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PRELIMINARY ASPECTS OF THE PERFORMANCE IN CAPTIVITY OF OREOCHROMIS NILOTICUS AND CHERAX QUADRICARINATUS IN A RAS SYSTEM: A BIOENERGETIC APPROACH

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ABSTRACT: Recirculating aquaculture systems (RAS) and polycultures with a polytrophic approach using Nile tilapia (*Oreochromis niloticus*) and crayfish (*Cherax quadricarinatus*) offer alternatives for sustainable water use and reduced feed costs in aquaculture production systems. Few studies on this topic exist in Mexico. Therefore, this research aimed to analyze the feasibility of managing both species in a pilot RAS system designed in the Aquaculture Laboratory of the Faculty of Sciences, UNAM. Water quality indicators were within the reported standards for both species, except for ammonia and nitrite in the initial phase. Growth, energy metabolism, and tissue energy content of *O. niloticus* differed significantly from that of *C. quadricarinatus* during the experimental phase (64 days), indicating faster growth for *O. niloticus* in the system. The protein content on a dry matter basis for both species was 50% and 38%, respectively. This preliminary study demonstrates the potential of *C. quadricarinatus* as a companion species for *O. niloticus* in aquaculture systems focused on bioenergetic complementarity, sustainability, utilization of secondary resources, and aquaculture diversification.

KEYWORDS: Polyculture, RAS, sustainability, *Oreochromis niloticus*, *Cherax quadricarinatus*.

Introduction

RAS system is a technology for cultivating aquatic organisms where water is treated and reused. These systems are characterized by recirculating between 90% and 99% of the water in the rearing ponds, minimizing the water footprint of the culture system (Ahmed & Turchini, 2021; Badiola

et al., 2012; Kamali et al., 2022; Murray et al., 2014). These procedures stand out for their advantages in recreating an optimal growth environment, which helps maximize organism growth and reduce losses due to pathogens. The efficiency that maximizes optimal production in a RAS lies in the key components of the system, which are: the culture tank, a mechanical filter that helps remove solid waste such as feces and uneaten food, a biofilter that houses bacteria that convert nitrogenous waste into nitrite and subsequently into nitrate, and a reservoir that returns the treated water to the rearing tank (Holan et al., 2020).

On the other hand, polytrophic systems are those in which organisms from different trophic levels interact, exploiting their synergies so that food not consumed by the primary species can be used by secondary species, maximizing energy transfer from the supplied feed and creating a more efficient and sustainable system (Chopin, 2013; Frouz & Frouzová, 2022). The integration of these two systems produces positive interactions in terms of production and cost reduction. The expected success of a polytrophic RAS is closely linked to the selection and compatibility of the species to be cultivated. In this study, two commercially important species were chosen: *O. niloticus* as the primary target species for the supplied feed and *C. quadricarinatus* as the organic extractive species integrated into the system.

On the other hand, the main approach to studying increased animal production is bioenergetics, a field that deals with the analysis of how organisms acquire, transform, and use energy from ingested food for their vital processes. This is essential for aquaculture. To express this in terms of

energy flow, there is a bioenergetic model widely accepted by the scientific community (Grodzinski, 1975).

$$C = P + R + F + U$$

Where C is the energy ingested in food, R represents the energy used in respiration (metabolism), U is excretion via urine and gills, F is the energy excreted in feces, and P is the production or retained energy. This model allows for the quantification of energy transfer and compartmentalization in cultured organisms and the calculation of their efficiencies to maximize biomass production in the production system.

Considering the above, the objective of this study was to evaluate the coexistence and trophic interaction between *O. niloticus* and *C. quadricarinatus* in a RAS system, to optimize resource utilization, minimize byproducts, and generate added value through an efficient and sustainable multi-species culture.

Materials and Methods

RAS System

This system included two 1000 L tanks (organism reservoirs) and three 120 L containers interconnected in the following sequence: a mechanical filter (shade mesh and polyester fiber), a biofilter (containing 250 bio-balls and 250 K1 pieces), and a filtered water reservoir equipped with pumps for recirculation to the rearing tanks. PVC pipes were installed inside the tanks as shelters for *C. quadricarinatus*, and a false floor was installed to separate the two species. The temperature was controlled with a precision thermostat ($25 \pm 1^\circ\text{C}$), oxygenation with Venturi aerators, and the water recirculation

system allowed for 4 – 6 water exchange cycles per day. The RAS was monitored and calibrated before the introduction of the organisms.

Water Quality Indicators

Physicochemical parameters (temperature, dissolved oxygen, pH, total solids, conductivity, and salinity) were measured Monday through Friday using an EcoSense ODO-200 oximeter ($0.0 - 20.0 \pm 0.1$ mg/L, temperature $0 - 50 \pm 0.1^\circ\text{C}$) and an Eutech PCSTestr 35 multi-analyzer. Ammonium and nitrite concentrations were measured twice a week: Monday and Friday using API test kits.

Organism Acquisition and Acclimation Phase

Two hundred male Nile tilapia fingerlings were obtained through a donation from the Zacatepec, Morelos fish farm, a SAGARPA-affiliated facility. The 36 individuals of *C. quadricarinatus* of both sexes were acquired from a rustic farm in the state of Morelos. All specimens were transferred to the aquaculture laboratory of the Faculty of Sciences, UNAM, where they were acclimatized in 600-liter ponds separated by species to prevent the spread of diseases, for 14 days prior to the growth trial.

Experimental Design

The research was conducted in two 1,000-liter tanks (with an operating volume of 900 L), interconnected to a specially designed RAS system in our laboratory. Stocking densities in the RAS system were $n = 60$ for *O. niloticus* and $n = 18$ for *C. quadricarinatus*, with one replicate. Four biometric measurements of *O. niloticus* ($n =$

15–20) and *C. quadricarinatus* ($n = 5$) were performed every three weeks for 64 days, measuring wet weight (WW) and total length (TL) for tilapia, and cephalothorax length (CL) and total length for *Cherax*. The organisms were fed daily at 7% of their body weight (initial phase, divided into two rations: 50–50%, morning–afternoon), which was adjusted according to the average weight obtained from the biometric measurements. Formulated feed for tilapia (“El Pedregal Silver Cup” with 35% protein) was used.

Growth Phase

During this stage, growth curves (T_0 to T_{64d}), specific growth rate (SGR, %) and survival were evaluated (Chapman, 1978).

Energy Balance Indicators (P and R)

At the end of the experimental phase, the organisms energy expenditure (routine metabolism) was measured to estimate the R value of the energy balance equation using a semi-closed respirometry system (Latournerié et al., 2023). Based on the final biometry data, the size ranges of the specimens were determined, and two small, two medium, and two large organisms were selected from each RAS. Two rectangular containers (water baths: $25 \pm 1^\circ\text{C}$) were set up with experimental chambers and a control chamber (without organisms).

Four oxygen measurements were taken per chamber during two time periods (10–12 h and 14–16 h). In each period, the initial oxygen level was measured, the chamber was closed, and after two hours, the final oxygen level was measured. The chambers were then aerated for one hour, and this procedure was repeated for the second cy-

cle. At the end of this period, biometry was performed on all organisms, recording the previously mentioned meristic indices.

Oxygen consumption (VO_2) was calculated from the difference between the initial and final values in each period, the duration in the chambers, and the body weight of the organisms. Respiratory rate data were converted to QO_2 based on the dry weight of 1 g of tissue on a wet basis for both species (*O. niloticus*: 31.3% and *C. quadricarinatus*: 33.3%). These were then converted to calories (g dry weight/h) using an oxy-caloric coefficient (QO_x) of 3.32 calories/mg O_2 consumed (Bradfield & Solomon, 1972; Latournerié, 2007). To calculate retained energy (P), the previously obtained growth rate results were converted to calories using the energy content values of the specimens: *O. niloticus* (5,658.6 cal/g DW) and *C. quadricarinatus* (3,325.5 cal/g DW), measured in a Parr calorimeter. From the P, R, and tissue energy content data for both species, feed assimilation efficiency was calculated in %, (Brett & Groves, 1979). In addition, proximate tissue composition analyses were performed on samples of organisms from both species at the end of the experimental stage.

Statistical Design

The design involved a fixed-effects factorial design: two species, four time periods, with interaction and one replicate in multivariable mode, with mean comparisons by point estimation (Tukey’s post hoc test, $p < 0.05$) and by 95% confidence intervals (95% CI).

Results and Discussion

Water Quality of the RAS System

Table 1 presents a summary of the eight water quality variables measured in the RAS system. It can be observed that the water quality indicators were within the reported range for both species, except for ammonium and nitrites. This is explained by the fact that the bacteria in the biological filter had not yet reached their maximum activity during the first two weeks, and from the third week onward, their values stabilized, reaching zero in both cases.

Regarding the growth of both species, Table 2 presents the mean growth rates with their respective confidence intervals and indicates the comparison of means by point estimation.

In the case of *O. niloticus*, all means indicated significant progressive growth. This dynamic may have been affected in its initial stage by anomalous concentrations of nitrogenous products, given that the specific growth rate was 3.3% at 21 days and 3.9% at 42 days, reflecting the adjustment of the limiting growth factor. The cumulative average during the entire phase was 3.1%. In the case of *C. quadricarinatus*, its growth was slow compared to *O. niloticus*, showing significant results only in the final phase of the trial. This trend is shown in Table 2, and Figure 1 displays the 95% confidence intervals (CI) of the WW means for both species.

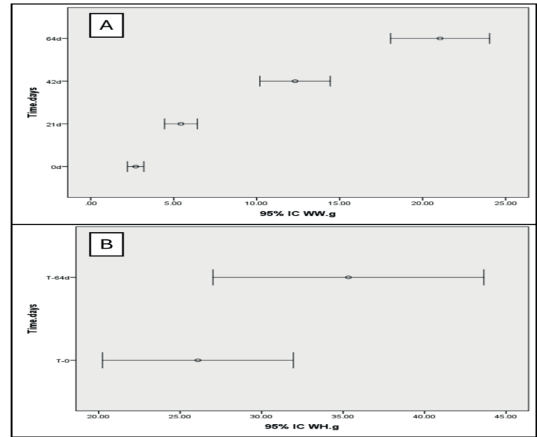


Fig. 1. 95% IC of body weight of *O. niloticus* (A) complete 64-day phase and *C. quadricarinatus* (B) (initial – final weight), in a polytrophic RAS system.

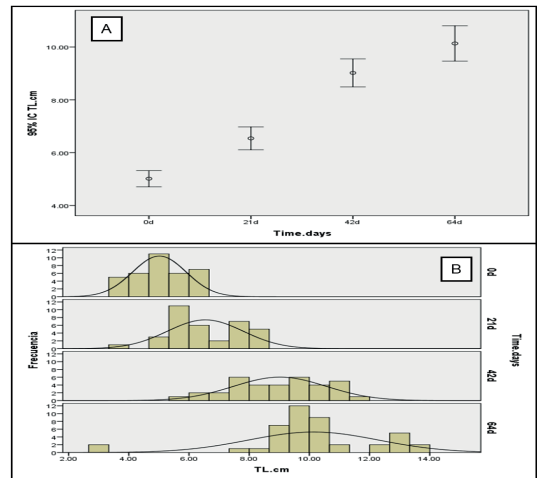


Fig. 2. Box plot (A) and TL size distribution (B) of *O. niloticus* during the experimental phase.

Furthermore, in a more detailed analysis of the growth of *O. niloticus*, Fig. 2 presents the box plot of the total length growth curve and the corresponding size distributions, showing that from 21 days onwards there is a tendency towards asymmetry of sizes and weights, indicating the beginning of the hierarchies in the experimental group.

This condition is relevant for the management of the organisms under culture conditions, since it allows adjustments to

Water quality	Mean ± DS	IC _{95%} lower	IC _{95%} upper	Guideline <i>Tilapia</i>	Guideline <i>Cherax</i>
T. °C	24.9 ± 0.82	24.5	25.2	25 - 32	23 - 32
O ₂ mg.l	5.7 ± 0.3	5.6	5.9	3 - 5	3 - 5
pH	8.01 ± 0.26	7.91	8.1	6.5 – 9.0	7 – 8.5
Cond. mS	605.1 ± 44.9	587.3	622.8	50 -500	-
T. S. mg.l	433.2 ± 28.2	418	448.3	100 - 300	100 - 300
Salin. mg.l	301.2 ± 38.7	285.9	316.5	500 - 1000	0 – 5 ppt
NH ₄ mg.l	1.37 ± 0.5	0.1	0.65	0 – 0.5	0 – 0.25
NO ₂ mg.l	0.14 ± 1.19	0.78	2.1	0 - 1	0 – 0.3

Table 1. Water quality (WQ) variables in the RAS system during the experimental phase, comparison with the regulations for both species.

SPECIES	Time, d	N	Mean ± DS	CI _{95%} lower	CI _{95%} upper
<i>Oreochromis niloticus</i>	0	34	2.7 ^A ± 1.4	2.2	3.2
	21	35	5.4 ^B ± 2.8	4.4	6.4
	42	35	12.3 ^C ± 6.1	10.1	14.4
	64	43	20.4 ^D ± 9.3	17.5	23.3
<i>Cherax quadricarinatus</i>	0	10	26.1 ^A ± 8.2	20.2	31.9
	21	13	28.4 ^A ± 10.1	22.2	34.7
	42	14	29.2 ^A ± 9.6	23.6	34.7
	64	7	34.6 ^B ± 7.5	27.6	41.5

Table 2. Comparison of growth (WW) for both species in the RAS system. Data are Mean ± SD and 95% CI. Means with different letters as superscripts are statistically different, Tukey post hoc (p<0.05).

be made in the size control (separating the hierarchs from the group), to readjust the growth of the organisms and maximize the production of biomass in the culture system.

In the case of *C. quadricarinatus*, growth was more moderate and stable, increasing from 26.09 g to 34.6 g during the same period. Weight gain was more significant than length gain, suggesting increased muscle mass and improved feed efficiency.

During the experimental phase, female *C. quadricarinatus* were observed to carry eggs in their abdominal area. Five egg-be-

aring females (28% of the initial sample), with a mean ± SD of 44 ± 21.1 eggs per specimen were removed to avoid stress. Although not quantified, the recurring presence of egg-bearing females indicates a high reproductive rate, considering that they are first-time breeding females. The culture environment was favorable for reproduction, demonstrating that the conditions (temperature, feed, water quality) favored gonadal maturation, which is positive for future breeding programs or multi-stage culture.

PERFORMANCE INDEX	<i>O. niloticus</i>	<i>C. quadricarinatus</i>
Initial WW: g	2.7 ± 1.4	26.1 ± 8.2
Final WW: g	20.5 ± 9.3	34.6 ± 7.9
Growth rate. g ww/day	0.28	0.14
SGR, %	3.1	0.47
Survival, %	100	100
M. R. (VO ₂) mg O ₂ /g. ww/day	3.24 ± 0.74	1.22 ± 0.41
M. R. (QO ₂) mg O ₂ /g. dw/day	10.13 ± 2.3	3.7 ± 1.36
Production (P). cal/g. dw/day	507.0	153.6
(R). cal/g. dw/day	33.6	12.3
Assimilation efficiency (P+R)/(ET), %	9.6	5.0
Energy tissue. (ET) cal/dw.g	5,658.6	3,325.5
Maintenance ration. (R/ET), %	0.6	0.36

Table 3. Comparison of performance indicators and bioenergetic indices of both species.

Relating to the proximate composition analysis of both species, *O. niloticus* showed higher tissue protein and fat content values than *C. quadricarinatus* (50.7% and 28%, and 38%, 7.7% respectively). This resulted in an enrichment of energy content in *O. niloticus* (5,658.6 kcal/g dry weight, compared to 3,325.5 kcal/g dry weight) for the secondary species. In summary, the captivity performance resulting from this study is presented in Table 3.

The indicators show that *O. niloticus*, as the main species, exhibits accelerated growth, with higher growth rates, biomass production, assimilation efficiency, and energy channeling in tissue than *C. quadricarinatus*, although with higher costs due to metabolism and maintenance ration. Nevertheless, the inclusion of *C. quadricarinatus* in the system offers aquaculture advantages such as supporting biomass production, with a focus on bioenergetic complementarity, sustainability, utilization of secondary resources, and aquaculture diversification.

Conclusions

This study suggests that integrating *C. quadricarinatus* with *O. niloticus* into recirculating aquaculture systems (RAS) offers multiple benefits. Bioenergetic complementarity refers to how these two species can interact to optimize energy use within the system, utilizing the debris of one species as a resource for the other. This contributes to sustainability by reducing dependence on external resources and minimizing byproducts. The utilization of secondary resources relates to the ability of *C. quadricarinatus* to use the litter of *O. niloticus*, creating a more efficient and partially self-sufficient system. Thus, aquaculture diversification implies the possibility of producing two commercially important species in the same system, which can increase the profitability and resilience of the procedure system.

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