

Systematic literature review of *Hyphessobrycon eques*: A support for the improvement of captive breeding

Revisão sistemática de literatura de *Hyphessobrycon eques*: suporte para melhoria da criação em cativeiro

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Highlights

First systematic literature review carried out on *Hyphessobrycon eques*.
Of 966 articles published between 1997 and 2020, only 25 were selected.
Information gaps on the cultivation of the species were analyzed.

Abstract

Hyphessobrycon eques has a great prominence in fishkeeping and scientific research. In this context, a systematic literature review was performed to assist in the improvement of captive breeding of this species and to identify knowledge gaps. Notably, of the 966 articles published between 1997 and 2020 found in an initial screening of the digital libraries of Google Scholar, PubMed, SciELO, Science Direct, and Scopus, only 56 represented research on the species according to the established criteria. Considering aspects pertinent to captive reproduction, 25 studies were selected. These studies addressed information regarding reproduction; embryonic, larval, and juvenile development of the species; parasitic fauna; limiting concentrations of prophylactic agents; food preferences; and dietary supplements already used for these fish. Only seven studies reported information on the physicochemical characteristics of water in natural habitats, with great variations in several parameters, mainly those related to temperature. In 5 of the 25 selected studies, information related to reproductive characteristics and/or embryonic, larval, and juvenile development was addressed. However, only two studies developed experimental studies with information relevant to the captive breeding of the species. Studies to identify parasitic fauna and to analyze the use of prophylactic agents were also found. As for the information gaps on the cultivation of the species, there are no studies related to nutrition seeking to determine the species nutritional needs, including feeding

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frequencies, feeding durations, and specific amounts of food. Therefore, this review offers a great support for the breeding of *H. eques* by highlighting opportunities for new scientific studies that could enrich and collaborate in the breeding of this ornamental fish.

Key words: Aquaculture. Fish Farm. Ornamental Fish.

Resumo

Hyphessobrycon eques tem grande destaque na aquicultura e na pesquisa científica. Nesse contexto, foi realizada uma revisão sistemática da literatura para auxiliar no aprimoramento da reprodução em cativeiro desta espécie e verificar lacunas de conhecimento. Notadamente, dos 966 artigos publicados entre 1997 e 2020 que foram encontradas na triagem inicial das bibliotecas digitais do Google Scholar, PubMed, SciELO, Science Direct e Scopus, apenas 56 representaram pesquisas sobre a espécie de acordo com os critérios estabelecidos. Considerando os aspectos pertinentes à reprodução em cativeiro, foram selecionados 25 estudos. Esses estudos abordaram informações sobre reprodução; desenvolvimento embrionário, larval e juvenil da espécie; fauna parasitária; a concentração limitante de agentes profiláticos; preferências alimentares; e suplementos dietéticos já usados para esses peixes. Apenas sete estudos relataram informações sobre as características físico-químicas da água em habitats naturais, com grande variação nos parâmetros, principalmente relacionados à temperatura. Em 5 dos 25 estudos selecionados, foram abordadas informações relacionadas às características reprodutivas e/ou relacionadas ao desenvolvimento embrionário, larval e juvenil. No entanto, apenas dois estudos desenvolveram estudos experimentais com informações relevantes para a reprodução em cativeiro da espécie. Foram observados estudos de identificação da fauna parasitária e pesquisas utilizando agentes profiláticos. Quanto às lacunas de informação sobre o cultivo da espécie, não existem estudos relacionados à nutrição que busquem determinar suas necessidades nutricionais, incluindo frequências e tempos de alimentação e quantidades específicas de alimentos. Dessa forma, foi possível oferecer um maior suporte à criação de *H. eques*, destacando oportunidades para novos estudos científicos enriquecerem e colaborarem com a produção deste peixe ornamental.

Palavras-chave: Aquicultura. Peixe Ornamental. Piscicultura.

Introduction

Hyphessobrycon eques (Steindachner 1882), popularly known in Brazil as "Mato Grosso" (Santana et al., 2019), is a member of the genus *Hyphessobrycon*, one of the most important genera in the family Characidae and order Characiformes (Park et al., 2014; Berchielli-Morais et al., 2015; Dagosta et al., 2016). Species of this genus probably originated in the Amazon, Guaporé, and Paraguay river basins (Weitzman & Palmer,

1998). However, these fish have been found in several regions of South America (Aguinaga et al., 2014), mainly in Brazil, where they are usually seen in schools of 20 to 30 individuals (Casatti et al., 2003) in places with macrophytes (Teresa et al., 2011), as well as in lentic and semi-lentic areas (Esgúicero & Arcifa, 2010). Examples of such locations include streams, rivers, lagoons, and reservoirs (Fujimoto et al., 2013; Gonçalves et al., 2013; Acosta & Silva, 2015; Fiori et al., 2016; Dias et al., 2017; Santana et al., 2019).

The species is small sized (Gonçalves et al., 2013), generally reaching a total length between 4 and 6 cm, with colors ranging from light reddish-brown to bright or dark red (Cole & Haring, 1999; Fujimoto et al., 2013). In addition, it has a laterally flattened body with black spots on the upper part of the pectoral fin, the dorsal fin, and the edge of the anal fin (Park et al., 2014). The pelvic and anal fins are dark red with white accents, whereas the caudal fin has a solid red color on its outermost edges (Cole & Haring, 1999). Because of its considerable beauty, fair adaptability, easy handling, and fair resistance to stress, *H. eques* plays a significant role in commercialization as an ornamental fish for aquariums in Brazil and worldwide (Park et al., 2014; Berchielli-Morais et al., 2015; Çelik & Cirik, 2019; Santana et al., 2019). This fish species is equally important in scientific research because, in addition to the characteristics already mentioned, it is adapted to the varied environments of South America and is relatively sensitive to various substances (Silva et al., 2015; Marcon et al., 2016; Mansano et al., 2018; Abe et al., 2019).

Studies on *H. eques* have been carried out using various approaches, such as toxicological corrections (Marcon et al., 2016; Mansano et al., 2018; Abe et al., 2019), assessment of eating behavior (Carvalho & Del-Claro, 2004; Pelicice & Agostinho 2006), reproduction and development (Park et al., 2014; Çelik & Cirik, 2019), parasitic fauna (Santos et al., 2017; Narciso et al., 2019), genetics and cytogenetics (Martinez et al., 2012; Piscor & Parise-Maltempa, 2015; Costa-Silva et al., 2018), food preferences (Miraldo & Pecora, 2017; Carniatto et al., 2020), and dietary supplementation (Pan et al., 2011; Berchielli-Morais et al., 2015). However, deficiencies in the information concerning this fish have

been frequently addressed (Carvalho & Del-Claro, 2004; Park et al., 2014; Acosta & Silva, 2015; Çelik & Cirik, 2019; Santana et al., 2019). Furthermore, there is still no compilation with sufficient scientific information to assist in the breeding of this species, and few studies have focused on research-based knowledge regarding its cultivation. Therefore, this area needs to be enriched with scientific studies and technical information.

Considering the current context, this study aimed to identify, categorize, and address this lack of information, in an unprecedented way, through a systematic literature review of the studies on the ornamental fish *H. eques* published between 1997 and 2020. In addition, we highlight the gaps in knowledge about the species as well as the perspectives for future research to collaborate in the improvement of the captive breeding of this ornamental fish.

Materials and Methods

Strategies for research selection

Through this systematic literature review, we aimed to answer the following questions: "What studies have been carried out on *H. eques*?" "Which aspects of these studies could assist in the captivity-based cultivation of this species?" For this purpose, five databases related to the scientific productions of Agrarian Sciences were selected: Google Scholar, PubMed, SciELO, Science Direct, and Scopus. Two distinct searching stages were also determined: initially, using the keywords "*Hyphessobrycon eques*" and, secondly, using the following search string: ("Ornamental fish" or "Aquarium fish" or "Pet fish" or "Ornamental

aquaculture" or "Ornamental fish farming") and ("*Hyphessobrycon eques*" or "Mato Grosso" or "Serpae tetra" or "Jewel tetra" or "Blood tetra").

The initial screening was conducted in December 2019 and was updated in July 2020. Screening was performed in accordance with the particularities of each database. For example, in the SciELO digital library, the search string was applied separately; otherwise, no results were obtained. Regarding the Google Scholar digital library, the search options "include patent" and "include citations" were not selected at either stage of search. Additionally, in the step where "*Hyphessobrycon eques*" was used as keyword, we selected the option "find articles with the exact phrase '*Hyphessobrycon eques*';" and such phrase had to be included in the title of the articles. After searching in all databases, the results were stored and manipulated using the program "StArt" version 2.3.4.

Certain criteria were applied for research eligibility, these included: studies based on experimental or exploratory analyses on the species *H. eques*, published either in a "scientific article" or "short communication" format, written either in Portuguese, English, or Spanish, and published until July 2020. Because of the divergences among the libraries in the selection of results, the criteria were verified only after the initial screening. Subsequently, specific criteria were applied for the research inclusion stage. These criteria allowed the inclusion of studies with information that could assist in the captive breeding of *H. eques*, such as works on reproduction, development, and possible prophylactic agents, as well as studies related to parasitic fauna and different aspects of

nutrition, such as food preferences, eating behavior, and nutritional diets.

Research selection process

The research was conducted according to the PRISMA recommendations in four distinct stages: 1. Identification, 2. Selection, 3. Eligibility, and 4. Inclusion. These steps were performed according to the methodology described by Galvão et al. (2015). In the identification step (step 1), all search results from the five databases were grouped, with the subsequent exclusion of duplicated studies. At this stage, studies that were not found in the initial screening but were present in several references of the selected studies (backward snowballing) were also included.

Thereafter, the results were selected (step 2) by reading the titles and abstracts of each study. In doing so, two researchers independently analyzed each study according to the first established criteria, ensuring non-interference in the selection stage. The selected results were then subjected to a second round of analysis consisting on reading the entire articles and verifying the availability of access to each study (eligibility phase or step 3). Finally, in the inclusion phase (step 4) a screening of the studies already specific to *H. eques* was done to include only those works with information that would assist in the cultivation of the species in captivity according to the specific criteria outlined in the selection step.

The results obtained in the selection process by each researcher were compared using the Kappa test (Cohen, 1960) as a statistical method to assess the agreement

between them. Using the method proposed by Landis and Koch (1977), the result obtained was $\kappa = 0.75$, which represents a substantial level of agreement between the researchers ($\kappa = 0.61$ – 0.80). This demonstrates the high level of reliability in the evaluation of the articles selected for this systematic review. Subsequently, disagreements over pieces of research were jointly defined by the researchers.

Extraction of selected research data

All studies selected in step 3 were categorized according to the main objectives of each study. Thus, it was possible to ascertain what kind of knowledge has been obtained and published about *H. eques* thus far (according to the digital libraries used in the review). Subsequently, information relevant to management conditions, concerning water quality parameters, was extracted from these studies, both under natural conditions and controlled experiments with *H. eques*.

As for the works included in the last phase of the selection (step 4), all information related to specific criteria that could contribute to improving the captive breeding process of *H. eques* was extracted, analyzed, categorized, and later discussed. The following categories were established: 1. reproductive and developmental aspects; 2.

health aspects; and 3. food aspects. All notes were only related to *H. eques*, regardless of the presence of other species in the studies. Finally, based on the approaches analyzed in the studies selected from steps 3 and 4, the main knowledge gaps, which could have contributed to the process of breeding this species in captivity, were discussed.

Results and Discussion

A total of 966 publications were identified during the initial screening, with the subsequent addition of 3 publications as a result of backward snowballing (Weitzman & Palmer, 1996, 1998; Cole & Haring, 1999). Of these publications, 56 presented information on *H. eques* with studies based on either experimental or exploratory analyses of the species, representing all the works published up to July 2020 according to the digital libraries used in the review (Table 1). Most of the studies were exploratory analyses of natural habitats and population structure ($n = 12$) as well as toxicological experiments ($n = 11$) on *H. eques*. The publication dates ranged from 1997 to 2020, with several fluctuations over these 23 years. Notably, since 2005, there has been an increase in works published per year, with significant increases in 2014, 2015, and 2018, which recorded the highest incidence of studies per year.

Table 1
Mapping of the studies selected in steps 3 and 4 of the systematic literature reviews and classified according to the main aspects addressed in each research on the ornamental fish species *H. eques*

Aspects Covered	Step Selection 3	Step Selection 4	Study
Anatomy	1	0	Datovo and Castro (2012)
Social behavior	1	0	Nijman and Heuts (2000)
Behavior/ Food Preference	9	9	Casatti et al. (2003); Carvalho and Del Claro (2004); Pelicice and Agostinho (2006); Crippa et al. (2009); Santana-Porto and Andrian (2009); Carniatto et al. (2014); Fiori et al. (2016); Miraldo and Pecora (2017), and Carniatto et al. (2020)
Creation and Production	1	1	Cole and Haring (1999)
Physiology	1	0	Gonzalez et al. (2018)
Genetics, Cytogenetics and Phylogeny	7	0	Javonillo et al. (2010); Martinez et al. (2012); Paz et al. (2014); Piscor and Parise-Maltempi (2015); Shimabukuro-Dias et al. (2016); Costa-Silva et al. (2018), and Piscor et al. (2020)
Natural habitat / Population structure	12	0	Weitzman and Palmer (1997, 1998); Pelicice and Agostinho (2005); Pelicice et al. (2005); Gonçalves and Braga (2008); Esguícero and Arcifa (2010); Teresa et al. (2011); Penha et al. (2014); Severo et al. (2018); Tondato et al. (2018); Nunes et al. (2020), and Pereira et al. (2020)
Nutrition	4	4	Wang et al. (2006); Pan et al. (2010, 2011), and Berchielli-Morais et al. (2015)
Reproduction and Development	5	2	Andrade et al. (2008); Gonçalves et al. (2013); Park et al. (2014); Çelik and Cirik (2019), and Santana et al. (2019)
Health	4	4	Fujimoto et al. (2013); Acosta and Silva (2015); Santos et al. (2017), and Narciso et al. (2019)
Toxicology	11	5	Russo et al. (2007); Sotero-Santos et al. (2007); Carraschi et al. (2011); Aguinaga et al. (2014); Silva et al. (2014); Carraschi et al. (2015); Silva et al. (2015, 2016); Marcon et al. (2016); Mansano et al. (2018), and Abe et al. (2019)

The publications included in the last stage of the selection had to present information pertinent to the captive breeding of *H. eques*. Only 25 studies were considered eligible (Table 1), making it possible to

address information on the following topics: reproduction and embryonic, larval, and juvenile development of the species (Park et al., 2014; Çelik & Cirik, 2019); parasitic fauna already reported in these fish (Fujimoto et al.,

2013; Acosta & Silva, 2015; Santos et al., 2017; Narciso et al., 2019); and lethal concentrations of potential prophylactic agents (Russo et al., 2007; Aguinaga et al., 2014; Silva et al., 2014; Carraschi et al., 2015; Marcon et al., 2016). In addition, the following topics were included: fish food preferences under natural and experimental conditions (Casatti et al., 2003; Pelicice & Agostinho, 2006; Crippa et al., 2009; Santana-Porto & Andrian, 2009; Carniatto et al., 2014; Berchielli-Morais et al., 2015; Fiori et al., 2016; Miraldo & Pecora, 2017; Carniatto et al., 2020) and nutritional additives already evaluated in this species (Wang et al., 2006; Pan et al., 2010, 2011; Berchielli-Morais et al., 2015).

Conditions related to water quality parameters for H. eques

Only seven studies reported information on the physicochemical characteristics of the water found in those natural habitats where *H. eques* was either analyzed or collected (Table 2). According to the data, there was a wide variation in the measured parameters, mainly in those related to temperature. Though several studies have reported the values for different water quality parameters under experimental conditions (Table 3), no study has carried out specific experiments for comparing different values of these parameters while working with *H. eques*. Such a study would make it possible to identify the ideal growing conditions for the species.

In natural environments, conditions are diverse, especially in relation to temperature, with variations from 12.2 °C in winter (Esguícero & Arcifa, 2010) to more than 30 °C

in the summer (Pelicice et al., 2005; Penha et al., 2014). Regarding pH, slightly acidic values have been found (Gonçalves & Braga, 2008; Carniatto et al., 2014), and dissolved oxygen is normally above 5.0 mg L⁻¹ (Casatti et al., 2003; Pelicice et al., 2005; Gonçalves & Braga, 2008; Esguícero & Arcifa, 2010; Carniatto et al., 2014; Penha et al., 2014).

Regarding water quality parameters under experimental conditions or during production, there are reports of temperatures ranging from 20 °C (Santos et al., 2017) to 28.5 °C (Park et al., 2014), slightly acidic or even neutral pH (Carvalho & Del-Claro, 2004; Park et al., 2014; Silva et al., 2014; Berchielli-Morais et al., 2015; Silva et al., 2015; Marcon et al., 2016; Silva et al., 2016; Santos et al., 2017; Mansano et al., 2018; Miraldo & Pecora, 2017; Çelik & Cirik, 2019), and dissolved oxygen above 5.0 mg L⁻¹ (Wang et al., 2006; Sotero-Santos et al., 2007; Pan et al., 2010; Carraschi et al., 2011; Pan et al., 2011; Aguinaga et al., 2014; Park et al., 2014; Silva et al., 2014; Berchielli-Morais et al., 2015; Silva et al., 2015; Marcon et al., 2016; Santos et al., 2017; Miraldo & Pecora, 2017). It should be noted that the conditions maintained in the experiments or in the cultivation of the species are similar to those observed in natural environments. Nevertheless, there is still no scientific research directly related to the determination of ideal cultivation conditions for the optimal growth and well-being of *H. eques* to optimize its breeding. According to Pérez et al. (2003), knowing the preferences and tolerances of a species, such as the ideal temperature range, is essential to defining which parameter values are the most suitable for cultivation, as well as to determining the level of fish survival in a given space.

Table 2
Water quality parameters in natural habitats of the species *H. eques*

Temp. (°C)	Parameters			Location / Geographic Coordinates	Collection period	Study
	pH	OD (mg L ⁻¹)	Cond. (µS cm ⁻¹)			
24.7 – 27.5	6.11 – 8.86	7.0 – 7.9	50.0 – 56.9	Right bank of the Paranapanema River (Reservoir Rosana - SP, Brazil) / 22°36'45.7" S; 52°15'19.5" W	†/July/2000; †/Set/2000; †/Dec/2000; †/Mar/2001	Casatti et al. (2003)
30.53 ± 0.23	7.42 ± 0.12	7.26 ± 0.24	41.4 ± 0.61	Six samples in the Paranapanema River (Rosana Reservoir, tributary of the Upper Paraná River, PR and SP - Brazil) / three in 22° 34 ' 07 " S, 52 ° 33 ' 34 " W and three in 22 ° 38 ' 29 " S, 52 ° 47 ' 16 " W	15 a 21/ Jan/2003	Pellicice et al. (2005)
17 – 21	5.2 – 6.6	0.54 – 7.00	†	Four lagoons (Catingueiro, Barrinha, Pedra and Fundão), right bank of the Mogi Guaçu River (Mogi Guaçu Reservoir, SP- Brazil) / 22 ° 16 'and 22 ° 18' S, 47 ° 09 'and 47 ° 12' W	†/Aug/2005 to †/July/2006	Gonçalves and Braga (2008)
12.2 – 19.9	5.5 – 7.6	6.19 – 7.62	49.1 – 138.7	Four habitats in the Jacaré-Guaçu Basin, right bank of the Tietê River (Alto Rio Paraná, Brazil) / 21° 50 '46" S, 48° 29 '22" W; 21° 51 '59" S, 48° 16 '42" W; 21° 51 '53" S, 48° 16 '42" W; 21° 51 '52" S, 48° 16 '09" W	22 to 25/ Jun/2008; 21 to 24/ Jun/2009	Esguícero and Arcifa (2010)
22.3 ± 1.2/ 22.2 ± 1.1	6.8 ± 0.4/ 6.6 ± 0.3	7.8 ± 1.2/ 7.7 ± 2.3	61.1 ± 3.6/ 59.2 ± 3.6	Three samples on the Canal (Alto Rio Paraná, Brazil) / 22° 47 '30 "S, 53° 24 '37" W	6 and 7/ Aug/2009	Carniatto et al. (2014)
17 - 32	7	> 5.0	†	Marginal lagoons of the Cuiabá River (Alto Pantanal, MT - Brazil) / between coordinates 16 ° and 17 ° S and 56 ° and 57 ° W	†/†/2005 to †/†/2008	Penha et al. (2014)
23 - 25	†	†	†	Olho d'Água River, a tributary of the Miranda River (Upper Paraguay River Basin, MS - Brazil) / †	†/May/2011	Nunes et al. (2020)

Temp.: Temperature, OD: Dissolved Oxygen, Cond.: Conductivity
† Values not reported.

Table 3**Main parameters of water quality in experimental conditions with *H. eques***

Temperature (°C)	Study
21.0 ± 1.5 / 22.4 ± 2.6 / 23.6 ± 3.7	Santos et al. (2017)
22.0	Nijman and Heuts (2000)
22.8	Aguinaga et al. (2014)
23.4 ± 1.68	Russo et al. (2007)
24.5 – 27.0	Carvalho and Del-Claro (2004)
25.0	Sotero-Santos et al. (2007)
25.0 ± 1.0	Miraldo and Pecora (2017); Marcon et al. (2016)
25.0 ± 2.0	Silva et al. (2014); Carraschi et al. (2015); Silva et al. (2015); Silva et al. (2016); Mansano et al. (2018)
25.0 – 26.0	Carraschi et al. (2011)
25.0 – 27.0	Gonzalez et al. (2018)
26.0 ± 0.5	Çelik and Cirik (2019)
26.0 – 28.0	Wang et al. (2006); Pan et al. (2010, 2011)
27.07 ± 0.04	Berchielli-Morais et al. (2015)
27.5 – 28.5	Park et al. (2014)
Ph	Study
6.5 ± 1.2 / 6.0 ± 3.2 / 7.2 ± 0.9	Santos et al. (2017)
6.25 ± 0.25	Park et al. (2014); Çelik and Cirik (2019)
6.5 – 7.0	Carvalho and Del-Claro (2004)
6.5 – 7.5	Silva et al. (2014, 2015, 2016)
6.8 ± 0.3	Miraldo and Pecora (2017)
6.8 ± 0.9	Marcon et al. (2016)
7.0 ± 1.0	Mansano et al. (2018)
7.04 ± 0.01	Berchielli-Morais et al. (2015)
7.0 – 8.0	Carraschi et al. (2011); Gonzalez et al. (2018)
7.1 – 7.8	Sotero-Santos et al. (2007)
7.3 – 8.3	Aguinaga et al. (2014)
7.5 – 8.0	Wang et al. (2006); Pan et al. (2010, 2011)
8.0	Russo et al. (2007)
Dissolved Oxygen (mg L ⁻¹)	Study
> 4.0	Silva et al. (2016)
> 5.0	Silva et al. (2014, 2015); Miraldo and Pecora (2017)
5.0 – 6.0	Carraschi et al. (2011)
6.3	Aguinaga et al. (2014)
5.3 ± 1.6 / 6.0 ± 3.4 / 6.8 ± 2.0	Santos et al. (2017)
6.0 – 7.0	Wang et al. (2006); Pan et al. (2010, 2011)
6.73 ± 0.29	Park et al. (2014)
6.9 ± 0.35	Marcon et al. (2016)
6.8 – 7.5	Sotero-Santos et al. (2007)
7.94 ± 0.02	Berchielli-Morais et al. (2015)

Reproductive aspects and embryonic, larval, and juvenile development of H. eques

In 5 of the 25 selected studies, information on the reproductive characteristics and/or embryonic, larval, and juvenile development of *H. eques* was addressed (Andrade et al., 2008; Gonçalves et al., 2013; Park et al., 2014; Çelik & Cirik,

2019; Santana et al., 2019). However, only two experimental studies have been conducted with information relevant to the captive breeding of the species (Table 4). Furthermore, no studies have defined the most appropriate conditions for the management of *H. eques* in any of these phases or determined what should be the main precautions when breeding this fish in captivity.

Table 4
Aspects related to the embryonic, larval and juvenile development of *H. eques* under experimental conditions

Study	Çelik and Cirik (2019)	Park et al. (2014)
Spawning Aquarium	15 L	500 mL (cup)
Aquarium (larval and juvenile)	15 L	75 L
Temperature	26 ± 0.5 °C	28 ± 0.05 °C
Egg diameter (mm)	0.94 ± 0.04	0.92 ± 0.01
Hatching (hours)	20 – 21	12 – 16
Larval length - 1st day (mm)	2.64 ± 0.21	2.89 ± 0.16
Development (eyes / mouth / anus)	3° – 4° day after hatching	3° day after hatching
Yolk sac absorption / Home exogenous feeding	4° / 3° – 4° day after hatching	3° day after hatching
Swimming bladder / Free swimming	4° – 5° day after hatching	3° day after hatching
Beginning of the youth stage	28° day after hatching	45° day after hatching
Juvenile length - 1st day (mm)	10.7 ± 0.27 (28° a 30° day)	12.5 ± 1.60 (45° day)

The reproduction and development of *H. eques* have been experimentally verified in only two studies thus far. Through these studies, the stages of embryonic and larval development have been described, from fertilization to the beginning of the juvenile period, providing important information for the captive breeding of this species. Regarding the physical characteristics of the species, there is no sexual dimorphism related to color; however, females are usually larger than males (Santana et al., 2019) and

have a larger abdomen, whereas males may have shorter anal fins that are sharp at the end (Park et al., 2014). In light of this, the most appropriate technique for the cultivation of the species would be to keep the breeders in groups for the duration of the reproductive period, as it is difficult to distinguish between male and female fish to form couples (Cole & Haring, 1999).

During spawning, it is essential to provide the best environmental conditions, prepare the animals, and observe their

behavior. According to Cole and Haring (1999), to ensure that spawning starts in the shortest possible time, *H. eques* need to receive a wide variety of live foods and good-quality diets. Park et al. (2014) observed the spawning behavior of this species and reported that the process started only at temperatures above 27 °C, generally occurring in the morning, with two or three males chasing each female and repeatedly stimulating her lower abdomen. After changing their color to dark green, the females spawned and the males released the sperm; this fertilization process was a few seconds long, but was repeated five to six times. In the study by Çelik and Cirik (2019), the authors only reported that spawning occurred at dawn and lasted 1–3 h, similar to what was reported by Cole and Haring (1999). Through the transference of this knowledge, it might be possible to maintain adequate breeding stock ratios, *i.e.*, a correct proportion between males and females, so that fertilization occurs more effectively. This is important because, with fewer males, there could be eggs left unfertilized.

Egg quality, larval growth, and survival are crucial factors in the captive breeding of ornamental fish species (Çelik & Cirik, 2019); these features are directly related to egg size. Park et al. (2014) reported that the eggs of *H. eques* were 0.91 to 0.93 mm (mean 0.92 ± 0.01 mm) in diameter, larger than those of other species in the same family. The eggs were also demersal, with a sticky appearance, round shape, and red color. Similarly, Çelik and Cirik (2019) found that the fertilized eggs of the species have spherical aspects and are transparent, demersal, and adhesive, with an average diameter of 0.94 ± 0.04 mm. Regarding the stickiness or adhesiveness of the eggs, an interesting alternative for

handling at the time of spawning would involve the use of substrates to help in their collection. Importantly, egg collection would prevent cannibalism, as adult fish usually consume the newly fertilized eggs after spawning is complete (Cole & Haring, 1999). Noteworthy, studies evaluating egg size determining factors could be helpful to optimize the size of the eggs of *H. eques*, given the lack of scientific knowledge in this regard.

The larvae can hatch approximately 24 h after fertilization at temperatures between 20 °C and 24 °C (Cole & Haring, 1999) or 20 to 21 h after fertilization when kept at a temperature of 26 ± 0.5 °C. The total length of the newly hatched larvae is 2.64 ± 0.21 mm in average (Çelik & Cirik, 2019). In their study, Park et al. (2014) reported that larvae generally hatched 16 h after fertilization when maintained at 28.0 ± 0.05 °C, and that such larvae had an average total length ranging from 2.76 to 3.05 mm (mean 2.89 ± 0.16 mm). From these data, it is clear that temperature significantly influences the hatching time of *H. eques*.

During larval development, the water temperature and type of food offered are closely linked to the growth and duration of the transformations (Anjos & Anjos, 2006). Çelik and Cirik (2019) reported that, on the third day after hatching, the mouths of the larvae opened and, on the fourth day, the yolk sac was completely consumed. Therefore, the larvae started to consume exogenous food even before the complete absorption of the yolk sac (5–10 nauplii mL⁻¹ of newly hatched *Artemia salina*). The authors also noted that the larvae began to swim freely only on the fourth or fifth day, on the same period at which the swim bladder began to function. This is similar to the period found for most ornamental

freshwater fish larvae (3–6 d). Park et al. (2014) observed that, on the third day after hatching, there was complete resorption of the yolk sac, opening of the mouth, completion of the development of the swim bladder, and initiation of an exogenous diet (consisting of 5–10 rotifers mL⁻¹ of *Brachionus rotundiformis* for 15 days, 10–15 nauplii mL⁻¹ of *Artemia* sp. from day 15 to day 35, and 200–310 µm live larvae mL⁻¹ from the 30th day on).

The transition from the larval to juvenile stage is characterized by the completion of morphological development; as in the larval stage, development is related to several factors, particularly temperature and nutrition (Çelik & Cirik, 2019). Additionally, according to Çelik and Cirik (2019), for *H. eques*, the beginning of the juvenile phase occurred between days 28 and 30 at a temperature of 26 ± 0.5 °C in fishes that had a total length of 10.7 ± 0.27 mm. In contrast, Park et al. (2014) observed that the average fish length was 10.2 ± 1.20 mm at 40 days, and that the juvenile stage of *H. eques* began at day 45, in fish measuring 12.5 ± 1.60 mm and maintained at 28.0 ± 0.05 °C. From these studies, it can be said that *H. eques* starts its juvenile stage within 28 to 45 days, and that the total length of the fish at this stage can vary between 10 and 15 mm. These differences are probably due to variations in the experimental conditions, such as water temperature and the type of food provided. Nevertheless, there is a lack of studies evaluating different cultivation conditions for each stage of *H. eques* development, such as water quality parameters or those related to nutritional aspects.

Remarkably, there is little scientific knowledge regarding the reproduction and initial developmental period of *H.*

eques. More detailed information on the reproductive dynamics of species native to South America, including *H. eques*, might guarantee sustainability in commercialization (Andrade et al., 2008) as well as provide a better understanding of the determining factors in embryonic and larval development. This improved understanding can enhance and optimize commercial production, which will then support the development of more assertive methodologies for the captive breeding of the species (Çelik & Cirik, 2019). Therefore, new publications and technical information addressing these issues will enrich the production of *H. eques*.

Behavioral aspects relevant to H. eques

Parasitic fauna in *H. eques* was identified through four surveys, which reported the presence of certain parasites for the first time. Among these parasites, *Quadrigyrus nickoli* (Acanthocephala) (Fujimoto et al., 2013), *Hysterothylacium* sp. (Nematoda: Anisakidae) (Acosta & Silva, 2015), and *Bragana nasuta* (Isopoda: Cymothoidae) (Narciso et al., 2019), were identified in animals collected from their respective natural habitats. Only one study verified the presence of parasites in *H. eques* collected directly from fish farms. The parasites found in this study included *Ichthyophthirius multifiliis*, trichodinids, and digeneans (Santos et al., 2017).

Some studies have used potential prophylactic agents in *H. eques* aquaculture (Table 5), including hydrogen peroxide (H₂O₂) (Russo et al., 2007), a bark extract from *Uncaria tomentosa* (Aguinaga et al., 2014), copper sulfate (CuSO₄) (Silva et al., 2014), florfenicol (FLO), enrofloxacin (ENR), thiamethoxan (TH), toltrazuril (TOL) (Carraschi et al., 2015), and

diflubenzuron (Dimilin) (Marcon et al., 2016). However, most of these studies only used *H. eques* to measure its resistance to these compounds, aiming to determine the lethal

concentrations or searching for pathological and behavioral changes in the animals, but not for controlling their pathogens.

Table 5

Potential prophylactic agents used in studies with the species *H. eques* to determine the pathological and lethal concentrations in the species

Agent	Maximum Recommendation (mg L ⁻¹)	Pathological Concentration (mg L ⁻¹)	Lethal Concentration (CL50) (mg L ⁻¹)	Study
Hydrogen peroxide (H ₂ O ₂)	5.6 (24 hours) 17.0 (1 hours)	6.6 ± 0.9 (1 hora)	26.9 ± 6.1 (1 hours)	Russo et al. (2007)
Plant bark Uncaria tomentosa	†	20.0 ml L ⁻¹ (24 horas)	18.16 ml L ⁻¹ (48 hours)	Aguinaga et al. (2014)
Copper sulphate (CuSO ₄)	†	†	0.16 ± 0.03 (96 hours)	Silva et al. (2014)
Florfenicol (FLO) / Enrofloxacin (ENR)/ Thiamethoxan (TH)/ Toltrazuril (TOL)	†	†	> 100 / > 100 / 49.78 / 6.22 (48 hours)	Carraschi et al. (2015)
Diflubenzuron (Dimilin)	†	0.01 (96 hours and 17 days)	†	Marcon et al. (2016)

† Values not reported.

Regarding studies on the parasitic fauna of *H. eques*, three studies found infected animals in their respective natural habitats. Acosta and Silva (2015) reported larval infection by nematodes of the genus *Hysterothylacium* in fish collected from the JurumirimreservoirintheParanapanemaRiver. Fujimoto et al. (2013) verified the occurrence of *Quadrigyrus nickoli* in the species after a collection in the Chumucuí River, Pará, Brazil. Furthermore, Narciso et al. (2019) observed that the ectoparasite *B. nasuta* parasitized this species in the Natural Heritage Reserve, Mato Grosso do Sul, Brazil. Santos et al. (2017) analyzed the parasitic fauna of ornamental fish in fish farms located in the state of Santa

Catarina, Brazil, and reported the presence of protozoan ectoparasites, such as *I. multifiliis* and trichodinids, in the gills and mucus on the surface of *H. eques*. In addition, digeneans (the most abundant parasites of the phylum Platyhelminthes) were present in the muscles of the animals. Additionally, according to the study, the prevalence of parasites may have been influenced by a lack of control of the fish population and of routine monitoring of water quality.

Studies on the parasitic fauna of ornamental fish have gained importance in recent years, largely due to economic losses in the cultivation and commercialization of

these animals (Narciso et al., 2019). With the intensification of fish farming, this occurrence is becoming increasingly prevalent (Aguinaga et al., 2014); therefore, constant monitoring during breeding is essential to guarantee the early diagnosis of pathogens (Santos et al., 2017). In addition, a better knowledge of the pathogens of ornamental species may also contribute to preventing infections and facilitating the optimal treatment of each species (Acosta & Silva, 2015).

As previously mentioned, some studies have used *H. eques* to evaluate the lethal concentrations of potential prophylactic agents that could be applied in aquaculture. These include the aqueous extract of the bark of the medicinal plant *U. tomentosa*, which has immunostimulatory, antiviral, and anti-inflammatory properties. In addition, Aguinaga et al. (2014) evaluated hydrogen peroxide, a chemical that is effective in controlling viral and bacterial diseases, fungi, and ectoparasites such as the flagellated ectoparasite *Ichthyobodo* spp. Russo et al. (2007) noted copper sulfate, which is known as an agricultural herbicide that is effective in controlling the growth of algae and macrophytes in nurseries, and also works as an agent in controlling bacterial and ectoparasitic diseases in fish farming (Silva et al., 2014). Similarly, the insecticide diflubenzuron, which is also used to control ectoparasites, has been tested on *H. eques* (Marcon et al., 2016), along with other drugs that have already been proven to be effective in the treatment of various diseases present in fish farms. These drugs include FLO, ENR, TH, and TOL (Carraschi et al., 2015).

According to these studies, hydrogen peroxide, at concentrations above 3.1 and 6.5 mg L⁻¹, proved to be effective in

treating external bacteria and *Ichthyobodo* spp., respectively. However, after 1 h at concentrations of 6.6 ± 0.9 mg L⁻¹, this agent caused abnormal behavioral changes in *H. eques* and, at concentrations of 26.9 ± 6.1 mg L⁻¹, the fish mortality rate reached 25%. Thus, the maximum recommended concentration for the species is 5.6 mg L⁻¹ for 24 h or 17.0 mg L⁻¹ for 1 h (Russo et al., 2007). Regarding the use of *U. tomentosa* bark extract, clinical and behavioral changes were only observed at concentrations above 20.0 mg L⁻¹ after 24 h, whereas, after 48 h, the LC50 was verified at 18.16 mg L⁻¹. Thus, its use was considered safe for prophylactic purposes with *H. eques* at low concentrations (Aguinaga et al., 2014). In a study done by Carraschi et al. (2015), the authors found that, after 48 h, the LC50 was > 100 mg L⁻¹ for FLO and ENR. Therefore, they could be used to breed *H. eques*. However, for TH and TOL the LC50 was 49.78 mg L⁻¹ and 6.22 mg L⁻¹, respectively, showing that the species is sensible to these two compounds; thus, great care should be taken when using these compounds in pathogen treatments. In contrast, the use of copper sulfate and diflubenzuron around *H. eques* is not recommended, due to the serious damage and high mortality rate of the animals after using small concentrations of any of these compounds, which are considered highly toxic to the species (Silva et al., 2014; Marcon et al., 2016).

Considering the aspects addressed in these studies, *H. eques* has been mostly used to determine the lethal concentrations of the compounds under study or the amounts that would cause pathological and behavioral changes in the fishes. Therefore, there is still a lack of studies on the use of potential prophylactic agents for the treatment of the pathogens present in *H. eques*.

Food aspects related to *H. eques*

The food preferences of *H. eques* were identified in nine studies (Table 6). Among these studies, it was possible to highlight the predominance of microcrustaceans and/or insect larvae consumption. Regarding

the feeding behavior of *H. eques*, these animals have diurnal eating habits and are characterized as opportunistic forage species, so they are classified among fish that are constantly turning over the substrate (Carvalho & Del-Claro, 2004; Pelicice & Agostinho, 2006).

Table 6
***H. eques* food preferences under natural and experimental conditions**

Diet composition <i>H. eques</i>	Study
- Insect larvae (chironomids and tipulidae); Microcrustaceans (Copepoda, Cladocera and Ostracoda); Parasites (nematodes)	Casatti et al. (2003)
- Microcrustaceans (Copepoda and Ostracoda); Algae and Bryophytes	Pelicice and Agostinho (2006)
- Microcrustaceans (Cladocera and Copepoda)	Crippa et al. (2009)
- Microcrustaceans (Cladocera)	Santana-Porto and Andrian (2009)
- Insect larvae (ephemeroptera and diptera)	Carniatto et al. (2014)
- Microcrustaceans (Cladocera and Copepoda)	Berchielli-Morais et al. (2015)
- Insect larvae (chironomids); Debris; Fish scales	Fiori et al. (2016)
- Insect larvae (<i>Aedes aegypti</i>)	Miraldo and Pecora (2017)
- Microcrustaceans (Copepoda and Cladocera); Insect larvae (efemerópteros and quironomídeos)	Carniatto et al. (2020)

Four studies evaluated the effects of additives in the diet on *H. eques*. In three of these studies, the same types of additives were used (the carotenoids astaxanthin and β -carotene) to verify their effects on several fish characteristics, such as survival, growth, pigmentation, and antioxidant capacity (Wang et al., 2006); the resistance of animals to stressful conditions caused by ammonia (Pan et al., 2011) or hypoxia during transport (Pan et al., 2010) was also determined. In a study by Berchielli-Morais et al. (2015), the consumption of diets with

microalgae (*Ankistrodesmus gracilis* and/or *Haematococcus pluvialis*) was evaluated and either compared with one another or with the consumption of zooplankton, to verify their effects on survival, growth, and pigmentation of the animals. Noteworthy, no studies have been conducted on the specific nutritional requirements of *H. eques* fish or on a method to determine the most appropriate food management techniques for the species, such as feeding frequency, feeding duration, and specific feeding amounts.

When verifying the food preferences of *H. eques* under natural and experimental conditions, it is possible to point out similarities in the diet of the species, despite the fact that studies under natural conditions were done in different regions. Berchielli-Morais et al. (2015), after supplementing the diet of *H. eques* with zooplankton, found that the consumption of *Diaphanosoma birgei*, Cladocera (59.5%), and *Argyrodiaptomus furcatus*, Copepoda (34.4%) was higher than that of other food options. Miraldo and Pecora (2017) used several species of ornamental fish to evaluate the efficiency of controlling larvae of the *Aedes aegypti* mosquito and considered *H. eques* as an adequate tool for such control due to the significant levels of consumption of the larvae.

According to Pelicice and Agostinho (2006), under natural conditions, the species almost exclusively consumed zooplankton (Ostracoda and, mainly, Copepoda) and fed on relatively smaller amounts of algae and bryophytes. Carniatto et al. (2020) observed that the eating habits of *H. eques* change according to the macrophytes they inhabit. In particular, greater consumption of microcrustaceans such as Chydoridae, Calanoida, and Cyclopoida (82% of the diet) was seen around native macrophytes, whereas, near invading macrophytes, the consumption of insects (including Ephemeroptera and Chironomidae) and microcrustaceans (mainly Daphniidae and Bosminidae) comprised 50% and 45.3% of the diet, respectively. Similarly, Crippa et al. (2009) and Santana-Porto and Andrian (2009) classified *H. eques* as zooplanktivores because of the predominance of microcrustaceans, such as Cladocera and Copepoda, in their diets.

In contrast, Casatti et al. (2003) found predominance in the consumption of dipteran larvae (Tipulidae and Chironomidae), Copepoda, and Cladocera (Chydoridae and Daphniidae) in *H. eques* collected during the dry season in the Rosana reservoir of the Paranapanema River. Higher consumption of Copepoda, Cladocera, and Ostracoda was observed in specimens collected during the rainy season, in addition to the presence of nematode parasites in some individuals. Fiori et al. (2016) also observed a prevalence in the consumption of aquatic invertebrates (Chironomidae) in one lagoon and of fish debris and scales in another lagoon, both located on the Miranda River (Pantanal of Mato Grosso do Sul). Carniatto et al. (2014) evaluated the diet composition of fish species inhabiting two different types of macrophytes in the Upper Paraná River. The researchers concluded that the diet of *H. eques* consisted predominantly of aquatic invertebrates such as Ephemeroptera insects around the macrophyte *Hydrilla verticillata*, and of larvae of Diptera and Ephemeroptera near the macrophyte *Egeria najas*. Therefore, because *H. eques* shows a significant preference for zooplankton, mainly microcrustaceans and/or insect larvae, these foods could be incorporated into the diet of these fishes when bred in captivity.

Regarding its feeding, *H. eques* partakes during daytime activity. In addition to being known as an opportunistic forager, it remains at the bottom of its habitat and constantly pushes substrates against macrophyte roots to capture small prey (Casatti et al., 2003; Carvalho & Del-Claro, 2004; Pelicice & Agostinho, 2006). In light of these observations, considering the natural characteristics of the species to determine

adequate management practices in captivity will certainly be very important. Thus, the provision of food during daylight hours seems to be the most appropriate feeding time and would contribute to achieving the best feeding practices for these fishes. Regarding the welfare of the individuals, the presence of macrophytes or artificial plants in the pond might represent a good solution. However, these hypotheses should be confirmed through scientific experiments to prove their viability for production.

When bred in captivity, ornamental fish are normally fed feed only, which requires carotenoid supplementation, as they are unable to synthesize it themselves (Wang et al., 2006). In three studies, supplementation of *H. eques* with the synthetic carotenoids astaxanthin and β -carotene was evaluated (Wang et al., 2006; Pan et al., 2010, 2011). In a study conducted by Berchielli-Morais et al. (2015), the authors noted the lack of available information on the use of a mixed diet and live food for ornamental fish native to Brazil. The authors also evaluated a control diet and a diet with microalgae (*A. gracilis* and/or *H. pluvialis*) in addition to a diet of zooplankton and zooplankton with microalgae for *H. eques*. This was done to assess the ability of these diets to provide energy and essential nutrients, such as natural carotenoids, to fish.

The main functions of synthetic and natural carotenoids are related to animal pigmentation (Pan et al., 2011). However, in the cultivation of several species, fish farmers have not yet been able to create an ideal diet to improve the color of the fish (Berchielli-Morais et al., 2015). This compromises commercialization efforts, as one of the main quality criteria that determines the value of ornamental fish in the market is beauty, which

is largely determined by color (Wang et al., 2006; Pan et al., 2010; Berchielli-Morais et al., 2015). Furthermore, these compounds have also been proven to collaborate for more effective protection of *H. eques* by providing increased antioxidant capacity (Wang et al., 2006), which bolsters the resistance of animals under stressful conditions caused by ammonia (Pan et al., 2011) or hypoxia during transport. In relation to this, Pan et al. (2010) showed a relationship between a reduction in dissolved oxygen, from 6.5 mg L⁻¹ to 1.0 mg L⁻¹, and carotenoid use. Additionally, in both the studies by Wang et al. (2006) and Pan et al. (2011), the authors found that after supplementation the animals converted β -carotene from the diet into astaxanthin within the body for storage. This may make the diets for this species more economical, as synthetic astaxanthin has greater commercial value than β -carotene, without having negative effects on fish survival or growth.

In contrast, Berchielli-Morais et al. (2015) reported that *H. eques* either received a control diet, a diet with microalgae (*A. gracilis* and/or *H. pluvialis*), a diet composed of zooplankton, or a diet composed of zooplankton with microalgae and obtained satisfactory results on pigmentation in all groups, with no mortality. Regarding the growth of animals, the groups that received only the artificial diet or the diet supplemented with microalgae showed increases in weight gain and total length. This was probably due to the reduced time and energy necessary for the fish to ingest readily available diets when compared to the intake of the same volume of zooplankton. This is because the crude protein content of the diet (with or without microalgae) was similar to that of zooplankton, which varied from 30% to

54% in microcrustaceans and Copepoda, respectively. This demonstrates that *H. eques* responds positively to food rations supplemented with microalgae; therefore, this method might be successfully implemented in the captive breeding of *H. eques*.

It is possible to observe that the main focus of the aforementioned studies was the evaluation of the effects of additives on color. As a result, there is a lack of studies evaluating nutritional requirements and dietary management, as well as dietary alternatives that could contribute to improved productivity and health of *H. eques*, regardless of the challenges attributed to breeding in captivity.

Aspects regarding the cultivation of H. eques that have not been covered in the literature

Concerning information gaps in those aspects related to cultivation that could enhance the production of *H. eques*, there are no studies on nutrition. This applies to both the determination of the specific nutritional requirements of the species and the adequacy of management practices, such as feeding frequency, feeding duration, and amount of food. In addition, concerning the development of the species, there is still no consensus regarding the duration of each phase as well as which management conditions are most appropriate for each of these phases. Furthermore, in terms of sanitation, experimental research has not yet been conducted to compare and determine the best prophylactic agent, especially for combating pathogens already identified in the species. Therefore, there are several research opportunities that could contribute to improve the captive breeding of *H. eques*.

Conclusion

More than 20 years of published research on the ornamental fish *H. eques* have been verified and compiled for the first time in this systematic literature review. However, there are also important notes related to a lack of knowledge about this promising fish, mainly in studies related to development, health, nutrition, and management in the cultivation of the species. In this way, it was possible to link fundamental information to assist in the captive breeding of the species, highlighting areas of opportunity so that future research can enrich the current information and collaborations between new and existing knowledge on *H. eques*.

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