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Research Article

Contribution to some aspects of the digestive tract anatomy and food evacuation of the Pacific fat sleeper *Dormitator latrifrons*

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ABSTRACT. The development of feeding protocols and the study of gastric evacuation or passage of food through the digestive tract is essential to generate information on the digestive physiology of organisms, as they help to quantify the daily ration to avoid any poor diet. In this sense, the present work aims to demonstrate some basic aspects of the fish *Dormitator latifrons* digestive physiology by evaluating the transit of food through the intestinal tract as a function of time. In a completely randomized standard design, juveniles of *D. latifrons* were fed *ad libitum* with commercial pellets. Five conditioned organisms were sacrificed every hour after a single feeding. The food bolus was collected at each sampling point, using polynomial regression models to analyze the passage of food through time in the digestive tract. The passage of food showed a nonlinear behavior in time, being the simple exponential decay model of two parameters ($R^2 = 0.630$; $P < 0.0001$) the best to explain the evacuation of food through the digestive tract. This knowledge will generate better feeding protocols for this species with commercial importance and aquaculture potential.

Keywords: *Dormitator latifrons*; native fish species; passage of food; gastric evacuation; feeding protocols; gut motility, passage rate

INTRODUCTION

Given the decline of fisheries and the continued high demand for fish, aquaculture plays an increasingly important role in the global production of fish and shellfish (SOFIA 2020). However, many aquaculture products negatively affect biodiversity and biosecurity due to introducing exotic species into natural ecosystems (FAO 2010). In Mexico, the study of native species with aquaculture potential has focused mainly on marine species, while inland aquaculture is based on exotic freshwater species such as tilapia and carp (Basto-Rosales et al. 2019, 2020, Vega-Villasante et al. 2021). There are inland aquatic species with high aquaculture potential, such as *Dormitator latifrons* (Richardson, 1844), that play an important role in regional fishing and gastronomical traditions in coastal communities in southern Mexico, such as Guerrero and Oaxaca, as well as South America, mainly in Ecuador (Flores-Nava & Brown 2010, Vega-Villasante et al. 2021). The reason is that this fish species has very good flavor and texture, as well as white meat with no intramuscular bones (EcoCostas 2006). It is also worth noting that the nutritional profile of *D. latifrons* is similar to that of other species of high commercial value (López-Huerta et al. 2018, Basto-Rosales et al. 2020).

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Despite the potential commercial importance of *D. latifrons*, there is still insufficient knowledge to produce it using aquaculture technology (Aréchiga-Palomera et al. 2022). Knowledge is especially scarce on developing appropriate feeding protocols and gastric evacuation (GE) or the passage of food through the digestive tract. Studying these essential subjects would be required to understand the digestive physiology of the species thoroughly, which would, in turn, support the development of aquaculture techniques that are suitable for this species since this knowledge would help to determine the optimal daily ration to avoid any overfeeding or underfeeding, both of which pose dangers to the health and the economic viability of aquaculture systems (Seyhan et al. 2020). In aquaculture, overfeeding causes degradation of water quality (e.g. ammonia poisoning, low oxygen, and pH levels) and increases feces production (biochemical oxygen demand); it is also a waste of expensive feed (Fateh et al. 2005, Seyhan et al. 2020). Insufficient feeding can result in poor growth and even death (Seyhan et al. 2020).

The stomach has multiple functions in gastric fish, including initial (temporary) food storage and protein digestion initiation under acidic conditions (Wilson & Castro 2010). The storage function of the stomach allows the fish to ingest larger meals, which is particularly important for animals that swallow large prey items whole (Le et al. 2019). However, the functions and capabilities of fish stomachs have changed (they have even been lost in some species) as fish evolved (Castro et al. 2014). As is the case of some species of the sleeper family, such as *D. latifrons*, which, according to some reports, lacks a true stomach and has detritivorous (Morelos-Castro et al. 2019) and omnivorous habits too (Freire-Lascano 2016). In general, the study of the passage of food through the digestive tract of fish suggests that, in addition to the specific anatomical-physiological characteristics of each species, there are factors that influence the transit time of food, such as culture temperature, stress, intestinal size and length, body size, hardness and composition of diets (Logothetis et al. 2001, Booth et al. 2008, Uscanga et al. 2010, Andersen 2012, Khan & Seyhan 2019).

There is a diversity of models that explain the rate of digestive evacuation in fish, of which the linear, exponential, and square root models are the most used ones (Álvarez et al. 2010). The variables used in these models are the food in the stomach (g) and time (h). Notably, these models were developed mainly for carnivorous species with a true stomach, so they would not necessarily apply to agastric species or those with relatively long intestines. The present work aimed to analyze some basic aspects of *D. latifrons* digestive physiology by evaluating the feeding and transit of food through the intestinal tract as a function of time. Some basic aspects of the digestive morphophysiology of *D. latifrons* were also analyzed to generate predictive models of the gastric evacuation rate, which could serve as the basis for generating better feeding protocols for this species with aquaculture potential.

MATERIALS AND METHODS

Organisms and pre-experimental phase

Wild specimens of *D. latifrons* were captured in the El Quelele Estuary, located at 20º43'25.43"N and 105º18'03.63"W, in Nayarit State, Mexico (July 2021). The fish were transported to a container (400 L) with constant aeration (O_2 concentration of 4 mg L^{-1}) within the facilities of the Experimental Aquaculture and Water Quality Laboratory (University of Guadalajara). Juveniles of *D. latifrons* with similar weight and size (n = 106, weight 60.13 ± 14.92 g; P = 0.405, length 16.38 ± 1.33 cm; P = 0.359) were selected for the pre-experimental phase. The fish were kept in a 400 L tank in a recirculating system with dechlorinated water at a temperature of 27-29°C, with a 12:12 h light:dark photoperiod and conditioned for 30 days to a single feeding (at 8:00 h) of 3 mm commercial pellets (Grow fish® for tilapia in development containing: protein min. 35.0%, fat min. 3.5%, moisture max. 12.0%, fiber max. 5.0%, ashes max. 8.0% and ELN min. 34.5%).

For the experiment, fish were fasted for 48 h to ensure empty digestive tracts. The method used was based on Uscanga et al. (2010), who recommend two considerations: i) using fish of similar size for repeated sequential sampling; ii) using a feeding protocol that guarantees that all the fish receive a similar amount of food. The passage of food (from a single *ad libitum* feeding) through the digestive tract was evaluated, that is, the function of the weight of dry food chyme collected over time. The feed was the same commercial feed used for the previous conditioning phase. From observations before the experiment with organisms of similar weights and sizes, it was decided that the feces were removed every 3 h to prevent the fish from consuming their fecal remains so that at time zero (T0), all the organisms were hungry and had an empty digestive tract. For this, conical experimental systems were used, which allow the exit of sediments or fecal feces in the lower part of the system.

Experimental design

The experimental design consisted of three fish per experimental unit (tank) in triplicate (n = 9). All organisms were fed *ad libitum* simultaneously (n = 108). Five of the nine fish per hour were randomly selected and sacrificed in sequence every hour for 12 h (n = 60). The fish were euthanized by immersion of fish in water prepared with dilutions of clove oil (eugenol 200 µL L^{-1}). All animals euthanized were submitted to biometric measurements: weight, size, and volume. Volume was measured using the indirect measurement method by displacement of water in a 1000 mL graduated cylinder, considering that volume is a measure of the amount of space that a liquid occupies in a container; in this case, the space occupied by the fish in the cylinder, therefore, the difference between the initial and final amount of water is the volume of the organism's weight (Hyslop 1980, Silva et al. 2014). Taking the necessary precautions to reduce displacement by pressure or movement, the entire digestive tract was dissected. It was then extended on a table with a millimetric scale to measure its length and, where appropriate, make a photographic record of the movement of food through the digestive tract. The alimentary chyme was recovered, dried (at 60°C, 24 h), and weighed (Nimbus Adam® Balance with 0.0001 g precision). Four hours after ingestion, sediment was removed from the experimental tanks every 30 min for 5 s to monitor the excretion of feces by the fish.

Statistical analyses

The biometric variables of the fish (weight, size, and volume) were subjected to linear correlation coefficient analysis to assess the association between these variables. Various nonlinear regression models were used to assess the relationship between the changes in the weight of the alimentary chyme (g) and the sampling hours. The model developed by Booth et al. (2008) was also applied. This model, called relative stomach or intestinal content, is as follows: (g dry matter 100 g BW^{-1}) = (dry matter extracted from stomach or intestine (g) / wet body weight of fish (g)) × 100, where BW is the weight of fish (Booth et al. 2008). The sampling hours were computed with a Mann-Whitney rank sum test. All analyses were conducted using the Sigma Plot 10 software.

RESULTS

No mortality was recorded during the entire experiment. There was a significant correlation (R = 0.861; $P < 0.001$) between the weight and size of these organisms (Fig. 1). There was also a significant correlation (R = 0.92; $P < 0.001$) between weight and volume (Fig. 2), and a low (R = 0.467) but significant ($P < 0.001$) correlation between volume and size (Fig. 3). A multiple linear regression model indicated a high correlation between the weight, volume, and size of the organisms (R = 0.951, $P < 0.001$). The intestinal tract had an average length of 28.69 ± 7.8 cm. A significant correlation was found between the weight of the fish and the length of the digestive tract (R = 0.485; $P <$ 0.001) and between the volume of the fish and the length of the digestive tract (R = 0.471; $P < 0.001$) (Fig. 4).

The *ad libitum* feeding period of 5 min was sufficient to satisfy hunger and ensure that all organisms ingested the pellets. During the first 10 h, the passage of the food could be appreciated and quantified thoroughly. By the final sampling at 12 h, it was already difficult to determine the weight of the alimentary chyme. Previous assays had already shown that the first fecal evacuations occurred 4 h after feeding (feed intake); likewise, it was observed that the organisms returned to eat food after the first hour of feeding; however, in the present study, experiments were performed with a single feeding.

The model that best explained the passage of food through the digestive tract of *D. latifrons* was the simple exponential decay model with two parameters (Fig. 5). Under the established experimental conditions, the passage of food showed an acceptable correlation, with an R of 0.776 ($P < 0.001$). The next best models, according to the value of R^2 and significance ($P <$ 0.0001), were the quadratic polynomial model (R^2 = 0.551) and the linear polynomial model (R^2 = 0.3292). The polynomial parameters of other models were not sufficiently significant (Table 1). Based on this analysis, the stomach or intestinal relative content index was used in a 2-parameter simple exponential decay model, which resulted in a significant correlation with an R of 0.704 ($P < 0.001$). The highest evacuation differential was recorded in the first 3 h, according to the model of gastrointestinal content as a function of the following hours ($P < 0.05$). It was calculated from differential evacuation registered in the first hours since there is a decrease in the weight of the chyme related to the first feces evacuations. The first hour had 0.31 g of dry matter 100 g BW^{-1} h^{-1}, the second had 0.103 g of dry matter 100 g BW^{-1} h^{-1}, and the third hour had 0.074 g of dry matter 100 g BW^{-1} h^{-1} (Fig. 6).

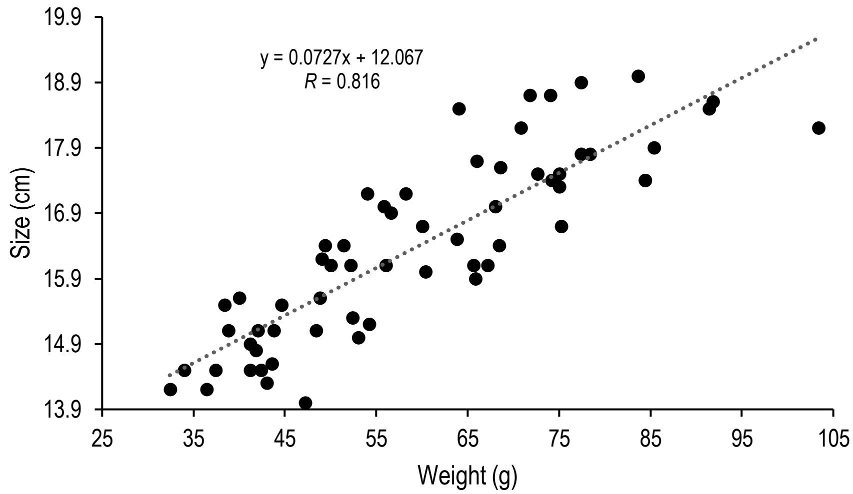

Figure 1. Weight (g) and size (cm) ratio in experimental organisms of *Dormitator latifrons* used to analyze gastric evacuation.

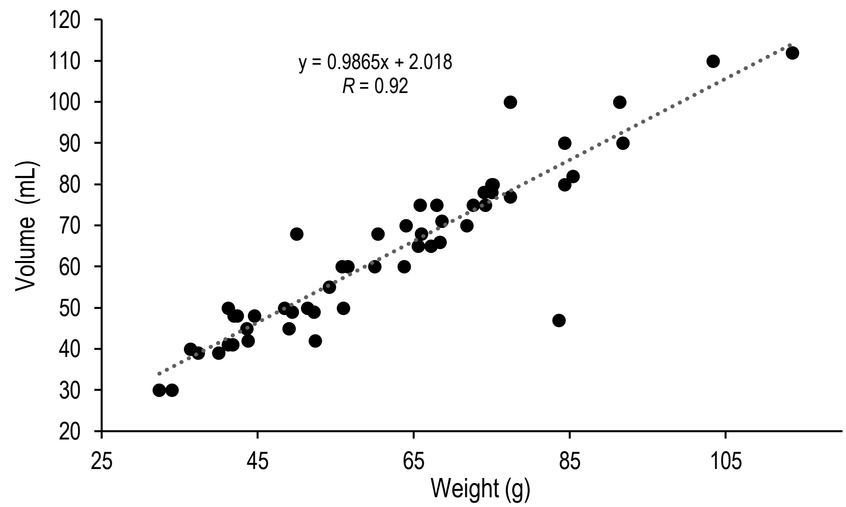

Figure 2. Weight (g) and volume (mL) ratio in experimental organisms of *Dormitator latifrons* used to analyze gastric evacuation.

DISCUSSION

Using growth models to assess the development of farmed fish can help decide which morphological parameters would be most appropriate to evaluate the development of fish at different life stages (wild or farmed). Delgadillo-Calvillo et al. (2012) explained that estimating the allometry coefficients allows for determining the type of growth of the organisms, which can be used as a practical index to evaluate the condition of the fish as a function of the availability of food, health status, sex, gonad development and spawning period, among other factors. In the present study, farmed specimens of *D. latifrons* showed a significant relationship between size and weight, a similar result to that reported by Sandoval-Huerta et al. (2015) for wild fish in their natural habitat, using a logarithmic regression. That study also reported positive allometric growth for *D. latifrons*, unique among the species of the Eleotridae family. In natural populations, isometric growth is considered when weight and length increase in the same proportion with time.

In contrast, sideways growth is considered positive or negative allometric growth (Chicaiza & Flores 2016). This last characteristic of *D. latifrons* can be explained by its morphology since it has a short and robust body, with a protruding abdominal section that gave rise to one of its common names: Pacific fat sleeper (Eigenman et al. 1885). This fact may explain

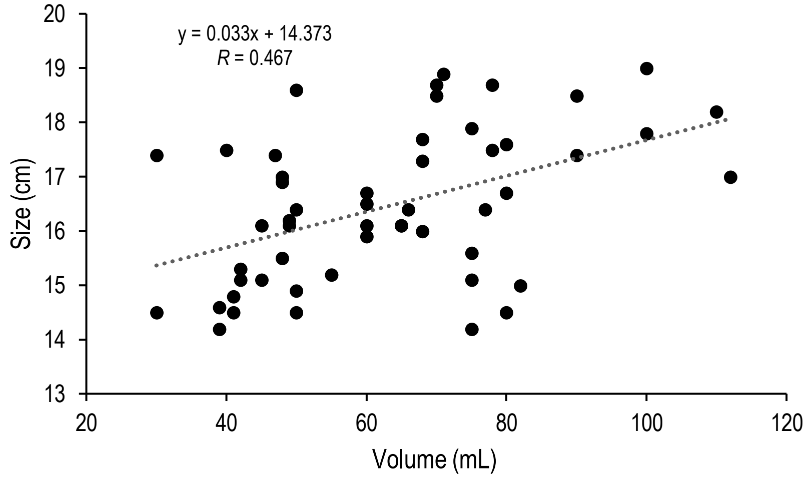

Figure 3. Volume (mL) and length (cm) ratio in experimental organisms of *Dormitator latifrons* used to analyze gastric evacuation.

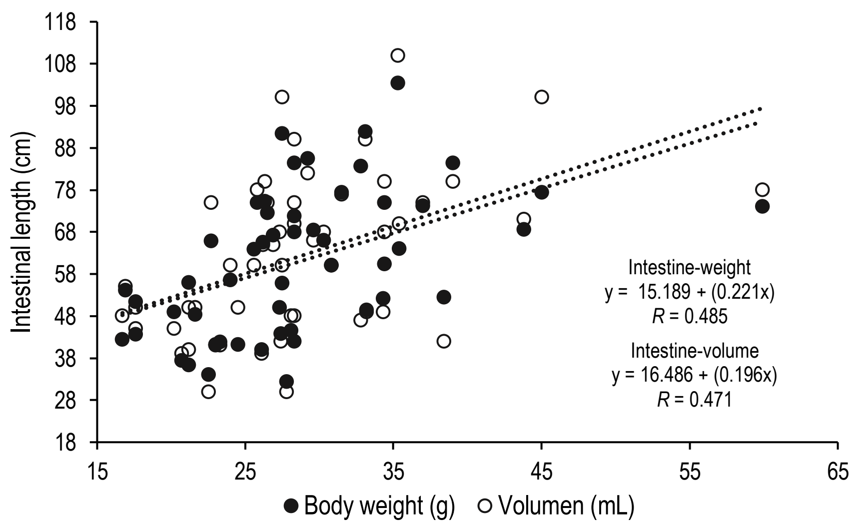

Figure 4. Relationship of the total length of the intestine and the weight and volume of experimental organisms of *Dormitator latifrons* used to analyze gastric evacuation.

why there were larger variations in weight (60.13 ± 14.92 g) and volume (61.21 ± 17.89 mL) than in size (16.38 ± 1.33 cm). Although the present study does not correspond to a study similar to the one mentioned, this coincides with the results of the organisms subjected to experimentation. Although acclimatized for 30 days, they were captured from the wild. The information from natural populations can help and have important implications for farmed organisms, in this case, with the population structure of *D. latifrons*. In this sense, it is important to carefully select organisms of homogeneous weights in the study of passage or evacuation of food to reduce possible biases and deviations in results.

This work is the first to address the issue of food transit in *D. latifrons*; that is, food transit was indirectly evaluated through the dry weight (g) of the food bolus (chyme) sampled over the hours with a single feeding. The best model that explains the food transit in *D. latifrons* was the simple exponential decay model with two parameters simultaneously, the intestinal relative content index. This exponential decay regression model was developed in this study and applied by Booth et al. (2008) to the fish *Pagrus auratus* to evaluate the gastric evacuation rate from a single feeding. In that study, a complete evacuation of the gastric chamber was recorded 16 to 20 h after feeding.

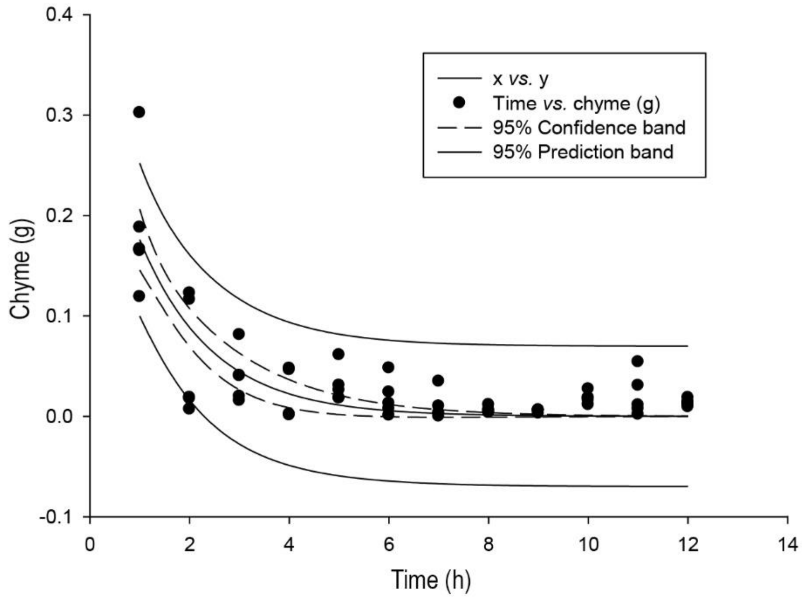

Figure 5. Nonlinear regression analysis of simple exponential decay model with two parameters to evaluate the passage of the food concerning alimentary chyme weight (g) over time (h). Test was carried out with 200 iterations. $R^2 = 0.603$; $P < 0.001$; f = a*exp(-b*x).

Table 1. Nonlinear regressions tested in the study of food transit in *Dormitator latifrons*. In all models, chyme weight (g) was the dependent parameter, and time (h) was the independent parameter. All tests were performed with 200 iterations. Ordered from highest to lowest according to R^2 and significance ($P < 0.0001$).

Model	Function	R^2	Coefficients				
			Parameter	Estimate	Standard error	t	*P*
Simple exponential decay, two parameters	f = a*exp(-b*x)	0.6303	a	0.3499	0.0597	5.8637	<0.0001
			b	0.6871	0.1138	6.0378	<0.0001
Quadratic polynomial	f = y0+a*x+b*x^2	0.5515	y0	0.1756	0.0179	9.7944	<0.0001
			a	-0.0422	0.0063	-6.6528	<0.0001
			b	0.0025	0.0005	5.3139	<0.0001
Linear polynomial	f = y0+a*x	0.3292	y0	0.099	0.0129	7.654	<0.0001
			a	-0.0094	0.0018	-5.3357	<0.0001
Inverse first-order polynomial	f = y0+(a/x)	0.6779	y0	-0.0096	0.006	-1.5944	0.1163
			a	0.1843	0.0167	11.0482	<0.0001
Cubic polynomial	f = y0+a*x+b*x^2+ c*x^3	0.6181	y0	0.2396	0.0264	9.0702	<0.0001
			a	-0.0917	0.0169	-5.4265	<0.0001
			b	0.0117	0.003	3.9442	0.0002
			c	-0.0005	0.0002	-3.1266	0.0028

In fish that do not have a true stomach (some omnivores and herbivores species), the role of pepsin is replaced by alkaline proteases, which are active at alkaline pHs (De Silva & Anderson 1995), as is the case in tilapia, where the digestion of food proteins occurs in the intestine under alkaline conditions (Klahan et al. 2009). It is known that, in species with a stomach, the passage of food is significantly affected by the speed of acid digestion. In contrast, in species without a stomach, the passage of food through the digestive tract responds to factors such as the length of the intestine and the weight and consistency of the food (Opuszynski & Shireman 1991). Generally, the time required to evacuate the food completely was lower than other carnivorous species and more similar to omnivorous or stomachless species. In the cod *Gadus morhua*, stomach

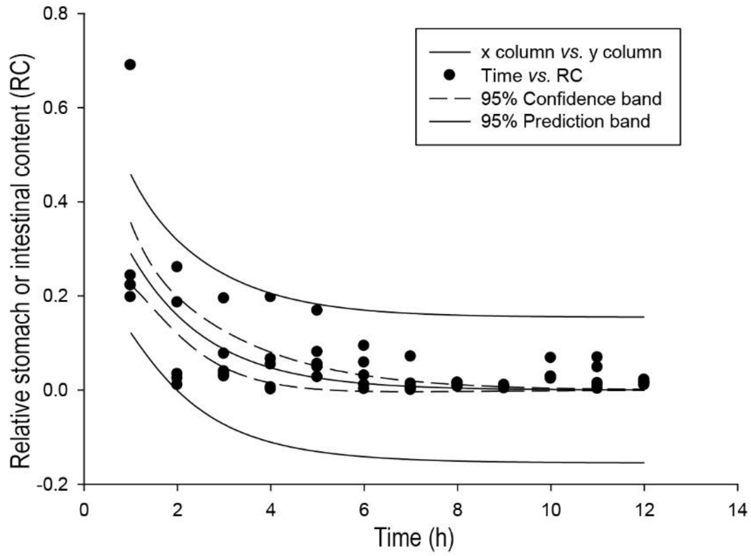

Figure 6. Nonlinear regression analysis of simple exponential decay model with two parameters to evaluate the passage of food concerning the relative content of the stomach or intestine over time (h). Test was carried out with 200 iterations. $R^2 = 0.6303$; $P < 0.001$; f = a*exp(-b*x).

content emptying was estimated to occur 27 h after feeding (Andersen 2012). In the trout *Salvelinus fontinalis*, total evacuation of food occurred more than 40 h after feeding (Khan & Seyhan 2019). The total food evacuation time in the Nile tilapia *Oreochromis niloticus* was 7.15 h (Uscanga et al. 2010). In *Atherinops affinis*, the intestinal passage time was just over 2 h, the shortest time measured in herbivorous fish, including stomachless species (Logothetis et al. 2001). In these studies, the average values of evacuation time or food passage may be linked to variations in the length of the intestines of the same species.

As mentioned, this comparison does not indicate a general rule since the passage of food, gastrointestinal evacuation, and the emptying of chyme are difficult to compare since they are conceptualized differently and measured using different methods, experimental designs, and models. In addition, the passage of food can be affected by various factors such as water temperature, diet type, and formulation, the frequency and time of feeding, or the species biology. The latter includes the dimensions of the fish, their life stage, digestive anatomy, and physiology, among others (Logothetis et al. 2001, Booth et al. 2008, Uscanga et al. 2010, Andersen 2012, Khan & Seyhan 2019, Le et al. 2019). In turn, these variables can affect studies of the digestive enzyme system throughout the entire distribution of the digestive system, as occurs in *O. niloticus*, with alkaline proteases in the passage of food (Uscanga et al. 2010), opening an opportunity to develop similar protocols in *D. latifrons* where the study of digestive enzyme activity is involved.

The results obtained under the experimental conditions used in the present study indicate that *D. latifrons* exhibits an omnivorous-type digestive behavior, as mentioned in the literature: annelids, copepods, and other microfauna, nevertheless, detritus and some plant remain also been recorded in its diet, thus corresponding to a primary consumer of the detritivore type (Vega-Villasante et al. 2021), although it is important to specify that the diet administered was commercial pellets, a feed formulated for growing fish. It is necessary to confirm this finding with different protocols and more detailed experimental designs that allow us to analyze in greater detail the effect of different foods (live and artificial), temperatures, life stages, sex, and sizes. Even so, the results of the present study can serve as a basis for further research on the digestive physiology of *D. latifrons*, including the most appropriate feeding frequency and quantity for this fishing resource.

CONCLUSION

The fish *D. latifrons* showed rapid passage and evacuation of food. This finding could develop new experiments and feeding protocols to evaluate different feeding frequency strategies over 24 hours.

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