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Zootechnical performance of juvenile *Pomacea haustrum* (Revee, 1856) under different diets and salinities[1]

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ABSTRACT - The development of aquaculture through the cultivation of what are considered unconventional species is an option with the potential to solve the problem of food scarcity. The aim of the present study was to evaluate the zootechnical performance of juvenile *Pomacea haustrum* fed different commercial diets, and subjected to different levels of salinity. After seven *P. haustrum* spawn had hatched in the laboratory, the juveniles were randomly selected and transferred to the rearing containers. Two different commercial diets were offered: rabbit feed with a high proportion of ingredients rich in crude vegetable protein (VP), and dog food containing balanced levels of ingredients rich in crude vegetable and animal protein (MP). The selected juveniles were randomly stocked at different salinities (0, 2 and 4 parts per thousand - ppt). The experiment lasted 65 days. Twenty-one hours after the start of the experiment, 100% of the individuals reared at a salinity of 4 ppt had died. There was a significant difference ($p \leq 0.05$) between the results for zootechnical performance (average final weight, weight gain, average final growth, specific growth rate, protein efficiency and apparent feed conversion) for animals reared in fresh water or at a salinity of 2 ppt, regardless of the type of diet. The origin of the protein in the diet had no significant effect on the zootechnical performance of the individuals; however, the salinity of the water impaired development of the animals in direct proportion to its concentration.

Key words: Cultivation. Feed. Development.

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INTRODUCTION

Among animal protein production chains, aquaculture is seen as a fast-growing activity that helps generate jobs and income, and has the potential to reduce hunger and poverty in various places around the world (Siqueira, 2018).

Even with the COVID-19 pandemic, global aquaculture continued to grow in 2020, producing 87.5 million tonnes of aquatic organisms for human consumption, 700 tonnes of shells and pearls for ornamental use, and 35.1 million tonnes of algae used for food or other purposes, giving a total of 122.6 million tonnes produced in 2020, representing a growth of 6.7 million tonnes compared to the 115.9 million tonnes recorded in 2018 (FAO, 2022). The revenue generated by production in 2020 is estimated at USD 281.5 billion, an increase of USD 18.5 billion compared to 2018, and USD 6.7 billion compared to 2019 (FAO, 2022).

Traditionally used in aquaculture, many molluscs are species with high economic value, in addition to performing important ecological functions in numerous ecosystems (Liu *et al.*, 2015). Mollusc aquaculture production exceeded 17.7 million tons in 2020 (USD 81.5 million), representing 25.8% of the total global aquaculture production (FAO, 2022).

Phylum Mollusca is a member of the invertebrate group, characterised by the presence of a soft body, and is the second most diverse phylum in the animal kingdom (more than 100,000 species), surpassed only by phylum Arthropoda (Escoubas *et al.*, 2016; Matthews-Cascon; Martins, 2001).

Molluscs are grouped into eight classes: Monoplacophora, Caudofoveata, Solenogastres, Polyplacophora, Gastropoda, Scaphopoda, Cephalopoda and Bivalvia (Hickman *et al*., 2016).

Within class Gastropoda is family Ampullaridae, which is divided into nine genera, with *Pomacea* being the most important in terms of species diversity (Hayes *et al*., 2008).

Genus *Pomacea* is native to South America, but is currently widely distributed, having been introduced into Asia, North America and Europe (Hayes *et al*., 2008). Although there are no reports of the commercial production of *Pomacea haustrum*, the successful adaptation of this genus has encouraged research on the taxonomy (Hayes *et al.*, 2008), reproduction (Coelho; Calado; Dinis, 2012) and management of the species, in addition to various behavioural studies (Heiler *et al.*, 2008).

Freshwater and land snails are accepted as food in many parts of the world (Ghosh; Jung; Meyer-Rochow, 2017). In various cultures, humans have consumed shellfish since prehistoric times (Hill *et al*., 2015), demonstrating the potential of these organisms as an alternative source of animal protein (Ghosh; Jung; Meyer-Rochow, 2017).

Human consumption of these animals as food, and their relevance in several areas such as ornamental aquaria and ecology, have led to numerous studies in various locations around the world. However, despite their importance, information regarding their biology in their natural habitat is still scarce (Coelho; Calado; Dinis, 2012).

Considering the potential for commercial production of these molluscs, the aim of the present study was to evaluate the zootechnical performance of juvenile *Pomacea haustrum* fed different commercial diets and exposed to different levels of salinity.

MATERIAL AND METHODS

Location of the experiment and collection site

The study was carried out at the Experimental Zoology Laboratory of the Biology Department of the Science Centre at the Federal University of Ceará, from 5 February 2018 to 10 April 2018.

Under collection permit No 13983-4 SISBIO, 24 adult individuals of *Pomacea haustrum* were collected from the Santo Anastácio Reservoir (3°44’36’ S, 38°34’15’ W) on the Pici Campus of the Federal University of Ceará, on 25 January 2018 (Figure 1).

The environmental conditions when the animals were collected were determined by measuring the water temperature, dissolved oxygen, pH and salinity, which showed values of 26 ºC, 4.6 mg L^{-1}, 6.2 and 0 ppt, respectively.

After being caught, the individuals were placed in plastic containers with water from the collection

Figure 1 - Santo Anastácio Reservoir, Pici Campus, Fortaleza, Ceará

site, and transported to the Experimental Zoology Laboratory, where they were acclimatised and then transferred to an aquarium (working volume of 50 L) containing water with a temperature, dissolved oxygen, pH and salinity of 27 ± 1.15 ºC, 5.5 ± 0.6 mg L^{-1}, pH 7.1 ± 0.3 and 0 ppt, respectively (Figure 2).

Water from the public network was used during the experiment. This was first stored for 48 hours in four water tanks (2 of 500 L and 2 of 150 L) until the chlorine had completely volatilised. In the first two days after acclimatisation, there were 12 spawnings, seven of which hatched completely after 11 days. No further eggs hatched after this period.

After hatching, the juvenile *P. haustrum* were randomly selected and transferred to the rearing containers. The newly hatched individuals were then measured and weighed with the aid of digital callipers and a precision digital balance, presenting an average length of 2.3 mm and average weight of 0.005 g (Figure 3).

Experimental design

Thirty circular plastic containers with a working volume of 500 mL (vivaria) were used in a completely randomised experimental design, where the position of each vivarium was randomly determined. Animals from 15 of the vivaria were fed commercial rabbit feed containing a high proportion of ingredients rich in crude vegetable protein (VP), while animals from the other 15 vivaria were fed commercial dog food containing a balanced number of ingredients rich in crude vegetable and animal protein (MP).

Of the 30 vivaria, 10 were supplied with water at a salinity of 0 parts per thousand (ppt), another 10 were supplied with water at a salinity of 2 ppt, and the remaining 10 were supplied with water at a salinity of 4 ppt. Most individuals of genus *Pomacea* are stenohaline and like freshwater. The experimental salinities were deliberately low, although it was known they might give different results. The saline solutions were prepared by diluting common salt in water with the aid of a portable hand-held refractometer (KASVI K52-100).

The present study resulted in 6 different treatments: 2 different diets x 3 different salinities, with 5 replications per treatment, giving a total of 300 animals.

The stocking density was 10 individuals/vivarium. Food was offered once a day ad libitum, six days a week throughout the experiment, except on Sundays and every 16 days when the individual animals were weighed and measured using a precision digital balance and digital callipers.

The water in each vivarium was completely changed daily before supplying the food, to remove leftovers and animal waste in order to improve the quality of the water. The experiment lasted 65 days.

Diets and composition of the feed

Diets based on VP (AgroMix Campestre for rabbits) and MP (Coldog Chips for adult dogs) were obtained from a local supplier. The VP diet included the following ingredients: calcitic limestone, sodium chloride (common salt), soybean bran, wheat bran, dicalcium phosphate, ground whole maize, sodium selenite, copper sulphate, iron sulphate, manganese sulphate, zinc sulphate, folic acid, pantothenic acid, choline chloride, calcium iodate, vitamin A, vitamin B1, vitamin B12, vitamin B2, vitamin B6, vitamin D3, vitamin E, vitamin K3, chlorhexidine hydrochloride, DL-methionine, L-lysine, additive and biotin.

The VP diet included the following possible substitutes: extruded soybean meal, whole soybean meal (toasted grains), calcined bone meal, wheat flour, ground whole sorghum, millet, cashew nut meal, babassu meal, Protenose®, rice bran, sunflower bran, Refinazil®, rice grits, mineral premix and vitamin premix, with the following guaranteed levels of product

Figure 2 - Collected parents of *Pomacea haustrum*

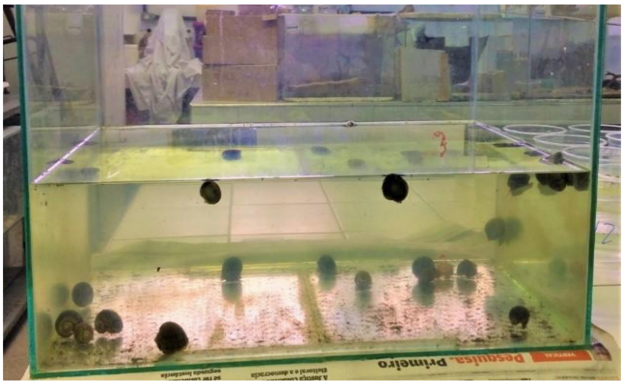

Figure 3 - Juvenile *Pomacea haustrum*

per kilogram: moisture (max.) 120 g/kg, crude protein (min.) 140 g/kg, ether extract (min.) 20 g/kg, crude fibre (max.) 200 g/kg, calcium (max) 15 g/kg, calcium (min) 12.75 g/kg, mineral matter (max) 140 g/kg, phosphorus (min) 3 g/kg and acid detergent fibre 150 g/kg.

The basic ingredients of the MP diet consisted of poultry offal meal, meat meal, ground whole maize, soybean meal, sorghum, vegetable oil, blood meal, sodium chloride (common salt), chicken hydrolysate, calcium propionate, butylated hydroxytoluene (BHT), folic acid, pantothenic acid, biotin, choline chloride, calcium iodate, niacin, pyridoxine, riboflavin, sodium selenite, cobalt sulphate, copper sulphate, manganese sulphate, zinc sulphate, ferrous sulphate, thiamine, vitamin A, vitamin B12, vitamin D3, vitamin E, vitamin K, tartrazine yellow, burgundy red and titanium dioxide.

The MP diet included the following possible substitutes: maize gluten 21%, maize gluten 60%, maize germ, chicken oil, liver hydrolysate and wheat bran, with the following guaranteed levels per kilogram of product: omega 6 fatty acids (min) 18 g/kg, omega 3 fatty acids (min) 1.1 g/kg, calcium (min) 10 g/kg, calcium (max) 21 g/kg, chlorine (min) 4.8 g/kg, ether extract (min) 60 g/kg, phosphorus (min) 6 g/kg, fibrous matter (max) 50 g/kg, mineral matter (max) 110 g/kg, crude protein (min) 200 g/kg, sodium (min) 3.2 g/kg and moisture (max) 120 g/kg.

Water quality parameters

Every morning before changing the water in the vivaria, the temperature and dissolved oxygen were monitored in each of the experimental units with the aid of a portable digital oximeter (MO-910); the pH was monitored using a digital pH meter (HANNA-HI98127).

Zootechnical performance parameters

Using the weight and length data obtained when the individuals were measured, the following zootechnical parameters were determined: average final weight, weight gain, average final growth; biomass; biomass gain, apparent feed conversion, condition factor, specific growth rate, feed efficiency, protein efficiency index and survival. The zootechnical parameters were evaluated using the following equations:

$$AW = \frac{\sum P}{N} \quad (1)$$

where: AW = average weight (g); P = sum of the weight of the sampled individuals (g); N = number of sampled individuals.

$$WG = AWf - AWi \quad (2)$$

where: GP = weight gain (g); AWf = average final weight (g); AWi = average initial weight (g).

$$AG = Lf - Li \quad (3)$$

where: AG = average growth (mm); Lf = average final length (mm); Li = average initial length (mm).

$$B = AW \times N \quad (4)$$

where: B = biomass (g); AW = average weight (g); N = number of sampled individuals.

$$GB = Bf - Bi \quad (5)$$

where: GB = gain in biomass (g); Bf = final biomass (g); Bi = initial biomass (g).

$$AFC = \frac{FI}{GB} \quad (6)$$

where: AFC = apparent feed conversion (g of feed per g of individual); FI = feed intake (g); GB = gain in biomass (g).

$$CF = \frac{W}{L^3} \times 100 \quad (7)$$

where: CF = condition factor; W = weight of the animal (g); L = total length of the animal (mm).

$$SGR(\%) = \frac{\ln Wf - \ln Wi}{t} \times 100 \quad (8)$$

where: SGR = specific growth rate (%); ln = Napierian logarithm; Wf = average final weight (g); Wi = average initial weight (g); t = length of the experiment (days).

$$FE(\%) = WG \times \frac{100}{QF} \quad (9)$$

where: FE = feed efficiency (%); WG = weight gain (g); QF = quantity of feed consumed (g).

$$PEI = \frac{WG}{QF} \quad (10)$$

where: PEI = protein efficiency index; WG = weight gain (g); QF = amount of feed consumed (g).

$$S(\%) = \frac{Nf}{Ni} \times 100 \quad (11)$$

where: S = survival rate (%); Nf = final number of individuals; Ni = initial number of individuals.

Statistical analysis

The statistical analyses were carried out using the BioEstat 5.3 software. The data on the zootechnical performance parameters and water quality were subjected to two-way analysis of variance, with the mean values and standard deviations determined for the five replications of each experiment. Before applying the analysis of variance (ANOVA), the Shapiro-Wilk test was used to test the normality of the data, and the test of homoscedasticity to evaluate the variability of the variances. The mean values of the treatments were compared using Tukey’s test. The significance level was 5%.

RESULTS AND DISCUSSION

Water quality parameters

Fifteen hours after the start of the experiment, 90% mortality was observed in the animals reared at a salinity of 4 ppt, which rose to 100% after 21 hours. In the freshwater, some of the animals stretched their tentacles and respiratory siphon normally, whereas in the vivaria at higher salinities, this behaviour was reduced or non-existent, possibly due to salt stress. For each of the salinities under study, the animals responded by climbing up the edges of the vivaria, which were covered to prevent them from escaping. After excluding the ten vivaria with water at a salinity of 4 ppt, the remaining twenty were redistributed in a completely randomised experimental design (Figure 4).

The physical and chemical parameters measured during the experiment (temperature, dissolved oxygen and pH) showed no significant difference in mean value between treatments ($p > 0.05$) (Table 1). The values of the parameters remained within the ideal range for aquaculture. (Bernatis; Mcgaw; Cross, 2016; Yoshida *et al*., 2014).

Keeping the various water quality parameters within their respective ideal ranges for aquaculture is essential for the successful cultivation of the most diverse of aquatic organisms, since unsuitable conditions may harm the various metabolic processes, such as growth, reproduction, health and survival, reflecting in the final quality of the harvested product (Wang *et al*., 2022).

Figure 4 - Final layout of the vivaria where the animals were kept

Dissolved oxygen is the most important water quality parameter in aquaculture because its presence is essential in accessible concentrations that enable the cultivated organisms to carry out processes that are necessary for survival, such as breathing, locomotion, feeding and biosynthesis (Wang *et al*., 2022). Most aquatic animals are able to thrive at dissolved oxygen levels of at least 4 mg L^{-1} (Lima *et al*., 2013).

Little is known about dependence on breathing air in *Pomacea*; however, these gastropods are able to remain in good condition even when access to atmospheric air is blocked. Among the Ampullariidae, genus *Pomacea* stands out for making the best use of atmospheric air (Seuffert; Martin, 2013).

In aquaculture, pH values in the range of 6.5 to 9 are typically ideal. Both adult and juvenile specimens of *P. haustrum* show a high tolerance to variations in pH, and will develop normally in the range of 5.5-9.5 for a period of 28 days. They are also resistant to desiccation. In humid environments, newborns can survive up to 49 days and adults up to 365 days. During the dry period, there is a significant reduction in heart rate, which drops from 64-66 bpm to 2-4 bpm (Bernatis; Mcgaw; Cross, 2016; Yoshida *et al*., 2014).

Together with dissolved oxygen and pH, water temperature is one of the most important parameters in the development of ectothermic animals in general, as it directly interferes with the function and speed of metabolic activity and, consequently, in the efficiency and effectiveness of assimilating any nutrients in the diet (Guerreiro *et al*., 2012). The interrelationship between these parameters is well known, so that changing one of them can, by a chain reaction, change the others. It is therefore necessary to maintain each of these parameters within the ideal range for the cultivated species, in order to avoid future losses.

Within genus *Pomacea*, the species *P. canilaculata* and *P. maculata* represent the most widespread invasive ampulariae. In general, the mobility of these animals varies with the water temperature, the ideal temperature estimated at 25 °C. They are, however, capable of

Table 1 - Water quality parameters when rearing juvenile *Pomacea haustrum* under different diets (VP and MP) and salinities (0 and 2 ppt) over 65 days

Variable	Treatment			
	VP0	VP2	MP0	MP2
Temperature (ºC)	25.16 ± 0.34	25.42 ± 0.32	25.23 ± 0.43	25.29 ± 0.27
Dissolved oxygen (mg L^{-1})	5.7 ± 0.46	5.5 ± 0.57	5.8 ± 0.51	5.5 ± 0.49
pH	7.15 ± 0.25	7.06 ± 0.24	7.14 ± 0.21	7.10 ± 0.21

The values show the mean values ± standard deviation for each variable surviving for short periods in extreme situations, such as for 10 to 14 days in environments with a temperature range between -4 °C and 40 °C; albeit the eggs do not develop below 16 °C. The ability to resist cold is related to the resistance to desiccation. Interestingly, young specimens of intermediate length (10 - 20 mm) show more resistance to cold than do smaller juveniles or adults (Bernatis; Mcgaw; Cross, 2016; Yoshida *et al*., 2014).

Zootechnical performance parameters

The diets differed, not only in terms of the main protein sources, but also in the protein concentration itself, and probably in other variables. However, regardless of the source of the feed, the performance of animals reared in salt water was worse.

At the end of the 65 days of the experiment, a negative trend was seen in the effect of water salinity on the zootechnical performance of the juvenile *P. haustrum*, showing significant differences between treatments ($p \leq 0.05$); the different diets, however, had no effect on the results ($p > 0.05$). Further studies are necessary to confirm this trend, or the present study can be replicated over a longer period (Table 2).

Most individuals of genus *Pomacea* are stenohaline, having low resistance to salinity, with young individuals able to withstand approximately 28 days in salinities of up to 8 ppt. At a salinity of 8 ppt, the mortality rate is very high among newborns during the first four days. Adults survive for a very short time when exposed to salinities greater than 16 ppt (Bernatis; Mcgaw; Cross, 2016; Yoshida *et al*., 2014).

Earlier research on *Pomacea canilaculata* by Yang *et al*. (2018) found that salinity has a significant effect on the survival and growth of these individuals. With increasing salt stress, the snails exposed for longer periods showed a marked increase in mortality. About half of the animals died within 48 h of exposure to high salinities, whereas in water at 5 ppt and 10 ppt, optimal growth was obtained compared to fresh water, suggesting that *P. canilaculata* can tolerate low salinities (Yang *et al*., 2018). The salinity of the water also had an effect on the survival of *Pomacea m*aculata, with 100% survival in freshwater, gradually decreasing as the salinity increased (Martin; Valentine, 2014).

Disregarding the two treatments maintained at a salinity of 4 ppt (VP4 and MP4), there was no significant difference between the survival rates ($p > 0.05$), which were excellent for the other treatments; this result can be attributed to the quality of the management and the water. The other parameters showed significant differences between the treatments in fresh water (0 ppt) (VP0 AND MP0) and brackish water (2 ppt) (VP2 and MP2) ($p \leq 0.05$).

It can be concluded that in fresh water the nutrients supplied by the MP diet were more suitable than those in the VP diet. In the brackish water this trend was reversed. However, despite the trends, there was no statistical difference between treatments at the same salinity ($p > 0.05$). Weight loss may be seen at moderate to high salinities and is possibly related to water loss. There was a statistical difference ($p \leq 0.05$) in average final growth (cm) between treatments VP0[a] and MP0[a] in relation to VP2[b] and MP2[b], ranging from 4.41 ± 0.49 cm in treatment MP0 to 3.25 ± 0.87 cm in treatment MP2.

According to Mendoza *et al*. (1999), individuals of *Pomacea bridgesi* showed better growth when maintained on diets based on animal protein instead of vegetable protein, in line with the trend seen in the present study

Table 2 - Zootechnical performance of juvenile *Pomacea haustrum* under different diets (VP and MP) and salinities (0 and 2 ppt) over 65 days

Variable	Treatment			
	VP0	VP2	MP0	MP2
Survival (%)	100	96	98	96
Average final weight (g)	0.055 ± 0.022[a]	0.034 ± 0.011[b]	0.063 ± 0.018[a]	0.032 ± 0.015[b]
Weight gain (g)	0.050 ± 0.019[a]	0.029 ± 0.0009[b]	0.058 ± 0.017[a]	0.027 ± 0.012[b]
AFG (mm)	3.98 ± 0.77[a]	3.32 ± 0.74[b]	4.41 ± 0.49[a]	3.25 ± 0.87[b]
SGR (% dia)	3.75 ± 0.23[a]	2.99 ± 0.18[b]	3.96 ± 0.19[a]	2.90 ± 0.16[b]
Final biomass (g)	2.75 ± 0.03[a]	1.63 ± 0.04[b]	3.09 ± 0.07[a]	1.54 ± 0.03[b]
Gain in biomass (g)	2.50 ± 0.02[a]	1.38 ± 0.05[b]	2.84 ± 0.03[a]	1.29 ± 0.02[b]
PEI	0.013 ± 0.002[a]	0.007 ± 0.002[b]	0.015 ± 0.001[a]	0.006 ± 0.001[b]
Condition factor	0.087 ± 0.001[a]	0.093 ± 0.002[b]	0.074 ± 0.003[a]	0.093 ± 0.001[b]
AFC (g/g)	2.40 ± 0.26[a]	4.35 ± 0.33[b]	2.11 ± 0.21[a]	4.65 ± 0.31[b]

The values show the mean values ± standard deviation for each variable. Different letters on the same line indicate a significant difference between the mean values ($p \leq 0.05$). AFG – Average final growth; SGR – Specific growth rate; PEI – Protein efficiency index; AFC – Apparent feed conversion (although no significant difference was proven), and comparing the different diets in fresh water.

Bocanegra *et al*. (2015) reported that a supply of green water with a predominance of *Chlorella* and *Scenedesmus* microalgae had a positive effect on growth and survival during the initial stages of *P. maculata* compared to clean water, and that water with a shorter renewal period showed better results compared to water that was renewed late or in smaller proportions.

The specific growth rate also showed a significant difference (% day), varying from 3.96 ± 0.19% in treatment MP0 to 2.90 ± 0.16% in treatment MP2, confirming the positive performance of these parameters in individuals reared in freshwater.

There was a statistical difference ($p \leq 0.05$) in the following parameters: final biomass (g) between treatments VP0 and MP0 in relation to VP2 and MP2, varying from 3.09 ± 0.07 g in treatment MP0 to 1.54 ± 0 .03 g in treatment MP2; gain in biomass (g), varying from 2.84 ± 0.03 g in treatment MP0 to 1.29 ± 0.02 g in treatment MP2; and the protein efficiency index, varying from 0.015 ± 0.001 in treatment MP0 to 0.006 ± 0.001 in treatment MP2. Since protein assimilation is influenced by several variables, the protein efficiency index works as an indicator of the proportion of crude protein in the diet that was converted into body weight (Rossato *et al*., 2014).

The condition factor showed a significant difference ($p \leq 0.05$) between treatments VP0 and MP0 relative to VP2 and MP2, ranging from 0.074 ± 0.003 in treatment MP0 to 0.093 ± 0.002 in treatment VP2. Insufficient nutritional intake can lead to variations in the value of the condition factor, as the parameter results from the relationship between the length and body weight of the animal. The value of the condition factor is an alternative way of determining the physiological state of a species and its spawn (Bocanegra *et al*., 2015). The study showed that the values of the condition factor between the treatments reflected in positive allometric growth, with the increase being due to length.

Once again, there was a significant difference ($p \leq 0.05$) between treatments PV0 and PM0 compared to PV2 and PM2. Determining the apparent feed conversion is the most appropriate way of assessing the quality of a given diet since it relates the proportion of energy ingested and deposited in the tissues. In other words, how much of the feed was effectively assimilated for the animal's development in terms of growth and fattening.

CONCLUSIONS

1. The results of this study show that the protein content of the diet did not affect the development of juvenile *P. haustrum*, while higher levels of salinity in the water had a negative effect on development;
2. *P. haustrum* shows the potential to be cultivated on different scales (pilot, small or large), under different cultivation systems (extensive or intensive), and in joint cultivation (poly- or monoculture), as the animal is hardy, and requires little care in terms of water quality or feeding;
3. Feeding costs are considerably reduced compared to species that are usually reared commercially by aquaculture, since it is possible to choose a feed with a lower protein content and of plant origin (PV), without compromising the performance of the animals. The feed is also less expensive and is consumed in significantly smaller quantities.

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