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# The potential of *Hoplias malabaricus* (Characiformes: Erythrinidae), a Neotropical carnivore, for aquaculture

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## ABSTRACT

We describe key production-related traits of trahira (*Hoplias malabaricus*), an aquaculture candidate species, in a semi-intensive fish farming system. We quantified growth from hatch through grow-out at two stocking densities and evaluated fillet protein, lipids and fatty-acid content. We made 2430 observations of growth and morphometrics on 270 juvenile fish allocated to four fish ponds, two at 5 fish/m<sup>2</sup> (90 animals) and two at 10 fish/m<sup>2</sup> (180 animals) through 11 months. The fish reached an average length of 27.5 cm (± 0.38) and 27.0 cm (± 0.24), and weight of 263.0 g (± 10.54) and 246.7 g (± 6.89), respectively, at the low and high densities. There was no significant difference in weight ( $P > 0.05$ ) between the densities for any of the parameters assessed, and hence the same growth curve applied to both densities ( $A = 292.9$ ;  $B = 28.1$ ; and  $K = 0.65$ ). Standard length, however, differed significantly ( $P < 0.05$ ) between densities, with  $K = 0.35$  at the low and  $K = 0.29$  at the high density. At both stocking densities, total lipids increased throughout the trial, while protein content decreased. Oleic acid (C18:0) increased throughout the experimental period, while docosahexaenoic acid and the n3/n6 profile decreased, with no differences among stocking densities. *Hoplias malabaricus* may prove a viable aquaculture species in Neotropical regions, where development of native species may contribute to sustainable aquaculture production.

## 1. Introduction

Aquaculture development has driven many alien species introductions worldwide (Naylor, Williams, & Strong, 2001; Pérez, Alfonsi, Nirchio, Muñoz, & Gómez, 2003). Environmental risks posed by fish introductions may threaten native fish diversity; hence, evaluation of native aquaculture candidate species is strategic for development of sustainable fish farming (Ross & Beveridge, 1995). The Neotropical region is rich in native fish genetic resources potentially useful for aquaculture (Hilsdorf & Hallerman, 2017). Among those resources, species of the genus *Hoplias* (trahiras) comprise eight valid species (Oyakawa & Mattox, 2009) which occur throughout drainages from Costa Rica to Argentina (Froese & Pauly, 2017). The different trahiras are economically important for local artisanal fisheries in many Neotropical countries (Oliveira & Nogueira, 2000; Ramsundar, 2005). However, trahiras have not been farmed even at small scale in

Neotropical countries. For instance, the aquaculture production of all trahiras in Brazil in 2016 was just 806,365 kg (IBGE, 2016, p. 44).

*Hoplias malabaricus*, also known as wolf fish, tiger fish, or trahira (Image S1), is a candidate species for aquaculture production. It is a carnivorous fish that preferentially inhabits lotic environments, also occurring in lentic waters such as lakes and reservoirs (Azevedo & Gomes, 1943). *H. malabaricus* is well adapted to hypoxic waters, can maintain constant oxygen uptake down to a  $\text{PiO}_2$  of 20 mmHg (Rantin, Glass, Kalinin, Verzola, & Fernandes, 1993), and can reproduce in these hypoxic waters (Gomes, Honji, Tolussi, Ribeiro, & Moreira, 2015). Constraints to full development of aquaculture production of *H. malabaricus* include feeding costs, presence of intramuscular bones, and the continuing availability of market-sized fish from capture fisheries. However, new processing techniques, yielding boneless trahira fillets, high fillet yield – around 44%, and high nutritional quality of the meat (Santos, Melo, Lopes, & Malgarim, 2001) have attracted consumers to

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trahira as a delicacy fish. Despite the relatively high costs of producing carnivorous fish, high meat quality and attractive prices in local markets have made trahira a good choice for commercial fish farming. However, technical aquaculture limitations, such as commercial availability of feed-trained fingerlings (Bondad-Reantaso, 2007), cannibalism during the fingerling stage (Luz, Salaro, Souto, & Zaniboni-Filho, 2000), lack of nutritional studies (Vieira & Lopes, 2006), and lack of assessment of growth rate in farming systems hinder achievement of large-scale aquaculture production of trahira.

Characterization of the fish growth curve supports identification and adoption of management practices that optimize production, providing insight into the nutritional needs of each growth phase (Tholon & Queiroz, 2009). These curves can be used to determine the optimal age for slaughter (Luo et al., 2015; Mello et al., 2015). Growth models used to characterize the growth of fishes (Enberg, Dunlop, & Jørgensen, 2008, pp. 1564–1572) include the von Bertalanffy (1938), logistic (Richards, 1959), and Gompertz (1825) models.

Development of attractive aquaculture products also depends on their nutritional value. Consumption of fish and fish products is highly recommended by health authorities, not only for their protein content, but also as an important source of essential fatty acids (FAs) (Garaffo et al., 2011), mainly omega 3 (n3) polyunsaturated fatty acids (PUFAs). To develop an aquaculture candidate species, it is useful to combine profitable growth rate with high nutritional content, such as high levels of PUFAs (Ogata, Emata, Garibay, & Furuita, 2004).

Assessment of trahira production under culture conditions (Luz et al., 2000; Luz, Salaro, Souto, Reis, & Sakabe, 2001; Nogueira et al., 2005; Salaro, Luz, Nogueira, Sakabe, & Lambertucci, 2003; Salaro, Luz, Sakabe, Kasai, & Lambertucci, 2008) have mostly focused on aspects of producing the species *Hoplias lacerdae*. To gain insight into the potential of *H. malabaricus* for aquaculture in the Neotropics, we: (i) determined the best growth model for *H. malabaricus* through one year of semi-intensive production; (ii) quantified the growth rate of *H. malabaricus* from fry through grow-out under contrasting production densities; and (iii) assessed fillet quality in terms of total protein, lipid levels and fatty acid profile.

## 2. Material and methods

### 2.1. Fish and rearing conditions

A total of 270 *H. malabaricus* juveniles were obtained from the Brumado Fish Hatchery, Ltd. in the State of São Paulo, which had produced them from wild broodstock. To assure proper taxonomic identification, the broodstock was assessed morphologically based on diagnostic characters set out for the species by Oyakawa and Mattox (2009).

The growth trial was carried out at the Ponte Nova Fish Farm located in Salesópolis County, State of São Paulo, Brazil (23°31'85" S, 45°50'77" W, 798 m elevation). The region has a subtropical climate with mean annual temperature of 19 °C. Juveniles were stocked in four concrete ponds of 12.6 m<sup>3</sup> (4.5 m × 2.0 m × 1.4 m) in a completely randomized design. To assess the rate of growth models under contrasting stocking densities, fish were stocked at 5 fish/m<sup>2</sup> (90 juveniles) and 10 fish/m<sup>2</sup> (180 juveniles). Fish were fed exclusively using an extruded commercial diet (NutriPeixe TC 40, Purina, Brazil), and the feeding regime was based on that commercially proposed for carnivorous fishes (Tables S1 and S2). The growth trial lasted 11 months from September to August. Every 40 days through the experiment, all individuals were anesthetized with 0.01% benzocaine previously diluted in ethanol. Individuals were weighed using a balance with a 1-g precision and standard length was measured with the aid of an analog caliper.

Each day, we assessed the quality of continuously flowing inlet water by measuring the temperature (°C) and oxygen (mg/L) using a Model 55-12 FT meter (YSI, Yellow Springs, Ohio, USA), total ammonia

level using ammonia test strips (Tetra®, Blacksburg, VA, USA), and pH using colorimetric test strips (Macherey-Nagel®, Bethlehem, PA, USA).

### 2.2. Grow-out performance and growth model data analysis

To determine the most appropriate nonlinear model for characterization of growth of trahira at each stocking density, we tested: the Gompertz model, in which  $y = A \exp(-Be^{-Kx})$ ; the logistic model, in which  $y = A(1+Be^{-Kx})^{-1}$ ; and the von Bertalanffy model, in which  $y = A(1-Be^{-Kx})^3$ , where  $y$  represents the trait (weight or standard length);  $e$  is the base of natural logarithms; and  $x$  is the age in days. Generally, the fixed effects  $A$ ,  $B$ , and  $K$  represent the average values of the population parameters. These model parameters have the following biological interpretations:  $A$  is the superior asymptotic response trait,  $B$  is a constant without biological interpretation, and  $K$  is the ratio between the relative growth rate and the adult animal response traits (weight and standard length). The higher the value of  $K$ , the more precocious is the animal.

The choice of the best nonlinear model for each trait (weight and standard length) and each density (5 fish/m<sup>2</sup> and 10 fish/m<sup>2</sup>) was based on the Akaike Information Criterion (AIC) (Akaike, 1974; Sakamoto, Ishiguro, & Kitagawa, 1986), as suggested by Katsanevakis (2006), and the Bayes Information Criterion (BIC) (Schwarz, 1978). Using this approach, the smaller the criterion value, the better-fitting the model.

After the choice of the best-fitting nonlinear model for each trait, we used a mixed-model approach to obtain accurate estimates of function parameters of animal growth (Cao & Wang, 2011; Pinheiro & Bates, 1995; Schinckel & Craig, 2002; Sofaer, Chapman, Sillett, & Ghalambor, 2013). Then the model parameters ( $A$ ,  $B$ , and  $K$ ) were maintained as fixed or included as random in the most appropriate nonlinear model using the Likelihood-Ratio Test (LRT) as a criterion, and then estimated and compared among the density. A random effect was considered to have an express influence when its inclusion in the model caused significant increases in  $\ln$ . To determine the most adequate model, the likelihood-ratio test (LRT) was applied in reduced sequence models (Kimura, 1980; Rao, 1973, pp. 417–420).

The value obtained for the statistical LRT then was compared to the chi-square table ( $\chi^2_{\text{tab}}$ ) with the appropriate degrees of freedom: if  $LR > \chi^2_{\text{tab}}$ , the effect was considered to have a significant influence. The level of significance used was 5%. LRT was also applied for equality of the parameters  $A$ ,  $B$  and  $K$  between the densities studied (Regazzi, 2003) to assess whether a single curve would be adequate to describe the growth (weight- or length-at-age) of trahiras grown at different densities. The growth-model testing described above was performed using the packages, Stats version 3.4.1, nlme version 3.1–131, and car version 2.1–5 of R.3.4.1 (R Core Team, 2017).

For the traits we studied, AGR is the weight or length gain observed over a unit of time. As the age is updated, AGR represents the average growth rate of animals in the population. We calculated the absolute growth rate (AGR) at age from the first derivative of the adjusted growth model.

Growth parameters based on morphometric measures were calculated to assess growth performance and models, including: condition factor ( $K$ ) = total weight/total length<sup>3</sup> × 100; feed efficiency (FE) = biomass increment × 100/food consumed; specific growth rate (SGR) =  $100 \times [(\ln(W_f) - \ln(W_i))/T]$ , where  $\ln(W_f)$  and  $\ln(W_i)$  are the natural logarithms of the weight at the beginning ( $W_i$ ) and end ( $W_f$ ) of the experiment, and  $T$  is the time interval in days; and survival (S) = final number of fish × 100/initial number of fish. The coefficient of variation for weight (CV, %) within tanks was used to assess size variation and was calculated as: weight standard deviation/mean weight × 100. The coefficient of variation was used because body weight often shows a higher coefficient of variation in fish than in terrestrial livestock (Gjedrem, 1998). The length-weight relationship was estimated by logarithmic transformation of equation  $W = a \times L^b$ , where  $W$  = body weight (g);  $L$  = standard body length (cm);  $a$  = the

intercept; and  $b$  = the regression slope.

The growth performance metrics are expressed as mean  $\pm$  standard error. A one-way analysis of variance (ANOVA) was used to test the significance ( $P < 0.05$ ) of any difference in growth at the two stocking densities. All data analyses were performed using SIGMA Stat for Windows Version 2.0 (Jandel Corporation<sup>®</sup>).

### 2.3. Lipids and protein content profile

Ten fish from each experimental group (5 fish/tank) were collected at one month of age (initial sample), after 90 days (five months of age - intermediate sample), and at the end of the experiment (nine months of age - final sample) to analyze body composition. These animals were sacrificed by cervical dislocation after sedation (with benzocaine at 100 mg/L) with Institutional Animal Care and Use Ethics Committee approval (#2018/01). A sample of white epaxial muscle was collected from the anteriodorsal region and frozen at  $-80^{\circ}\text{C}$  until analysis.

Total proteins were extracted from muscle tissue by precipitation and solubilization according to Milligan and Girard (1993). Tissue protein content was analyzed by the colorimetric method of Lowry, Rosebrough, Farr, and Randall (1951), using bovine serum albumin to develop the standard curve (Sigma Diagnostics, St. Louis, MO, USA), and expressed as mg/g of dry mass. Total lipids were extracted from muscle and diet with chloroform: methanol: water (2:1:0.5) according to Folch, Less, and Stanley (1957), modified by Parrish (1999) for aquatic organisms. The total lipid content of tissues was quantified by the enzyme-colorimetric method described by Frings, Fendly, Dunn, and Quenn (1972) using cod liver oil (Sigma Diagnostics, Inc., Livonia, MI) for the standard curve and expressed as mg/g of mass.

Methylation of the lipid extracts was performed with acetyl chloride (5%  $\text{CH}_3\text{COCl}$  in methanol) (Christie & Han, 2010, p. 448), and fatty acid (FA) composition was determined as methyl esters using a gas chromatograph (GC) (Model 3900, Varian Analytical Instruments, Walnut Creek, CA, USA) coupled to flame ionization detection (FID). FAs were identified by comparing retention time, using a known standard of methyl esters (FAME) (Supelco, 37 components; Sigma-Aldrich; Larodan Malmö, Sweden, Mixture Me93, and Qualmix PUFA Menhaden Oil). The FAMEs were analyzed on a capillary column, CP Wax 52 CB, with 0.25  $\mu\text{m}$  thickness, 0.25 mm inside diameter, and 30 m length. Hydrogen was used as the carrier gas at a linear velocity of 22 cm/s. The temperature program was  $170^{\circ}\text{C}$  for 1 min followed by a  $2.5^{\circ}\text{C}/\text{min}$  ramp up to  $240^{\circ}\text{C}$ , and a final hold time of 5 min. Injector and flame ionization detector (FID) temperatures were 250 and  $260^{\circ}\text{C}$ , respectively.

The percentage of FA was used to calculate the indices of atherogenicity (AI) and thrombogenicity (TI), as proposed by Ulbricht and Southgate (1991):

$$AI = [(4 \times \text{C14:0}) + \text{C16:0} + \text{C18}) / (\Sigma \text{MUFA} + \Sigma \text{n6 PUFA} + \Sigma \text{n3 PUFA})];$$

$$TI = [(\text{C14:0} + \text{C16:0} + \text{C18:0}) / (0.5 \times \Sigma \text{MUFA}) + (0.5 \times \Sigma \text{n6 PUFA}) + (3 \times \Sigma \text{n3 PUFA}) + (\text{n3} / \text{n6})]$$

Values were expressed as mean  $\pm$  standard error. The comparison of growth parameters and body composition between stocking densities during the experiment were performed using one-way analysis of variance (ANOVA). For all the analyses, differences were considered to be significant when  $P \leq 0.05$ . These analyses were performed using the statistical software BioStat 2009 (AnalystSoft, Inc., Walnut, CA, USA).

## 3. Results

### 3.1. Test environment

The average water temperature during the spring/summer season (September to March) was  $24.3^{\circ}\text{C}$  ( $\pm 2.2$ ) and during the autumn/

**Table 1**

Akaike (AIC<sup>a</sup>) and Bayesian (BIC<sup>a</sup>) information criteria<sup>a</sup> for different growth models applied to the weight and standard length (SL) traits in relation to age for trahira, *Hoplias malabaricus*.

Model	5 fish $\text{m}^{-2}$			
	Weight	SL	AIC	BIC
Logistic	<b>7261.32</b>	<b>7312.26</b>	<b>2979.00</b>	<b>3029.94</b>
Gompertz	7287.53	7338.47	2985.4	3036.34
Von Bertalanffy	7307.54	7358.48	2988.48	3039.41
10 fish $\text{m}^{-2}$				
Logistic	<b>13290.19</b>	<b>13348.13</b>	<b>5242.54</b>	<b>5300.48</b>
Gompertz	13368.91	13426.85	5260.92	5318.86
Von Bertalanffy	13410.95	13468.90	5267.59	5325.53

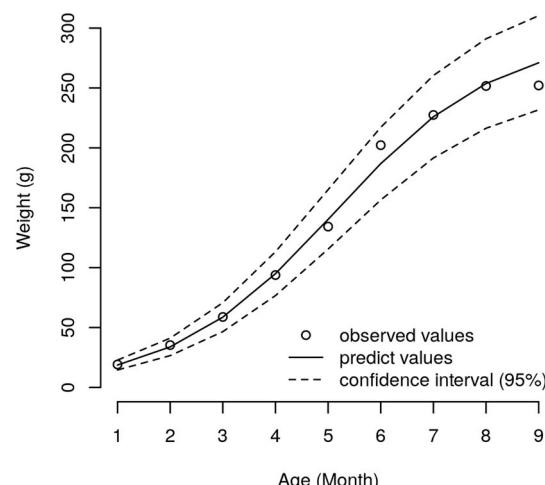
<sup>a</sup> Bold values indicate the best-supported model for each criterion.

winter (March to September) was  $19.6^{\circ}\text{C}$  ( $\pm 2.6$ ). The lowest temperature measured was  $16.1^{\circ}\text{C}$  and the highest  $28^{\circ}\text{C}$ . Dissolved oxygen ranged from 5 to 9 mg/L, the ammonium ion concentration ranged from 0.10 to 0.20 mg/L, and the pH ranged from 6.7 to 7.2. These water quality parameters were within the range for routine fish farming operations in the region.

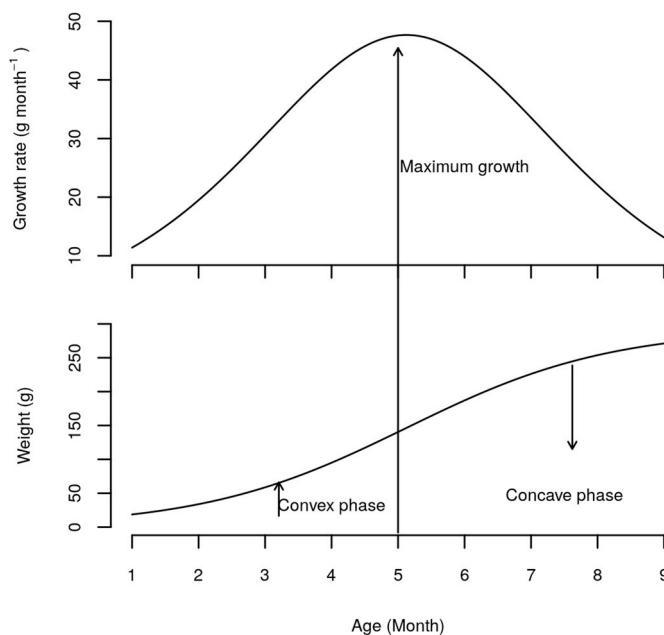
### 3.2. Growth model assessment

According to the AIC and BIC criteria (Table 1), for both weight and standard length at both stocking densities, the logistic model best fit the data for size-at-age of the animals. The likelihood ratio test for the weight trait indicated that the  $A$  and  $B$  parameters at both densities must be included as random parameters in the logistic model ( $P < 0.05$ ), while  $K$  must be included as a fixed parameter ( $P > 0.05$ ). The same outcomes were observed for the trait standard length (Table 1).

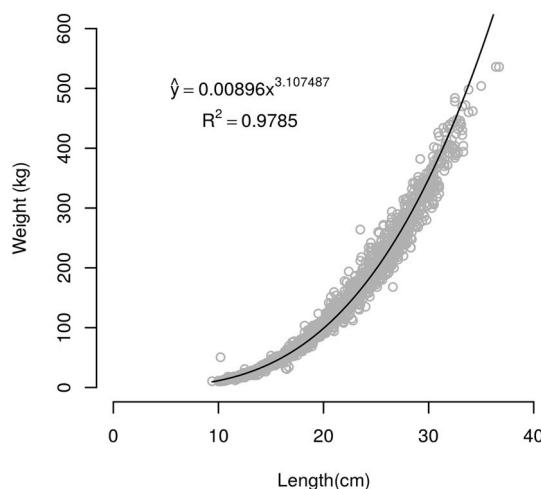
Comparing the growth curves for both densities, the parameter-equality tests applied to the logistic model for weight showed that the hypotheses  $A_1 = A_2$ ,  $B_1 = B_2$  and  $K_1 = K_2$  were not rejected (Table 1); hence, a single model can be used to describe weight-at-age for *H. malabaricus* (Fig. 1). Thus, the equation describing weight-at-age was estimated ( $A = 292.90$ ;  $B = 28.14$ ; and  $K = 0.65$ ). Regarding the parameters  $A$  and  $B$ , the equality hypothesis was not rejected, indicating that trahira has the same ultimate size ( $A$ ) and the same constant of integration ( $B$ ) at both stocking densities. Maximum increment



**Fig. 1.** Growth of weight based on the values observed and predicted by the logistic model, with the 95% confidence interval.



**Fig. 2.** Absolute growth rate and logistic curve adjusted to both stocking densities.



**Fig. 3.** Length-weight relationship for *H. malabaricus* under intensive farming management.

for weight-at-age, i.e., the highest absolute growth rate, occurred before the fifth month of age for the lower density (5 fish/m<sup>2</sup>) and the sixth month of age for the higher density (10 fish m<sup>-2</sup>) (Fig. 2). The length-weight relationship,  $W = 0.00896 \times b^{3.107487}$ ,  $R^2 = 0.9785$  (Fig. 1), showed a mean  $b$  value of 3.07, which differs slightly from the standard value of 3.0. Thus, *H. malabaricus* under semi-intensive farming conditions presents positive allometric growth, i.e., individuals increase in relative body thickness, becoming heavier at a given length as the fish grows (Tesch, 1968). The  $b$  value for trahira is between 2.5 and 3.5, as is found for most fish species (Froese, 2006). Equality tests for the parameters within the Huxley model regarding the length-weight relationship at both stocking densities did not differ significantly ( $P > 0.05$ ) (see Fig. 3).

### 3.3. Grow-out performance

Table 2 summarizes the growth performance of *H. malabaricus* under the two density conditions. Initial total length, final total length

**Table 2**

Growth rate parameters from *H. malabaricus* juveniles reared at two stocking densities (5 fish/m<sup>2</sup> and 10 fish/m<sup>2</sup>) under on-farm grow-out conditions\*.

Growth parameters	Stocking densities	
	5 fish/m <sup>2</sup>	10 fish/m <sup>2</sup>
Initial total length (cm)	12.0 ± 0.06 <sup>a</sup>	12.2 ± 0.04 <sup>a</sup>
Final total length (cm)	27.5 ± 0.38 <sup>a</sup>	27.0 ± 0.24 <sup>a</sup>
Initial body weight (g)	18.6 ± 0.26 <sup>a</sup>	19.4 ± 0.18 <sup>a</sup>
Final body weight (g)	263.0 ± 10.54 <sup>a</sup>	246.7 ± 6.89 <sup>a</sup>
Relative growth (%)	43.03 ± 12.51 <sup>a</sup>	39.58 ± 9.85 <sup>a</sup>
Specific growth rate (% /day)	0.36 ± 0.08 <sup>a</sup>	0.35 ± 0.07 <sup>a</sup>
Food conversion rate (%)	3.90:1 <sup>a</sup>	3.34:1 <sup>b</sup>
Condition factor (K)	1.23 ± 0.03 <sup>a</sup>	1.23 ± 0.30 <sup>a</sup>
Feed efficiency (%)	34.24 ± 8.22 <sup>a</sup>	37.05 ± 6.53 <sup>a</sup>
Coefficient of variation (%)	24.8 ± 1.29 <sup>a</sup>	23.67 ± 0.82 <sup>a</sup>
Survival rate (%)	69 <sup>a</sup>	76 <sup>b</sup>

\*Data are shown as mean ± standard error. Means in a row followed by the same superscript are not significantly different ( $P \leq 0.05$ ).

and body weight, as well as the majority of the growth parameters, were statistically equal at both stocking densities. Conversely, feed conversion rate and survival rate showed statistically significant differences between stocking densities by the end of the experiment. The coefficients of variation for growth traits were high and within the range found in fish and shellfish (CV = 17%–29%, Gjedrem, 1998).

### 3.4. Protein and lipid body profile of *H. malabaricus*

The total protein level of the fillet decreased between the initial sample and subsequent samples ( $P < 0.001$ , Table 3), but there were no differences between the treatments ( $P > 0.05$ ). Conversely, the lipid concentration of fillets increased in animals at both densities by the end of the experiment ( $P < 0.001$ ), and were not significantly different between the treatments.

The fillet FA profile of animals from both densities showed an increase in saturated fatty acids (SFA) at the end of the experiment ( $P = 0.002$  and  $P < 0.001$ , respectively, Table 3), mainly due to C18:0. n3 PUFA decreased through the experiment in both treatments, with docosahexaenoic acid (DHA) most responsible for this decrease. The decrease in n3 PUFA was associated with a decrease in the n3/n6 ratio at the end of the experiment at both stocking densities. No significant differences were found in AI and TI among stocking densities.

## 4. Discussion

Our results showed that stocking densities had little influence on growth rate, protein and lipid content, or fatty acid profile of fillets of *H. malabaricus*.

### 4.1. Growth model and grow-out performance

Successful cultivation of an aquaculture candidate species depends on genetic make-up and physiological plasticity so that the species can adjust to culture conditions and show a viable growth rate for commercialization (Avault, 1993). Hence, modeling fish growth is a first step for assessing an aquaculture candidate species because it supports predictions of how well the fish will grow under various production systems.

High-productivity fish farming systems monitor physical and chemical water quality because it directly affects physiological processes of fish. The physical and chemical water quality parameters did not change in a way that would affect growth or fillet composition, except for water temperature. Total ammonia concentration and pH remained constant and within fish-farming standards throughout the experiment. pH values remained between 6 and 8. The saturation level of DO was

**Table 3**

Total protein, lipid (% wet weight) and fatty acid profiles (% of total lipids) of *H. malabaricus* juvenile fillets produced at two stocking densities (5 fish/m<sup>2</sup> and 10 fish/m<sup>2</sup>).

	Initial	Intermediate		Final	
		5 fish/m <sup>2</sup>	10 fish/m <sup>2</sup>	5 fish/m <sup>2</sup>	10 fish/m <sup>2</sup>
Total proteins	32.6 ± 1.41 <sup>a</sup>	24.0 ± 1.34 <sup>b</sup>	22.4 ± 1.12 <sup>b</sup>	23.9 ± 0.75 <sup>b</sup>	21.9 ± 1.05 <sup>b</sup>
Total lipids	1.4 ± 0.11 <sup>a</sup>	1.6 ± 0.32 <sup>a</sup>	1.4 ± 0.32 <sup>a</sup>	8.7 ± 1.21 <sup>b</sup>	6.5 ± 0.37 <sup>b</sup>
<b>Fatty acids</b>					
C17:0	0.4 ± 0.15	0.5 ± 0.11	0.6 ± 0.26	0.8 ± 0.66	0.6 ± 0.39
C17:1cis	0.4 ± 0.09	0.3 ± 0.11	0.3 ± 0.08	0.2 ± 0.04	0.2 ± 0.09
C18:0anteiso	0.1 ± 0.00	0.2 ± 0.12	0.2 ± 0.04	0.3 ± 0.16	0.3 ± 0.16
C19:0	0.1 ± 0.03	0.4 ± 0.24	0.2 ± 0.03	0.1 ± 0.03	0.1 ± 0.02
C23:0	Nd	0.1 ± 0.06	0.4 ± 0.34	1.4 ± 0.39	Nd
ΣOFA - BFA	0.6 ± 0.44	1.4 ± 0.73	1.2 ± 0.67	1.7 ± 1.42	1.1 ± 0.43
C14:0	1.5 ± 0.42	1.6 ± 0.41	1.9 ± 0.36	1.8 ± 0.29	1.7 ± 0.28
C16:0	19.3 ± 0.74	20.0 ± 0.73	20.7 ± 0.86	20.9 ± 1.84	21.2 ± 1.23
C18:0	7.9 ± 1.02 <sup>a</sup>	8.8 ± 1.14 <sup>ab</sup>	8.1 ± 1.17 <sup>bc</sup>	8.8 ± 1.08 <sup>c</sup>	9.2 ± 1.52 <sup>c</sup>
C20:0	0.3 ± 0.12	0.3 ± 0.06	0.3 ± 0.10	0.3 ± 0.03	0.3 ± 0.05
ΣSFA	29.1 ± 1.19 <sup>a</sup>	30.7 ± 0.52 <sup>ab</sup>	31.2 ± 1.41 <sup>bc</sup>	31.9 ± 1.63 <sup>c</sup>	32.6 ± 1.22 <sup>c</sup>
C16:1n7	4.1 ± 0.76	4.1 ± 0.93	4.6 ± 0.78	3.7 ± 0.97	3.5 ± 0.88
C18:1n7	2.4 ± 0.35	2.6 ± 0.26	2.5 ± 0.17	2.2 ± 0.16	2.2 ± 0.13
C18:1n9	25.5 ± 3.05	26.3 ± 4.87	28.8 ± 4.65	28.0 ± 6.20	27.3 ± 6.15
C20:1n9	0.7 ± 0.29	0.3 ± 0.28	0.4 ± 0.35	0.6 ± 0.63	0.2 ± 0.25
C20:1n11	0.6 ± 0.38	0.8 ± 0.05	0.8 ± 0.03	0.7 ± 0.06	0.9 ± 0.31
ΣMUFA	32.9 ± 3.88	33.9 ± 5.76	37.0 ± 5.21	35.2 ± 6.75	34.0 ± 6.91
C18:3n3	0.6 ± 0.22	0.6 ± 0.16	0.7 ± 0.17	0.8 ± 0.11	0.7 ± 0.18
C18:4n3	0.2 ± 0.07	0.4 ± 0.12	0.3 ± 0.13	0.3 ± 0.08	0.2 ± 0.06
C20:5n3	1.2 ± 0.11	1.3 ± 0.27	1.2 ± 0.18	0.8 ± 0.23	0.8 ± 0.15
C22:5n3	1.2 ± 0.20	1.4 ± 0.25	1.1 ± 0.18	1.0 ± 0.33	1.1 ± 0.36
C22:6n3	12.9 ± 3.45 <sup>a</sup>	11.0 ± 4.05 <sup>ab</sup>	8.2 ± 2.23 <sup>bc</sup>	6.6 ± 2.66 <sup>c</sup>	8.0 ± 3.94 <sup>c</sup>
Σ n3 PUFA	16.1 ± 3.54 <sup>a</sup>	15.0 ± 4.34 <sup>ab</sup>	11.8 ± 2.46 <sup>bc</sup>	9.5 ± 3.03 <sup>c</sup>	10.9 ± 4.24 <sup>c</sup>
C18:2n6cis	10.7 ± 1.91	10.0 ± 0.99	10.5 ± 1.58	12.0 ± 0.97	12.5 ± 0.76
C18:3n6	0.4 ± 0.09	0.4 ± 0.18	0.5 ± 0.05	0.8 ± 0.20	0.8 ± 0.14
C20:2n6	1.1 ± 0.45	1.0 ± 0.44	1.3 ± 1.34	1.5 ± 1.44	0.9 ± 0.32
C20:3n6	3.7 ± 0.82	2.9 ± 0.70	2.5 ± 0.58	2.5 ± 0.89	2.7 ± 0.70
C20:4n6	3.1 ± 0.88 <sup>a</sup>	2.9 ± 0.98 <sup>ab</sup>	2.3 ± 0.82 <sup>bc</sup>	2.5 ± 1.18 <sup>ab</sup>	2.9 ± 1.31 <sup>ab</sup>
C22:2n6	0.7 ± 0.36	0.3 ± 0.14	0.3 ± 0.15	1.9 ± 1.41	0.2 ± 0.04
C22:4n6	1.6 ± 0.48	1.2 ± 0.49	0.9 ± 0.39	0.9 ± 0.43	1.1 ± 0.63
C22:5n6	0.4 ± 0.34	0.5 ± 0.55	0.7 ± 0.10	1.2 ± 1.08	0.3 ± 0.33
Σ n6 PUFA	21.3 ± 1.27 <sup>a</sup>	19.0 ± 1.59 <sup>ab</sup>	18.8 ± 2.62 <sup>bc</sup>	21.6 ± 4.10 <sup>a</sup>	21.4 ± 2.32 <sup>a</sup>
Σ total PUFA	37.4 ± 3.72 <sup>a</sup>	34.0 ± 5.54 <sup>ab</sup>	30.5 ± 4.67 <sup>b</sup>	31.2 ± 6.80 <sup>b</sup>	32.3 ± 6.32 <sup>b</sup>
n3/n6	0.8 ± 0.17 <sup>a</sup>	0.8 ± 0.19 <sup>a</sup>	0.6 ± 0.10 <sup>a</sup>	0.4 ± 0.09 <sup>b</sup>	0.5 ± 0.15 <sup>b</sup>
AI	0.5 ± 0.02	0.5 ± 0.02	0.5 ± 0.04	0.6 ± 0.04	0.6 ± 0.03
TI	0.4 ± 0.05	0.4 ± 0.06	0.5 ± 0.04	0.6 ± 0.09	0.5 ± 0.09

\*Data are shown as mean ± standard error. Means in a row followed by the same superscript are not significantly different ( $P \leq 0.05$ ).

ΣOFA: odd fatty acids, ΣSFA: saturated fatty acids; ΣMUFA: monounsaturated fatty acids; ΣPUFA: polyunsaturated fatty acids; Σn6: omega 6; Σn3: omega 3; AI: atherogenic index; TI: thrombogenic index. \*Data are shown as mean ± standard error. Means in a row followed by the same superscript are not significantly different ( $P < 0.05$ ). Nd – not detected.

about 9.0 mg/L at 20 °C, and about 7.8 mg/L at 27 °C. Even at higher temperatures, observed DO concentrations throughout the experiment were appropriate for production of trahira. Variations in temperature above or below the optimum of a species can change its growth rate (El-Sayed, El-Ghobashi, & Al-Amoudi, 1996; Person-Le, Mahe, Le Bayon, & Le Delliou, 2004; Ribeiro, Moreira, Cantelmo, & Esposito, 2014; Tolussi, Hilsdorf, Caneppele, & Moreira, 2010). The temperature ranged between 16 and 28 °C depending on the season. Water temperature was below the range for warm water farmed fishes (26.06–31.97 °C, Boyd & Tucker, 1998, p. 700), which negatively influenced growth performance at both stocking densities.

Three nonlinear mathematical models relate fish growth to age; these models have three parameters, which at least theoretically have biological importance (Freitas, 2005; Enberg et al., 2008, pp. 1564–1572). Various authors have suggested the use of nonlinear mixed models using both fixed and random effects to increase their predictive accuracy (Schinckel & Craig, 2002; Cao & Wang, 2011). Using BIC and AIC tests (Table 1), we found that the logistic model best captured growth in weight- and length-at-age of *H. malabaricus*. The best-fitting adjusted model varies among species. For instance, in Nile tilapia *Oreochromis niloticus*, the logistic model best describes the morphometric growth under culture conditions (Ansah & Frimpong,

2015). In the Neotropical aquaculture species tambaqui *Colossoma macropomum*, Mello et al. (2015) concluded that the Gompertz nonlinear regression model was most appropriate. Growth models for Neotropical carnivorous species have been assessed only for wild populations, with the von Bertalanffy model best describing growth of spotted surubim *Pseudoplatystoma corruscans* (Mateus & Petre, 2004), duckbill catfish *Sorubim cf. lima* (Penha, Mateus, & Barbieri, 2004), *H. malabaricus* (Tordecilla-Petro, Sánchez-Banda, & Olaya-Nieto, 2005), and dorado *Salminus brasiliensis* (Zuliani et al., 2016). The divergence in the best-supported growth model is a result of the specific growth pattern of the species under study (Sarmento et al., 2006), and also the sample size and frequency (temporal resolution) of growth measurements; that is, when the inflection point of the curve ranges from 0 to 1, the best adjustment will be obtained with the models of Brody and Gompertz. However, when it oscillates between 1 and 2, it will be obtained with the Gompertz or logistic models (Richards, 1959).

Assessment of growth models for cultured *H. malabaricus* using the LRT showed that the mixed-model approach provided a better fit to the data than traditional modeling with only fixed effects. Cao and Wang (2011) suggested the use of mixed-effects model increases the accuracy of predictions about growth. Craig and Schinckel (2001) developed mixed nonlinear models for porcine growth and showed more accurate

estimates for parameters of functions for animal growth than traditional, fixed-effects models.

The typical lifetime growth curve of fish has a sigmoid shape, i.e., growth during early life is slow and is followed by a period of acceleration, and then growth reaches its maximum around puberty, when deceleration occurs (Berg & Butterfield, 1976, p. 240). Fig. 2 shows this pattern for weight growth in trahira, in which absolute growth and the logistic curve were adjusted for the two stocking densities. At the lower stocking density (5 fish/m<sup>2</sup>), the convex phase of the growth curve started earlier than at the higher stocking density (10 fish/m<sup>2</sup>). However, the growth rate in the fish at a higher stocking density increased until the sixth month of grow-out. Some authors have observed the same effect in other species, for example, Piaia and Baldisserotto (2000) for *Rhamdia quelen* and Salaro et al. (2003) for *Hoplias lacerdae*, reported that in captivity at high-density the fish tended to gather for feeding, decreasing territorial behavior and cannibalism. In our study of *Hoplias malabaricus*, the survival rate was 69% at a density of 5 fish/m<sup>2</sup> and 76% at 10 fish/m<sup>2</sup>.

Growth models for farmed fishes may be affected by environmental factors, particularly oxygen availability and temperature, as well as culture-dependent factors such as stocking density and feeding. To assess the growth curve for a candidate fish for aquaculture, such as *H. malabaricus*, it is important to understand and manage culture variables in order to improve growth rate, and to determine the optimal cost-benefit weight for harvest size (Kapetsky & Nath, 1997, p. 128; Gomiero et al., 2009). Grow-out performance of *H. malabaricus* during the one year-production cycle had similar outcomes for the two stocking densities, as can be seen in Table 2. This outcome has also been observed with the congeneric trahira species *Hoplias lacerdae* through 120 days of growth (Salaro et al., 2003). The coefficients of variation for growth of *H. malabaricus* were higher than for other farmed carnivorous species, such as *Clarias gariepinus* (ranging between 0.38% and 6.5%, (Hengsawat, Ward & Jaruratjamorn, 1997), and similar to other Neotropical species of the genus *Pseudoplatystoma* (CV ranging between 18 and 45%, Miranda & Ribeiro, 1997; Tura et al., 2009). The mean weight after 11 months of grow-out did not differ significantly between the two densities, 263.0 g and 246.7 g for 5 and 10 fish/m<sup>2</sup>, respectively. Although the specific growth rate of *H. malabaricus* was half that of *Pseudoplatystoma* catfish (sorobim) (Scorvo-Filho, Romagosa, Ayoza, & Frascá-Scorvo, 2008), an important Neotropical carnivorous farmed fish, we note that the juveniles we used originated from wild broodstock which had not undergone genetic improvement. The high coefficient of variation at the last weight measurement may reflect the full range of phenotypic variation for the trait. After the eleventh month of rearing, including the winter months, fish weighing more than 450 g were found among the trial fish. The market demand for an individual portion (plate size) and the growth rate achieved in this study indicate the commercially interesting weight for trahira is between 500 and 600 g. Observation of fish with higher growth rates under semi-intensive culture management suggests that *H. malabaricus* may have potential for improved growth by selective breeding. Domestication and selective breeding to improve feed conversion rate would address a factor critical for adoption of *H. malabaricus* for commercial aquaculture.

The extent of genetic variation found within and among populations provides the basis for a genetic improvement program, i.e., phenotypic variation and underlying heritability for growth rate are functions of relevant genetic variation (Gjedrem & Baranski, 2009). The establishment of a selective breeding program using genetically diverse populations has the potential to improve growth rate to market size for succeeding generations of *H. malabaricus*.

#### 4.2. Nutritional aspects of farmed *H. malabaricus*

Regarding the body composition, total protein concentrations in fillets decreased through the experiment at both stocking densities;

however, the values still remained higher than those for other species analyzed, such as Murray cod *Maccullochella peelii peelii* (Palmeri, Turchini, De Silva, 2007), gilthead sea bream *Sparus aurata* (Senso, Suarez, Ruiz-Cara, & Garcia-Gallego, 2007), Tiete tetra *Brycon insignis* (Tolussi et al., 2010), goldfish *Carassius auratus* (Dal Bosco, Mugnai, Mourvaki E., & Castellini, 2012), and Nile tilapia (Ribeiro et al., 2014; Vieira, Hilsdorf, & Moreira, 2012). The decrease in fillet protein may be due to utilization of protein as an energy source for growth, suggesting that use of a higher protein diet (> 40%) may be appropriate for growth-out of *H. malabaricus*.

Lipid content is also an important aspect of fillet quality (Quinton, McMillan, & Glebe, 2005). Fillets can be classified as “lean” when the lipid content is below 2.5% and as “semi-skimmed” between 2.5% and 10% (Bennion, 1980, p. 598; Jacquot, 1961). In the present study, high concentrations of lipids in the muscle of *H. malabaricus* juveniles were such that the fillet would be classified as high-quality, in the semi-skimmed range. The fillet fatty-acid profile showed few changes over time, but we noted an interesting content of SFAs and PUFAs. The major FAs in *H. malabaricus* juvenile fillets were: C16:0, C18:0, C18:1n9, C18:2n6 (linoleic acid, LA), ARA (C20:4n6), and DHA. FAs of aquatic origin are characterized by the predominance of PUFAs, especially EPA (C20:5n3) and DHA (Arts, Brett, & Kainz, 2009, p. 380), as seen in our results.

Despite the increase of SFA observed in the fillets of fish at harvest at both stocking densities, values remained lower than those found by Kasai et al. (2011) in *H. lacerdae* fingerlings. On the other hand, total PUFA presented a significant decrease between the initial and later samplings at both densities due to decreasing n3 PUFA content, specifically DHA. The percentages of total, n3 PUFAs/DHA, nonetheless is high and similar to those of *H. malabaricus* fillets from fish captured from the wild (Gutierrez & Silva, 1993; Petenuci et al., 2016; Torres, Zambiasi, Chiatone, Fonseca, & Costa, 2012). The DHA content in *H. malabaricus*, when its freshwater origin is considered, is higher than many other economically important freshwater fishes, including Nile tilapia (Vieira et al., 2012), common carp *Cyprinus carpio* (Živić et al., 2014), sábalo *Prochilodus lineatus* (Luzia, Sampaio, Castellucci, & Torres, 2003), pintado *Pseudoplatystoma corruscans* (Martino, Cyrino, Portz, & Trugo, 2002), peacock bass *Cichla* sp. (Inhamuns, Franco, & Batista, 2009), and spotted pim *Pimelodus maculatus*, threespot leporinus *Leporinus friderici*, and dorado *Salminus brasiliensis* (Andrade, Rubira, Matsushita, & Souza, 1995).

*H. malabaricus* juveniles had a high percentage of C18:2n6 (almost 90% of total PUFAs), suggesting that they tend to accumulate n3 PUFAs, mainly DHA, in the fillet, even at high stocking densities and with a relatively low percentage of this FA in the diet. This FA is mainly found in soybean, corn, and sunflower (Matsushita et al., 2006, pp. 125–131), suggesting the inclusion of a higher proportion of these ingredients in the commercial diet, together with the low animal protein content, results in a low percentage of EPA and DHA, the main FAs of fishmeal. Hence, the high percentage of C18:2n6 in the muscle tissue reflects that of the diet. This FA is considered essential to freshwater fishes and humans because it acts as the main precursor of arachidonic acid (ARA) (C20:4n6), also found in a reasonable percentage in the fillet of *H. malabaricus*. ARA is the precursor of many physiologically active compounds, such as prostaglandins, thromboxanes, and leukotrienes. In fishes, production of these hormones can influence growth, vitellogenesis, immune response, and physiological response to environmental stress (Bell, Tocher, MacDonald, & Sargent, 1995). Greater fish consumption is associated with increased n3 PUFA intake and with an increase in the n3/n6 ratio, which plays a critical role in improved cognition and cardiovascular health in humans (Liu et al., 2017; Marik & Varon, 2009).

In the present study, despite a decrease in the n3/n6 ratio at the end of the experiment, the values remained between 0.4 and 0.8. The optimal ratio of n3/n6 is not known for most organisms and is hypothesized to be species-specific (Sargent, 1995). AI and TI showed very low

values in comparison to those observed in other animals, such as Atlantic bluefin tuna *Thunnus thynnus* ( $AI = 0.7$ ) (Garaffo et al., 2011) and terrestrial livestock animals such as rabbit (0.7 and 0.99; Dal Bosco, Castellini, Bianchi, & Mugnai, 2004) and poultry (0.49 and 0.88; Castellini, Bosco, Mugnai, & Pedrazzoli, 2006). Among PUFAs, the main fatty acids with anti-atherogenic action (inhibiting platelet aggregation and decreasing plasma cholesterol concentration) are C18:2n6, C18:3n3, EPA and DHA, which are also known to have anti-thrombotic effects (preventing the formation of clots in blood vessels). On the other hand, SFAs such as C14:0 and C16:0 are known as health risk factors (Lajous et al., 2013; Ulbricht & Southgate, 1991).

## 5. Conclusions

The search for new native aquaculture species as an alternative to introduced ones has been on the agenda of ministries of agriculture in multiple Neotropical countries (Garcia-Trejo, Hurtado-Gonzalez, Soto-Zarazua, & Gutierrez-Yurrita, 2014; Ross & Beveridge, 1995). The trahira *Hoplias malabaricus* is distributed through many Neotropical countries. In Brazil, it has been captured in reservoirs and supplied to markets and after intramuscular bone removal, it is prepared for consumption using many different recipes. The results of this study indicated logistic as the best model to explain growth in weight- and length-at-age in trahira under aquaculture operations. And that density did not induce modified fillet protein or lipid deposition, including fatty acids. Trahira fillet composition showed high fatty-acid contents for n3 and n6 PUFAs, such as DHA, LA and ARA compared to other tropical aquaculture species. Therefore, market demand, adaptation to farming conditions, low requirement for dissolved oxygen, ready consumption of processed feed, considerable growth potential, along with high nutritional quality and superior meat flavor, indicate that *H. malabaricus* is a suitable candidate for development of profitable aquaculture operations in Central and South American countries.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.aaf.2019.01.002>.

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