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# Growth, Metabolic and Physiological Response of Juvenile Cheraxquadricarinatus Fed Different Available Nutritional Substrates

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## Abstract

There are many factors in intensive culture conditions which vary the physiological response of organisms in culture affecting performance such as growth, metabolic levels in blood, respiratory metabolism and the inflection point of dissolved oxygen concentration. The study was conducted under high density culture conditions with multi-trophic systems. Results show that the species can tolerate intensive culture conditions without affecting their growth and survival and has the ability to leverage multiple food sources. This work also shows that *Cheraxquadricarinatus* has the ability to maintain a low routine metabolic rate, is highly tolerant to limited dissolved oxygen conditions and thus uses energy efficiently. The metabolic rate in juvenile *C. quadricarinatus* was 0.07 ± 0.003 mg $O_2$/g/h while the critical level of oxygenconcentration, when the organism passes from regulator to conformer is 0.483 ± 0.002 mg $O_2$/L. This is significantly lower than previously reported for cultured decapod species.

**Keywords:** Crayfish; *C. quadricarinatus*; Metabolism; Oxygen; Glucose; Lactate

## Introduction

*Cheraxquadricarinatus* is an omnivorous species originated in Australia, with ideal characteristics for commercial culture, such as growth rate [1], that allows for harvest of commercial size organisms in 6-8 months [2]. It was introduced in Mexico in the 90´s [3] and is mainly cultured in the northeast region. Research studies have been conducted with the species, such as nutrition [4-6], reproduction and embryonic development [7-10], growth [11] and technology development for commercial culture [12]. Nevertheless, to optimize development during culture it is necessary to know and explore the limits of the physiological response of the organism under varying conditions. In particular, nutritional substrates have shown to produce a different response in terms of growth, and physiological response [13-16]. The metabolic rate is an index frequently used in aquaculture [17], where oxygen consumption determinations contribute to define energy use patterns and serves to determine stress related to environmental conditions [18] and the inflection point of dissolved oxygen concentration where the organisms switches from metabolic regulator to conformer [19]. Several authors [17-19,20-25] have reported results on the effect of environmental conditions, such as salinity and temperature, in the metabolic response of crustaceans. On the other hand, changes in hemolymph metabolites have been reported by several authors as a tool to define the physiological state of the organism, in relation to variations in temperature, salinity and oxygen concentration [26-30], reproductive performance [7,31-33], nutritional state [34-38], handling-stress [39,40], and disease infection [41]. In its natural habitat, *C. quadricarinatus* has been found in extreme temperatures and low oxygen concentrations [42,43], and has been reported to be very tolerant to environmental variations [1,2,44]. Nevertheless, the limits have not been established, the physiological response has not been defined, and the impact on production in culture conditions has not been measured [18]. This is of particular importance, as the current trends for aquaculture point towards intensification and the use of multitrophic systems [12,45-47]. Systems, using photoautotrophic or heterotrophic strategies [46,48-50] are based on the specific control of microalgae, plankton and bacterial populations to reduce undesirable metabolites, improve the use of supplemental nutrients and maximize system efficiency. The use of probiotics such as *Bacillus spp.* [46,51-53], *Saccharomycescerevisiae* [54], *Lactobacillus* [55,56] has been reported to improve water quality, improve growth rates on shrimp [57-59], tilapia [54,60,61], marine fish larvae [62], oyster [63] and reduce the impact of pathogens [46,53,64-66].This work presents the growth, metabolic and physiological response of juvenile *C.quadricarinatus* grown in systems with varying available nutrient substrates.

## Materials and Methods

### Experimental organisms and acclimation

*Cheraxquadricarinatus* juveniles (1.5 ± 0.3 g) were obtained from the experimental reproduction ponds at the Centro de Investigaciones Biológicas del Noroeste (CIBNOR) and placed in 1,000 l fiberglass tanks (1.00 m wide ×2.00 m long ×0.50 m deep) with filtered freshwater at 28 ± 1°C and constant aeration for acclimation.

### Culture conditions

An experimental system with fiberglass tanks measuring 0.26 $m^2$ (0.38×0.69 m) with 60 l was used. Twenty five juvenile *C. quadricarinatus*were randomly selected and placed in each unit at a density of 96 juveniles/$m^2$. The experimental units had filtered water at 28.0 ± 0.5°C; with oxygen saturation maintained at 7.37 ± 0.38

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mg $O_2$/l and a light cycle adjusted to 14 hours per day. Water quality (temperature, pH,oxygen saturation) was recorded daily. Water exchange of 20% was used to collect and eliminate feces and uneaten feed. Nylon mesh hiding places ($1m^2$) were introduced in each tank to avoid cannibalism [43]. Growth and survival were evaluated every 15 days for 60 days.

### Experimental treatments

Two treatments and one control group were tested with four replicates each: (1) Natural productivity from grow out ponds at CIBNOR. Daily supply of unfiltered water from culture ponds to the experimental units, to simulate primary productivity conditions during the trial; and (2) A commercial probiotic (Ecoterra®), which was prepared 24 hours prior to use, following the manufacturers recommendations. This commercial probiotic is composed of *B.licheniformis, B.subtilis, Nitrobacter,Nitrosomonas, Rizobium, S.cereviciae* and *T.oxidans*, which were identified through microscope and counted using a hemacytometer to determine the concentration of the prepared inoculum. Cell concentration in every experimental tank was determined after routine water exchange and probiotics were added to reach a total of 200,000 cells/liter. The control group (Filtered water) had units with filtered (5 micron) and UV treated freshwater. All the experimental units were fed a commercial shrimp pellet (35% crude protein) twice a day, for a total of 2% of the crayfish biomass [67]. Overall, the probiotic mix had a mean biochemical composition of 88.87 mg/L proteins, 33.08 mg/L lipids and 36.65 mg/L carbohydrates. The mean biochemical composition of primary productivity was of 9.06 mg/L proteins, 5.84 mg/L lipids and 4.34 mg/L carbohydrates.

### Growth trial

Juvenile performance was evaluated as follows:

a) Specific growth rate (average growth per day),$SGR=( \ln W_f - \ln W_i)/t \times 100$,

where

$W_f$ represents final weight (g),$W_i$ is the initial weight and t is time (days).

b) Apparent food conversion rate, FCR = Food fed (g)/Weight gain (g)

c) Survival %, S=(Final number of juveniles/ Initial number of juveniles)×100

### Biochemical analyses

At the end of the experiment, 20 juveniles were randomly selected from each treatment and hemolymph was extracted using 5% potassium oxalate as anticoagulant. Ten µl were used for hemocyanin analysis [68]. The rest was centrifuged at 3600 rpm and 10°C for 10 minutes to obtain plasma [69], to test for glucose (GOD PAP GL2623, Randox Method), lactate (LC2389 PAP Randox method), protein [70] and total lipids [71]. All the analyses were done using micro plates [69]. Additionally also performed these analyzes in hemolymph biochemical to a juveniles group from culture ponds which were not subject to the multi-trophic culture system to obtain baseline values.

### Metabolic rate and the inflection point of dissolved oxygen concentration (IP)

The metabolic rate was determined using a closed respirometer [22] for 20 randomly selected juveniles per treatment. After the experimental culture period, the organisms were starved for 24 hours to insure their digestive system was emptied [20,72]. The juveniles were placed individually in respirometers one hour before the evaluation, allowing the crayfish to acclimate. Temperature and aeration were maintained constant during this time. The metabolic rate was individually determined in water saturated with oxygen at 28 ± 0.5°C. A control blank (a respirometer without an experimental organism) was used for every 10 respirometers with experimental organisms. Oxygen saturation was recorded at the beginning of the trial and every 15 minutes for 1 hour, using a PreSens Precision System® (Regensburg, Germany) with a fiber optic oxygen sensor. The metabolic rate (MR) was determined using the equation:

d) $MR = DO/W/t$,

where *DO* represents milligrams of oxygen, W is weight (g) and *t* is time (hours). After the evaluation, the organisms were weighted (0.01 g) using a digital balance (Sartorius Portable PT600, precision ± 0.01 g, Gottingen, Germany), and a hemolymph sample taken for biochemical analyses, as described above. The inflection point of dissolved oxygen concentration (IP) was determined in independent trials using the respirometer described above. Oxygen saturation was recorded every 15 minutes until the variation between 3 recordings was not significant. After the evaluation, the organisms were weighted and a hemolymph sample taken for biochemical analyses, as described above. The inflection point of dissolved oxygen concentration (IP) was established as the intersection of best-fitlinear regressions above and below the inflexion point where the organism passes from regulator to conformer [22,73].

### Statistical analyses

Normality and homoscedasticity of data was tested before a one-way ANOVA test was used [74] to compare treatments. *A-posteriori* tests were used to determine statistical differences between treatments [75], using STATISTICA 6®.

## Results

### Overall performance

Results did not show statistical differences between treatments in terms of final weight, survival and FCR (Table 1), with acceptable growth rates for all treatments (mean = 2.1±0.1 g/week). Similarly, no differences were observed for survival that was around 70%.

### Biochemical analyses

Total lipids in hemolymph were similar for all treatments (Table 2) with juveniles from the probiotic treatment showing the highest mean value. After the metabolic evaluation in the respirometer, the lipid level for this treatment had the lowest mean value. Table 2 also shows that there were no significant differences in total proteins, hemocyanin and the proportion of hemocyanin/protein. Significantly higher levels of hemolymph glucose ($P<0.05$) were observed for the treatment with added probiotics (0.34 ± 0.08 mg/ml), compared to all other treatements (0.1 and0.15 mg/ml) (Figure 1a). A similar pattern was found for total lactate, where all treatments showed higher values than the baseline levels (0.04 ± 0.02 mg/ml), especially the treatment with added probiotic (1.86 ± 0.59 mg/ml) (Figure 1b). Limiting oxygen concentration during the metabolic trials in the respirometer produced a significant reduction in glucose and lactate levels in juveniles from the probiotic treatment (Figure 2). Conversely, glucose was highest

| Culture condition | Initial weight (g) | Final weight (g) | SGR (%/day) | FCR n | FCR n |
|---|---|---|---|---|---|
| | | | | | |
| Filtered Water | 1.47 ± 0.11 | 5.57 ± 1.91 | 2.2 ± 0.2 | 0.5 ± 0.10 | 47 |
| Primary productivity | 1.49 ± 0.07 | 5.26 ± 1.89 | 2.1 ± 0.1 | 0.4 ± 0.03 | 50 |
| Probiotics | 1.54 ± 0.04 | 5.45 ± 1.75 | 2.1 ± 0.1 | 0.4 ± 0.02 | 50 |

**Table 1:** Mean values ± standard deviation of initial and final weight, specific growth rate (SGR) and feed conversion rate (FCR) of juvenile Cheraxquadricarinatus for different nutritional substrates. (ANOVA p> 0.05, n = number of organisms per treatment).

| Juvenilecondition | Culture condition | Total Proteins | Total Lipids | Hemocyanin | Ratio |
|---|---|---|---|---|---|
| | | (mg/ml) | (mg/ml) | (mmol/L) | HC/TP (%) |
| Before | Baseline values* | 443.46 ± 5.4 | 2.30± 0.60 | 2.71 ± 0.61 | 39.0 ± 3.15 |
| standard | Filtered Water | | | | |
| metabolic | (Control group) | 483.27 ± 84.2 | 2.46 ± 0.86 | 3.14 ± 0.82 | 40.1 ± 5.39 |
| rate | Primary | | | | |
| evaluation | productivity | 498.4 2± 79.8 | 2.60 ± 1.46 | 3.18 ± 0.82 | 38.9 ± 6.38 |
| | Probiotics. | 488.53 ± 73.5 | 3.48 ± 0.87 | 3.55 ± 0.77 | 43.4 ± 4.91 |
| Post | Filtered Water | | | | |
| metabolic | (Control group) | 525,50 ± 44,1 | 3,05 ± 1,36 | 2,96 ± 0,34 | 34,9 ± 8,90 |
| evaluation | Primary | | | | |
| | productivity | 494.58 ± 44.6 | 3.10 ± 1.50 | 3.10 ± 1.50 | 54.3 ± 10.4 |
| | Probiotics. | 481.7 8± 37.0 | 1.90 ± 0.62 | 2.64 ± 0.82 | 37.1 ± 10.6 |

**Table 2:** Mean values ± standard deviation of the concentration of total protein (PT), total lipids and hemocyanin (HC) in the hemolymph of juvenile Cheraxquadricarinatus for different growing conditions, before standard metabolic rate evaluation and post metabolic evaluation. The ratios between hemocyanin andtotal proteins are presented as percentage ± standard deviation, n=20. (ANOVA p>0.05, n=number of organisms used for biochemical analysis). * Juveniles from culture ponds not subject to the multi-trophic culture system.

for the natural productivity treatment. In a general way, glucose and lactate levels increased after the metabolic trials, when compared with analysis made from random samples at the end of the growth trial in the treatments with filtered water and natural productivity.

## Metabolic rate and the inflection point of dissolved oxygen concentration (IP)

There were no significant differences in terms of standard metabolic rate (Figure 3a), though natural productivity and probiotic treatments showed a slightly higher mean. In terms of the inflection point of dissolved oxygen concentration (IP) the values shown are significantly lower than previously reported data, but there were no significant differences between treatments (Figure 3b).

## Discussion

The experimental treatments did not have an effect on production efficiency of juvenile *Cheraxquadricarinatus*, in terms of growth and survival. Juvenile growth reported in this work compares favorably

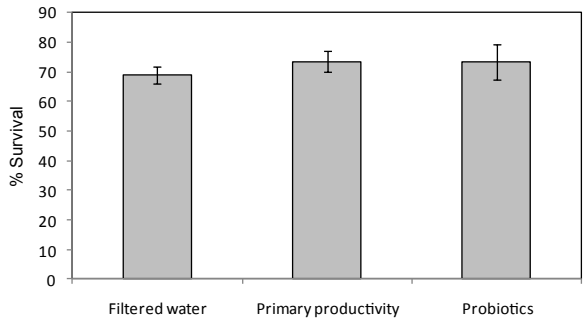


**Figure 1:** Survival (%; mean ± s.d.) during intensive culture of juvenile *C. quadricarinatus* for different treatments: (1) Filtered fresh water system, (2) Addition of primary productivity and (3) Addition of probiotics. (ANOVA p>0.05).

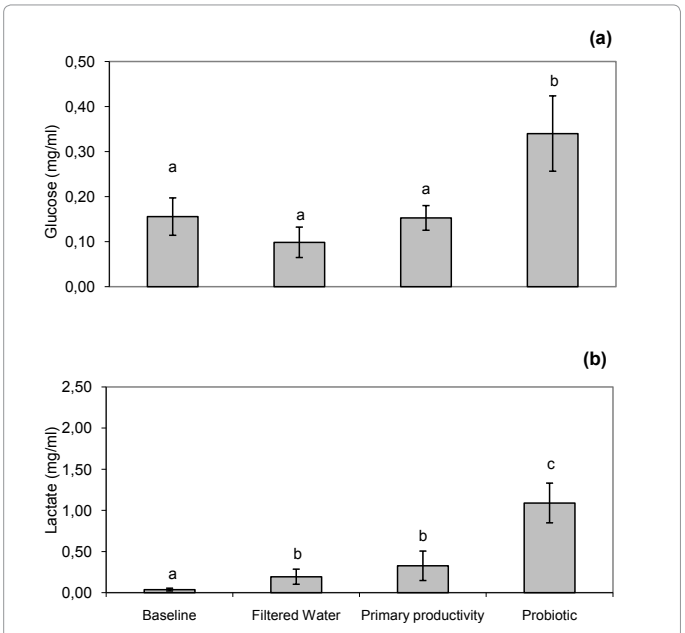


**Figure 2:** Concentration of glucose (a) and lactate (b) in the hemolymph of juvenile *Cherax quadricarinatus* for different nutritional substrates before standard metabolic rate evaluation. The letters denote significant differences between treatments. (ANOVA P<0.05).

with previous reports [67,76]. The meanfood conversion rates (0.4 ± 0.02) were significantly lower than those reported for this and other decapods. Some research [4,43,77] have reported experimental and commercial juvenile *C. quadricarinatus* FCR´s ranging from 0.8 a 1.21. This indicates that the species is energetically efficient, which is consistent with an omnivore with ample capacity to use several food sources for good performance [78], having the ability to use a diverse range of nutrient substrates [79], such as the multitrophic systems

that usually develops in outdoor culture systems [43]. It has been reported that freshwater crayfish increase their total consumption intake when offered a variety of feeds [78], increasing the total energy available. Also, reports indicate that the addition of diverse natural nutrient sources can improve the survival of cultured organisms [46,47]. In our trial, survival was slightly higher for treatments with added natural productivity or probiotics, and better than the reports by other authors [11,76].Variations in specific biochemical parameters in the haemolymph contribute to elucidate both the condition of the organism and adaptation pathways for culture. In this trial we could not determine statistical differences between treatments. Stored lipids are the main source of energy in crustaceans [80,81] and are usually mobilized via the hemolymph when required for reproduction [8,10]. In juveniles, energy demands are evidenced by their growth rates, but energy is also required when stress conditions develop during culture, such as in events of low oxygen concentration. Results show that juveniles exposed to probiotics are probably better prepared for sudden drops in oxygen concentrations as Figure 3 shows higher hemolymph levels of glucose and lactate. Pascual and coworkers [14] reported that total dietary lipids and proteins are usually reflected in hemolymph levels. This would be consistent with this trial, as the mean total glucose and lipid values in the probiotic were, respectively, 36 and 33 mg/, as opposed to 4.34 and 5.84 mg/l for natural productivity. It has been reported that proteins in hemolymph are constituted mainly by hemocyanin (60-90% of total shrimp proteins [35,36]. An increase in hemocyanin synthesis has been reported under limited oxygen availability, as a mechanism to increase the amount of $O_2$transport [82-84]. This was not the case for *Cheraxquadricarinatus*, probably because the exposure time to hypoxic conditions was too short. Indeed, for *Litopenaeusvannnamei*, the increase in hemocynin levels was observed after 2 weeks of exposure to hypoxia, in contrast to two

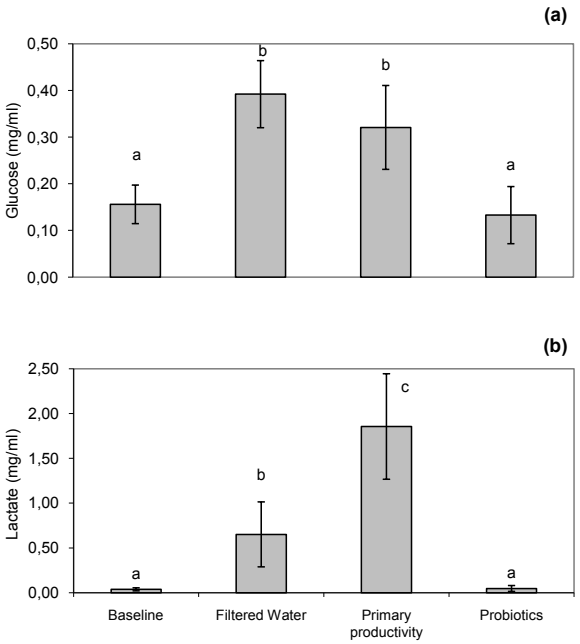


**Figure 3:** Concentration of glucose (a) and lactate (b) in the hemolymph of juvenile *Cherax quadricarinatus* for different nutritional substrates after metabolic evaluation. The letters denote significant differences between treatments. (ANOVA P<0.05).

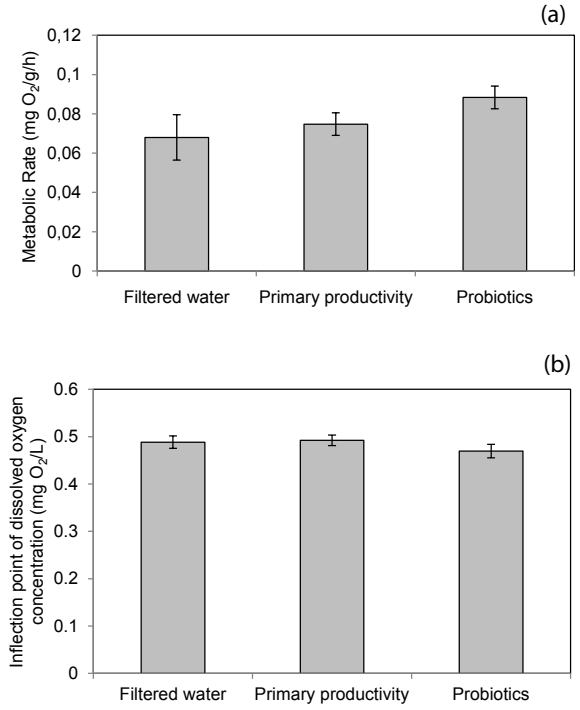


**Figure 4:** Metabolic rate (a) and the inflection point of dissolved oxygen concentration (IP) (b) of juvenile *Cherax quadricarinatus* for different treatments: (1) Filtered fresh water, (2) Addition of primary productivity and (3) Addition of probiotics. (ANOVA P>0.05).

days-exposure [27]. We obtained a hemocyanine/total protein index of 40%, which is consistent with levels reported for crabs and crayfish [37,85,86], and may represent an adaptive strategy by these organisms where they tolerate lower water oxygen concentrations for some time. This hypothesis is supported by the standard metabolism results and inflection point of dissolved oxygen concentration (IP) levels established in the experiment. Standard metabolic rate for juvenile *C. quadricarinatus* was 0.07 ± 0.003 mg.$O_2$/g/h. This compares favorably with reports for other crustacean species that range from 0.38 to 1.56 mg $O_2$/g/h [87-90]. In terms of IP, curves for oxygen consumption by *C.quadricarinatus* showed an inflection point at 0.483±0.002 mg $O_2$/L. Reports for shrimp show a limit in the capacity of the organism to control respiration at levels around 2.0-1.3 mg $O_2$/L [91-93]. According to these results *Cheraxquadricarinatus* is a highly tolerant species to limited oxygen conditions. This is consistent with reports for other species of crayfish, where the organism uses different adaptive strategies allowing them to maintain homeostasis [94], or the use of complex biochemical, physiological or molecular mechanisms of action [94,95] required for crustaceans to survive and tolerate limiting dissolved oxygen conditions. Organisms tolerant to hypoxia frequently control the metabolic rate in both catabolic and anabolic pathways in a coordinated way, reducing total ATP expenditure for extended periods of time [96,97]. This is consistent with low levels of metabolic rate found for *C. quadricarinatus*in this study (Figure 4).

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