



Nutrient removal in a recirculating aquaculture (RAS) for white shrimp intensive culture using a biological filter wetland-type



International seminar DAAD
La Paz, Bolivia 27th September 2018

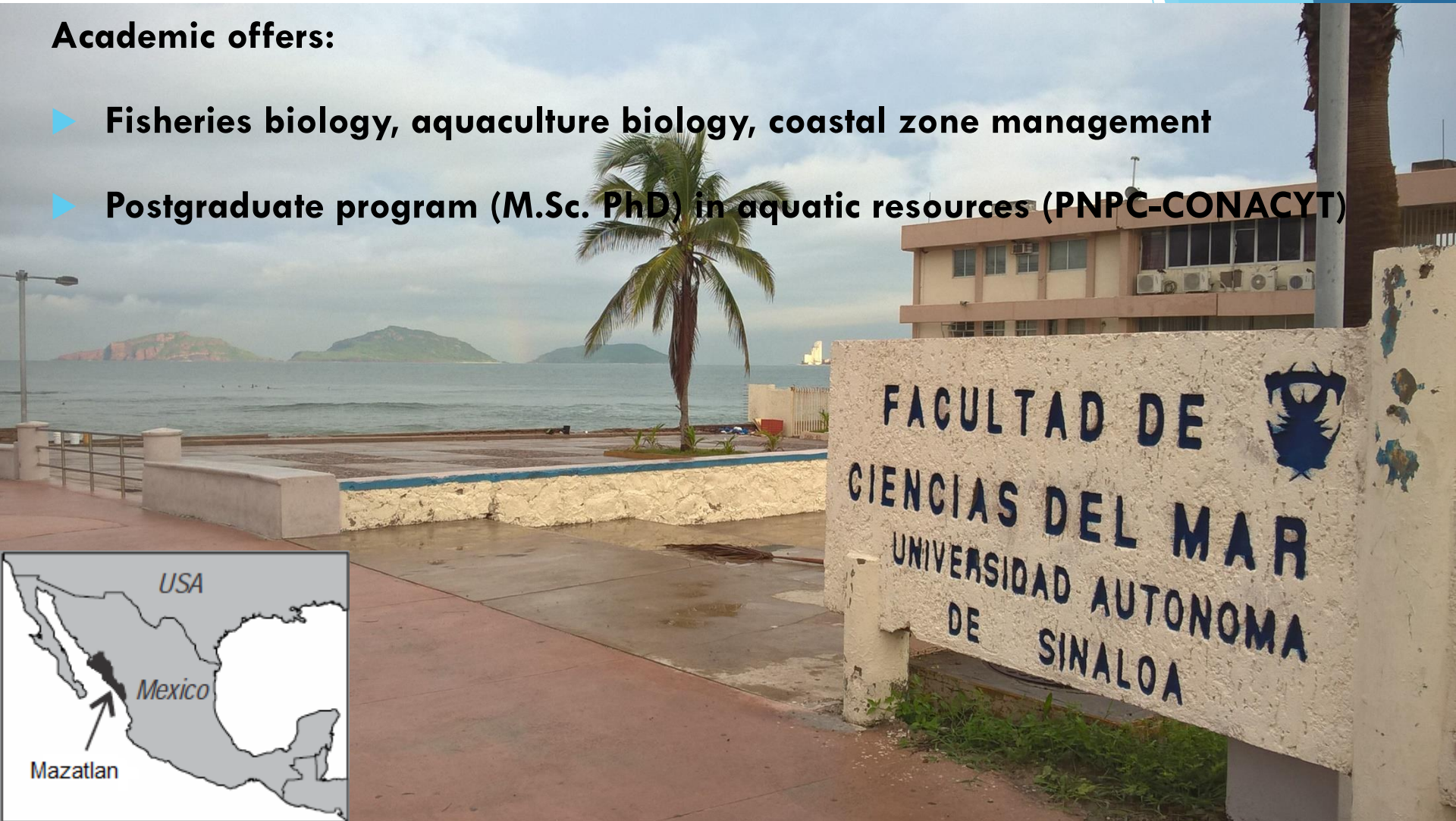
Dr. Otoniel Carranza Díaz

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The Autonomous University of Sinaloa

Academic offers:

- ▶ Fisheries biology, aquaculture biology, coastal zone management
- ▶ Postgraduate program (M.Sc. PhD) in aquatic resources (PNPC-CONACYT)



White shrimp production in Mexico 2016



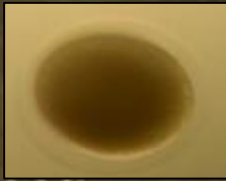
White shrimp reproduction facility



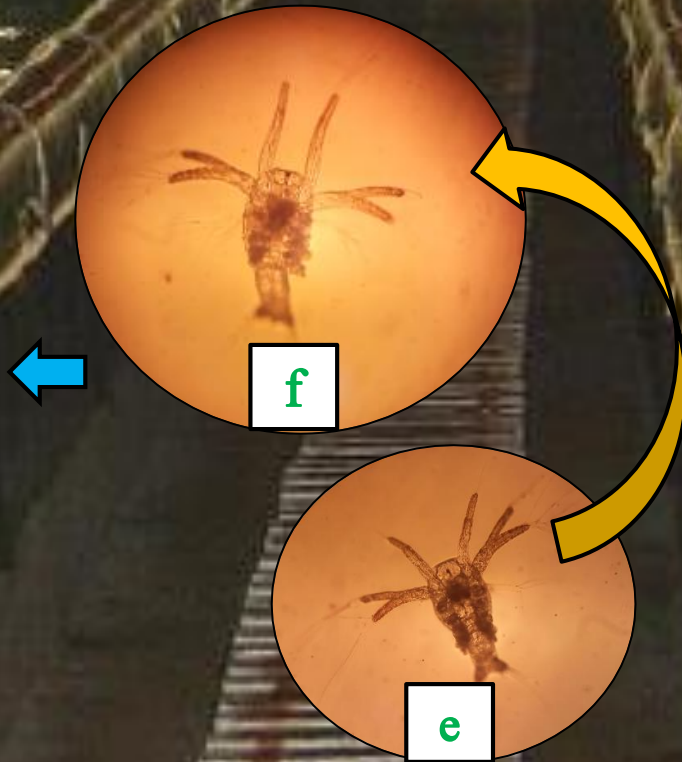
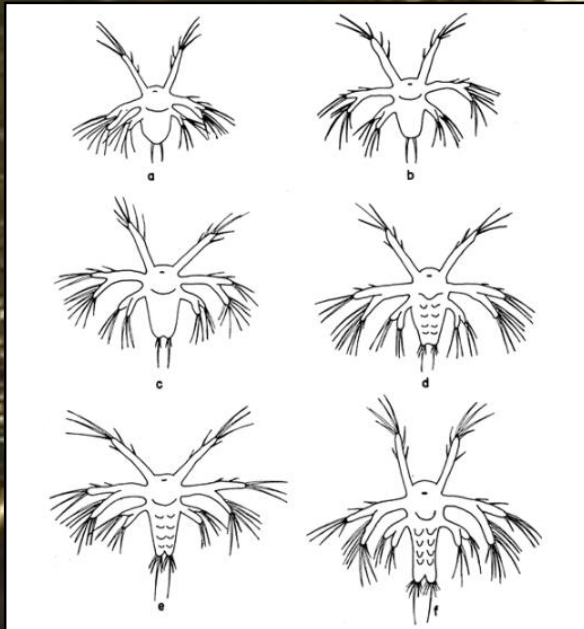
Picture taken by Osuna, (2017)

Madure female

Fertilized eggs



Shrimp larval stadium (Nauplii I a Nauplii VI advanced).



Picture: Lugo, (2017)

a. Nauplii I, b. Nauplii II, c. Nauplii III, d. Nauplii IV, e. Nauplii V, f. Nauplii VI (advanced)

Nauplii (6 stages)

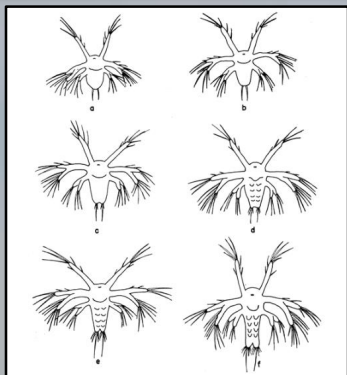
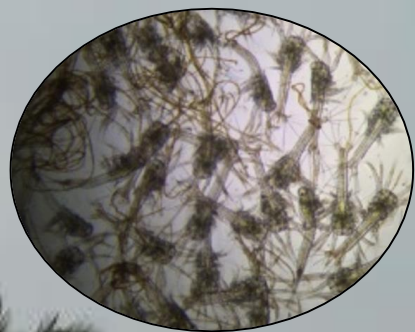


Foto: Lugo, (2017)

Zoea

27-29°C

Thalassiosira sp. y Chaetoceros sp.
29-30°C



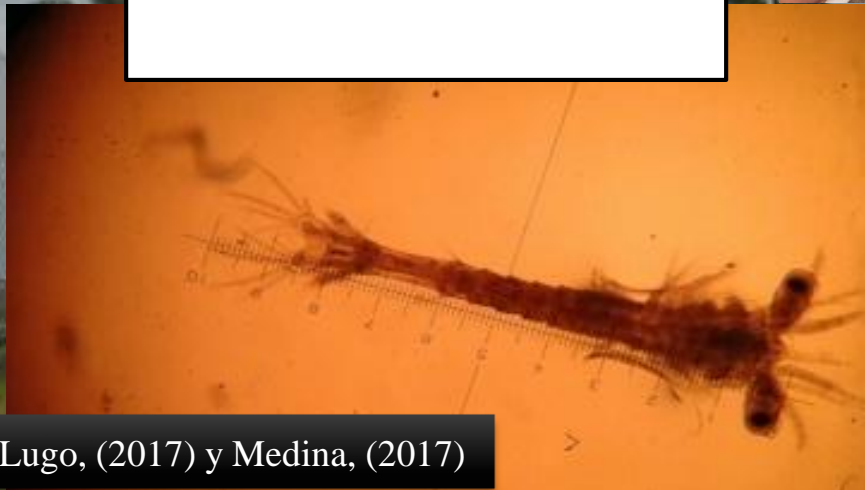
Mysis 1-3

30-31°C

Artemia nauplii and micropellets.

Postlarvae 1-16

31-33.5°C



Fotos: Lugo, (2017) y Medina, (2017)

Nurseries, green house cultures and transport to fattening units

Pre-grown facilities



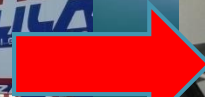
Nurseries
(environmental control)



Water quality



Intensive cultures → 500 orgs/m³



Pictures: Lugo, (2017) & Medina, (2017)

Aerial view of a white shrimp fattening production unit



Urías coastal lagoon

Mazatlan airport

**What happened when wastewater is discharge to the environment ?
Is there a risk for groundwater contamination?**

Environmental problems

Reproduction facilities,
Nurseries, green house
cultures

Demand energy
Discharge high
concentration of solids,
nutrients (N, P, K),
organic matter and
chemical substances

✓ **Aquaculture
industry needs a
lot of water**

Fattening

Deforestation
Lost of natural habitat
Discharge nutrients and
chemical substances
Modify the natural
drainage regime

Páez-Osuna, 2001

The effluents of shrimp farms deteriorate the environment (Páez-Osuna, 2001). Sustainable aquaculture. There is a need to improve water management and contaminant mitigation strategies.

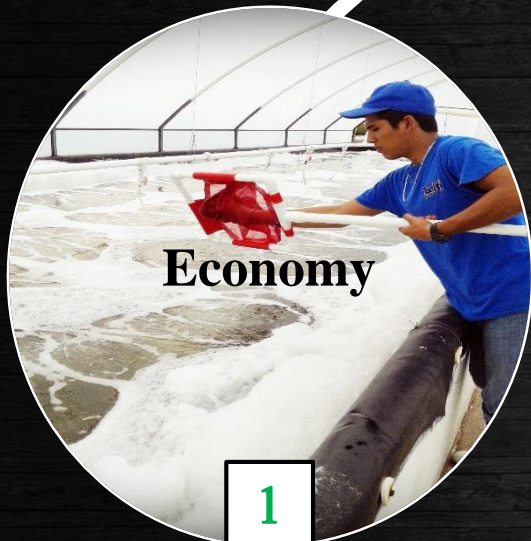
Environmental problems



Picture: Quiñonez-López, (2018)



Importance of aquaculture

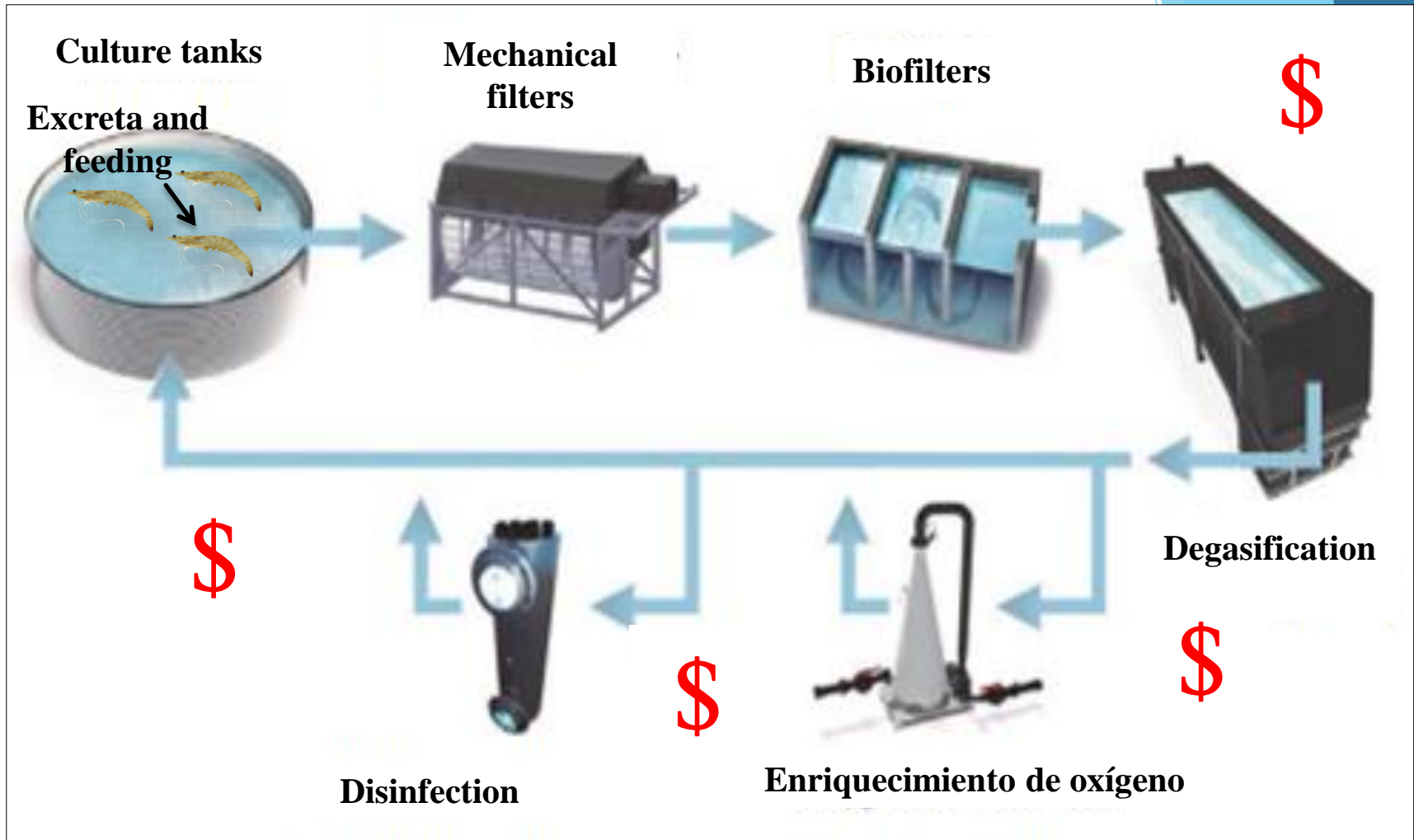


Environmental pollution

Research and development

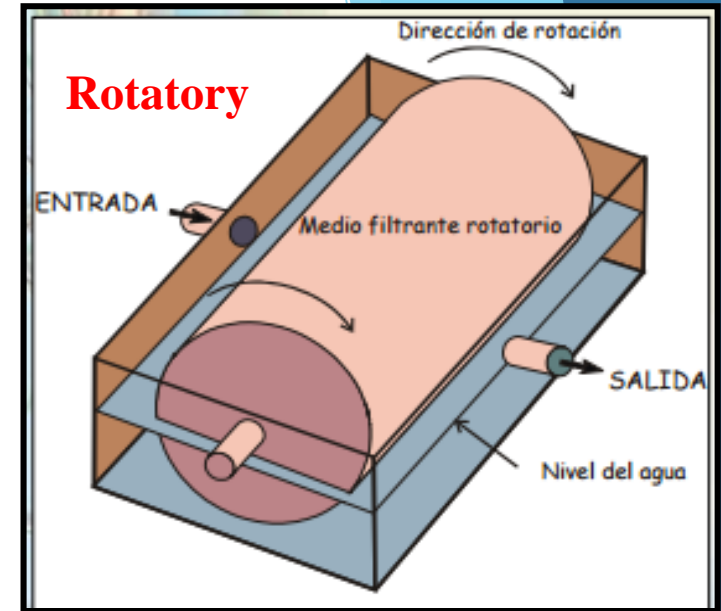
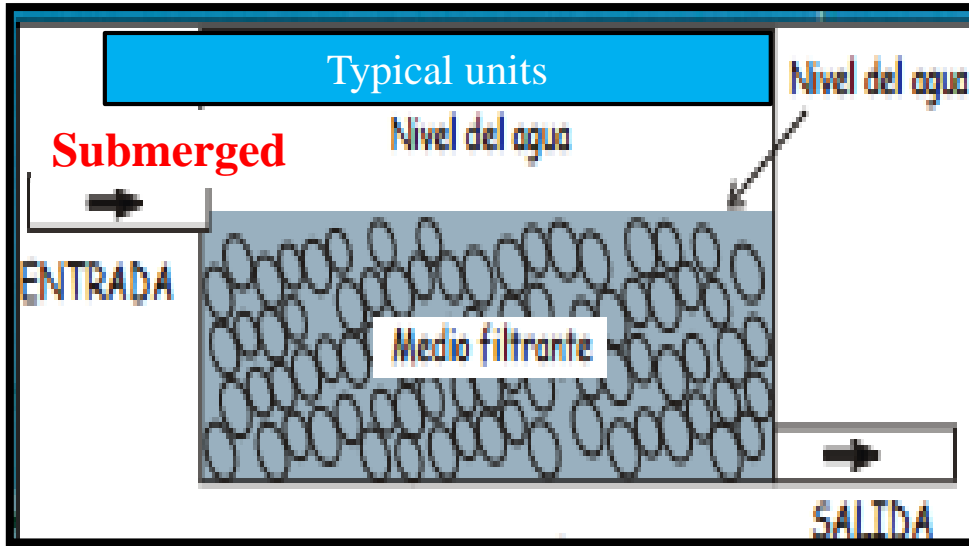
Water management

Recirculating aquaculture system (RAS)

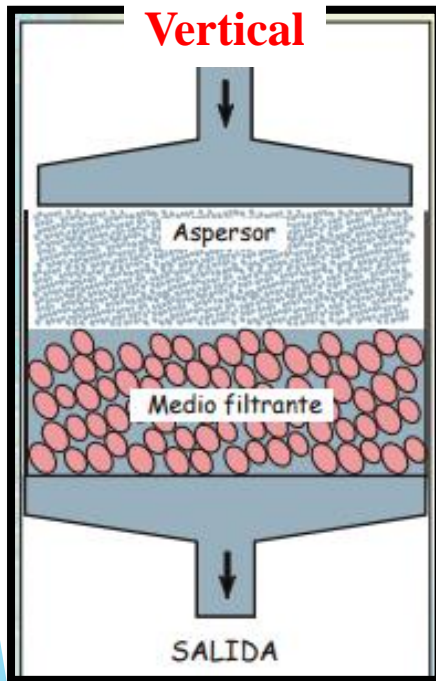


The wastewater treatment consist of mechanical filtration, biological treatment and disinfection.

Biofilters



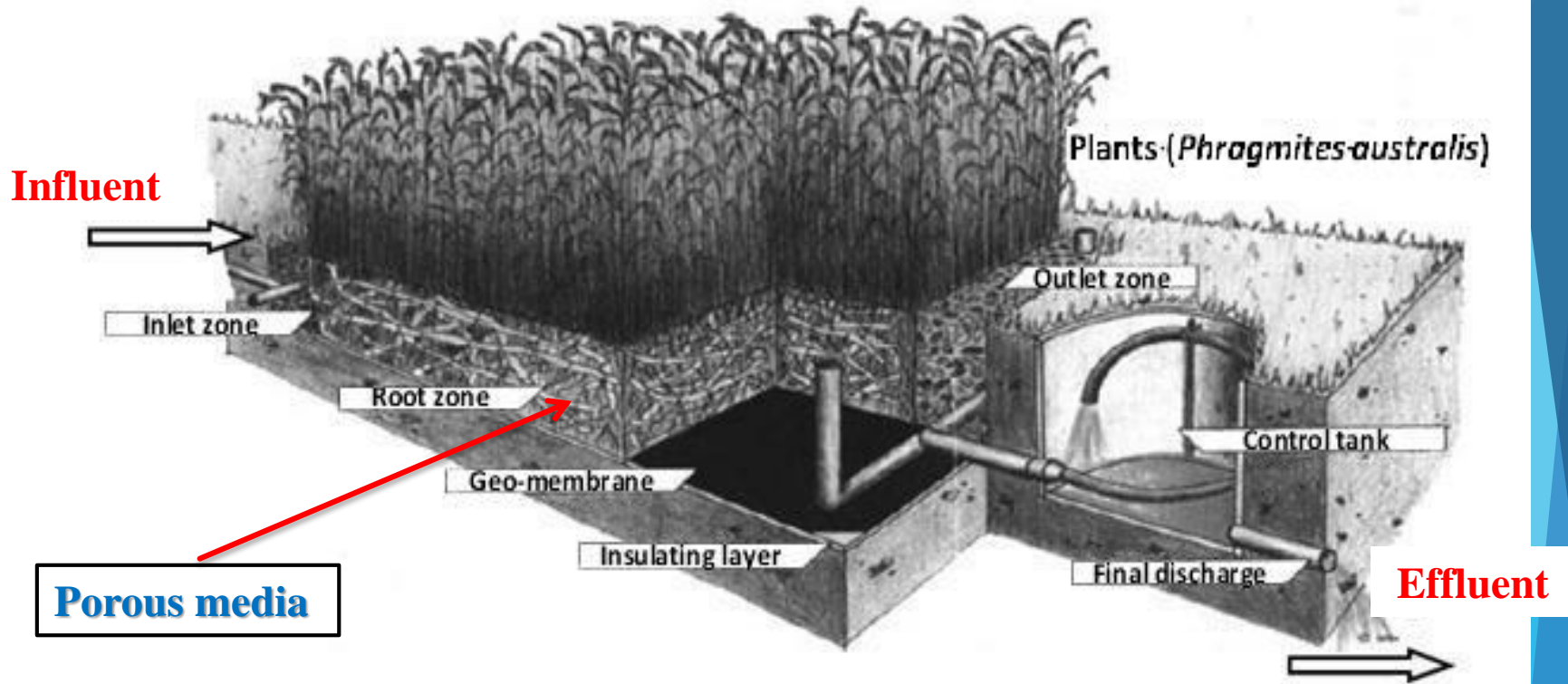
(Tilley *et al.*, 2002)



Timmons y Ebelinf, 2007.



Constructed wetlands (CW)



Mexico: CW for treatment of aquaculture effluents (Ramírez-Carrillo *et al.*, 2009)

Asia: CW in white shrimp RAS (Lin *et al.*, 2005; Zachritz *et al.*, 2008)

Constructed wetlands types (CW)

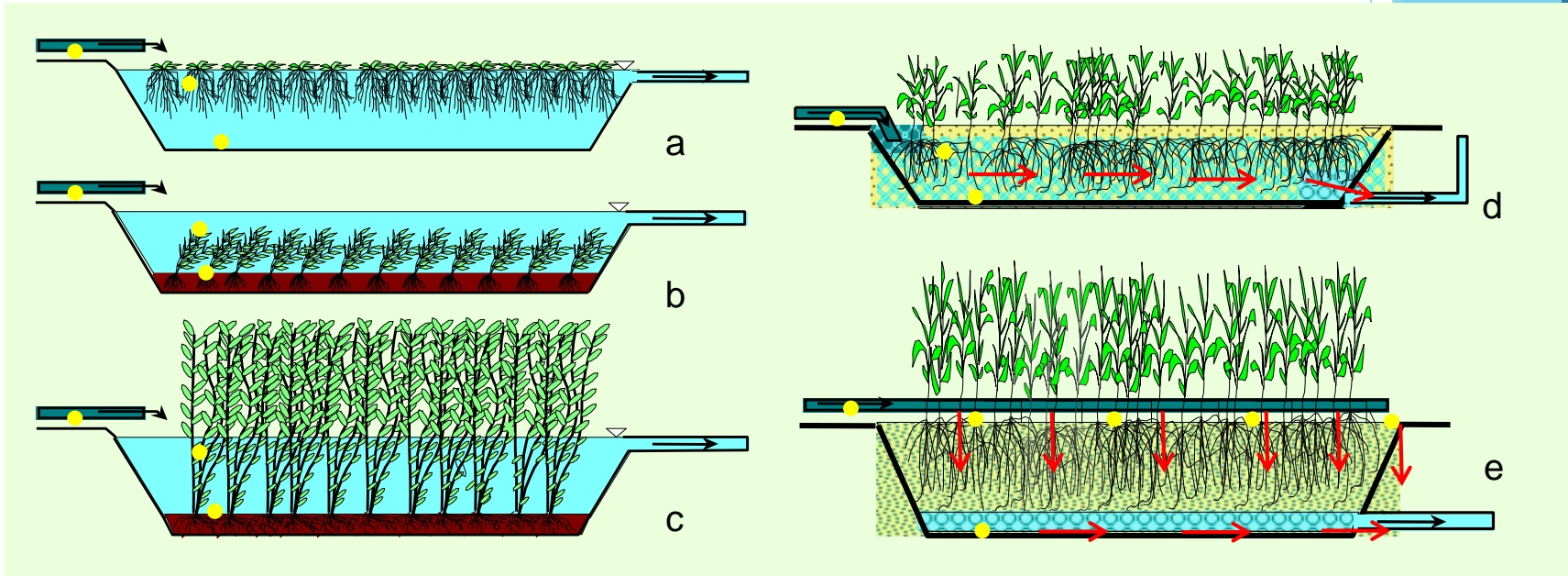
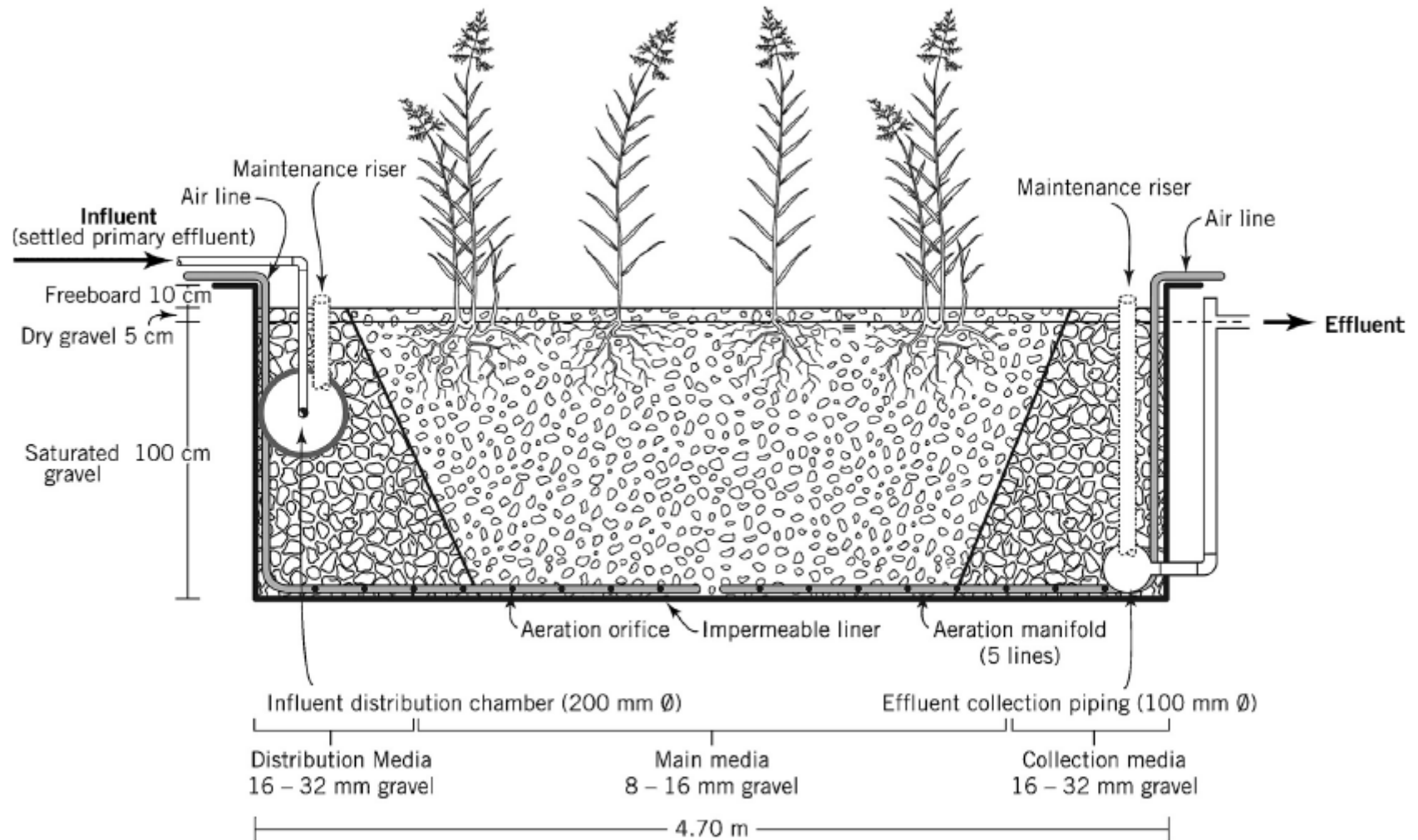


Figure Courtesy of Chen, (2012)

- a: Surface Flow, floating aquatic plants**
- b: Surface flow, submerged aquatic plants**
- c: Surface flow, emergent aquatic plants**
- d: CW, horizontal sus-surface flow**
- e: CW, vertical flow**

Intensified constructed wetlands

Total nitrogen removal was 22 % higher than not intensified unit



note: drawing not to scale

Biofilters in RAS

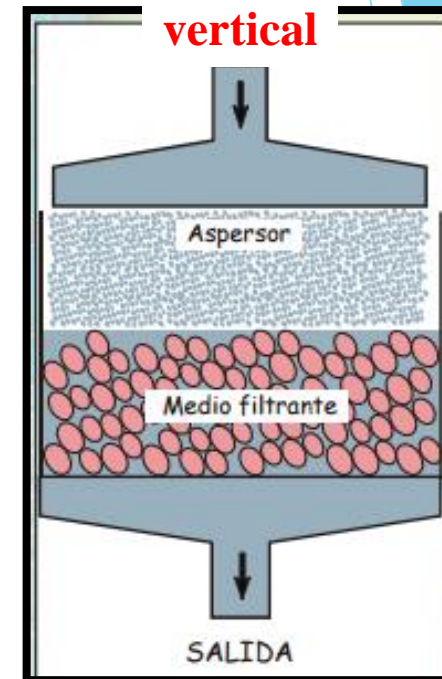
Constructed wetlands



Picture: Medina-Astorga, 2017.

Nutrient removal, solids, BOD
(plant uptake, biodegradation)
Required area and oxygen can be
limited

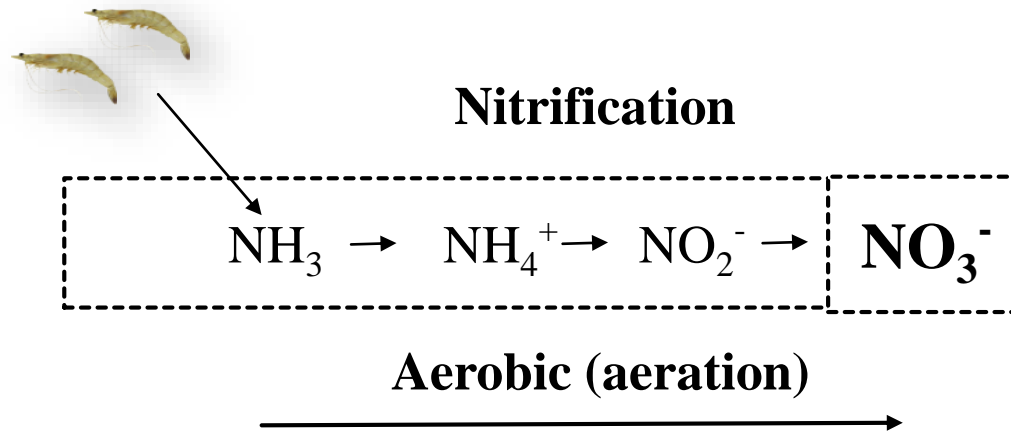
Other biofilters



Nitrification is achieved,
However nitrate accumulation

Nitrate accumulation process

The bacteria *Nitrosomonas* and *Nitrobacter* enable nitrification under aerobic conditions however increasing nitrate concentrations in shrimp cultures



- Concentration of $200 \text{ mg NO}_3^- \text{ L}^{-1}$ \rightarrow susceptibility to diseases and survival (Lee y Wickins, 1997, Frías-Espiricueta *et al.*, 1999; Vinatea y Carvalho, 2007).
- The recommended value of NO_3^- by Van-Wyk y Scarpa, (1999) for shrimp culture is 60 mg L^{-1} .

Nitrate removal process

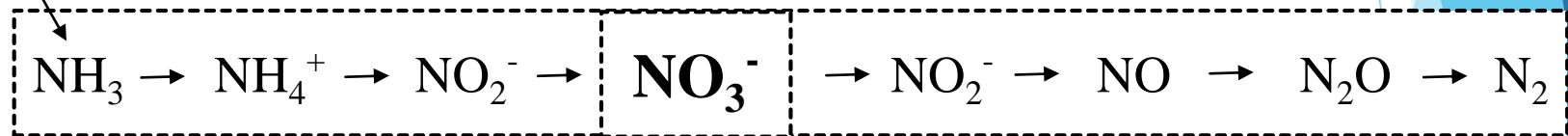
In denitrification the organic matter can be used by heterotrophic bacteria as carbon source to reduce nitrate to N_2 under anoxic conditions.



Constructed wetlands ?

Nitrification

Denitrification



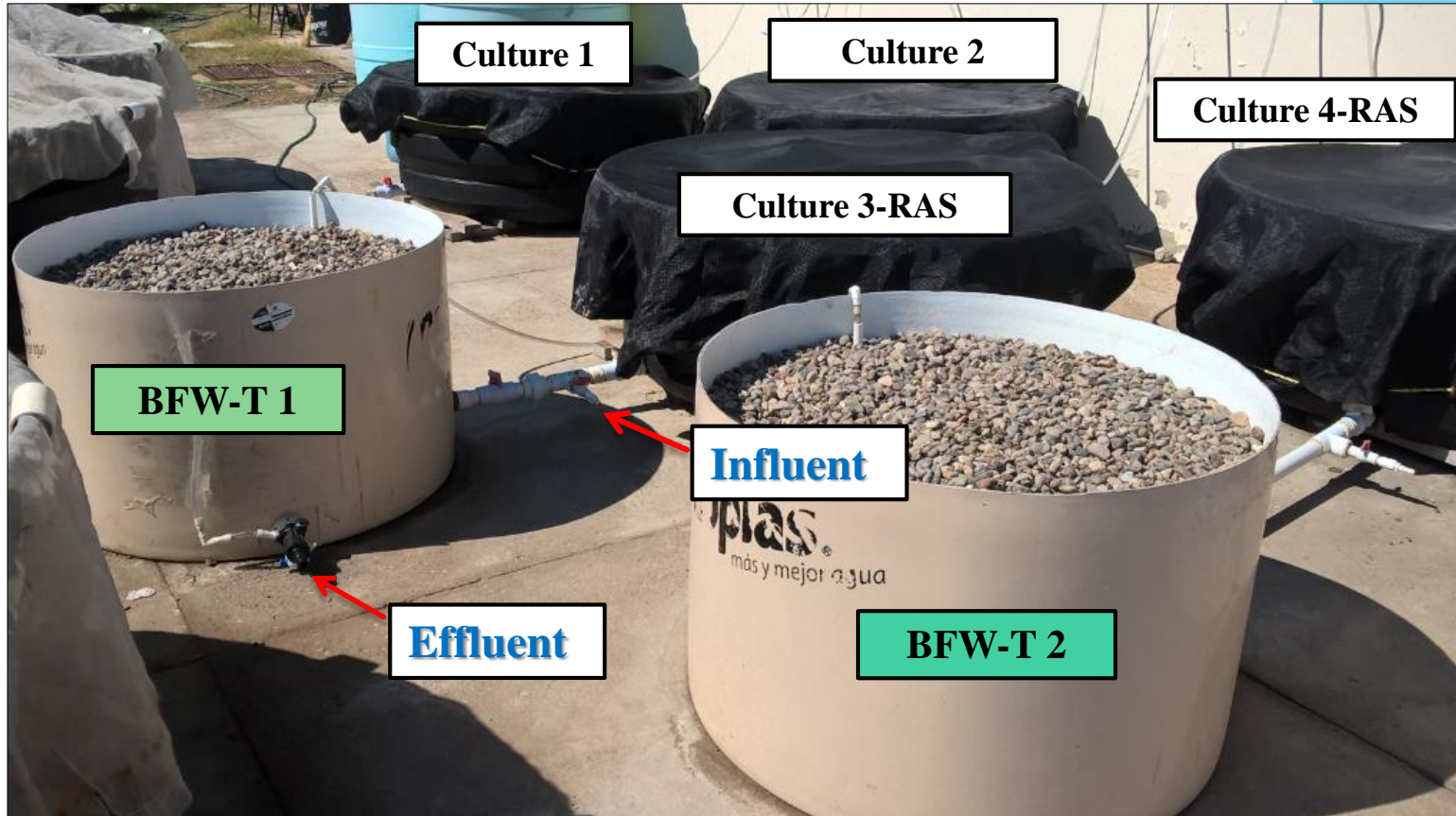
Aerobic (aeration)

Anoxic (absence de O_2)



Development of RAS in Mazatlan Mexico

Experiences at the Autonomous University of Sinaloa

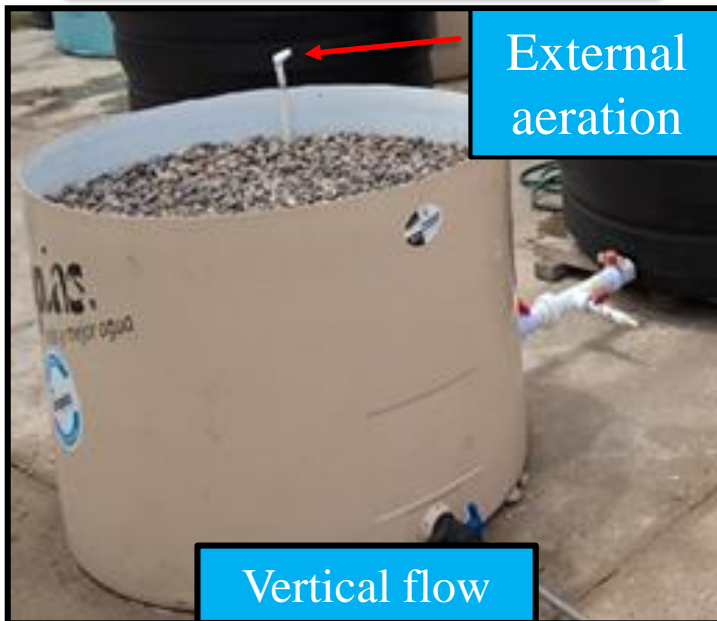


Biological filter wetland-type

The prototype works as an external wastewater treatment module incorporated in a RAS. It has an external aeration (on, off, intermittent).

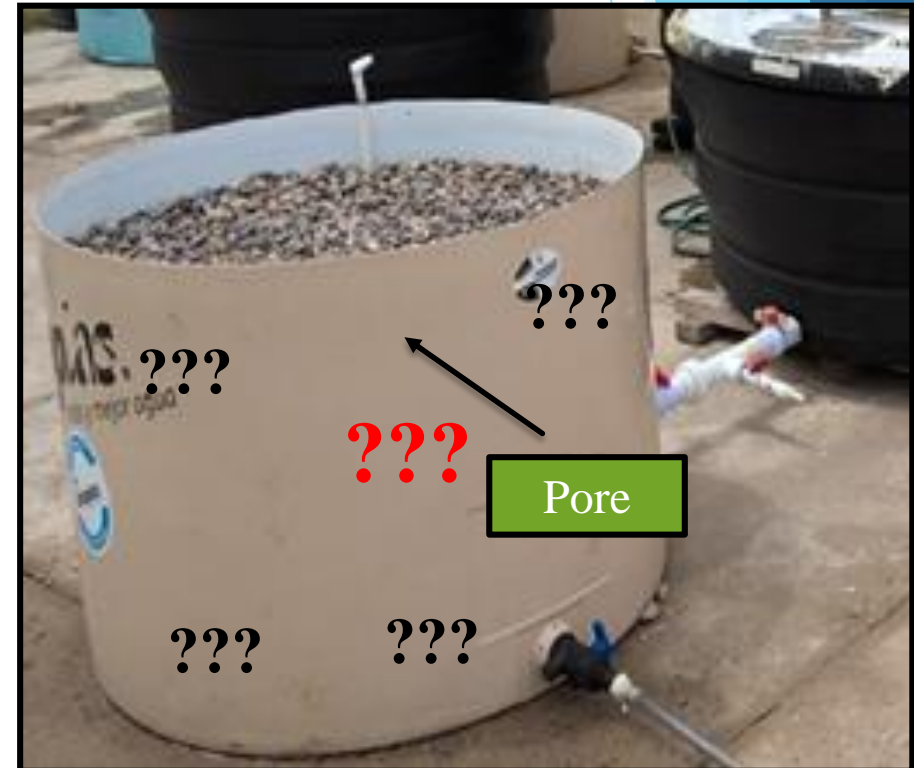
Implemented to enhance recirculating rate

Experimental prototype

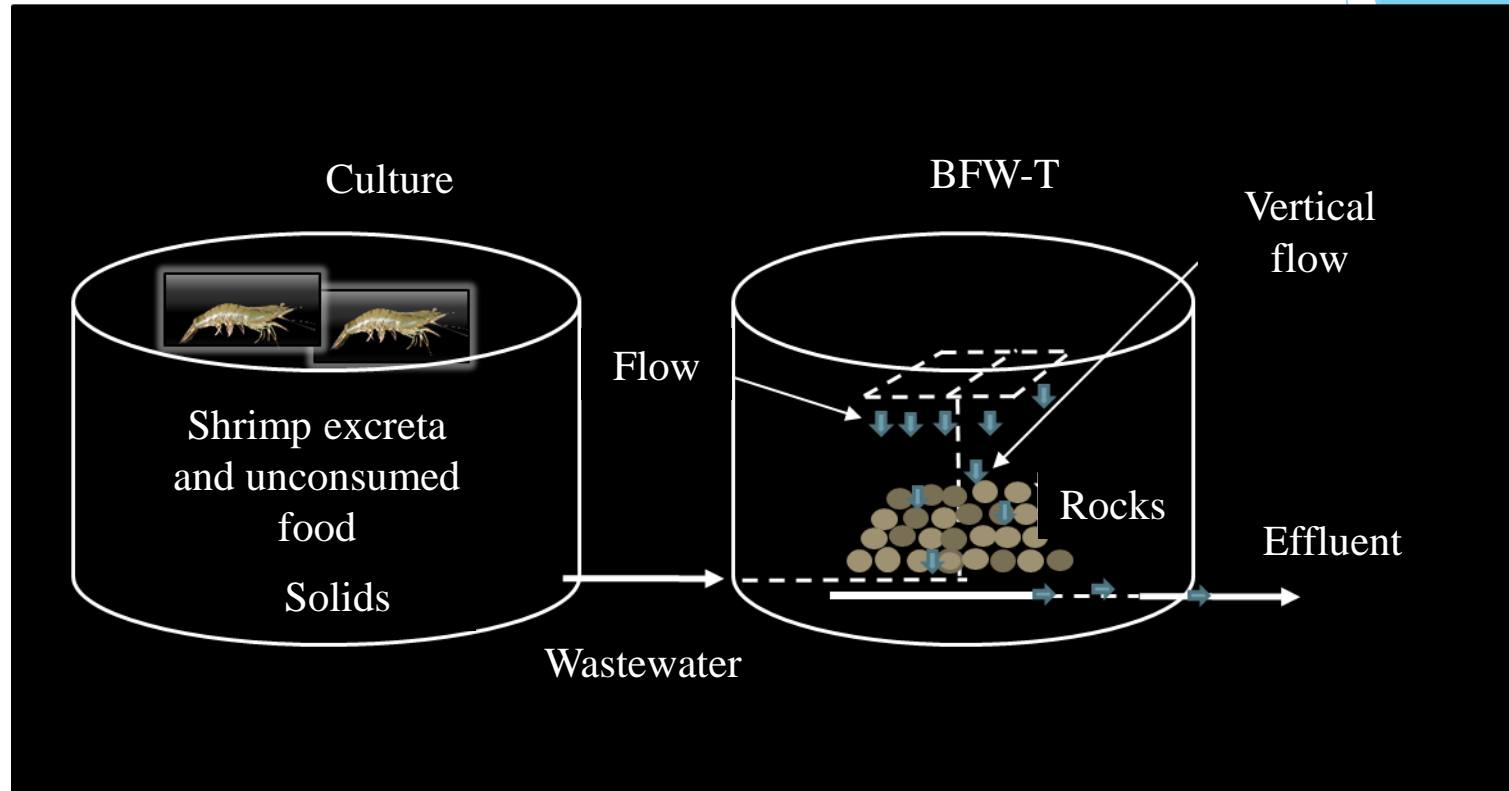


External aeration

Vertical flow



Description of the RAS coupled with BFW-T



La remoción de sólidos

Quiñones-López, (2018)

po por una

filtración física.

1. ¿Is the performance of the RAS culture (+ BFW-T) of *L. vannamei* comparable to a similar intensive culture using seawater exchange?.
2. ¿Does the BFW-T remove settleable solids?.
3. ¿Can selected nutrients be removed in the BFW-T when works i) with external aeration and ii) without external aeration?.

General

Investigating selected nutrient removal in RAS cultures of *L. vannamei* post larvae (nursery) using a Biological Filter Wetland Type.

Particular

1. Determine the performance of the RAS culture and compare it with an intensive culture.
2. Determine the settleable solids removal using the BFW-T.
3. Evaluate selected nutrient removal (NH_3 , NO_2^- , NO_3^-) (influent vs effluent of the BFW-T) in the RAS culture operating with external aeration.
4. Evaluate the response of nitrate (NO_3^-) in the pore when the BFW-T works without external aeration.

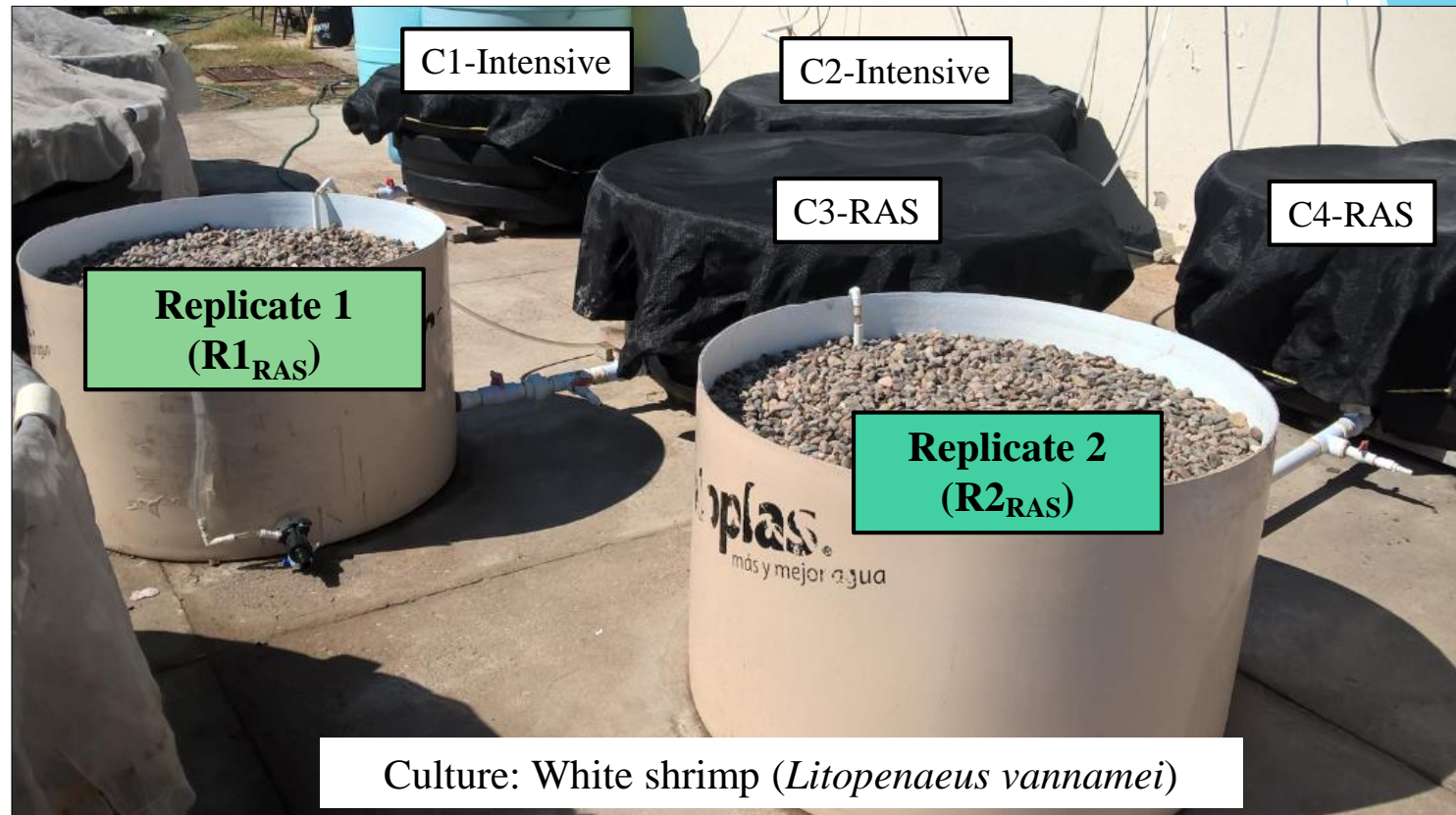


MATERIALS AND METHODS



Selected culture conditions

Density: 1000 organisms/m³. Initial W (mean): 0.008 g (PL₁₆). Food: 2 times/day. Culture time: 86 days. Two culture technologies (Intensive vs RAS). Two replicates each one (Culture: 1-intensive, 2-intensive, 3-RAS y 4-RAS). The BFW-T were operated in Batch after WQ parameters were detected.



Recirculating rate and seawater exchange

Intensive cultures: 2 seawater exchange/week (seawater + evaporation loss).

Water exchange: = 150 L (300 L/week).

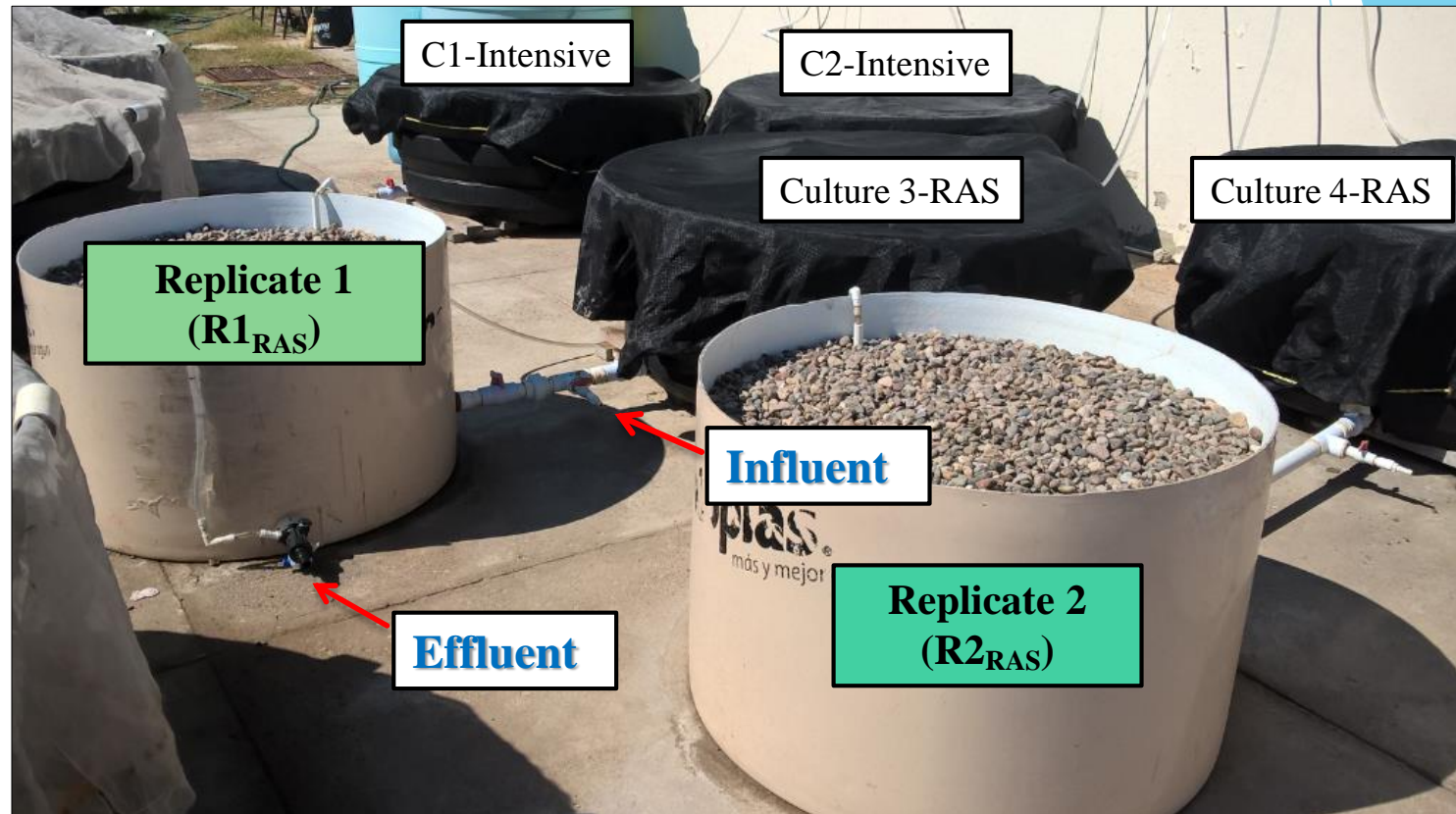
RAS-Cultures: 2 recirculating events/week (BFW-T + evaporation loss).

Recirculating rate = 150 L (300 L/week).

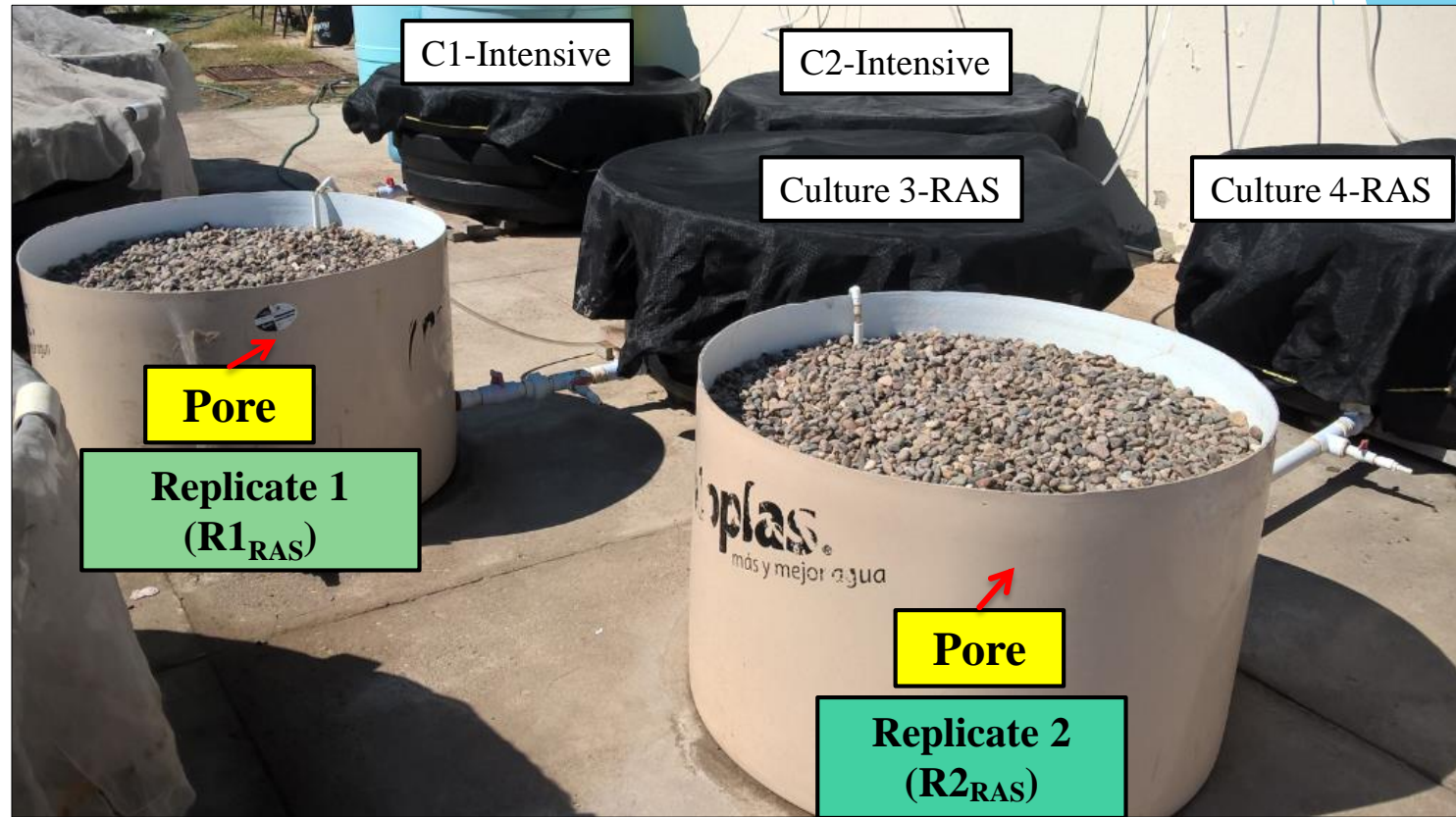


Sampling

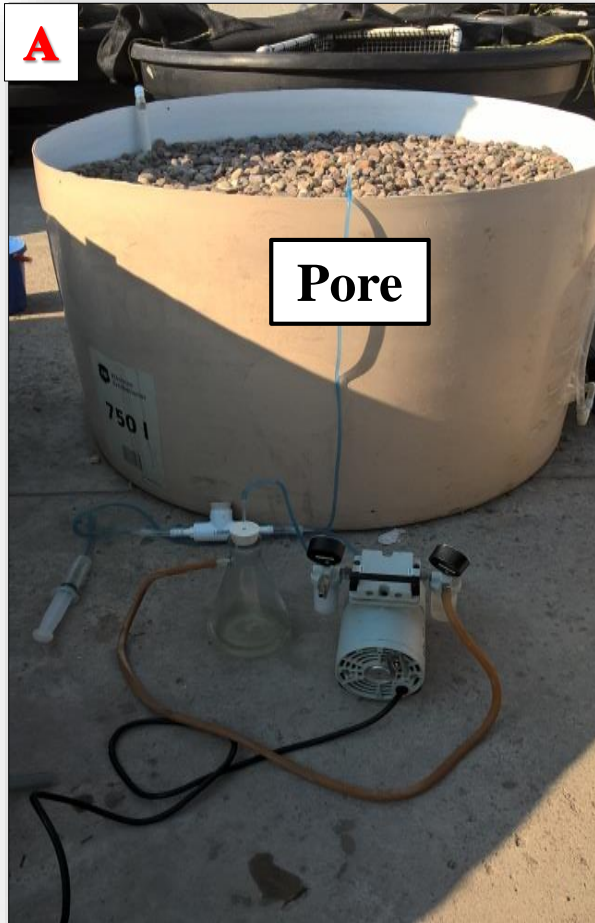
- 21 sampling events **influent, effluent** from 27.09.2016 to 13.12.2016



- 18 sampling events **in the pore** from 07.11.2016 to 21.12.2016



Pore water sampling

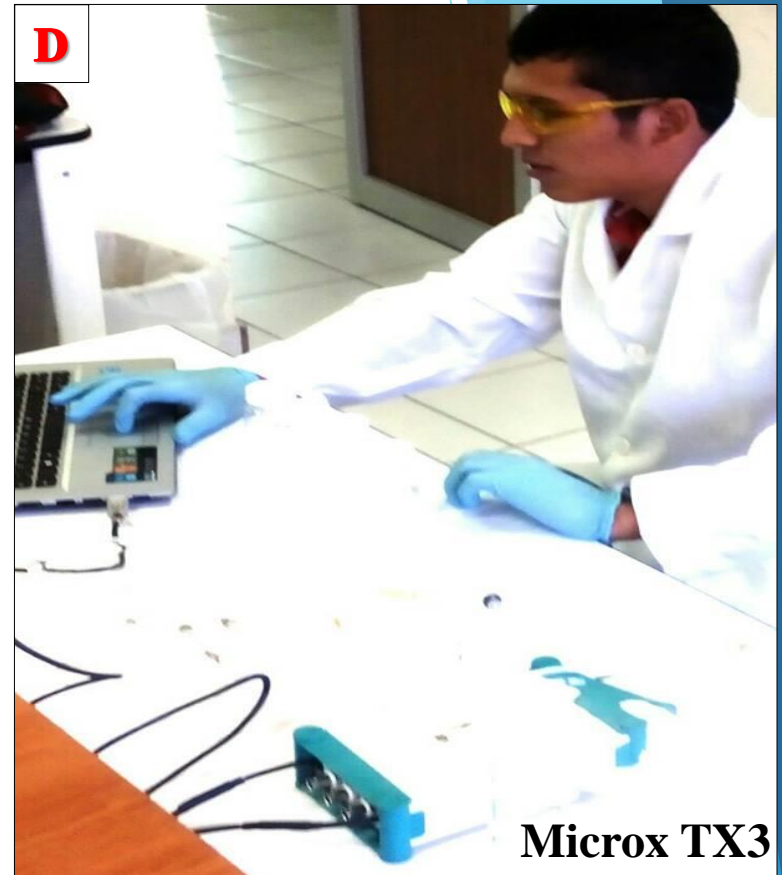
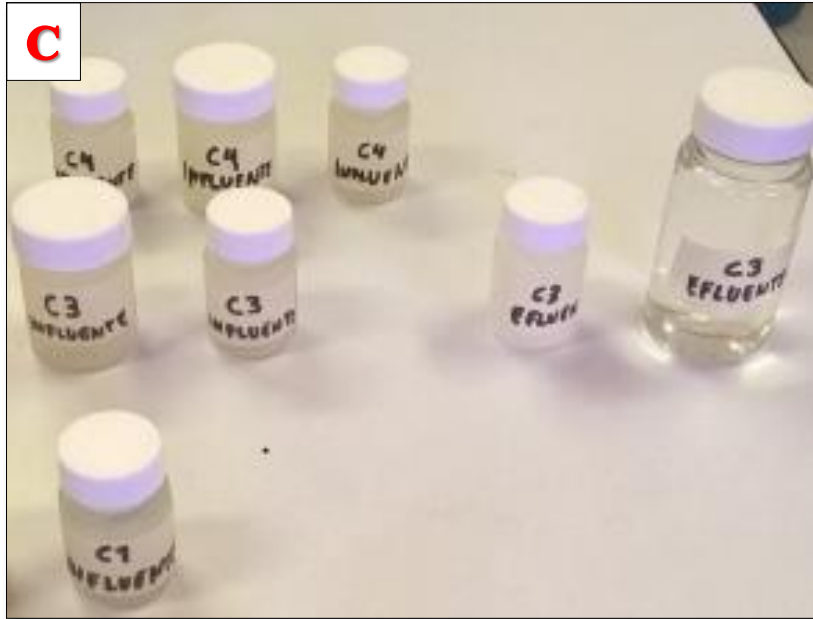


Dissolved oxygen and nitrate (NO_3^-) were measured

Without external aeration. 2 campaigns: Campaign 1 → 14/12/16 to 17/12/16 y

Campaign 2 → 19/12/16 to 21/12/16.

Plastic lance (25 cm), Vacuum pump, Erlenmeyer flask, Syringe



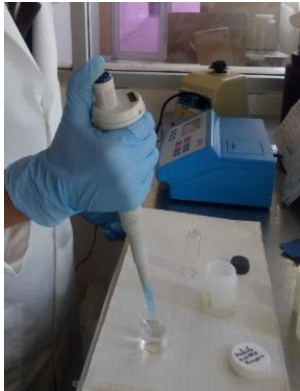
DO analysis: PreSens® (Limit of detection 1 µg/L)

Nutrient analysis: ammonia nitrogen, nitrite and nitrate

Nutrients (NH_3 , NO_2^- , NO_3^-) were analyzed using a photometer Hanna HI 82203.

Laboratory at FACIMAR, UAS

1



Water sample, dilution if needed

2



Sample without reactant (reference)

3



Reactant

4



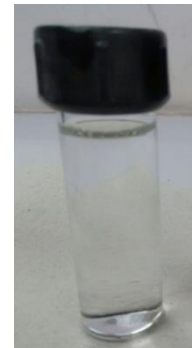
Sample preparation

6

Analysis using photometer Hanna HI 82203



5



Measurements of settleable solids



AWWA, (1992)

33

$$\text{Removal}(\%) = \frac{\text{Influent concentration} - \text{Effluent concentration}}{\text{Influent concentration}} \times 100$$

Concentration (mg/L) → nutrients
Volume (ml/L) → settleable solids

Culture performance: Intensive vs RAS

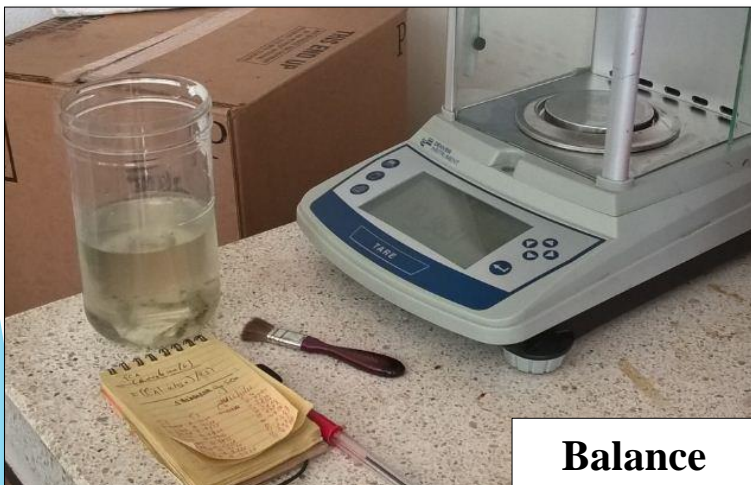
Biomass (g) was registered once per week. The water consumption along the whole culture was registered.



Biometry/week



Harvest: Final (g) and survival (%)



Mean comparison → Kruskal Wallis test

1. Dissolved oxygen concentrations between the four cultures.
2. Dissolved oxygen concentrations in the pore of BFW-T (aeration vs without aeration).
3. Volume of settleable solids influent and effluent of the BFW-T.

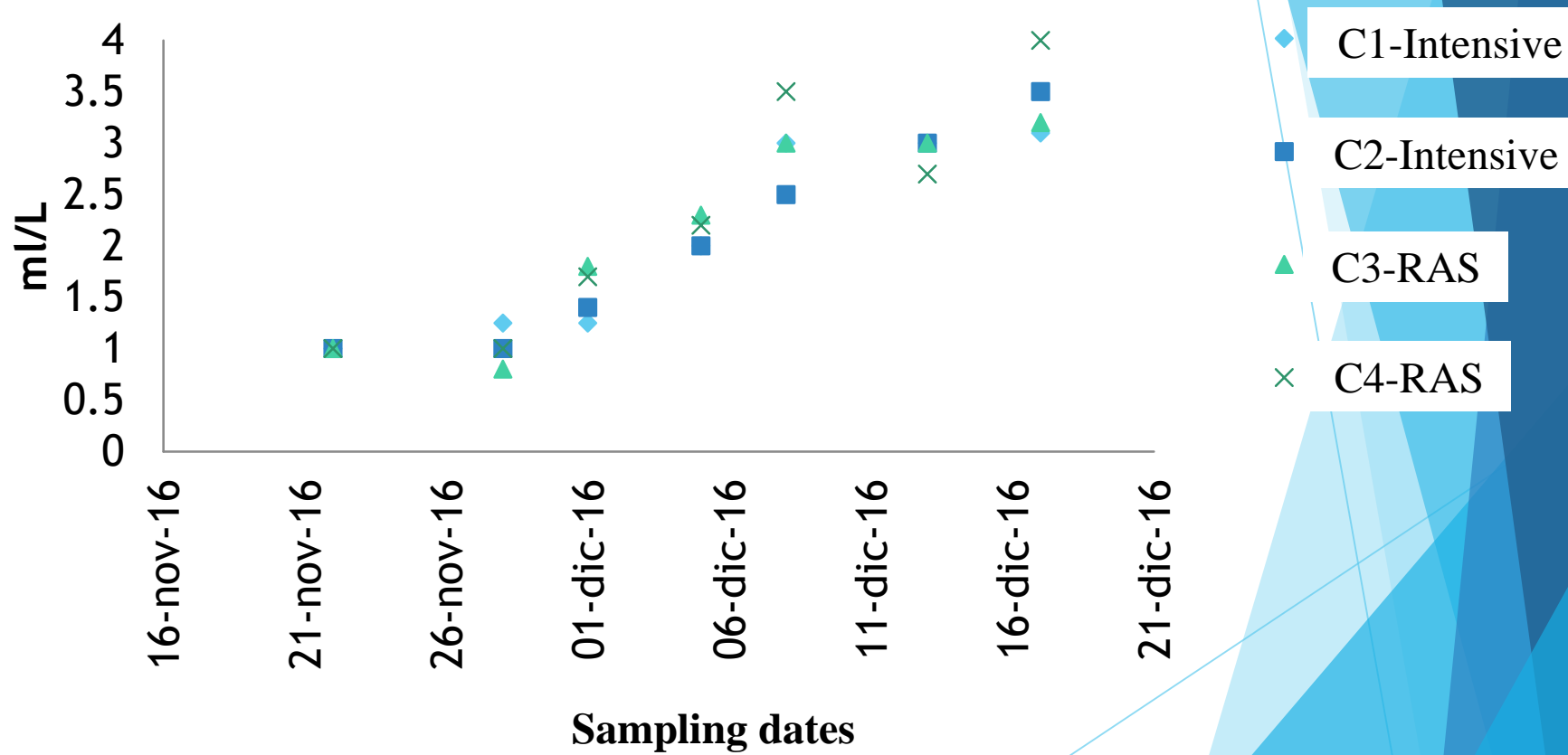
Significant differences were considered when $p < 0.05$.



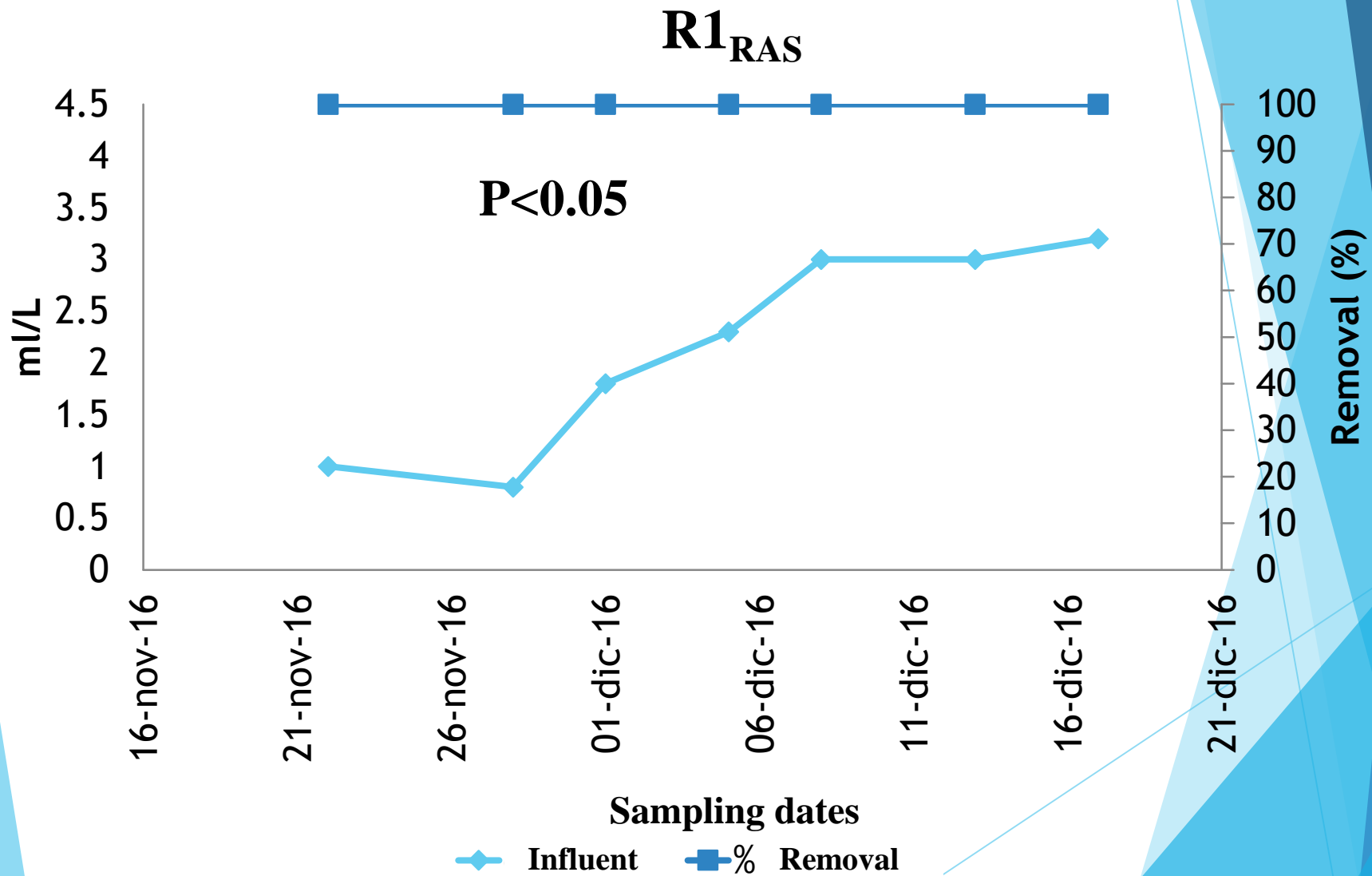
RESULTS



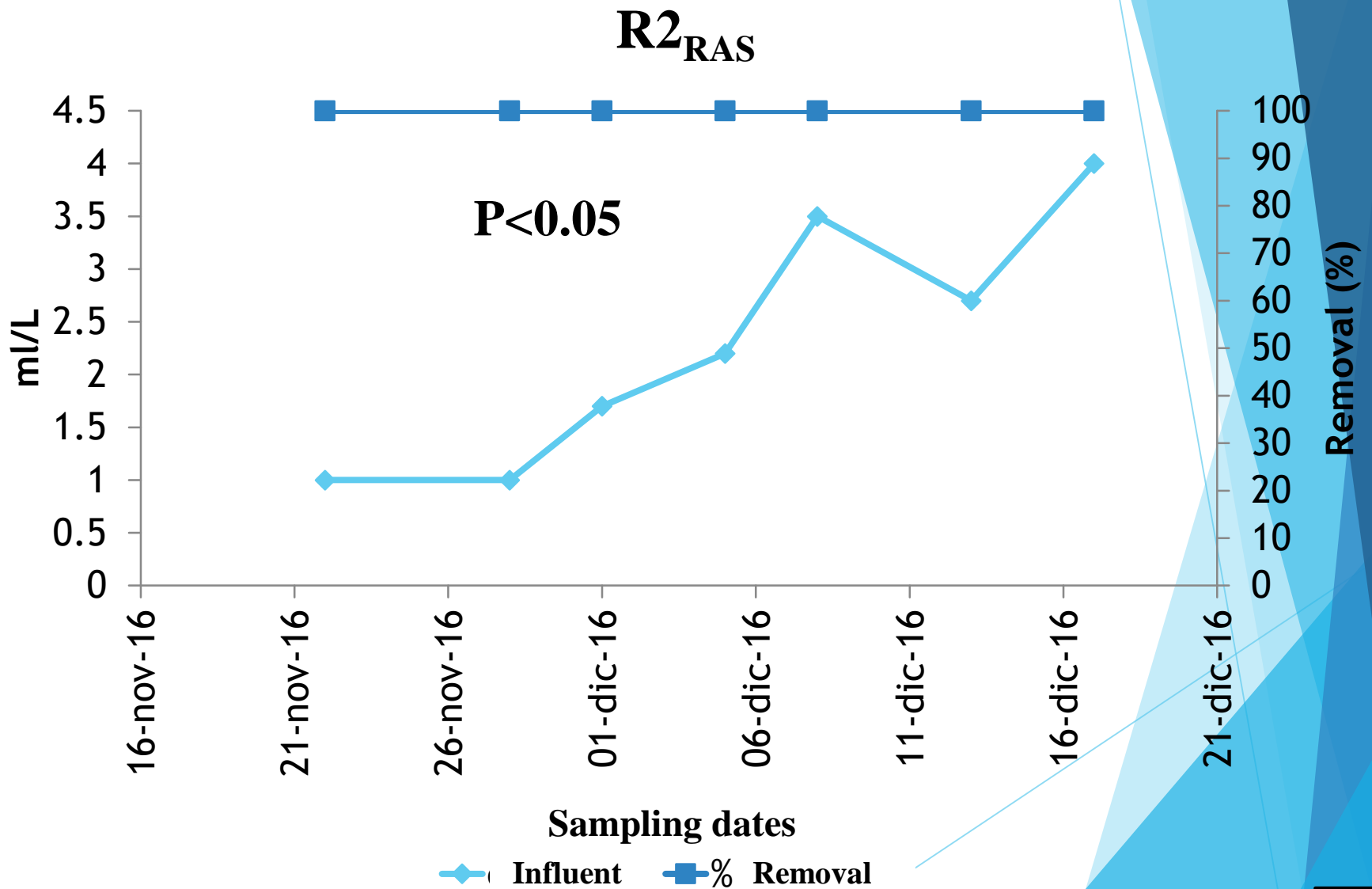
Settleable solids in the four cultures



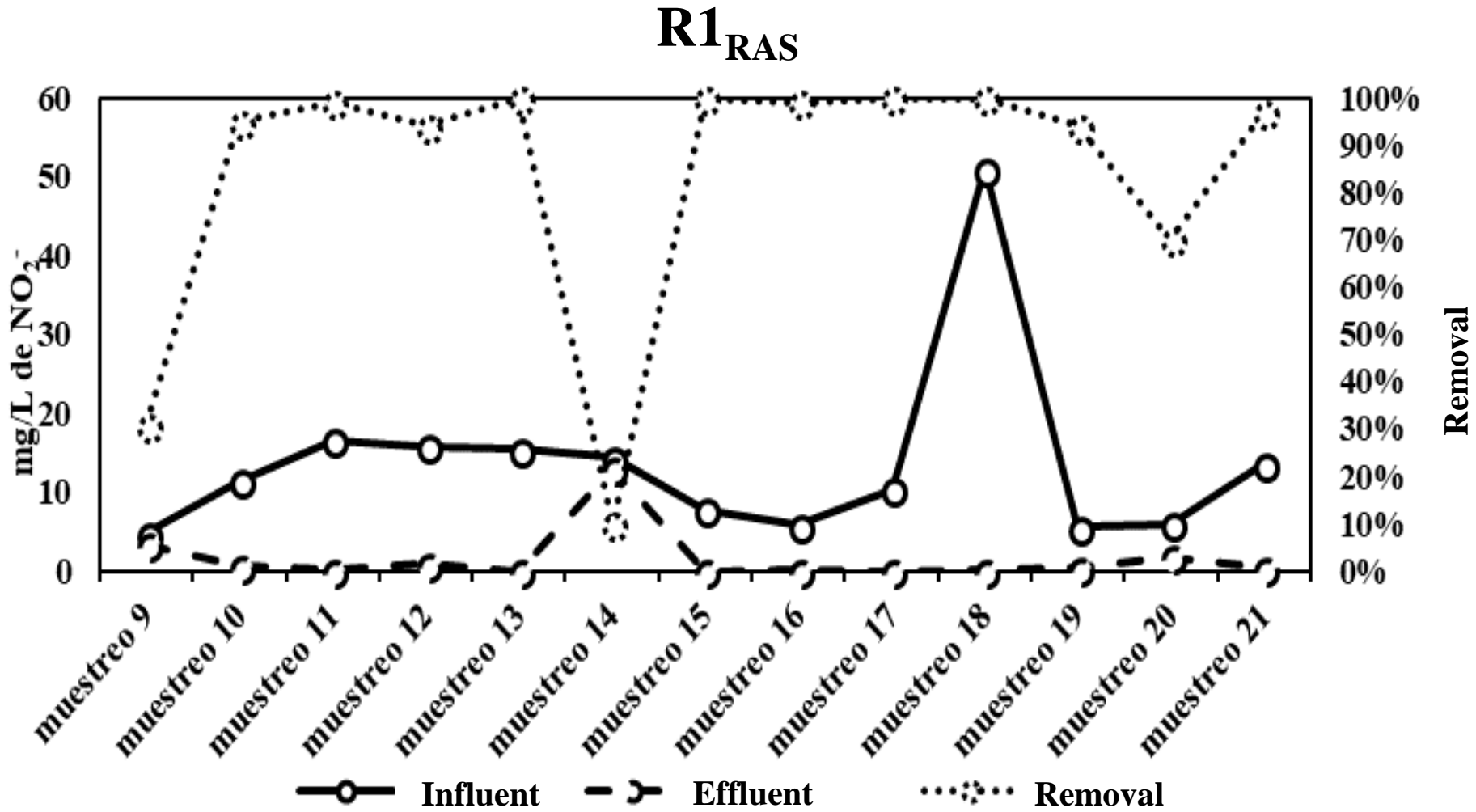
Settleable solids removal



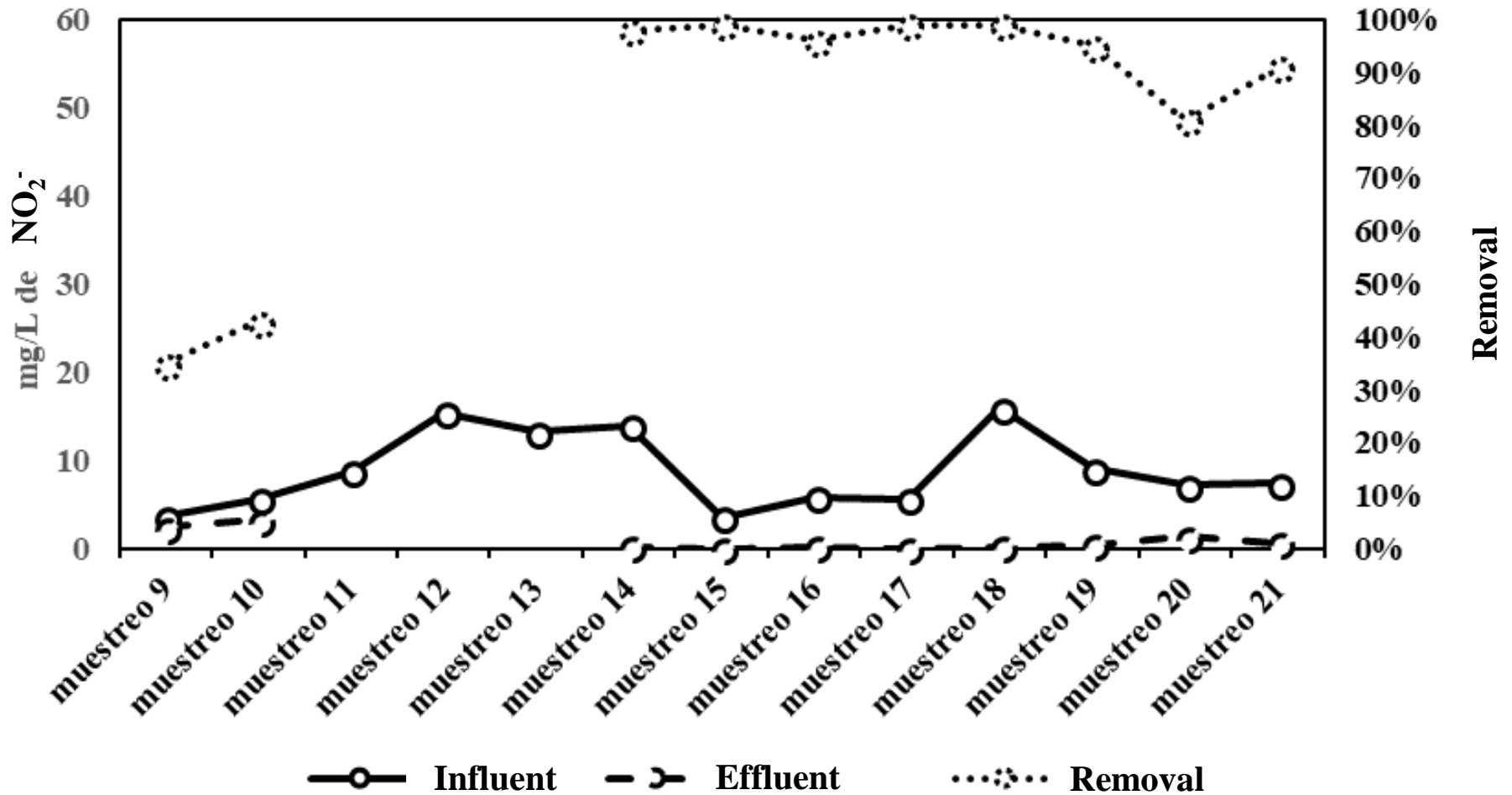
Settleable solids removal



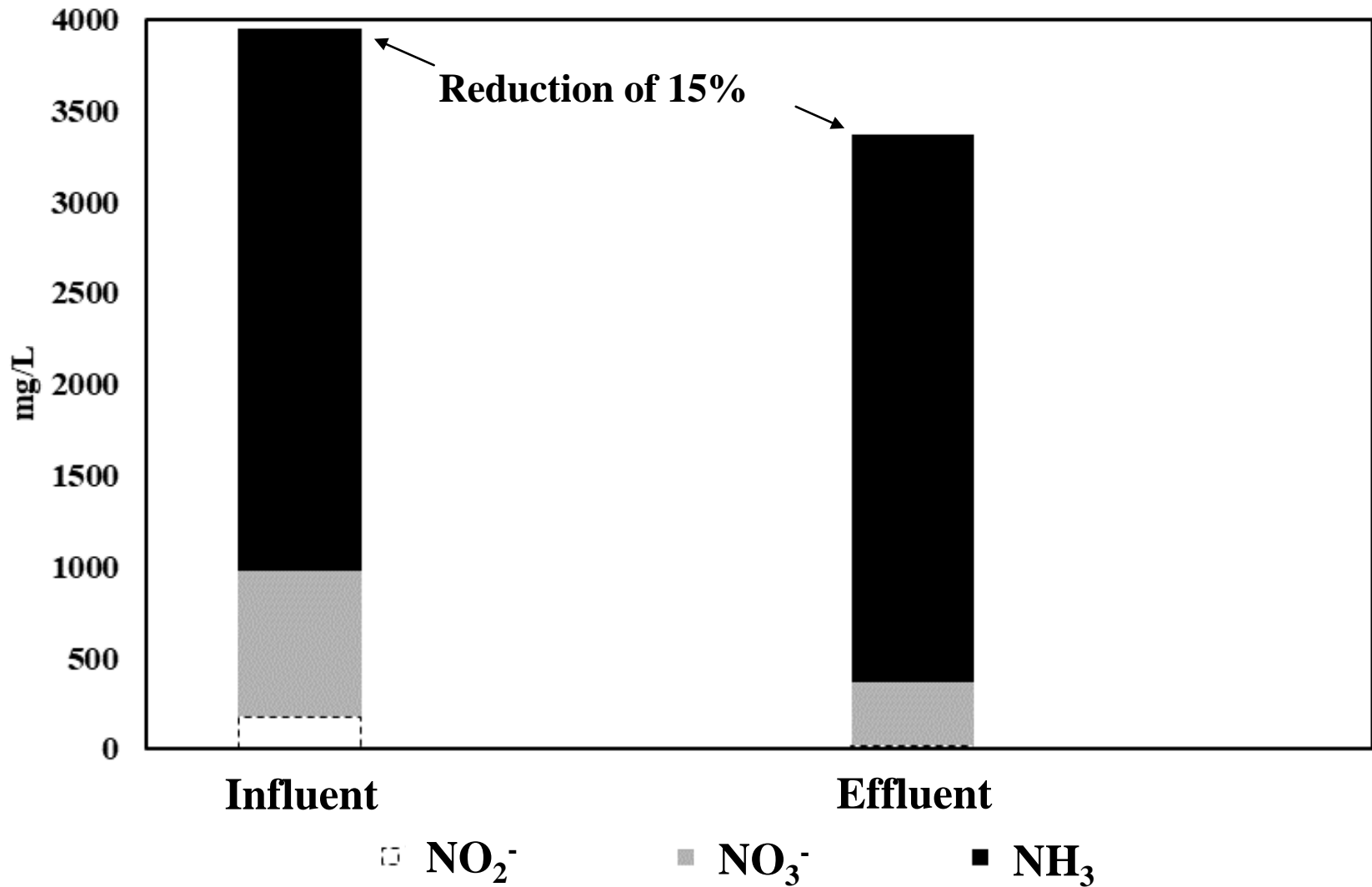
Influent and effluent nitrite (NO_2^-) concentration and removal in the BFW-T: with external aeration



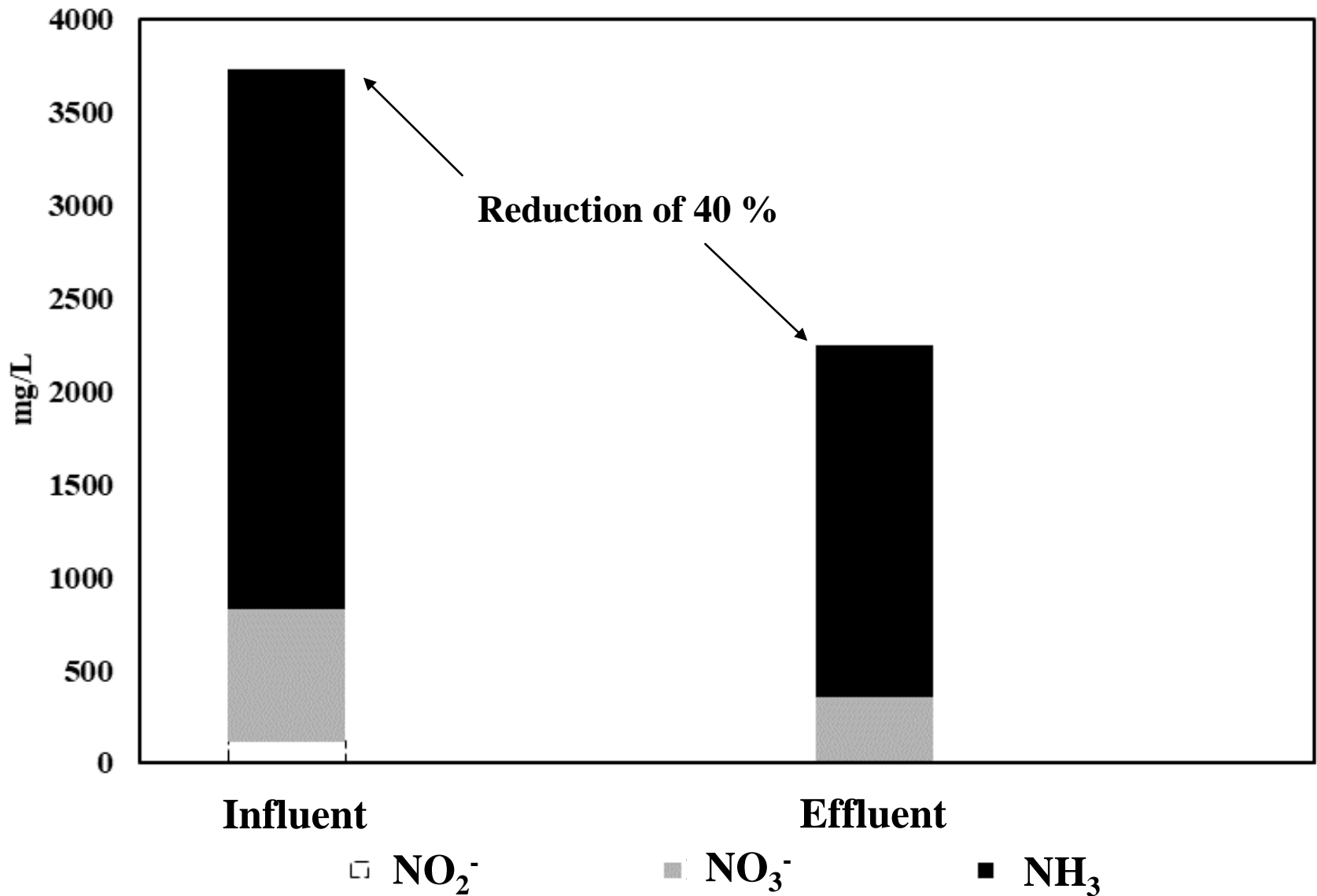
R^2_{RAS}



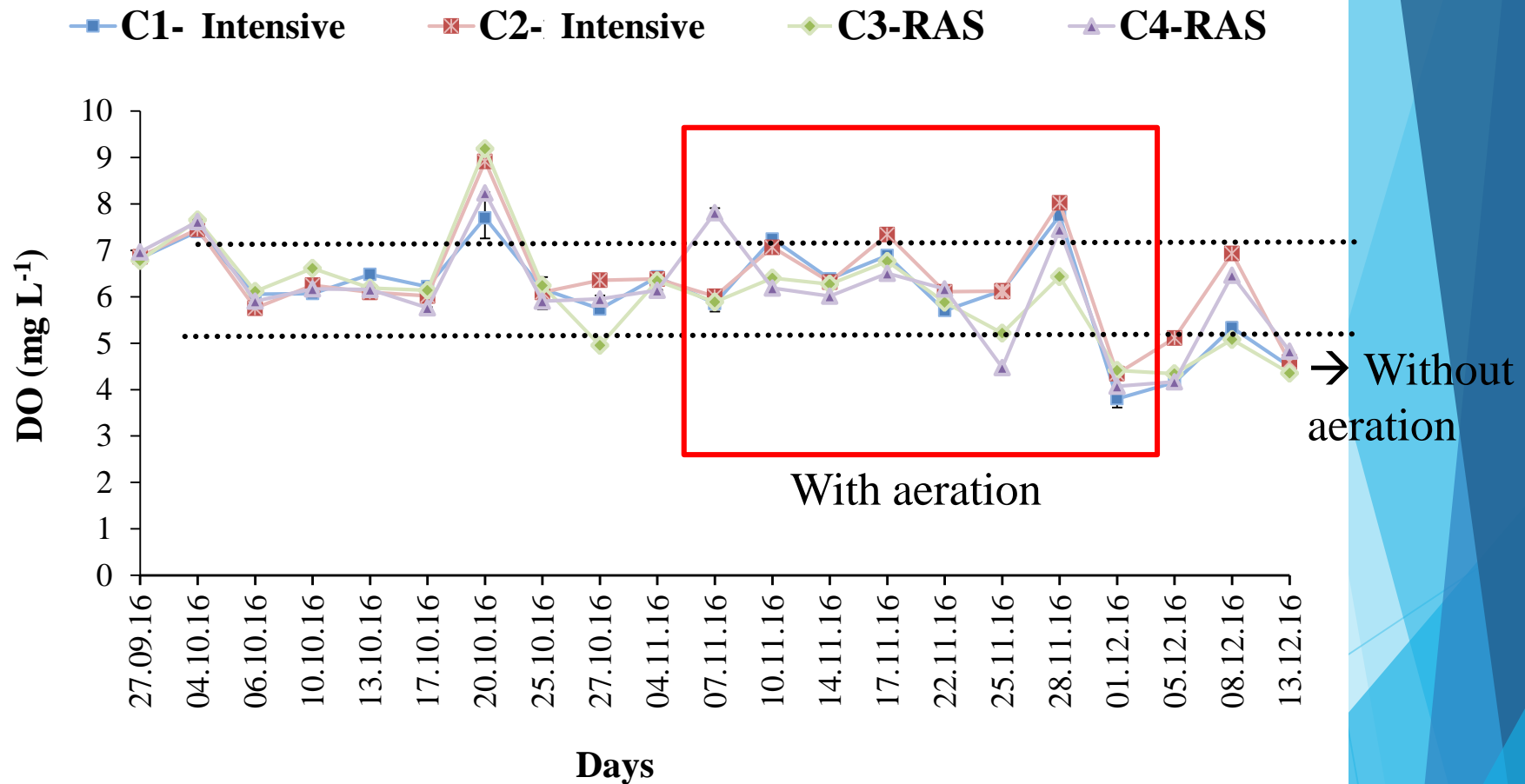
$R1_{RAS}$



$R2_{RAS}$



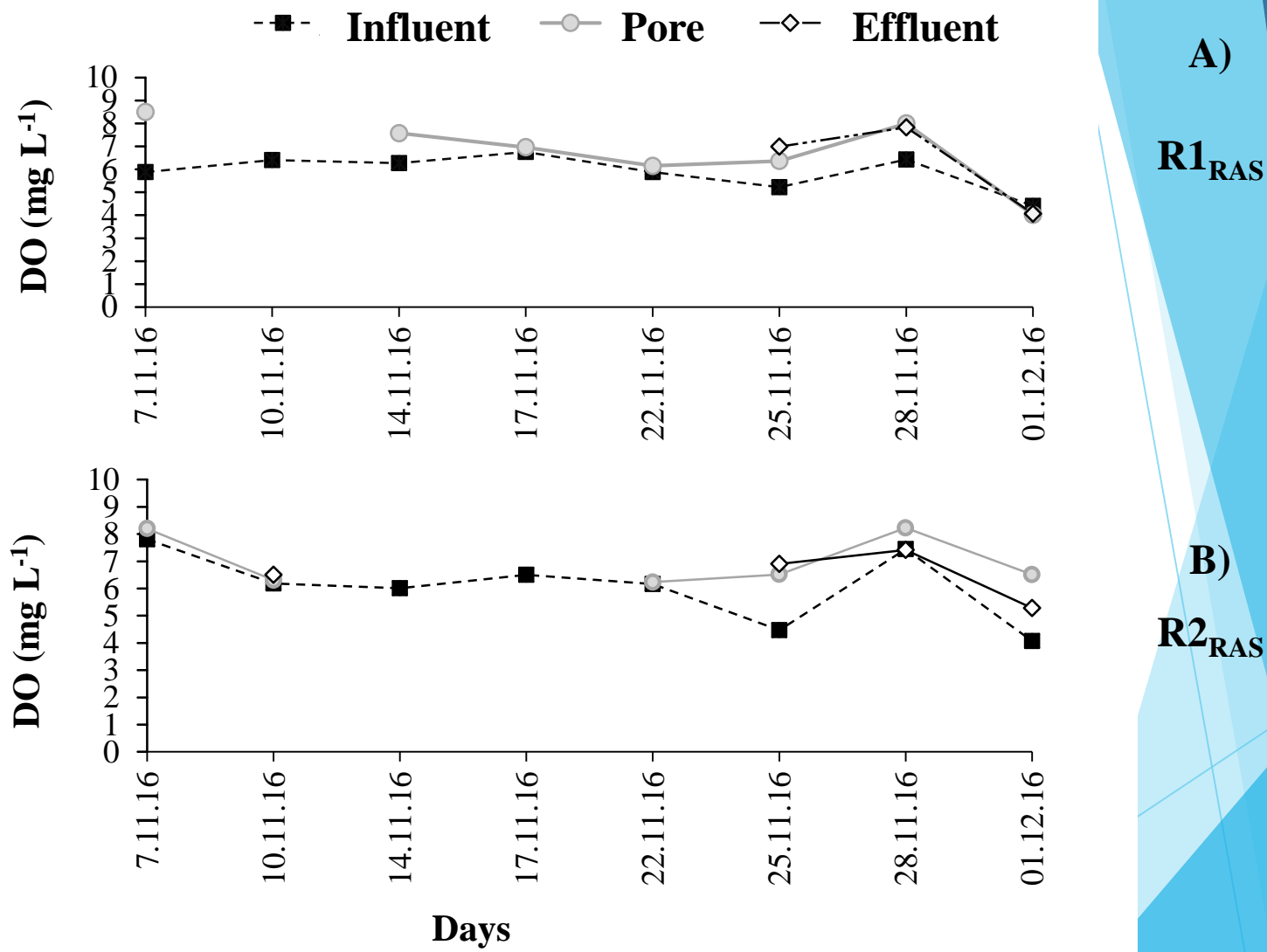
DO concentrations in the four cultures of white shrimp: intensive vs RAS



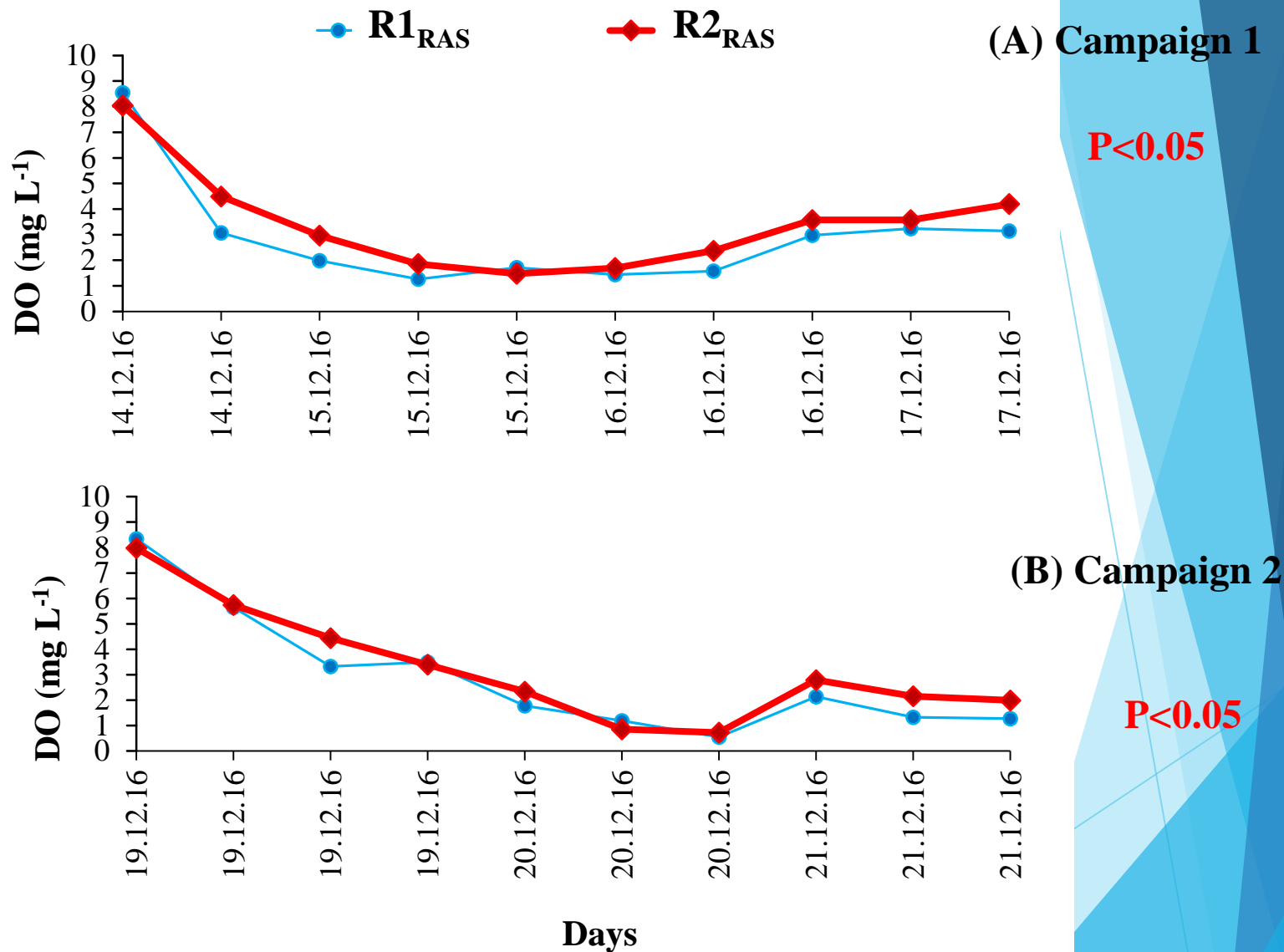
DO concentrations (mean, standard deviation). The dotted lines indicate the optimum DO interval of 5 a 7 mg L⁻¹ for white shrimp culture (Martínez-Córdova, 1999).

- DO: **1 mg/L** → mortality (Díaz y Rosenberg, 1995).

DO in the BFW-T ($R1_{RAS}$ y $R2_{RAS}$): with external aeration

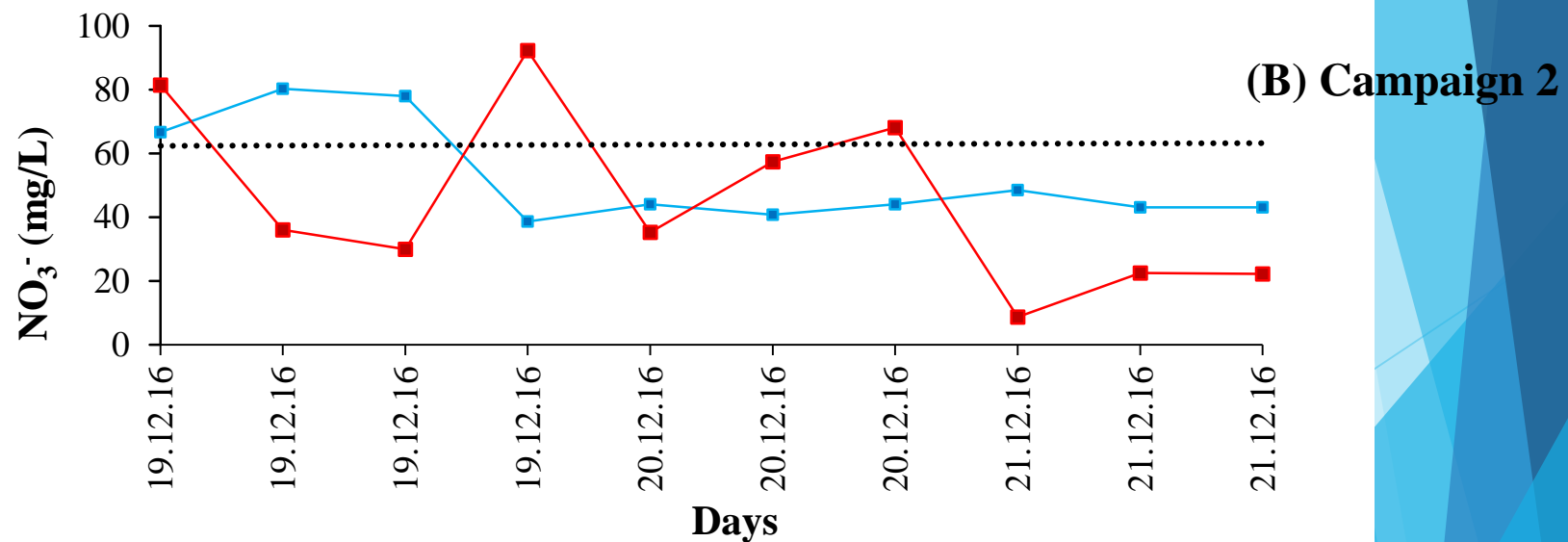
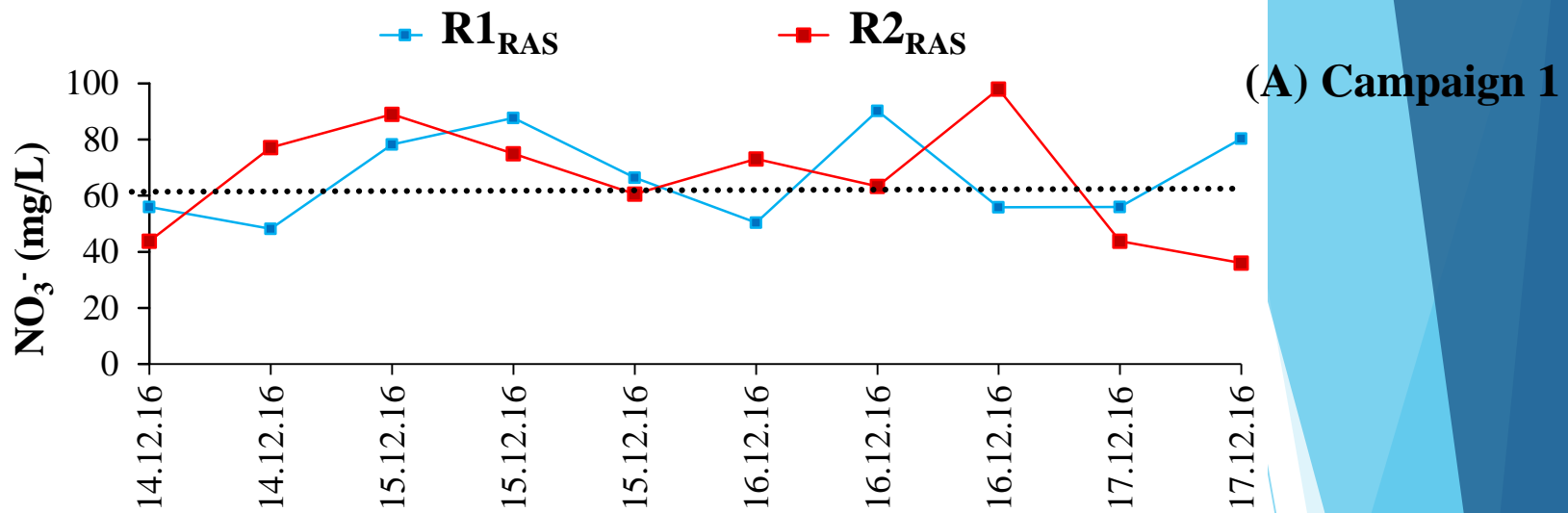


DO concentrations (mean) in the $R1_{RAS}$ (A) and $R2_{RAS}$ (B): with external aeration



DO concentrations (mean) in $R1_{RAS}$ y $R2_{RAS}$ during campaigns 1 (A) y 2 (B).

Nitrate in the pore of both BFW-T ($R1_{RAS}$ y $R2_{RAS}$): without external aeration



NO_3^- concentrations in $R1_{RAS}$ and $R2_{RAS}$ in both sampling campaigns: 1 (A) y 2 (B). The black dots indicate the maximum value of NO_3^- for the culture of *L. vannamei*: 60 mg L^{-1} (Van-Wyk & Scarpa,

Culture	Inicial W (g)	Final W (g)	Survival (%)	Biomass (g)	Final density (org/m³)
Intensive	0.008	1.881	68.45	1,288	685
RAS	0.008	1.434	82.30	1,180	823

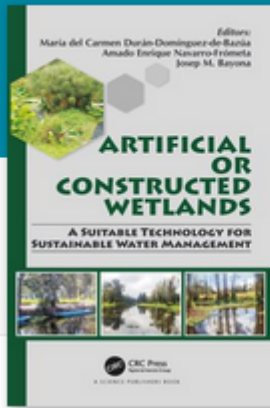
The relationship between survival-biomass has been documented in literature (Wasielesky *et al.* , 2013; Esparza-Leal *et al.*, 2015; Ray y Lotz, 2017).

	C1- Intensive	C2- Intensive	C3- RAS	C4- RAS
Seawater (L)	4600	4600	1000 + 150	1000 + 150

Evaporation losses were compensated with sea water in all systems

Water saving of 78 % in RAS

- The BFW-T were efficient for the removal of settleable solids (Removal > 99%).
- The BFW-T were efficient for the removal of nutrients ($\text{NH}_3 + \text{NO}_2^- + \text{NO}_3^-$) between 15 y 40 %.
- The Dissolved Oxygen concentration in the pore of the BFW-T significantly reduced without external aeration. Thus, nitrate concentrations in the pore were reduce as well.
- The *L. vannamei* survival was better in the RAS cultures than the intensive cultures. In contrast, the biomass (g) was higher in intensive cultures tan in the RAS.
- The water demand in the RAS culture of *L. vannamei* was 78% les than in the intensive cultures.



Artificial or Constructed Wetlands

A Suitable Technology for Sustainable Water Management

Edited By María del Carmen Durán-Domínguez-de-Bazúa, Amado Enrique Navarro-Frómata, Josep M. Bayona

Chapter 12

Bioremediation of Shrimp Aquaculture Effluents: The Convenience of Artificial Wetlands

By Otoniel Carranza-Díaz, José Guillermo Galindo-Reyes

Pages 16

[< Back to book](#)

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¡¡Thank you very much!!



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- Laboratorio de ecofisiología de organismos acuáticos y cultivos de apoyo para la acuicultura (UAS)
- Laboratorio de toxicología y contaminación (UAS)