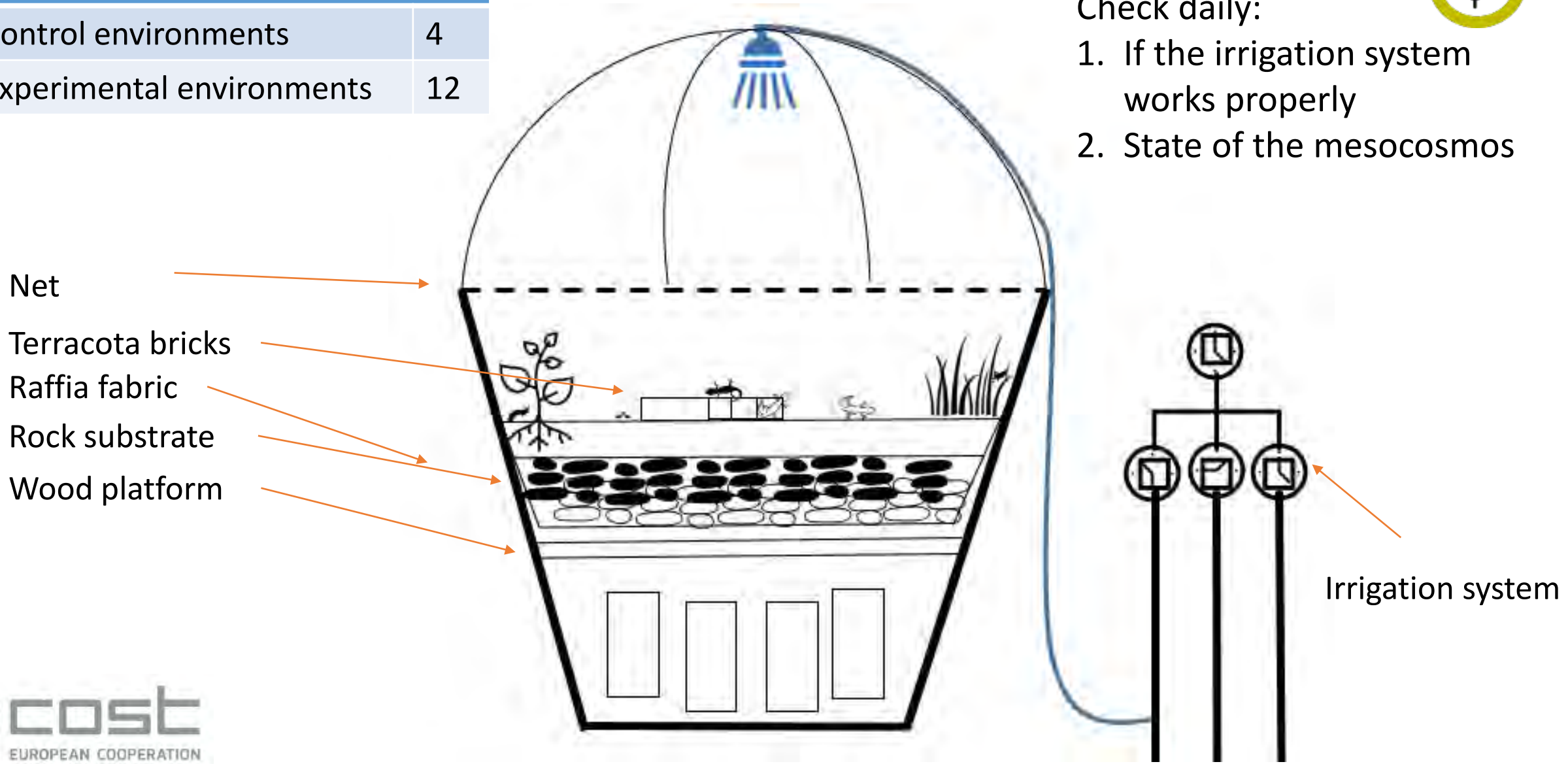


Mesocosm support

Mesocosm environments (n = 16)	
Control environments	4
Experimental environments	12

Check daily:

1. If the irrigation system works properly
2. State of the mesocosmos



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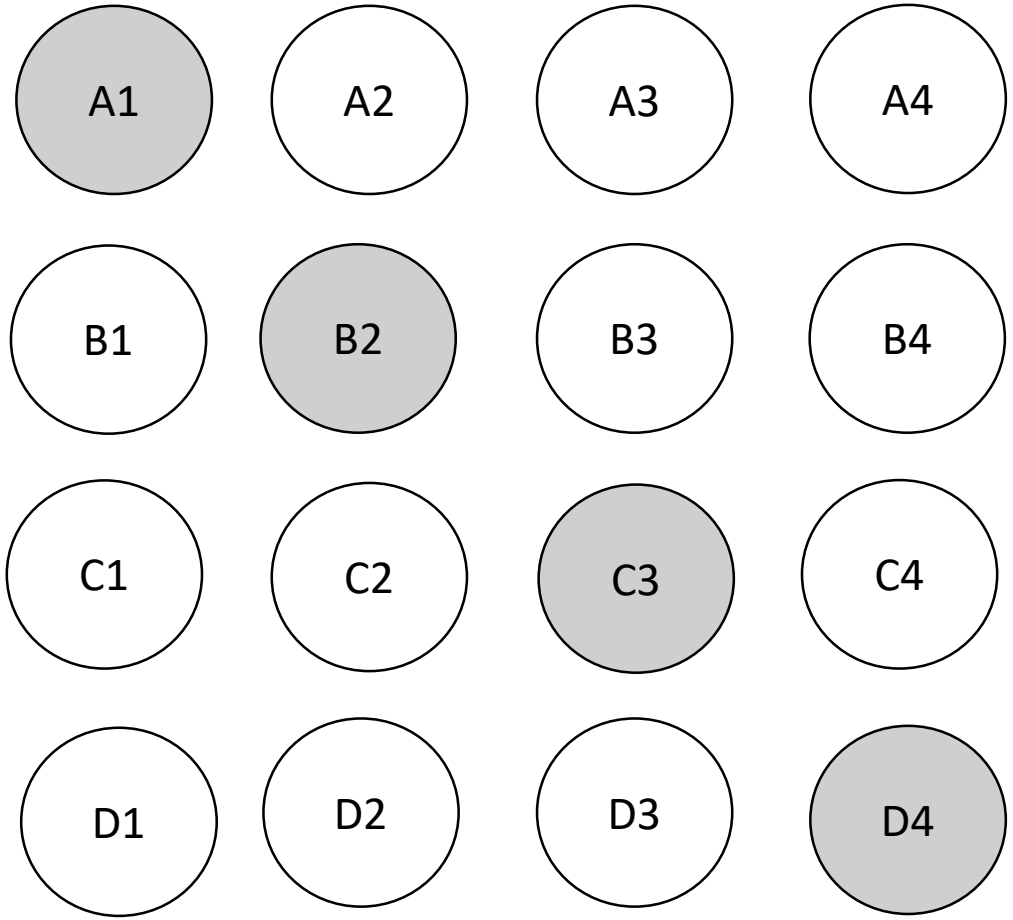
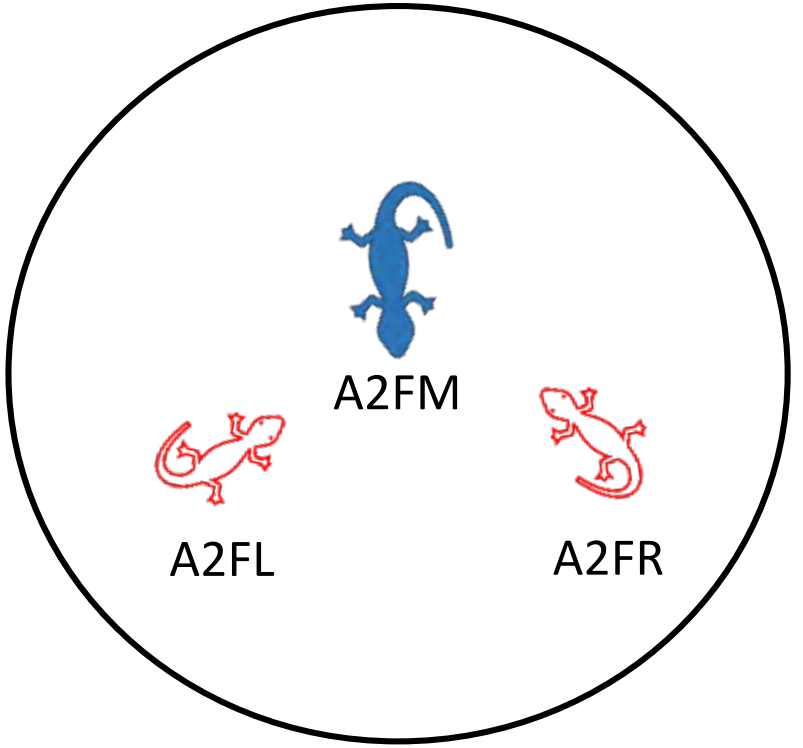
b) Animals

In 1 experimental environment: 1 male (FM) and 2 females (FR and FL)



Why 1 M and 2 F?
· To compose a social group
· To prevent the aggression between males



A2 mesocosm

Adults

Feeding: every 5 days

Tenebrio molitor larvae

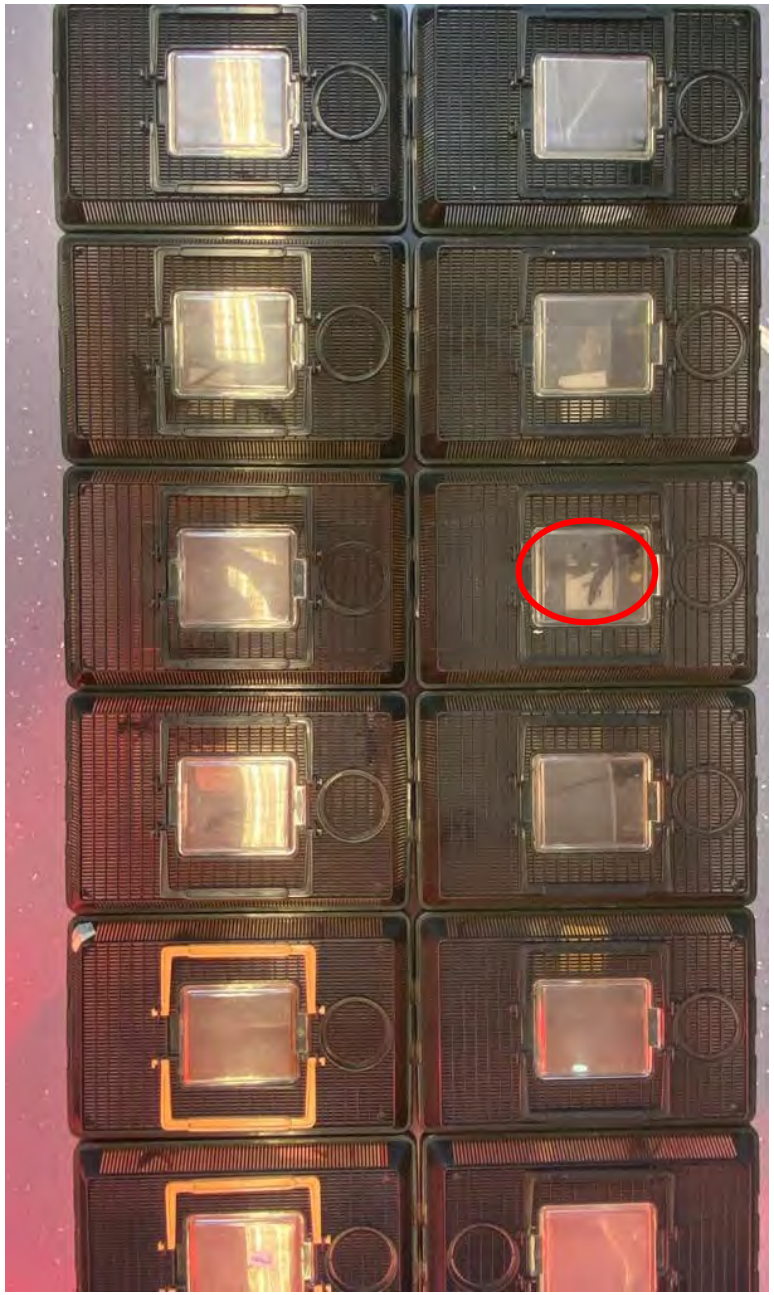
Measurements: every 18 days

Biometric parameters measured:

- Snout-vent length (SVL)
- Body mass (BM)

Why?

- Monitoring SVL and BM and making sure the animals are kept in good environmental condition



Boxes with animals from every mesocosm

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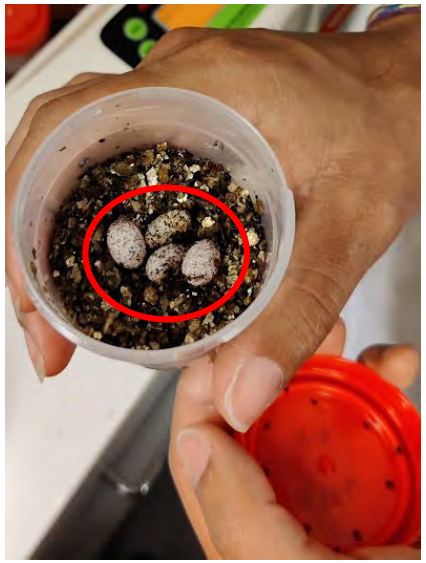
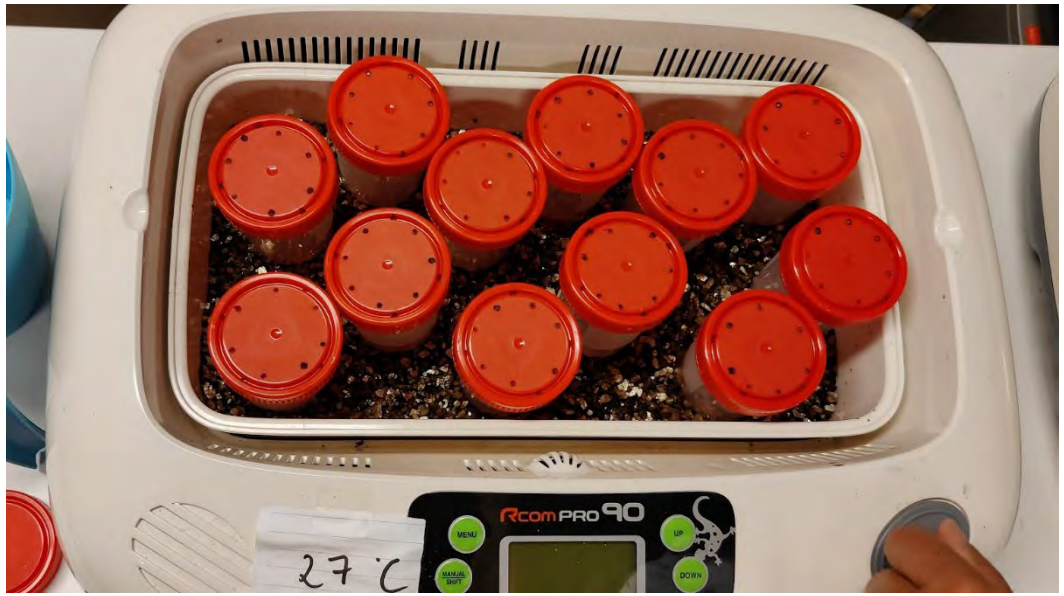
Pregnant females





1.Nest → 2.Eggs → 3.Incubator

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Juveniles

1. From incubator (n = 8)
2. Found hatched in mesocosmos (n = 19)

Initial box (smaller)

Bigger box

Feeding - juveniles

Daily – *Tenebrio molitor* larvae (smaller size) + crickets (small size)

Tenebrio molitor larvae

Acheta domesticus

Measurements

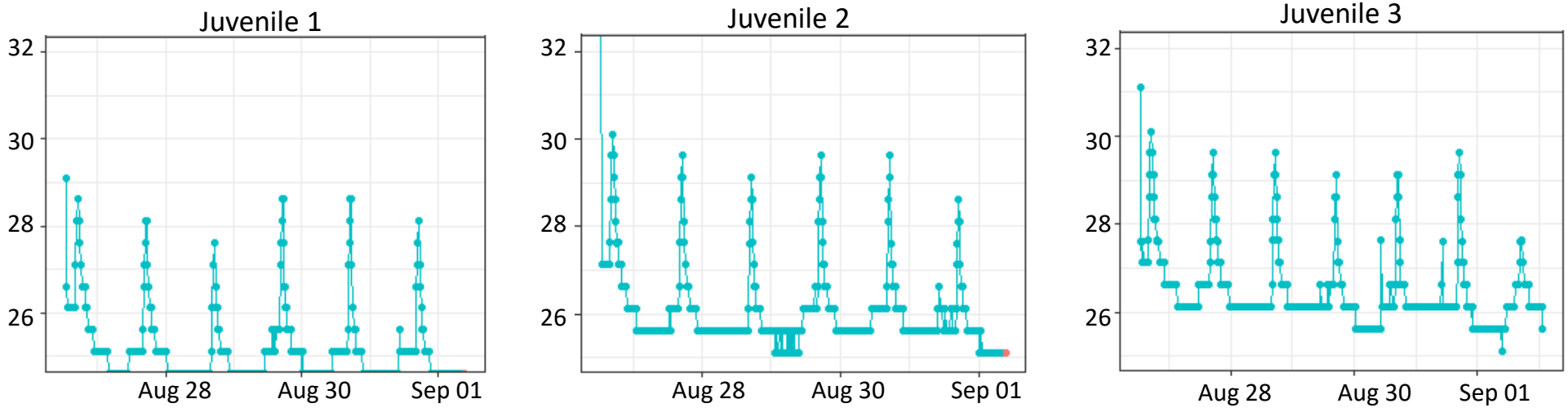
1. After 4 days of hatch in incubator/found in mesocosms
2. Proceeded the same as adults (every 18 days)

Juveniles found already hatched in mesocosms

Juvenile – *Podarcis bocagei*

Conditions inside boxes - juveniles (Air temperature + Humidity)

Air temperature



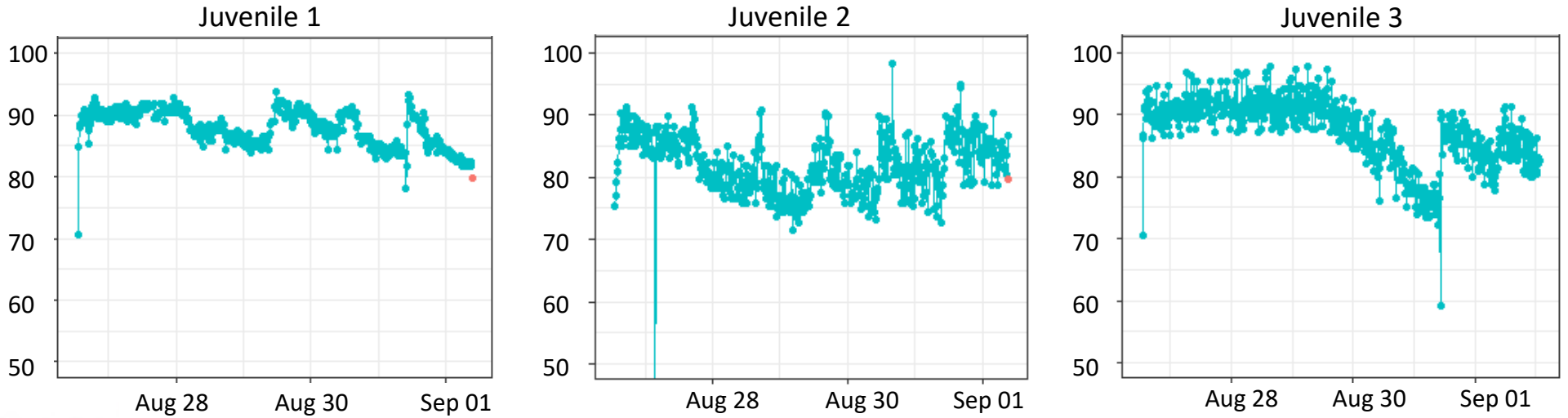
Legend

Dead0_vs_Alive1

0

1

Humidity



Date&Time (every 15 minutes)

3. Data management

Fred_ID_ Code	Sex	Mesocosmos	Nr_Ventral_ Scales	Date_Hatched (DD//MM/YYYY)	1st_Day_in_Trial (DD//MM/YYYY)	Maternal_ID	Clutch_Nr	Date_Deceased	Days_Alive	Weeks_Alive
B1FR	F	B1			06/05/2022					
B1FL	F	B1			06/05/2022					
A2FR	F	A2			06/05/2022					
A2FL	F	A2			06/05/2022					
C2FR	F	C2			06/05/2022					
C2FL	F	C2			06/05/2022			22/07/2022		
C1FL	F	C1			06/05/2022					
C1FR	F	C1			06/05/2022					
D1FL	F	D1			06/05/2022					
D1FR	F	D1			06/05/2022			22/07/2022		
A3FL	F	A3			06/05/2022					
A3FR	F	A3			06/05/2022			22/07/2022		
D2FR	F	D2			06/05/2022					
D2FL	F	D2			06/05/2022					
B3FL	F	B3			06/05/2022					
B3FR	F	B3			06/05/2022					
D3FL	F	D3			06/05/2022			22/07/2022		
D3FR	F	D3			06/05/2022					
A4FR	F	A4			06/05/2022			22/07/2022		
A4FL	F	A4			06/05/2022					
B4FL	F	B4			06/05/2022					
B4FR	F	B4			06/05/2022					
C4FR	F	C4			06/05/2022					
C4FL	F	C4			06/05/2022			14/06/2022		

ID control

Mother_ID (Fred_ID)	Clutch_1 Date_Found	Clutch_1_ Size	Clutch_1_ Hatched	Clutch_1 %_Success	Clutch_1 1st_Hatch_Date	Clutch_1 Incubation_Time	Clutch_2 Date_Found	Clutch_2_ Size	Clutch_2_ Hatched	Clutch_2 %_Success	Clutch_2 1st_Hatch_Date	Clutch_2 Incubation_Time	Clutch_3 Date_Found	Clutch_3_ Size
C2FR														
C2FL														
C1FL														
C1FR														
D1FL														
D1FR														
A3FL														
A3FR	01/06/2022	2	1	50	11/07/2022	40								
D2FR														
D2FL	04/07/2022	2		0										
B3FL	01/06/2022	4	0	0										
B3FR														
D3FL	30/05/2022	4	3	75	06/07/2022	37								
D3FR														
A4FR	24/05/22	4	4	100	04/07/2022	41								
A4FL	17/06/22	3		0										
B4FL	27/06/22	3		0										
B4FR														
C4FR														
C4FL	15/06/22	3		0										
C4FL1	22/06/2022	5												
D3FL1	25/07/2022	4												
CFA003W01	06/07/2022	1												
CFA001W02	22/06/2022	5												

Reproductive control

Day	Date_DD_MM_ YYYY	ID (Prem_ID)	ID (Fred_ID)	Maternal_ID	Clutch_Nr	MASS	SVL_mm	Mesocosmos	Sex	Age	Scars	Gravid	Laid	Spectra	Photo/ Scan	Blood_ Smear	BC_Mass VL
0	06/05/2022		B1FR			2.2016	58.55	B1	F	A				☑	☑	☐	0.038
0	06/05/2022		B1FL			3.3427	54.31	B1	F	A				☑	☑	☐	0.062
0	06/05/2022	48	A2FR			1.9210	49.01	A2	F	A				☑	☑	☐	0.039
0	06/05/2022		A2FL			1.5258	44.15	A2	F	A				☑	☑	☐	0.035
0	06/05/2022		C2FR			3.7125	55.42	C2	F	A				☑	☑	☐	0.067
0	06/05/2022		C2FL			2.3565	52.93	C2	F	A				☑	☑	☐	0.045
0	06/05/2022		C1FL			3.5182	60.46	C1	F	A				☑	☑	☐	0.058
0	06/05/2022		C1FR			1.6744	45.17	C1	F	A				☑	☑	☐	0.037
0	06/05/2022	A1FL	D1FL			3.7335	61.34	D1	F	A				☑	☑	☐	0.061
0	06/05/2022		D1FR			2.0121	51.64	D1	F	A				☑	☑	☐	0.039
0	06/05/2022	A2FR	A3FL			3.6641	57.29	A3	F	A				☑	☑	☐	0.064
0	06/05/2022		A3FR			2.5612	54.13	A3	F	A				☑	☑	☐	0.047
0	06/05/2022	B2FR	D2FR			2.7786	53.39	D2	F	A				☑	☑	☐	0.052
0	06/05/2022	B2FL	D2FL			3.4926	58.84	D2	F	A				☑	☑	☐	0.059
0	06/05/2022		B3FL			3.2787	61.28	B3	F	A				☑	☑	☐	0.054
0	06/05/2022		B3FR			3.1685	56.35	B3	F	A				☑	☑	☐	0.056
0	06/05/2022	47	D3FL			3.1508	56.55	D3	F	A				☑	☑	☐	0.056
0	06/05/2022	49	D3FR			2.2390	49.62	D3	F	A				☑	☑	☐	0.045
0	06/05/2022		A4FR			3.3503	58.89	A4	F	A				☑	☑	☐	0.057
0	06/05/2022		A4FL			2.3848	53.38	A4	F	A				☑	☑	☐	0.045
0	06/05/2022		B4FL			3.3244	58.54	B4	F	A				☑	☑	☐	0.057
0	06/05/2022		B4FR			2.9309	56.03	B4	F	A				☑	☑	☐	0.052
0	06/05/2022	50	C4FR			3.3922	56.01	C4	F	A				☑	☑	☐	0.061
0	06/05/2022	51	C4FL			2.9427	56.86	C4	F	A				☑	☑	☐	0.052

Body condition control

adults schedule mesocosm	
22/07/2022	17/08/2022
27/07/2022	22/08/2022
29/07/2022	26/08/2022
01/08/2022	29/08/2022
05/08/2022	02/09/2022
09/08/2022	07/09/2022
12/08/2022	12/09/2022

week	number of tenebrios larvae/day	friday
1	4	-
2	5	6
3	6	8
4	7	10
5	7	10
6	8	12
7	9	12
8		
9		

juvenile schedule	A4FRa	A4FRb	A4FRc	A4FRd	D3FLa	D3FLb	D3FLc	A3FRa	B3FRa	B3FRb	A3FLa	A3
04/07/2022	4	4	4	4								
05/07/2022	4	4	4	4								
06/07/2022	4	4	4	4	4	4	4	4				
07/07/2022	4	4	4	4	4	4	4	4				
08/07/2022	6	6	6	6	6	6	6	6				
11/07/2022	5	5	5	5	5	5	5	5				
12/07/2022	5	5	5	5	5	5	5	5				
13/07/2022	5	5	5	5	5	5	5	5				
14/07/2022	5	5	5	5	5	5	5	5				
15/07/2022	8	8	8	8	8	8	8	8				
18/07/2022	6	6	6	6	6	6	6	6				
19/07/2022	6	6	6	6	6	6	6	6				
20/07/2022	6	6	6	6	6	6	6	6				

Feeding schedule

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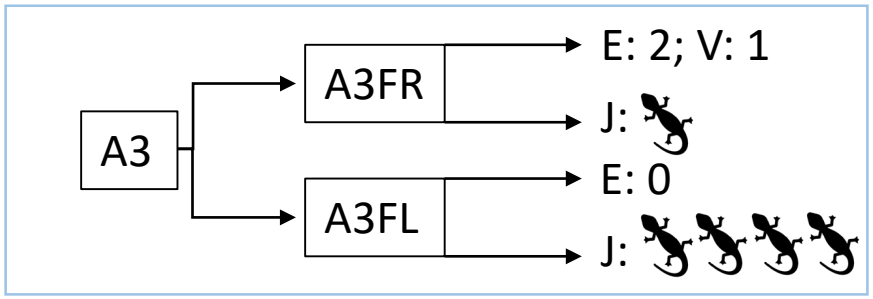
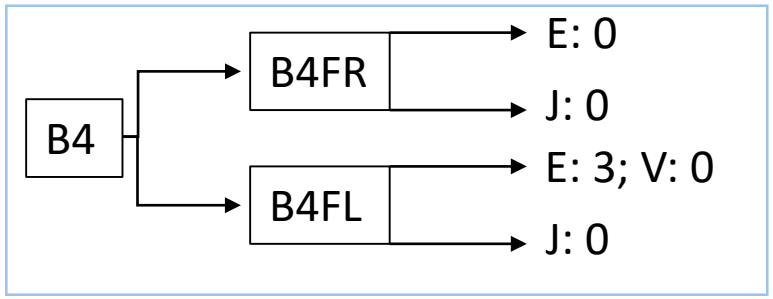
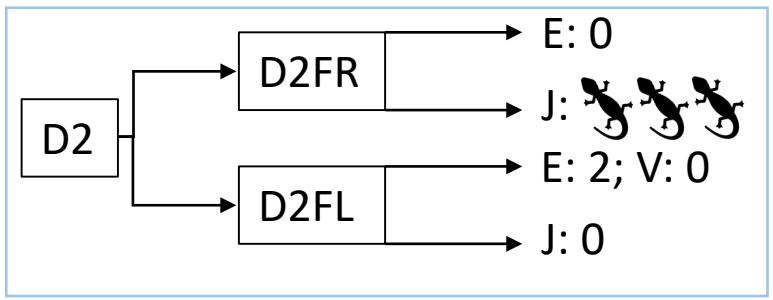
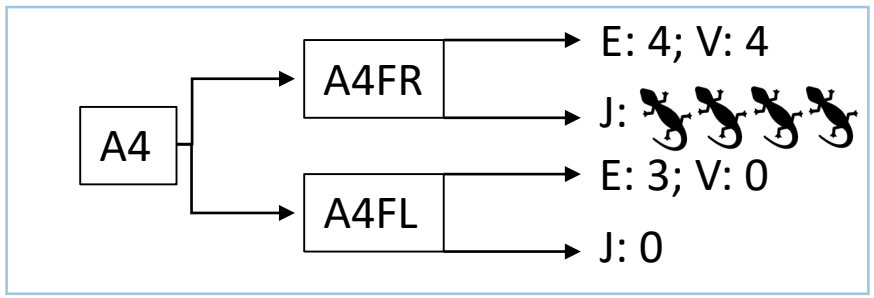
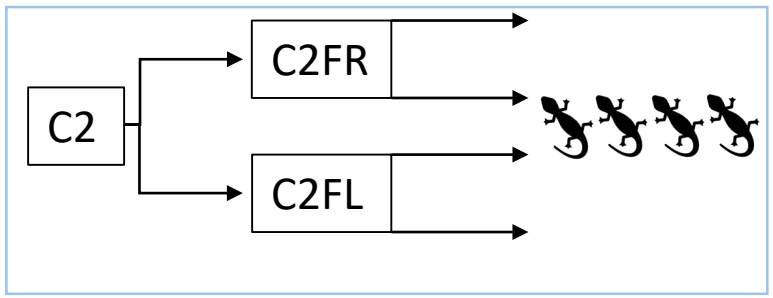
Eggs and Juveniles Situation

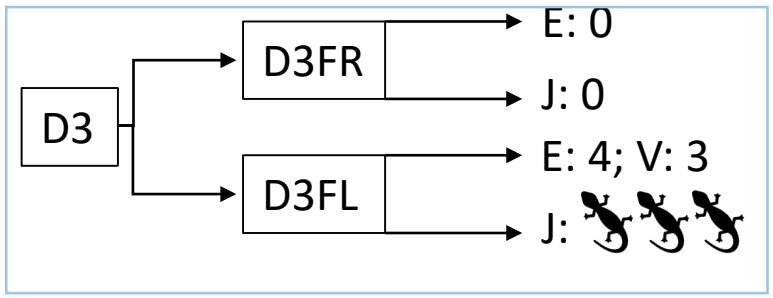


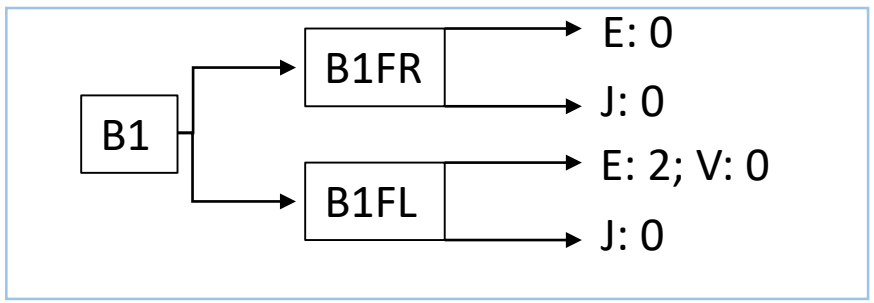


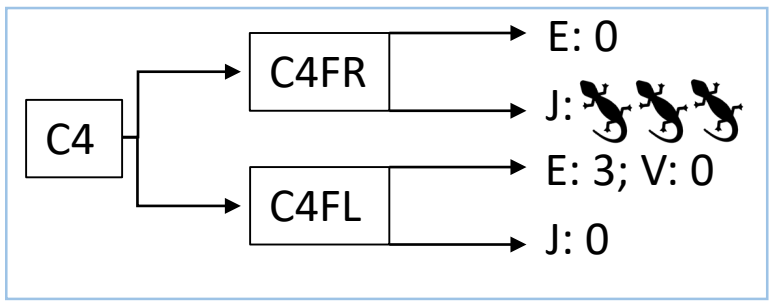


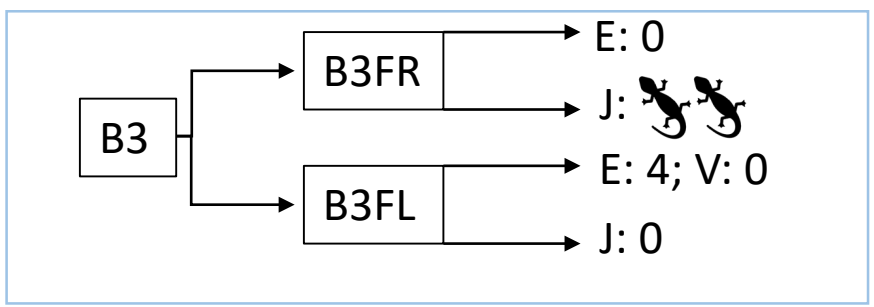


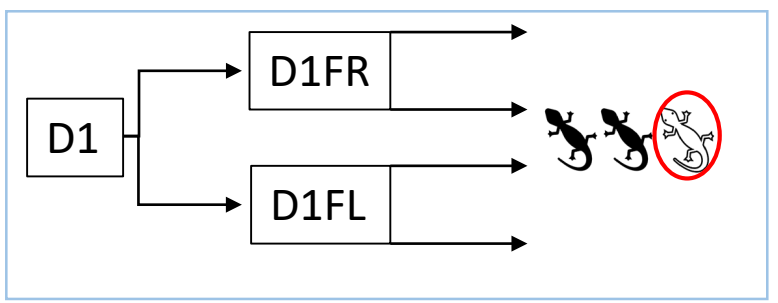






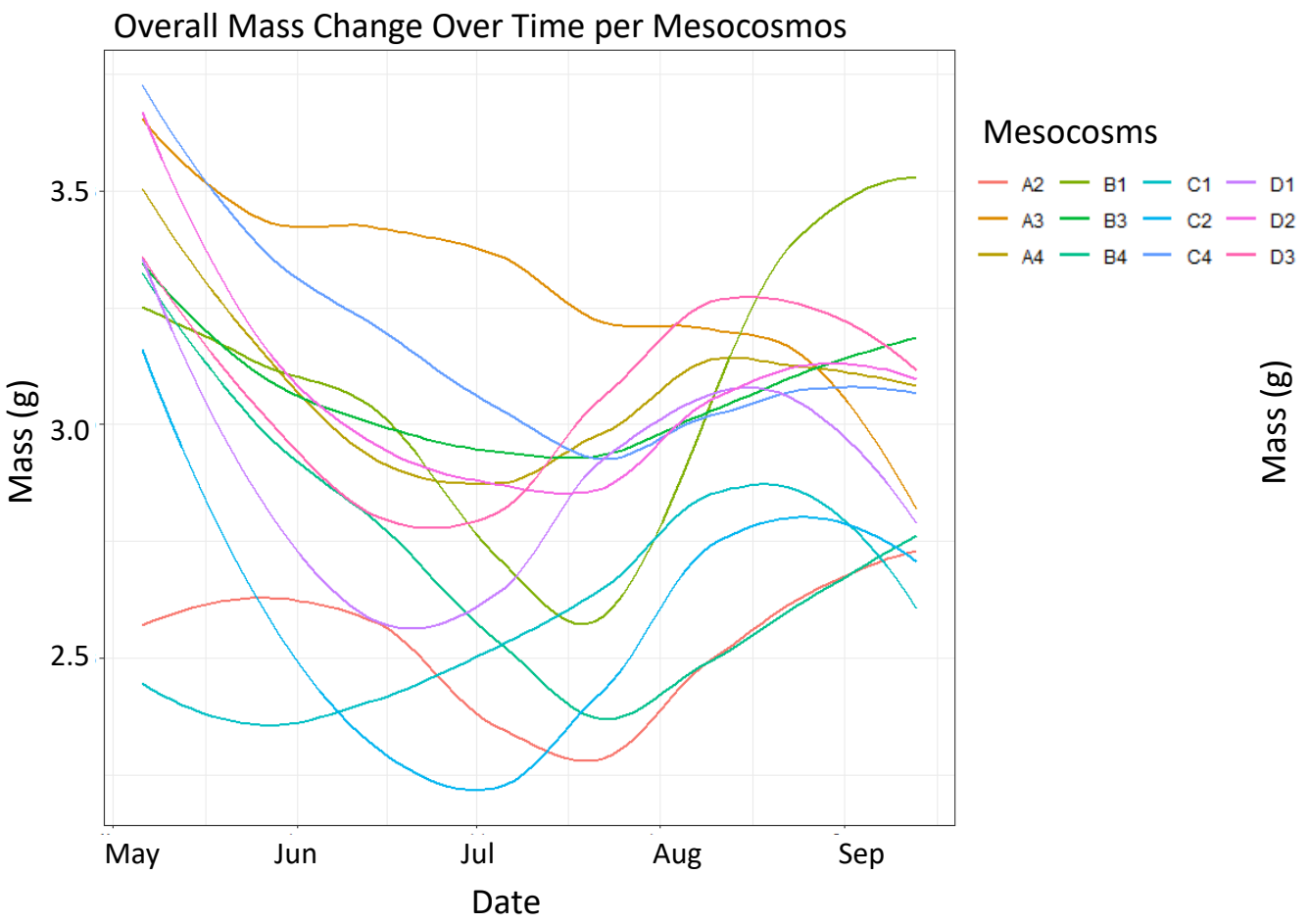




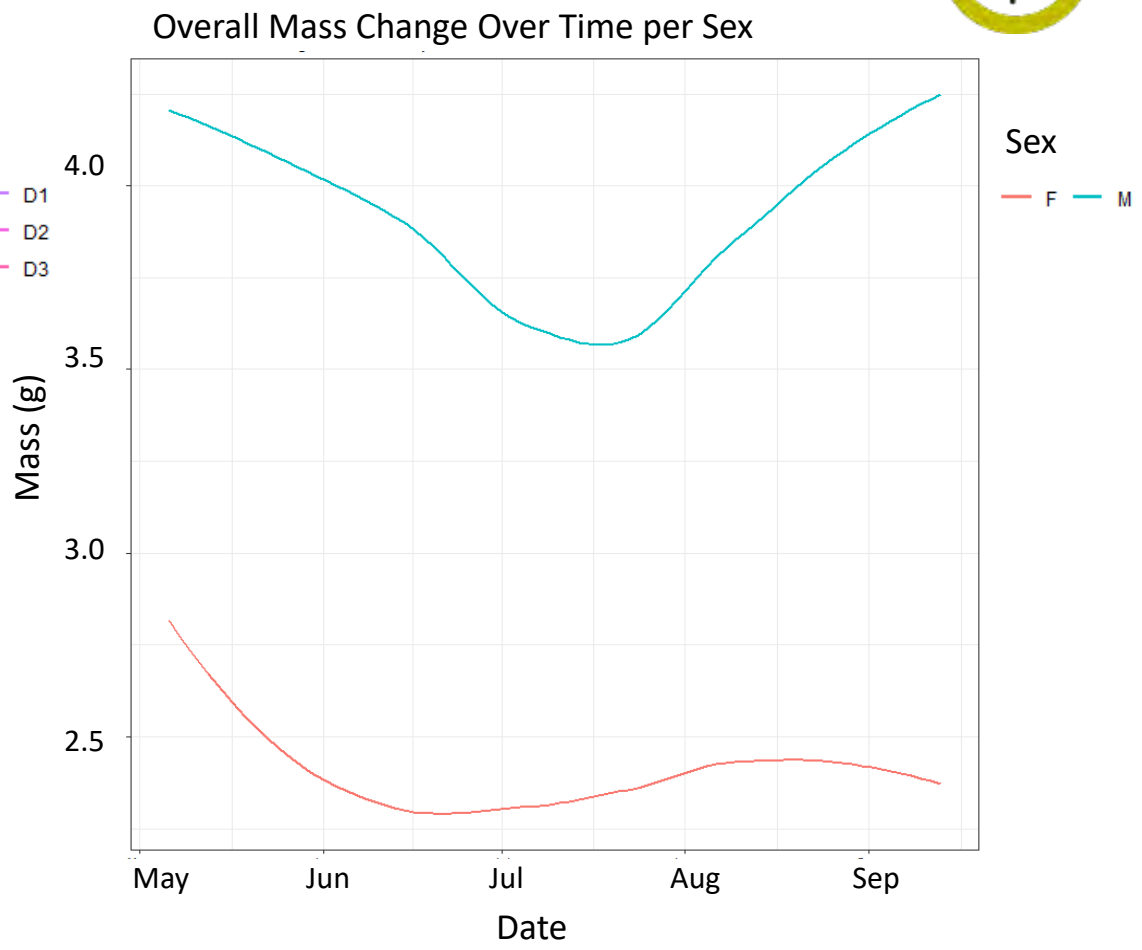


Legend:
E – Eggs
V – Viable eggs
Dead juvenile
Alive juvenile

4. Preliminary results - Adults

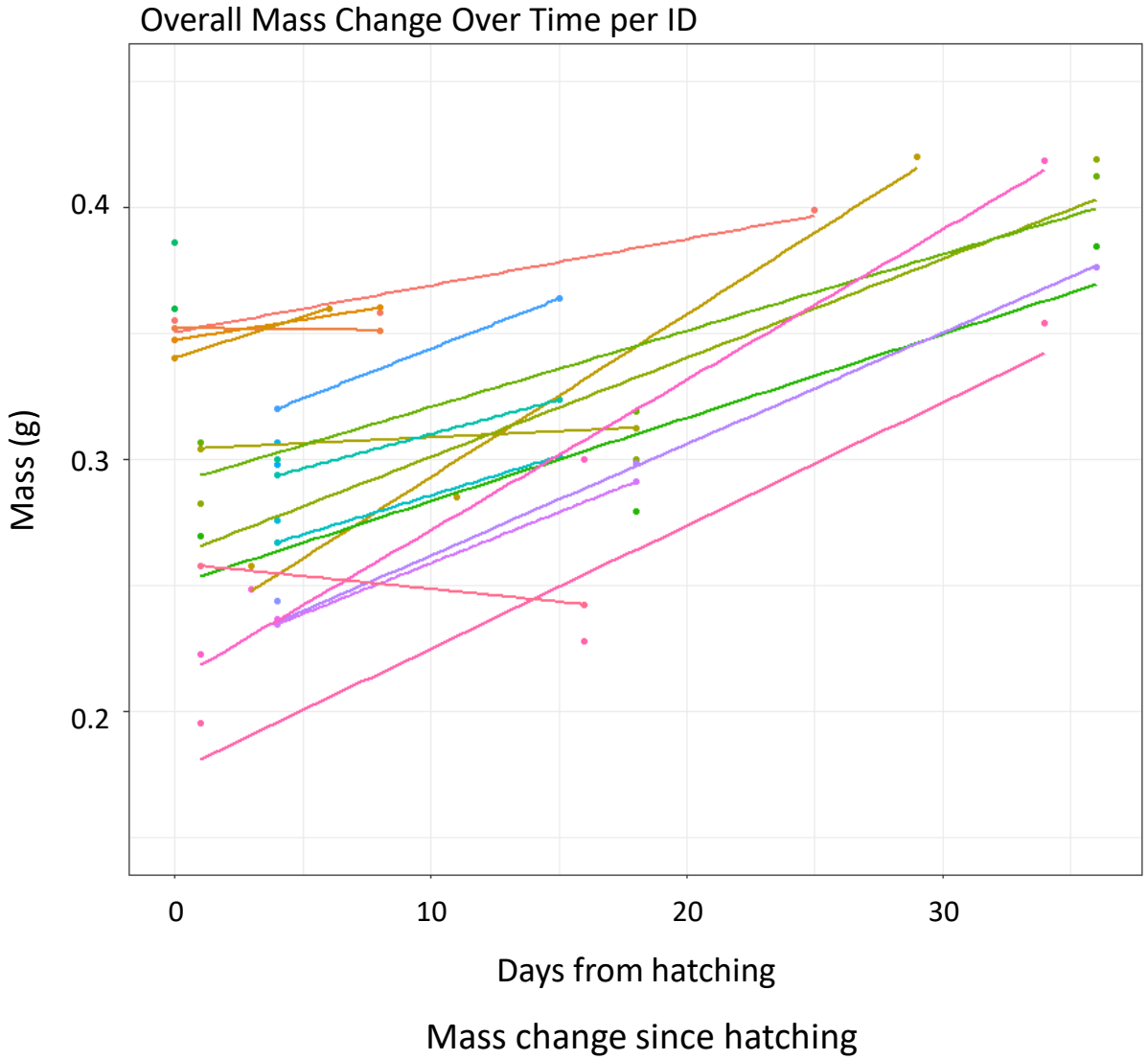


Mass change per every mesocosm



Mass change per sexes

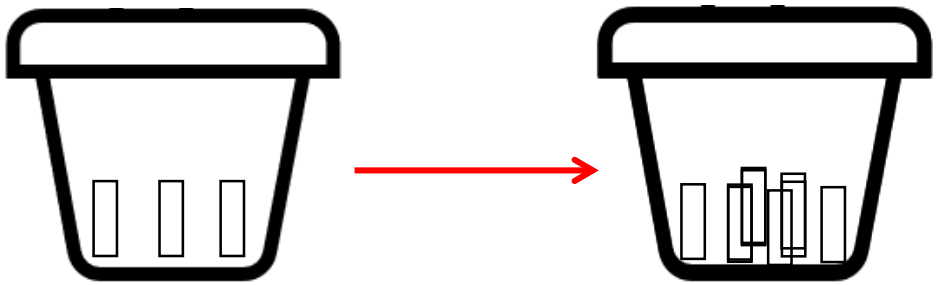
4. Preliminary results - Juveniles



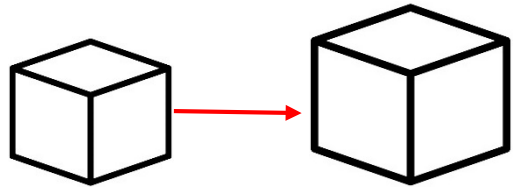
Mass change since hatching

5. Improvements

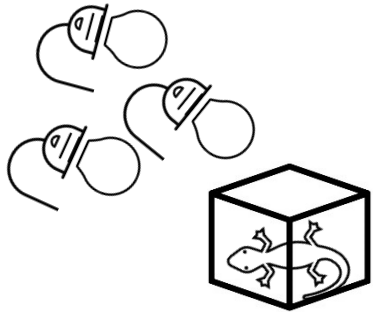
Regarding mesocosms
a) Use a more stable support by adding more bricks (In the beginning there were only 3 bricks, now are 8)



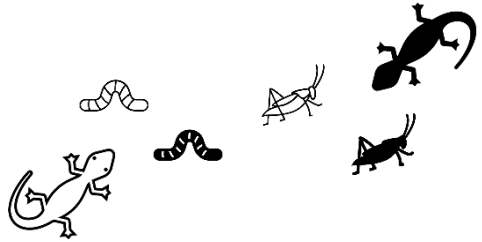
Regarding juveniles:
a) Used bigger boxes from the beginning with only half substrate and less humid than we made initially



b) Added basking lights that works for a certain time to give them the occasion to thermoregulate



c) Added crickets of small size in their diet for diversification



6. Conclusions

1. Mesocosm environments in experiments using adult lizards

Feasible for a long period

2. Breeding under environmental conditions in our mesocosms is possible.

It’s better to remove the eggs and juveniles from the mesocosms and to keep in lab conditions

3. Mesocosm design

Suitable for future modification by manipulating biotic and abiotic conditions

7. Issues to taking in consideration

1. Issues with incubation - fungi

2. Issues with juvenile husbandry - diversification of diet

3. Very important – daily checking all the animals

THANK YOU!

TEAM

- Frederico Maria: Experimental design and secondary coordination
- Lorenzo Papaleo: Experimental construction
- Lekshmi Sreelatha: Background support
- Luis Eduardo: Collecting the animals
- Gabriel Ene: Assistance in animal husbandry
- Laura Gautier: Assistance in animal manipulation

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