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Pós Graduação em AQUICULTURA Universidade Federal do Rio Grande - FURG

ESTAÇÃO MARINHA DE AQUACULTURA - FURG PROJETO CAMARÃO

HETEROTROPHIC VS MIXED BIOFLOC SYSTEMS: IMPACTS ON USE OF WATER, SUSPENDED SOLIDS PRODUCTION AND ZOOTECHNICAL PERFORMANCE OF *Litopenaeus vannamei*

Hellyjúnyor Brandão, Dariano Krummenauer, Íris Xavier, Gabriel Santana, Henrique Santana, and **Wilson Wasielesky***

LAS VEGAS, 2018

AQUACULTURE

SUPERINTENSIVE
INTENSIVE
EXTENSIVE

Biosecurity
Stocking Density
Production
Feed consumption
Excretion

NITROGEN COMPOUNDS
(as ammonia, e.g.)

CHEMOAUTOTROPHIC

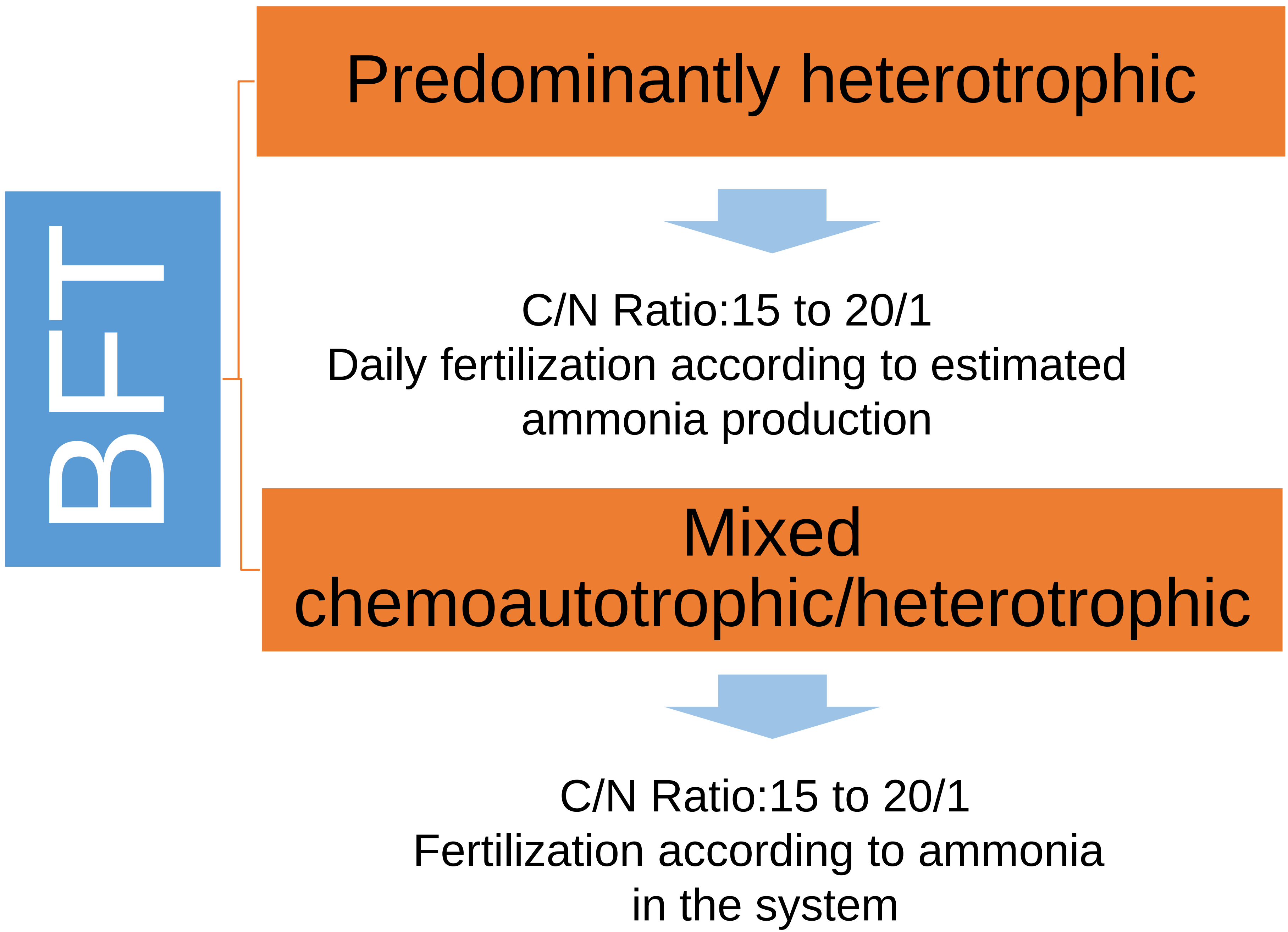
- Inorganic nitrogen consumption;
- Inorganic carbon consumption;
- Oxidation of ammonia to nitrite and then to nitrate;
- **Less biomass (more efficient);**
- **Slower growth;**

HETEROTROPHIC

- Inorganic nitrogen consumption;
- Organic carbon consumption;
- **Fast growth**
- **Increase the amount of total suspended solids (sludge);**

Type of fertilization

Bacteria degrade excess organic matter and allow successive cycles of shrimp production without the need for water renewal culture.


BFT
Predominantly heterotrophic
C/N Ratio:15 to 20/1
Daily fertilization according to estimated ammonia production
Mixed chemoautotrophic/heterotrophic
C/N Ratio:15 to 20/1
Fertilization according to ammonia in the system

Objective

To evaluate the effect of bioflocs formation techniques and their effects on the zootechnical performance of *L. vannamei*, on the use of water and production of suspended solids.

Material and methods

MARINE STATION of AQUACULTURE

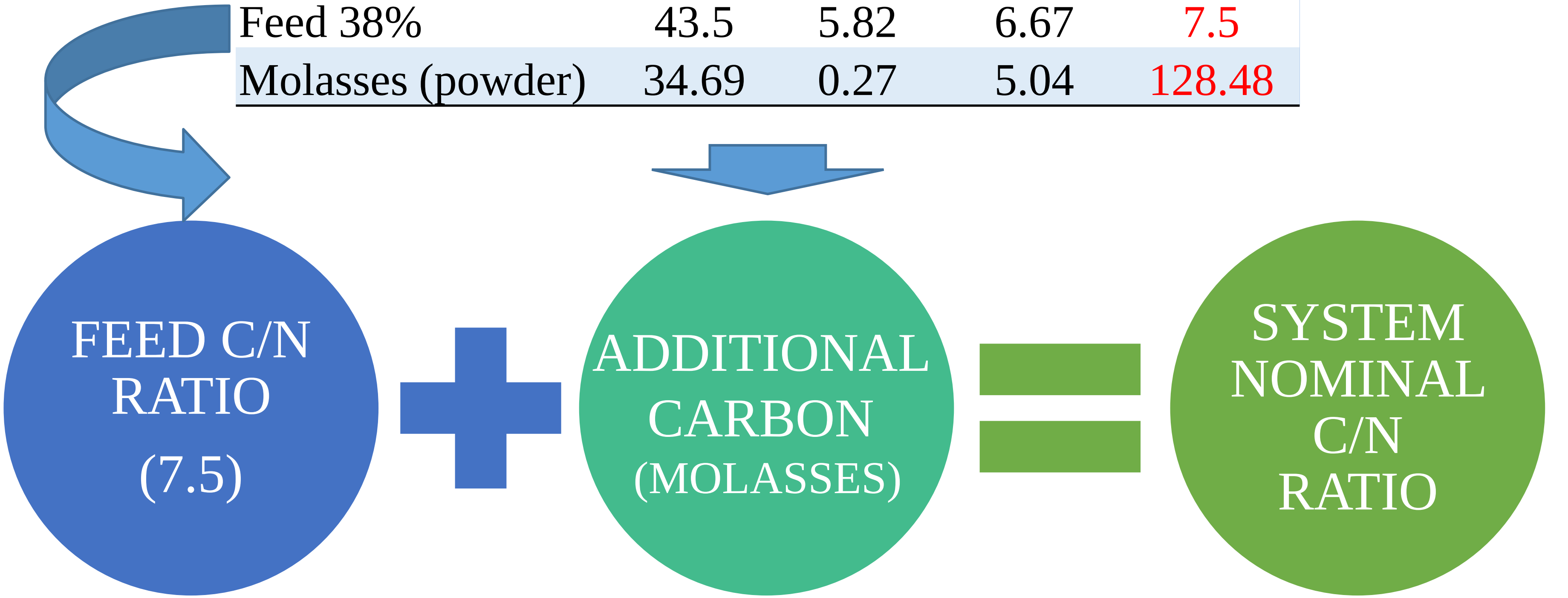
Federal University of Rio Grande
Southern Brazil

Material and methods

- ✓ 150 L experimental tanks
- ✓ 300 shrimps/m^3
- ✓ Initial weight 7.05 ± 1.37g
- ✓ 60 days

Percentage of carbon, nitrogen and hydrogen in feed and molasses were determined using a CNHS Elemental Analyser

	C	N	H	C/N
Feed 38%	43.5	5.82	6.67	7.5
Molasses (powder)	34.69	0.27	5.04	128.48



Treatments

No organic fertilization

Mixed - chemoautotrophic /heterotrophic

(fertilization according to the nominal ammonia reading)

Heterotrophic

(fertilization according to the estimated ammonia produced)

C/N ratio = 15/1

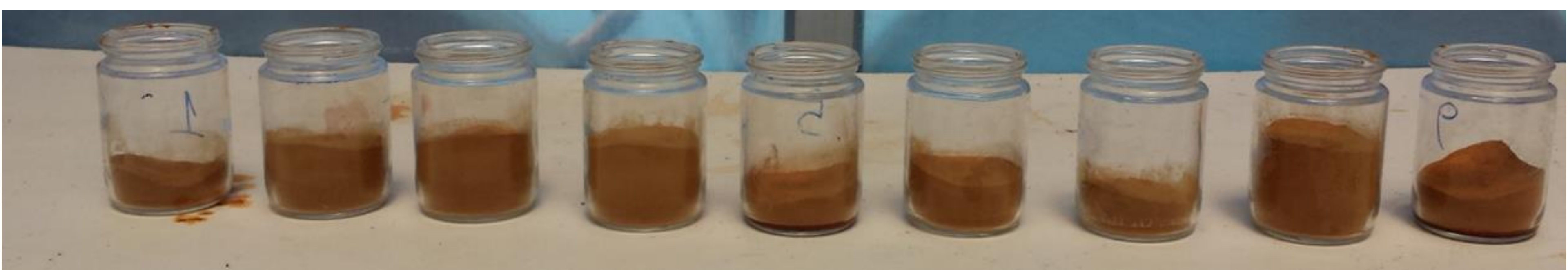
MIXED:

Organic fertilization was done every time total ammonia nitrogen (TAN) exceed 1.0 mg/L

HETEROTROPHIC:

Estimated Nitrogen production = Feed*% protein*0.144
(Ebeling, 2006)

QUANTITY OF MOLASSES (g) = [TAN]/0.3469*C/N RATIO*VOLUME*1.02/1000



Water use: Clarify or water exchange

- 20% of water renewal, in case:

TAN reached

7 mg/L

Nitrite reached

20 mg/L

SST

> 500 mg/L

(Gaona, 2011)

Twice the safe level of each one*
(approximate levels by Lin & Chen, 2001; 2003)

Water parameters

- **Temperature**
- **pH**
- **Dissolved Oxygen**

} Daily

Laboratorial analysis:

- **Total Ammonia Nitrogen** (UNESCO, 1983)
- **Nitrite** (Bendschnider & Robinson, 1952)

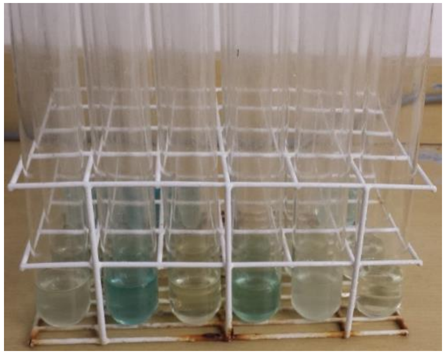
} Daily

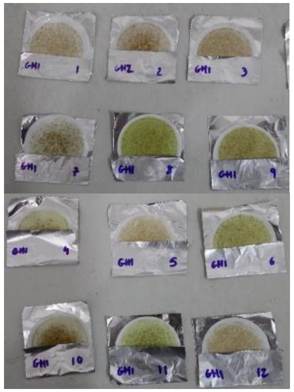
- **Alkalinity** (APHA, 1998)
- **Total suspended solids** (AOAC, 2000)

} Twice a week

- **Nitrate** (Aminot & Chaussepied, 1983)
- **Turbidity** (turbidimeter)

} Weekly





- ✓Feeding frequency: 2 times per day (08:00 a.m. and 5:00 p.m.);

- ✓Feeding trays with 10% of feed;

- ✓Feeding rate according consumption;

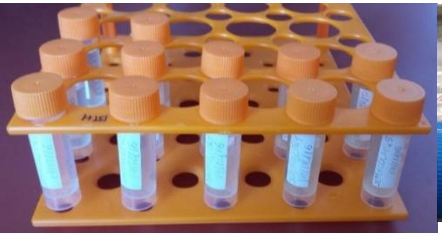
- ✓Monitoring: every 24 h.

Shrimp monitoring – every 7 days – 20 shrimps / tank were sampled and individually weighed

- Weekly growth rate (WGR)
  - WGR = (final weight / number of weeks of culture)
- Survival (S%)
  - S% = [(final biomass / average individual weight) / number of individuals stocked)] x 100
- Productivity
  - Prod = (biomass increment / tank volume)
- Feed conversion ratio (FCR)
  - FCR = offered feed / biomass increment

BFT water samples were collected and fixed in 2% paraformaldehyde (PFA) to detect the growth of the population of nitrifying and heterotrophic bacteria by Fluorescent *IN SITU* Hybridization (FISH) methodology, at Federal University of Juiz de Fora – MG - Brazil

- ✓ Culture–independent molecular biology technique
- ✓ Allows a direct and precise quantification of the pathogenic and probiotic bacteria cells at species or genus level

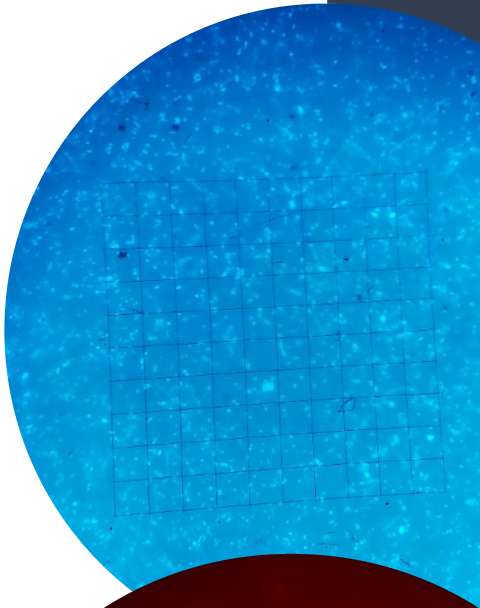


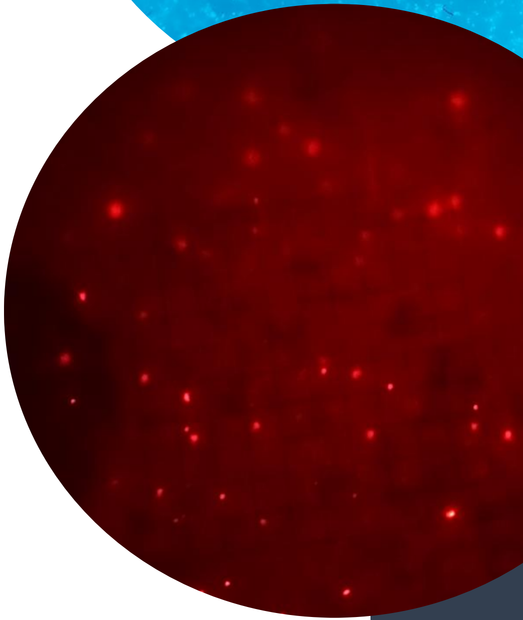

- Oligonucleotide probes rRNA-targeted will be used to identify the groups of bacteria.

- All probes will be labeled with the Cy3 fluorochrome.

- **The abundance of bacteria will be determined by direct counting at 1000× magnification using an epifluorescence microscope (Olympus® BX-60)**

- A negative control made with a probe without any specificity for bacteria will be used to evaluate the efficiency of hybridization.





Statistical analysis

- Homoscedasticity of variances and normality tests;
- One-way ANOVA - Detect possible differences between treatments and posteriori Tukey’s test ($\alpha = 0.05$).

Results

Resultados

Table 1 – Water quality parameters (mean ± standard deviation) in different BFT systems. Means in the same line with different letters are significantly different ($p < 0,05$).

	Heterotrophic	Mixed	No fertilization
Temperature (°C)	27,75±1,45	28,15±1,17	28,73±1,65
Dissolved Oxygen (mg/L)	6,15±0,55	6,18±0,25	6,21±0,34
pH	7,96±0,14 [a]	7,65±0,20 [b]	7,64±0,18 [b]
Total ammonia nitrogen (mg/L)	1,10±1,09 [a]	1,53±1,12 [a]	4,88±2,11 [b]
NO_2^--N (mg/L)	2,38±3,22 [a]	6,46±8,08 [b]	9,44±9,27 [b]
NO_3^--N (mg/L)	20,13±3,56 [a]	87,77±3,22[b]	79,21±2,88 [b]
Alkalinity ($CaCO_3$ mg/L)	321±22 [b]	135±11 [a]	144±17[a]
TSS (mg/L)	355±102[b]	199±85 [a]	119±66[a]

Physical and chemical parameters were withing the range reccomend for *L. vannamei* (Jiang and Pan, 2005; Ponce-palafox et al., 1997).

Higher pH and alkalinity in heterotrophic treatment due to less use of inorganic carbon by heterotrophic bacteria (Ebeling, 2006)

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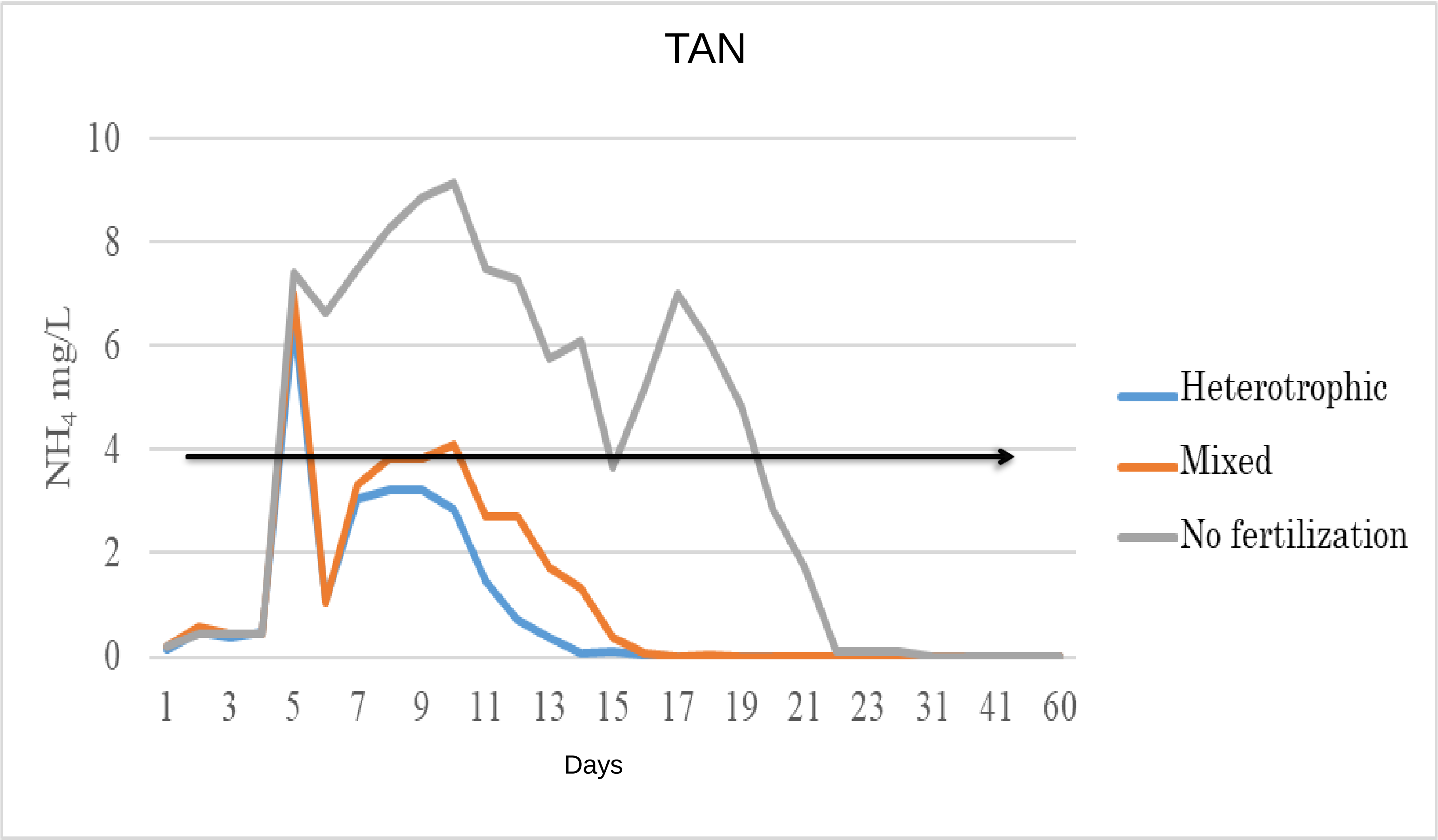
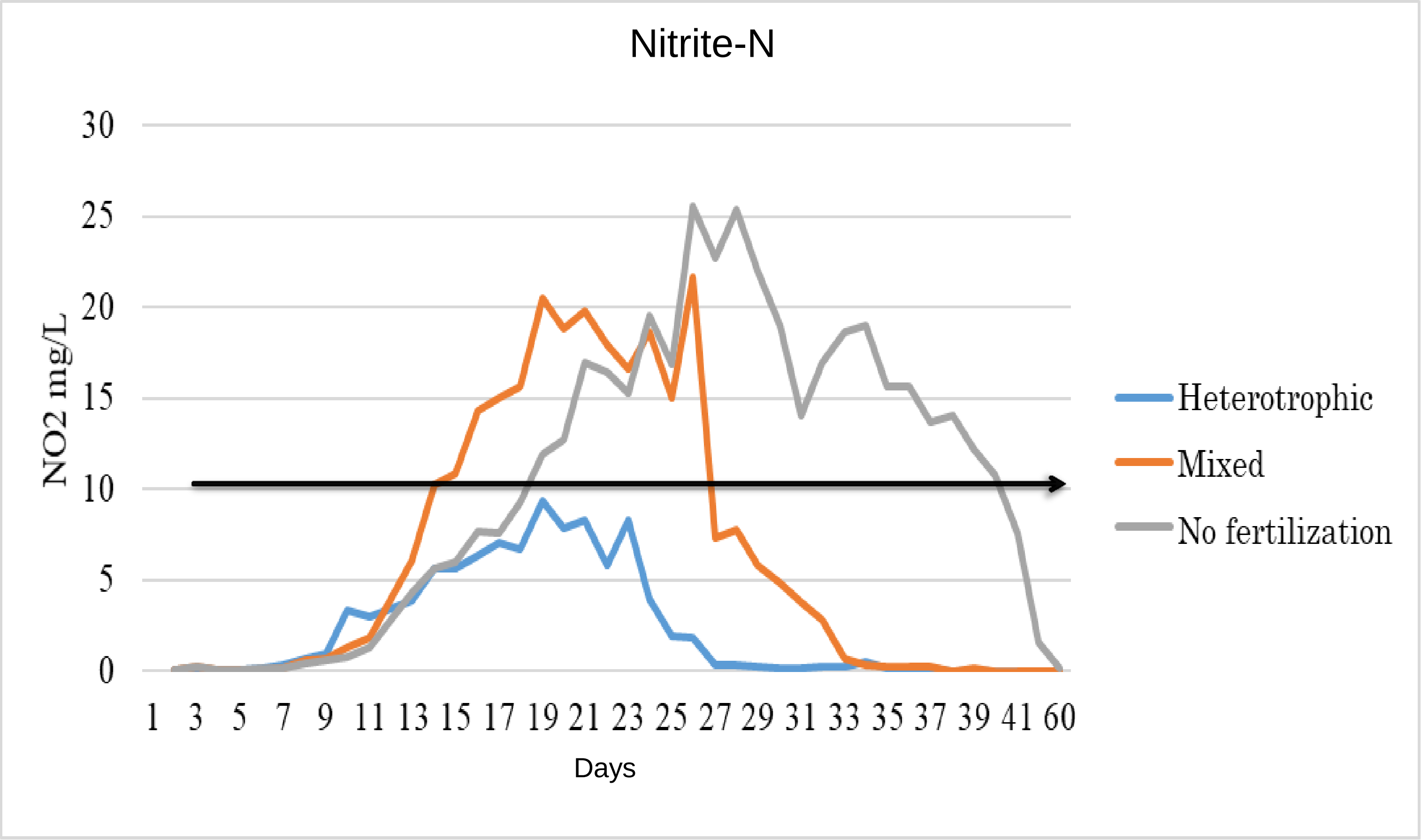
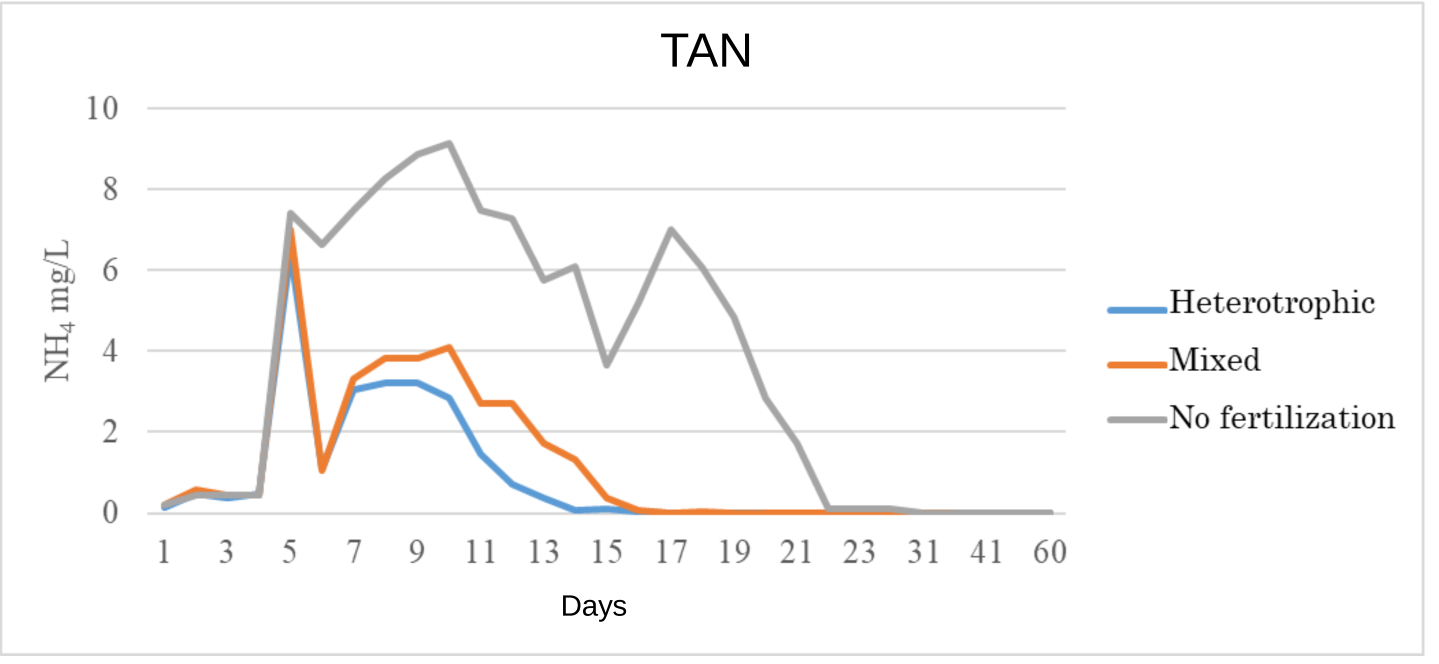


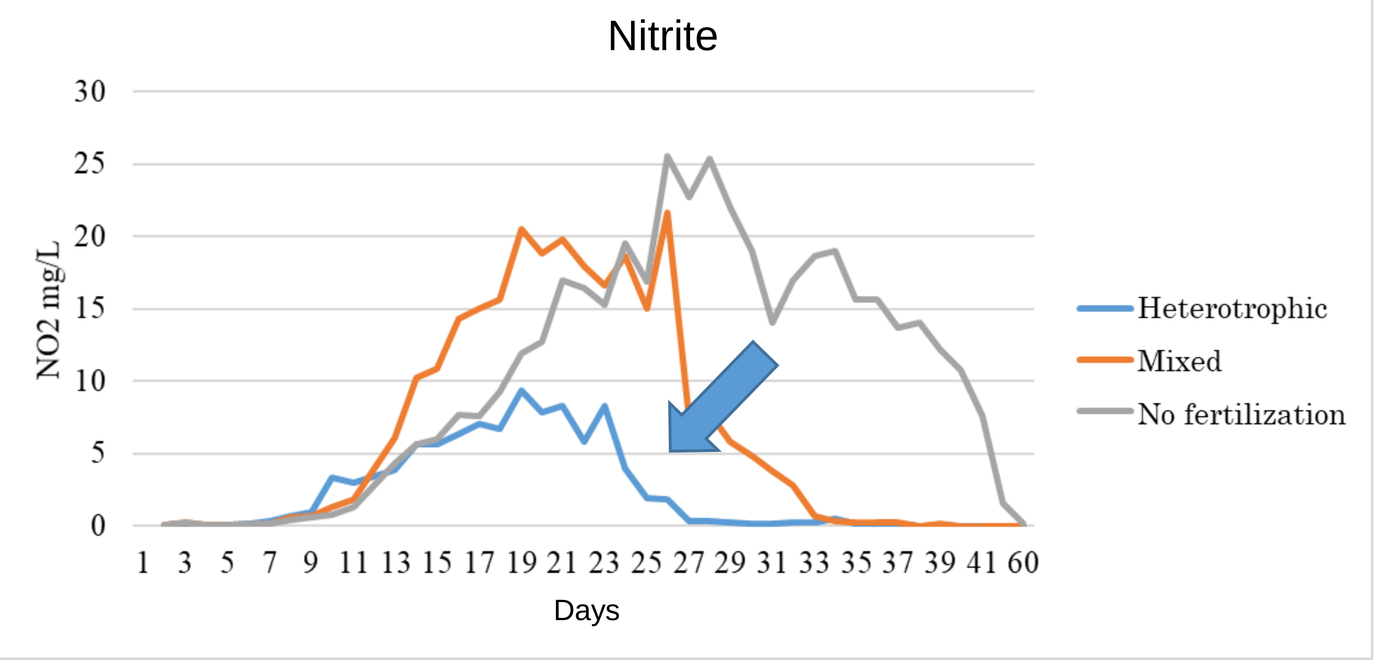
No fertilization treatment overcame safe concentrations



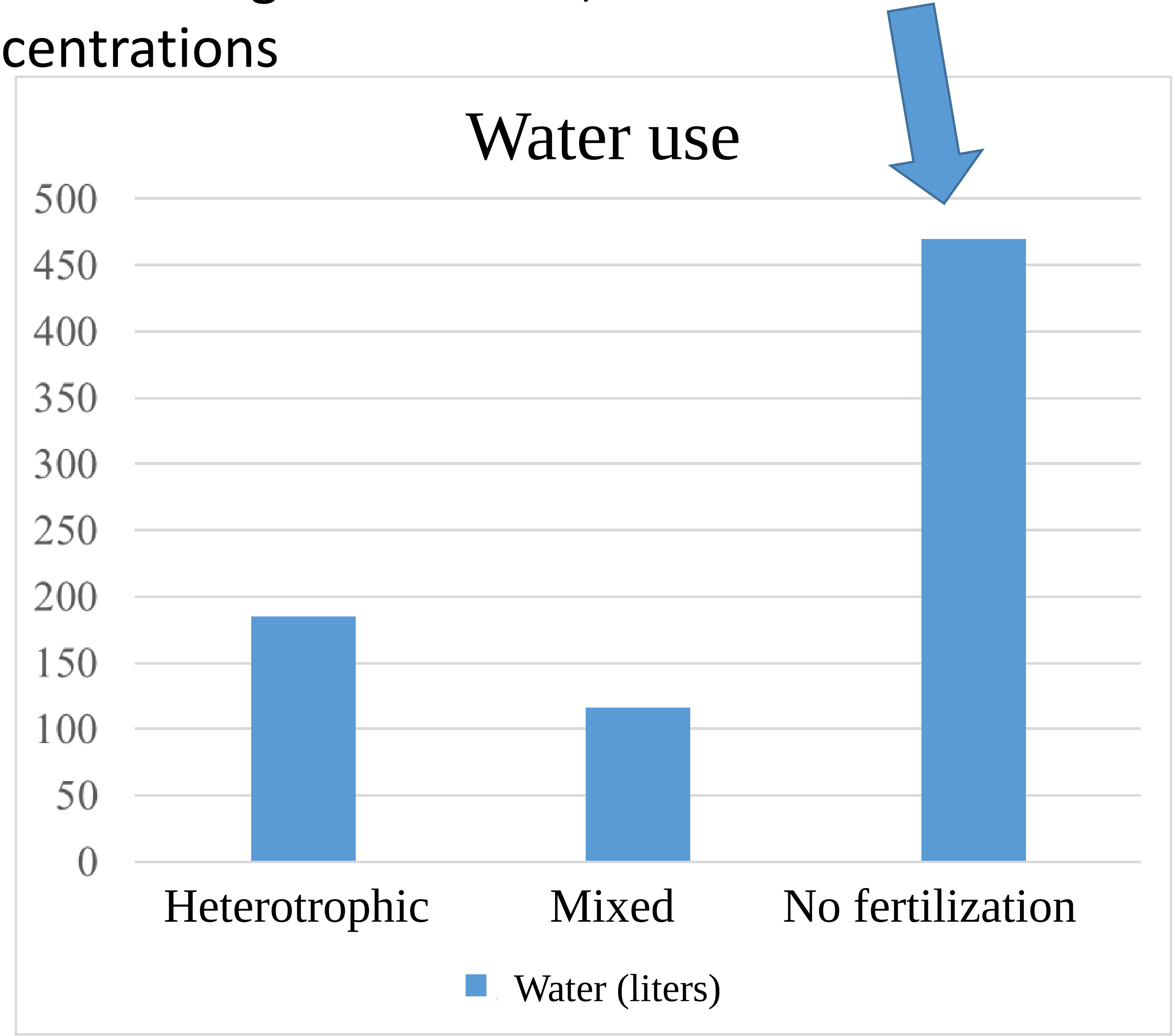
Nitrite-N
NO2 mg/L
30
25
20
15
10
5
0
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29 31 33 35 37 39 41 60
Days
Heterotrophic
Mixed
No fertilization



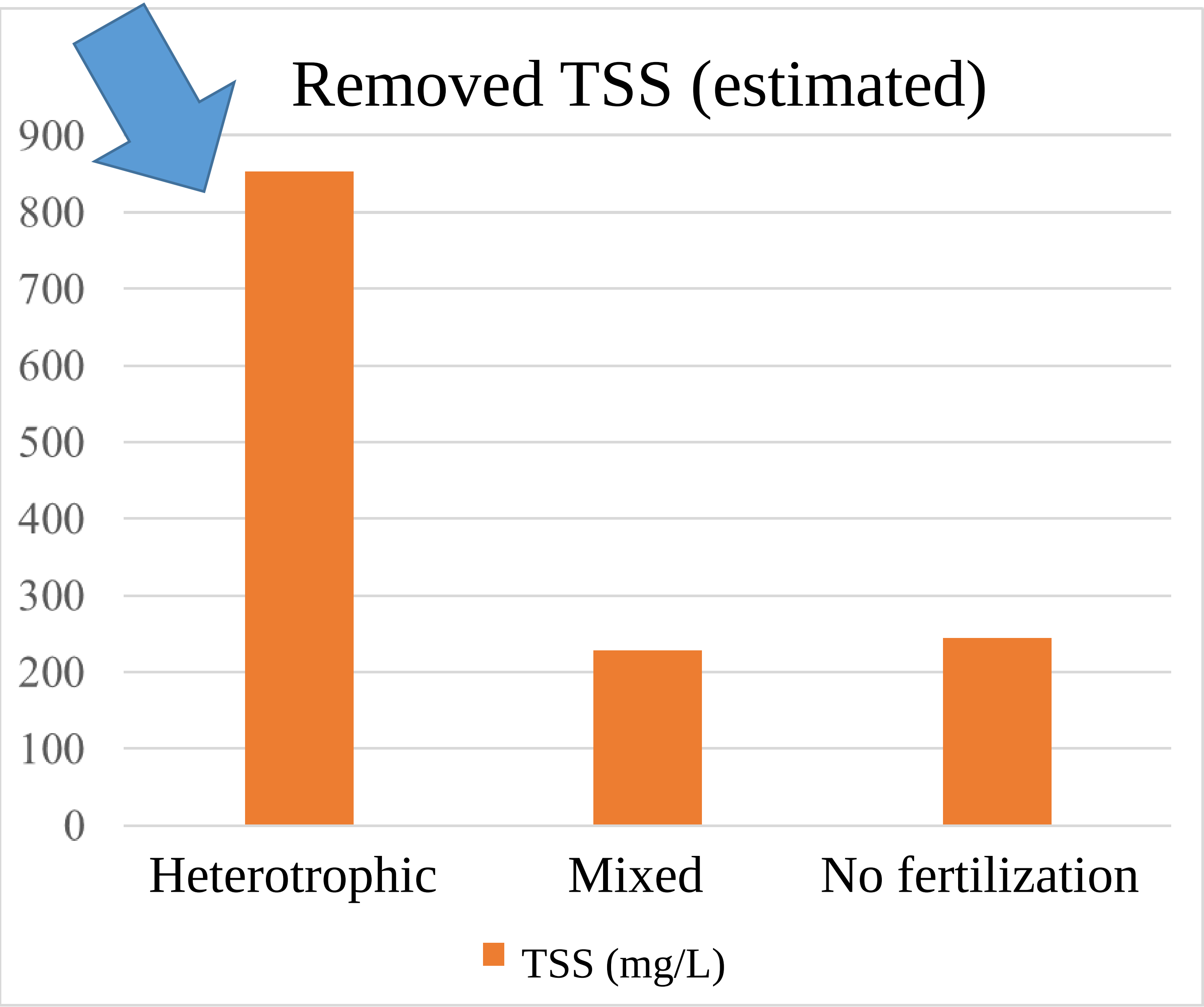
Nitrification supression in heterotrophic treatment.



Water exchange due to TSS, ammonia and nitrite concentrations



Heterotrophic bacteria (Hargreaves, 2006)



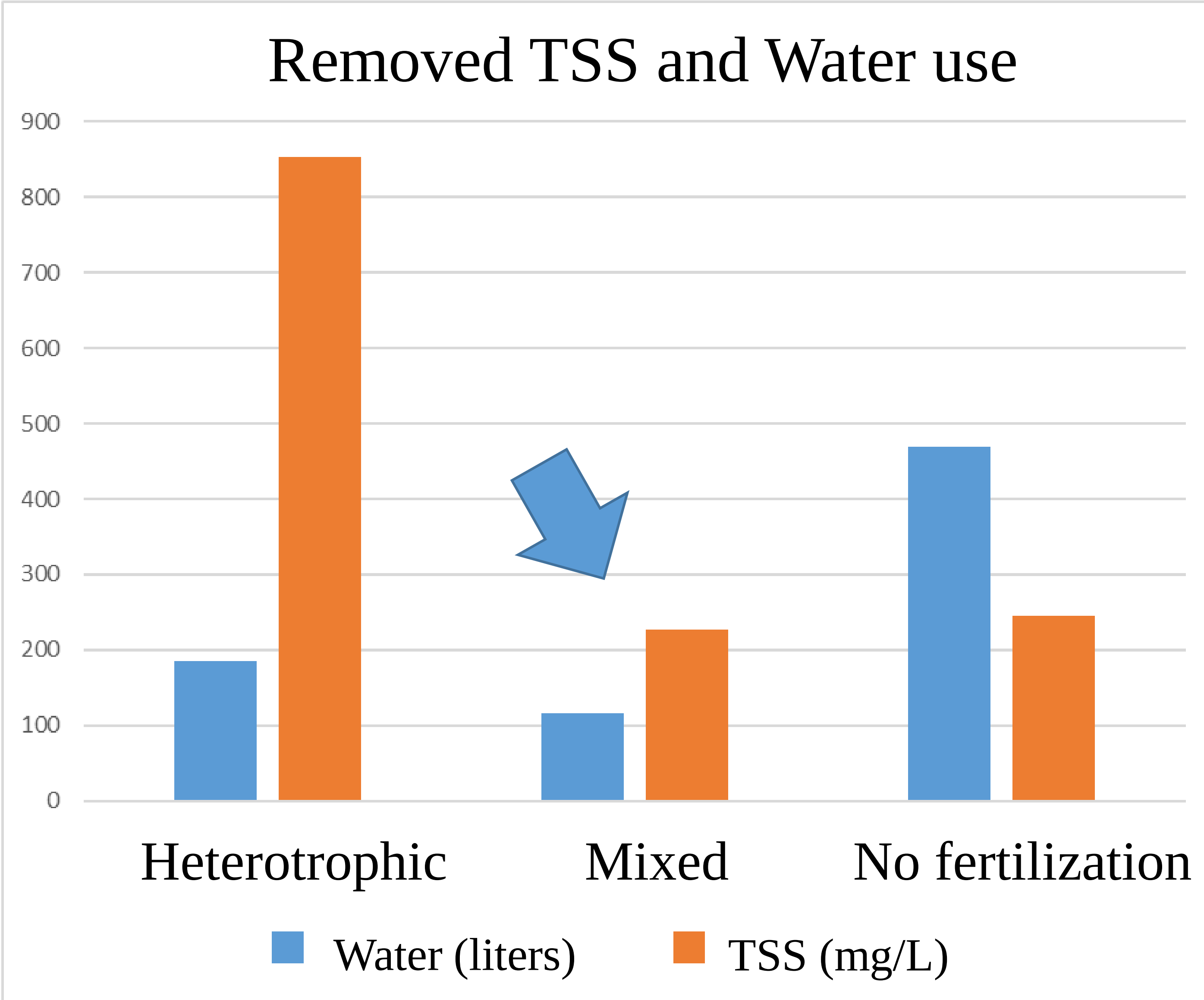

Removed TSS and Water use
900
800
700
600
500
400
300
200
100
0
Heterotrophic
Mixed
No fertilization
Water (liters)
TSS (mg/L)

Tabela 2 - Zootechnical performance indexes (mean ± standard deviation) of *L. vannamei* juveniles grown in tanks with different BFT systems. Means in the same line with different letters are significantly different ($p < 0.05$).

	Heterotrophic	Mixed	No fertilization
Initial weitght (g)	7.0±1.37	7.0±1.37	7.0±1.37
Final weight (g)	12.6±0.28[b]	13.8±0.68[a]	13.3±0.06[ab]
Survival (%)	87.4±5.13	93.3±3.85	94.07±3.39
Final biomass (g)	493.64±34.97	578.03±47.04	563.58±18.72
Biomass gain (g)	178.64±34.97[b]	263.03±47.04[a]	248.58±18.71[a]
Weekly growth (g/sem)	0.69±0.03[b]	0.84±0.08[a]	0.79±0.00[ab]
Final yield ($kg.m^{-3}$)	3.29±0.23[b]	3.85±0.31[a]	3.76±0.12a
FCR	2.24±0.41[b]	1.52±0.27[a]	1.58±0.11[a]

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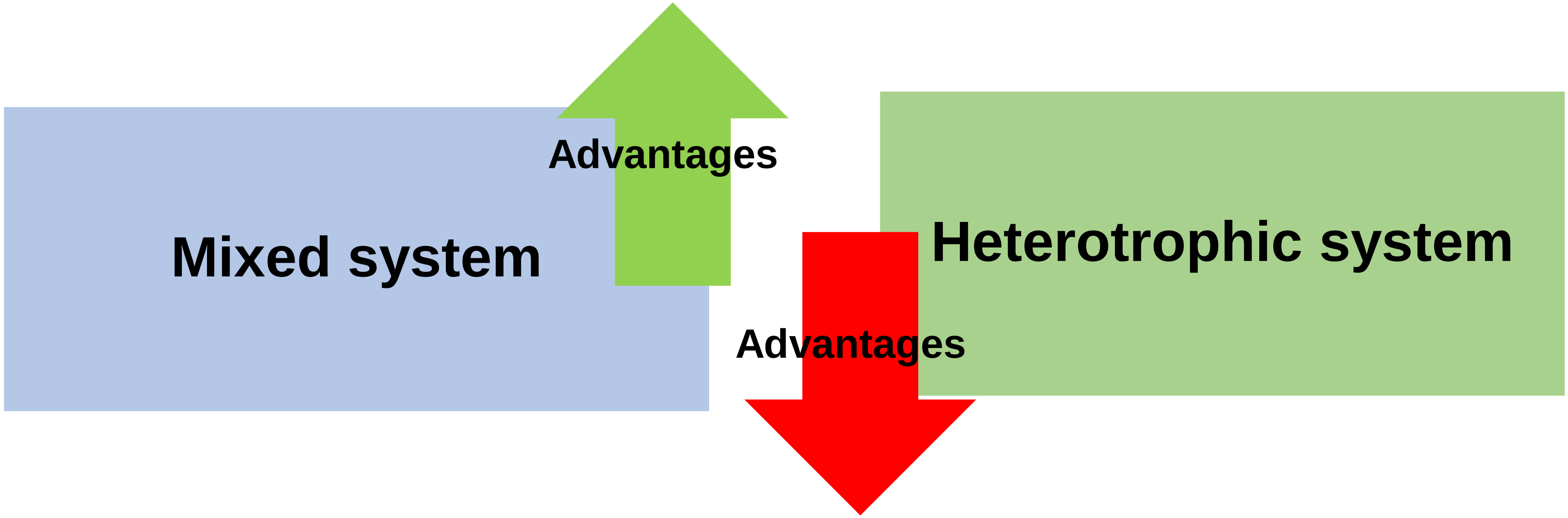
FCR	2.24±0.41[b]	1.52±0.27[a]	1.58±0.11[a]

Similar zootechnical results, however in no fertilization treatment the water consumption was 450% higher, waste water higher, and solids production higher.

Conclusions



C/N ratio = 15/1

The results showed the importance of adopting a mixed biofloc system to optimize the use of water and decrease the production of solids.

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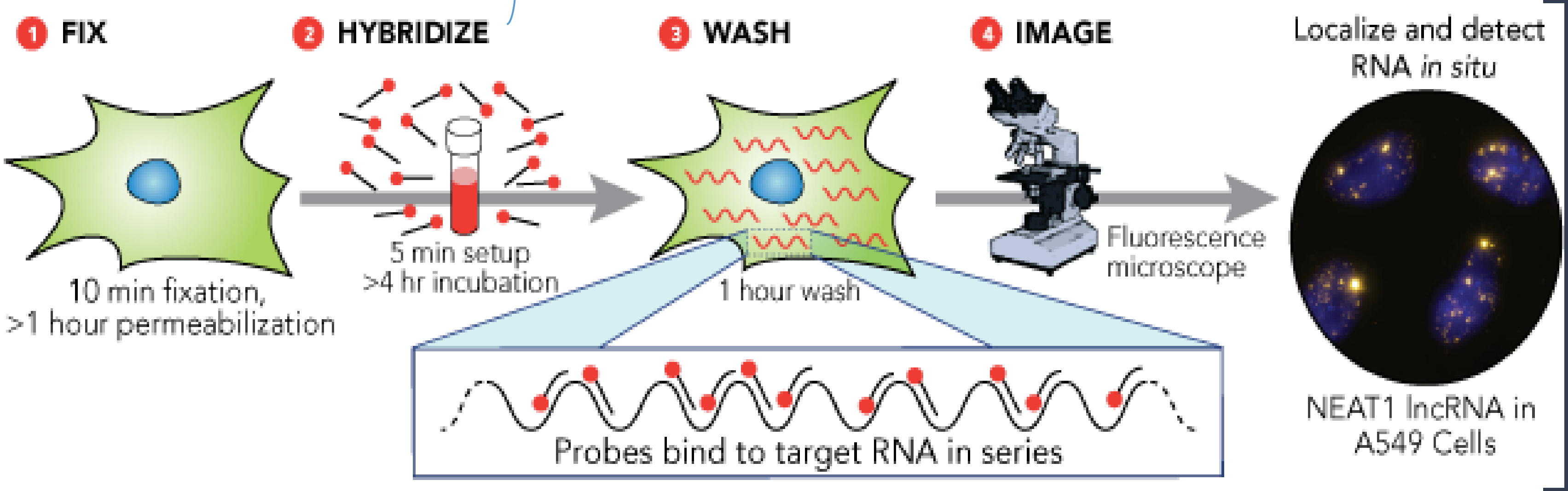
FISH – the tool

Probes that bind to the bacteria rRNA complementary sequences



Probe	Sequence of probe (5' - 3')	Place of destination (rRNA) and position	Specificity	*%FA	**NaCl (mM)	Reference
NON	TAGTGACGCCGTCGA	-	Negative Control	30	112	Yokokawa & Nagata (2005)
NIT3	CCTGTGCTCCATGCTCCG	16S (1030–1047)	Nitrobacter spp. – NOB	40	56	Wagner et al. (1996)
NITCOC 206	CGGTGCGAGCTTGCAAGC	-	Nitrococcus mobilis – AOB	20	225	Juretschko et al. (2000)
NSO 190	CGATCCCCTGCTTTTCTCC	16S (190–208)	Nitrosomonadales – AOB	35	80	Mobarry et al. (1996)
NSO 1225	CGCCATTGTATTACGTGTGA	16S (1224–1243)	Nitrosomonadales – AOB	35	80	Mobarry et al. (1996)
NSMR 76	CCC CCC TCT TCT GGA TAC	16S (132–149)	Nitrosomonas marina-like – AOB	20	225	Burrell et al. (2001)
NTSPA 685	CAC CGG GAA TTC CGC GCT CCT C	16S (664–685)	Nitrospira moscoviensis, Nitrospira marina – NOB	20	225	Burrell et al. (2001)
NTSPA 712	CGCCTTCGCCACCCGGCCTTCC	-	Phylum Nitrospira – NOB	50	28	Daims et al. (2001)
PAE 997	TCTGGAAAGTTCTCAGCA	16S (997-1014)	Pseudomonas spp. – Heterotrophic	35	80	Amann et al. (1996)

* Percentage of formamide (FA) in the hybridization solution. ** Concentration of sodium chloride in the wash solution.