# Differential ontogenetic effects of gut passage through fish on seed germination

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## ARTICLE INFO

## ABSTRACT

Passage through the digestive tract of frugivorous vertebrates likely influences seed dormancy, time to germination, and/or future seedling growth. Effects of gut passage on seed germination have been extensively investigated among frugivorous birds and mammals, however, few studies have focused on frugivorous fish. Here we present the results of a feeding experiment focusing on a broadly distributed Amazonian frugivorous fish, *Brycon falcatus*, and a fig tree, *Ficus gomelleira*. We investigated the effects of gut passage and ontogeny on seed germination probability and speed. The probability of seed germination and germination speed increased for seeds defecated by fish relative to control seeds. We also detected a negative effect of intestine length, where germination probability and speed decreased when seeds passed through larger fish with greater intestine to body length ratio. Such effects are probably the result of ontogenetic diet shifts, in which young fish transition from carnivory to frugivory as adults; a process only revealed by the inclusion of both small juvenile and large adult individuals in our study. As such, we advance knowledge of the mechanisms involved in frugivory as a feeding habit among fishes and on animal-fruit mutualisms.

## 1. Introduction

Elucidating how fruit- and seed-eating animals influence the germination patterns of endozoochorous plant species (i.e., those that depend on animals swallowing and dispersing their seeds) is pivotal to advance our understanding of the ecology and evolution of animal-plant interactions (Traveset et al., 2007). For instance, gut-passage often accelerates germination speed (Traveset et al., 2008; Fedriani and Delibes, 2009), enhancing the fitness of some plant species (Verdu and Traveset, 2005). But sometimes it can delay germination speed (e.g., Boedeltje et al., 2016), facilitating long-distance seed dispersal which in turn influences the demography and genetic pool of plant populations (Jordano, 2017). It is recognized that morphological and physiological traits of the digestive tract of fruit-eating animals exert a variety of effects on ingested seeds which ultimately determine their probability of germination (Herrera and Pellmyr, 2002). Effects of digestive tract characteristics on seed germination have been extensively investigated for birds and mammals and positive and negative effects on seed dormancy, the timing of seed germination, and/or seedling growth have been demonstrated (Traveset et al., 2007; Traveset and Verdú, 2002). Few studies have robustly assessed the impacts of seed ingestion by fish and report positive or neutral effects on germination probability and speed (see reviews in Correa et al., 2007; Correa et al., 2015a; Pollux, 2011). For example, it has been shown that a trade-off between mechanical and chemical digestion in fish with gastric mills (but low acid production) leads to a delay in germination speed of seeds that survive gut passage (Boedeltje et al., 2016). Additionally, locomotion during digestion could augment seed retention time by fish (Van Leeuwen et al., 2016), and fish and plant traits sometimes interact to influence the effect of gut passage on seed germination (Boedeltje et al., 2015; Van Leeuwen et al., 2016). Despite these insights there still remains a large gap in our understanding of the mechanisms that drive the effects of fish seed ingestion on seed germination and the potential for future seedling recruitment.

In an evolutionary and temporal perspective, frugivorous fish were likely the first vectors of dispersal of flooded forest and riparian plants (Correa et al., 2015a). Fish-fruit interactions are ubiquitous, but nearly half of the fish species that consume fruits during a portion of their life cycle (i.e., approximately 150 fish species) are distributed in the

Neotropical region (Horn et al., 2011). Studies of seed dispersal by fish (i.e. ichthyochory), like those focusing on other frugivore groups, usually explore fruit consumption in natural environments. For example, recent studies revealed greater efficiency of large-bodied fish as seed dispersers based on the volume and number of intact seeds, relative to damaged seeds, in stomach contents (Anderson et al., 2009; Correa et al., 2015a). The consumption of seeds by frugivores and the passage through their digestive tract causes chemical or physical scarification of seeds and promotes gas exchange between seeds and the gut environment, which leads to the elimination of germination inhibiting factors and facilitates the activation of metabolic processes (Traveset and Verdú, 2002). These effects may lead to an increase in the rate of seed germination and seedling vigor. Temporal aspects of germination, such as germination speed, have only been studied in a few plant species that are dispersed by fish (Boedeltje et al., 2016; Pollux et al., 2006; Van Leeuwen et al., 2016; Yule et al., 2016).

Frugivory researchers recently started to recognize that variability among individuals within seed disperser populations have disparate impacts on seed fate (Zwolak, 2018; Pollux, 2017). For instance, the social status of dispersers gives them differential access to preferred fruits during times of limited resource availability and leads to interspecific differences in the frequency of plant species dispersed (e.g., Tsuji et al., 2020). Fluctuating variability across individuals in other traits such as specialization, animal personality, and sexual dimorphism can affect multiple aspects of seed dispersal (reviewed by Zwolak, 2018). Additionally, many species of vertebrates undergo a dietary shift from carnivory to herbivory during ontogeny (Stevens and Hume, 1995). Some frugivorous fishes, for instance, switch from insectivory to frugivory with coupled ontogenetic changes in gut morphology and digestive enzyme activity (Drewe et al., 2004). As frugivory is not consistent across the life span of frugivorous fishes, differences in gut chemical composition at various ontogenetic stages may have different effects on seeds. This makes frugivorous fishes an ideal system to further advance our understanding of the effects of endozoochory, and intraspecific variability, on seed fate.

To further our understanding of the fish-fruit mutualistic relationship, we conducted a feeding experiment using an iconic frugivorous fish, *Brycon falcatus* (Bryconidae), and a common fig (*Ficus gomelleira*, Moraceae). This *Brycon* species is part of a diverse (44 species) and broadly distributed genus (from Mexico to the Rio de la Plata Basin, Argentina; Lima, 2017), represented by medium and large omnivorous species that feed on fruits, flowers, insects, other fish, and even small vertebrates (Goulding, 1980; Lima, 2017). Multiple *Brycon* species disperse seeds of numerous riparian plant species (Banack et al., 2002; Gomiero et al., 2008; Horn, 1997; Reys et al., 2008). *Brycon* species typically undergo a dietary shift during ontogeny; younger fish are carnivorous and have a short intestine, while older fish are predominantly frugivorous and have a longer intestine (Drewe et al., 2004). Of all frugivorous fish species found within in the middle stretch of the Teles Pires River, in Southern Amazonia, *B. falcatus* is the most important as it consumes the most fruit, relative to other fish species, within this system (Matos et al., 2016 and. L.N. Carvalho, unpubl. data). This *Brycon* species is currently under great threat as the region is impacted by deforestation, intensive agriculture, overfishing, and hydroelectric dam construction (Supplementary Information Fig. S1). Figs are abundant across tropical and subtropical humid ecosystems (Corner, 1965) and are among the most important plants for frugivores. That is because tropical frugivores generally eat more figs than fruits of other plant species (Bleher et al., 2003; Shanahan et al., 2001). Fig consumption by Neotropical fish has been reported in different watersheds (Correa and Winemiller, 2014; Reys et al., 2008; Souza-Stevaux et al., 1994; Weiss et al., 2016). In Central American riparian forests, *B. guatemalensis* are key seed dispersers of figs (Horn, 1997; Banack et al., 2002). Among the fruits consumed by the frugivorous fish assemblage of the middle Teles Pires River system, *F. gomelleira* is the second most consumed species (L. N.Carvalho, unpubl. data).

Here we investigated the effects of gut passage on the germination probability and speed of *F. gomelleira* seeds consumed by *B. falcatus* of various body sizes and ontogenetic stages. We predicted that seed passage through the gut of *B. falcatus* would increase germination probability and speed. Additionally, we predicted that germination probability and speed would be greater when seeds passed through the digestive tract of young carnivorous individuals compared to adult frugivorous individuals.

## 2. Methods

### 2.1. Fish collection and acclimatization

We captured 30 *B. falcatus* individuals in May 2018, with line and hook, in a tributary stream and in the main river channel of the middle Teles Pires River (between 11°36′25.6″ S 55°41′15.0″ W; see Supplementary Information Fig. S1, and Matos et al., 2016 for a map). Fish were individually placed in plastic bags where one third of the bag's volume was filled with water from the site of capture and the remainder was filled with ambient air. We transported fish in a cooler to our lab (Acervo Biológico da Amazônia Meridional – ABAM), located at the Federal University of Mato Grosso, Sinop Campus, Brazil.

The fish were then acclimated for 15 days in three 500 L reservoirs (10 fish each), equipped with submerged water recirculation pumps (i. e., 2000 L/h), and oxygenation pumps. Protective screens were installed to prevent fish from jumping out of the reservoir; a common behavior in this species. We kept a 12 h photoperiod and monitored water quality daily. Average values of dissolved oxygen, temperature, ammonia, and pH were 6 mg/L, 24.8 ± 0.6 °C, 0 ppm, and 6.3 ± 0.3, respectively, during the experimental period.

### 2.2. Feeding trial and fish-body metrics

*Ficus gomelleira* syconia, in the final stage of maturation, were collected ($N = 40$) from riparian forest trees ($N = 3$) along the Teles Pires River, in the same location where the fish were caught. To homogenize maturation stage, we selected ripe syconia for integrity (i.e., no obvious damage), standard size (mean diameter: 16 mm and mean weight: 1.8 g), and color (dark green). Syconia were then packed in an airtight plastic container and stored at 12 °C. For our experiment, 9000 seeds were manually removed from the collected ripe syconia. To homogenize maturation stage, we selected seeds of similar shape, size (mean ± SD: 1.30 ± 0.12 mm length, 0.73 ± 0.2 mm width, 0.71 ± 0.1 diameter, $N = 3600$), and color (dark yellow).

The seeds were stored in aluminum foil envelopes (100 seeds per envelope) and kept at 12 °C. To standardize the number of seeds of *F. gomelleira* supplied to each fish, we manually created pellets of ripe plantain pulp, which aggregated seeds well and were readily consumed by fish. One hour before each feeding trail, plantain pulp pellets were created, each containing 100 seeds of *F. gomelleira*. Each pellet weighed (mean ± *SD*) 2.3 ± 0.1 g and was 18.0 ± 0.6 mm long and 15.3 ± 0.5 mm in diameter. The number of seeds included in our artificial pellets was defined according to the approximate average number of seeds contained in one *F. gomelleira* syconium of similar size.

After acclimatization, one fish was transferred to an experimental 500-L tank containing two individuals of another fish species (*Astyanax* sp., Characidae). We used *Astyanax* sp. to mimic the natural shoaling feeding behavior (e.g., *Brycon* refused to eat for as long as 45 days when fish were kept alone during pilot feeding trials). Fish in the experimental tank were fasted for 48 h in order to allow emptying of the gastrointestinal tract. After fasting, feces were siphoned. Fish were fed twice a day with the first meal being the plantain pellets with seeds, which was offered at 0800 h. Once *Brycon* consumed the pellets, we removed the *Astyanax* sp. individuals (~10 min after offering the pellets). The second meal was a commercial ration (i.e. 32% protein and 3000 kilocalories), to ensure enough nutrient intake, which was offered at 1700 h. After

each feeding event, we inspected each tank for fecal material every 2 h and observed that feces were produced between 12 and 36 h after feeding. Thus, we collected fecal material by siphoning the bottom of the tanks at 12 h intervals, up to 36 h after feeding for each of the subsequent fish put through the feeding trial. At the end of each feeding-digestion-defecation process, we repeated the process, totaling three repetitions per individual fish. Collected feces were observed under a dissecting scope to assess number of seeds and seed integrity. The integrity of seeds was visually evaluated with the aid of a magnifying glass. Seeds were considered as damaged if they presented bite marks or were crushed.

At the end of the experiment, fish were euthanized by immersion in an Eugenol solution (Neiffer and Stamper, 2009). We recorded standard length (in mm, measured from the tip of the snout to the base of the hypural plates) and intestine length (to 0.01 mm precision using a digital caliper). The size at which *B. falcatus* achieve first maturation (L50) ranged between 20 and 23 cm SL (Matos et al., 2016 and L.N. Carvalho, unpubl. data). In our study, fish ranged between 7.2 and 32.0 cm standard length (SL) and the length of the intestine varied from 6.0 to 40.0 cm.

### 2.3. Germination experiment

Seeds retrieved from feces were washed with distilled water and pat dried with absorbent paper. Dry seeds were then placed on a double layer of Germitest paper, within petri dishes (14 cm in diameter), and placed in a vertical germination chamber (Biochemical Oxygen Demand) with a 12h photoperiod (78 μmol, supplied by a 15W white light fluorescent lamp) and 30 °C constant temperature. The paper was moistened daily with distilled water to saturation (excess water was pipetted). The number of germinated seeds was assessed daily within the germination chamber, with germination being defined as embryonic radicle emission. As a control, we also placed 3000 seeds of *F. gomelleira* directly removed from syconia in the germination chamber, following the same protocol used for feces-retrieved seeds. We used the same syconia to retrieve both experimental seeds, for the feeding trials, and control seeds. Germination typically occurred within 120 days, but we maintained the experiment for another 30 days, totaling 150 days of daily observation. We deposited a botanical specimen of *F. gomelleira* in the Centro-Norte-Mato-Grossense Herbarium (CNMT), Federal University of Mato Grosso (voucher CNMT-8051).

### 2.4. Data analyses

#### 2.4.1. Effects of passage through the fish digestive tract

To assess whether the germination probability of seeds ingested by fish and recovered from feces was different from that of control seeds not consumed by fish, we analyzed seed germination via a two-part modelling approach where each part described a different aspect of the germination process (Martin et al., 2005; Cameron and Trivedi, 1998). First, we examined the effect of fish consumption (i.e., fish treatment, $N = 6239$ seeds; control, $N = 3000$ seeds) on the probability of germination occurrence as a binary outcome (yes/no) using a generalized linear mixed model (GLMM; binomial family term). Then, for instances when germination occurred, we examined the time to germination (i.e., days) as a function of fish consumption using a generalized additive model (GAM; cox proportional family term). This modeling approach was chosen because an initial cox proportional hazard model indicated failure to comply with the assumption of proportional hazards (cox.zph test: $\chi^2 = 107.0$, P < 0.001).

#### 2.4.2. Effects of fish size

In *B. falcatus*, intestine length increases with fish size ($R^2 = 0.90$, $\beta = 1.50$, $P < 0.0001$, $N = 30$ fish). Thus, we investigated possible effects of seed passage through fish digestive tracts of various lengths on the probability of germination of ingested seeds. First, we examined the

probability of germination occurrence using a GAM (with a binomial family term), which is useful when predictors (i.e., intestine length) have non-linear relationships with the response variable (Yee and Mitchell, 1991). Then, in instances where germination occurred, we modelled the time to germination (i.e., days) as a function of intestine length using another GAM (with a cox proportional family term) due to time-varying effects within different intestine lengths. Additive models are useful substitutes to cox proportional hazard survival models when data cannot be stratified to deal with proportional hazard failures or there are time-varying effects (Lin and Ying, 1997; Abadi et al., 2011). Intestine length was included as a smoothed term in both GAMs.

All four models included individual ID as a random effect to account for the multiple measures per individual (i.e., multiple seeds retrieved from the same individual fish or syconia). All analyses were assessed using Wald chi-square ($\chi^2$) tests for significance of fixed effects, which are analogous to F-tests when the number of observations is large and allow for a refined means of dealing with large degrees of freedom (Agresti, 1990). Comparisons of means were considered marginally significant if $0.05 < P < 0.10$. All analyses were conducted in R v. 3.5.1 (R Core Team 2019) with the GLMM performed using the R-package lme4 (Bates et al., 2015), GAMs using the R-package mgcv (Wood, 2011), and the proportional hazards test using the R-package survival (Therneau, 2014).

## 3. Results

### 3.1. Effects of gut passage on germination probability

A total of 9000 fig seeds were feed to the fish, of which 69.3% (6239 seeds) were recovered intact from feces and, of these, 81.32% germinated (5074 seeds). Out of the 3000 control seeds submitted to germination, 68.3% germinated (2049 seeds). There was a positive effect of fish consumption on the probability of germination, with a 12.9 ± 2.1% ($\pm SE$) increase in germination probability when seeds were consumed by fish relative to controls ($\chi^2 = 43.3$, $P < 0.001$; Fig. 1a). There was also an effect of intestine length on the probability of germination, with a 10.1 ± 2.5% decrease in the probability of germination when seeds passed through longer intestines ($\chi^2 = 29.1$, $P = 0.01$; Fig. 1b). Additionally, the germination probability declined at a faster rate in fish with shorter intestines (≤20 cm) relative to those with longer ones (Fig. 1b). The probability of germination, however, was still greater for fish-ingested seeds, regardless of intestine length, compared to germination of control seeds (Fig. 1).

### 3.2. Effects of gut passage on germination speed

Germination occurred over a broad time-range for both treatments. Germination after gut passage began in the first 10 days after planting, reached a peak between 31 and 40 days, and the last seeds germinated between 81 and 90 days. In contrast, germination of control seeds began between the 30th and 41st day after planting, with a germination peak between the 61st and 70th day, while the last seeds germinated between the 111th to 120th day.

We recorded 7055 germination events with 63 right-censorships due to failure to germinate before the end of the observation period. Overall, it took an average ($\pm SE$) of 45.9 ± 0.3 days for seeds to germinate. Seed consumption by fish influenced germination time ($\chi^2 = 1192.0$, P < 0.001), with fish-ingested seeds germinating 47.9 days sooner compared to control seeds during the observation period (fish: 32.1 ± 0.1 days; control: 80.0 ± 0.4 days; Fig. 2a). Fish intestine length also influenced germination time ($\chi^2 = 29.3$, P = 0.001), with seeds going through shorter intestines (≤10 cm) germinating 6.8 days sooner compared to all other intestine lengths (≤10 cm: 26.7 ± 0.2 days; > 10 cm: 33.4 ± 0.1 days; Fig. 2b).
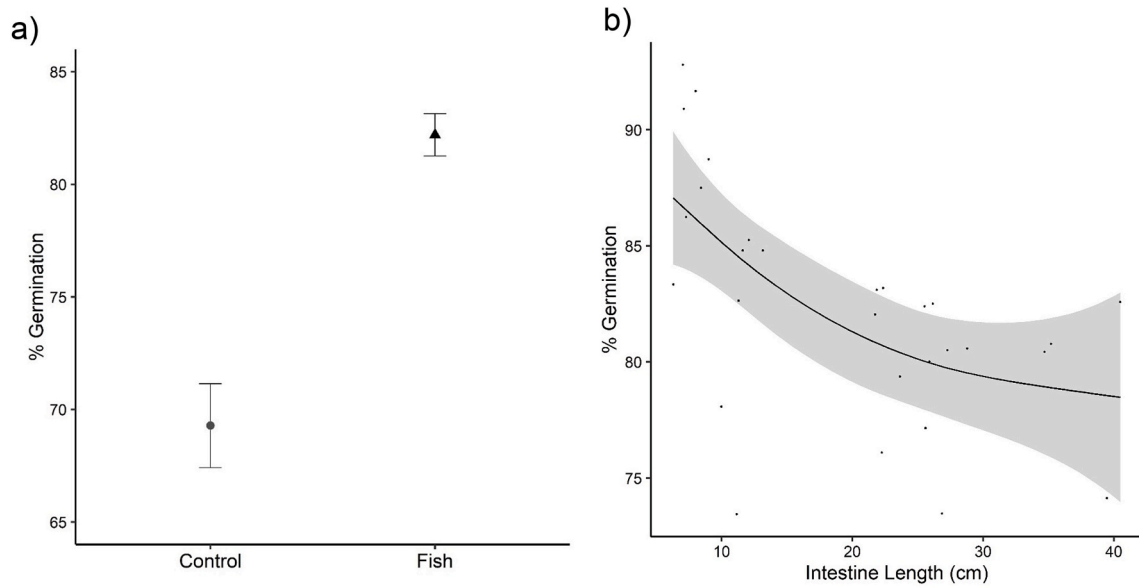
**Fig. 1.** Mean (±SE) proportion of seeds germinating as a result of (a) fish consumption relative to controls and (b) fish intestine length (cm). Data retrieved from fish feces after feeding trials where seeds of *Ficus gomelleira* were offered to *Brycon falcatus* of different sizes.
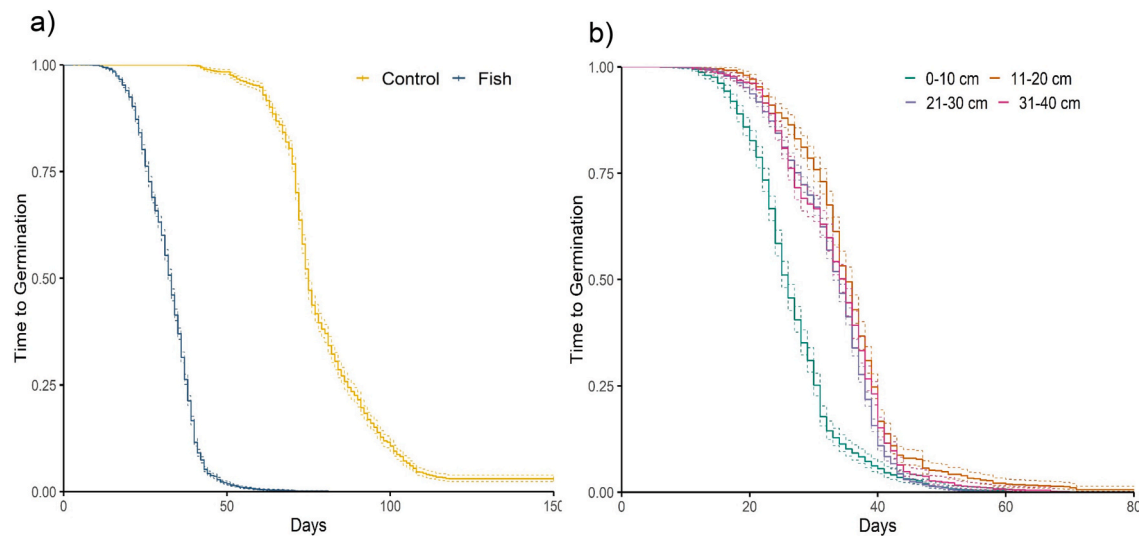


**Fig. 2.** The time to germination (shown as Kaplan-Meier curves) as a result of (a) fish consumption compared to control seeds and (b) fish intestine length (cm).

## 4. Discussion

### 4.1. Effects of gut passage on germination probability and speed

Seed passage through the gastrointestinal tract of fish can increase the probability of germination (Anderson et al., 2009; Pollux et al., 2009), have no effect (Adams et al., 2007), or even reduce germination (Pollux et al., 2006). These effects can be related to, not only the characteristics of the plant species consumed (Traveset et al., 2007), but also the anatomical and physiological characteristics of the consumer (Boedeltje et al., 2015). For example, characid fish, including *Brycon*, often cut, masticate and destroy many of the seeds they consume because of chisel-like and molariform dentition (Horn et al., 2011). The high percentage of intact seeds recovered during our feeding experiment, thus, must be due to the morphological traits of fig seeds. Their tiny size and spherical shape likely make them less susceptible to damage by masticatory movements compared to larger, elongated seeds of other plant species (e.g., Pollux et al., 2006). Additionally, the greater germination

probability observed among seeds retrieved from feces, relative to that of controls, suggests that passage through the gastrointestinal tract of *B. falcatus* does benefit fig seed germination. This may occur through two processes: 1) the removal of secondary germination metabolites present in fruit pulp, and 2) mechanical and chemical effects on the seed coat during the digestive process, which may enhance its turgor and thus stimulate cell division (Pollux, 2011). It is likely that one or both processes occurred in the present study; however, our treatments did not include seeds encased in natural fig pulp. Future studies should include this treatment to better discern the effects of seed passage through fish via pulp removal.

Seed passage through the digestive tract of frugivores has variable effects on germination speed. Our findings are similar to the acceleration in germination speed reported for birds and mammals (Traveset et al., 2007). Contrary to our findings, feeding experiments with *B. guatemalensis* demonstrated that seeds of *Ficus insipida* (=*glabrata*) that passed through the fish's digestive tract had a longer mean time until first germination relative to control seeds retrieved from syconia

collected from the forest floor or from the river (land and water treatments, respectively; Horn, 1997). The disparate results may be due to species-specific differences in germination speed among *Ficus* species. Germination speed is inherently variable across plant species and such variation remains even after gut passage (Traveset et al., 2007; Pollux et al., 2006). For example, the germination speed of seeds of 19 species of aquatic and riparian plants consumed by two fish species varied across plants species without a consistent effect (Boedeltje et al., 2016).

### 4.2. Effects of fish size on germination probability and speed

Empirical evidence from a broad array of fruit-eating taxa including birds, mammals, reptiles, and even invertebrates, demonstrates that ontogenetic variability can influence the feeding behavior of frugivores and thereby their effectiveness as seed dispersers (e.g., rates of fruit consumption, seed handling, and dispersal distance; Zwolak, 2018 and references therein). For instance, experiments with the giant alpine scree weta (Orthoptera) demonstrated that larger individuals not only consume a remarkably greater number of fleshy fruits, but are more effective seed dispersers; larger weta are more likely to disperse intact seeds and travel longer distances every night (Larsen and Burns, 2012) than smaller counterparts. Evidence from previous research on ichthyochory in Neotropical wetlands demonstrates that fish body size also influences multiple aspects of seed dispersal effectiveness. For example, smaller individuals of three *Brycon* species are more likely to masticate and damage seeds than larger individuals, which tend to swallow seeds intact, resulting in higher seed predation rates (Correa et al., 2015a). Additionally, a positive relationship between fish size and the volume of intact seeds retrieved from fish digestive tracts has been reported for multiple fish species with a 58.0% increase in the chance of dispersion for each 1 cm increase in the standard length of *Brycon amazonicus*, 2.7% for small-scaled pacu (*Piaractus mesopotamicus*), and 1.2% for red hook pacu (*Myleus torquatus*; Correa et al., 2015b). Similar positive trends with number of intact seeds and the probability of seed germination also hold across other species, including pacu (*Piaractus brachypomus*) and tambaqui (*Colossoma macropomum*; Anderson et al., 2009), likely due to longer gastrointestinal retention times in larger fish (Anderson et al., 2009; Correa et al., 2015a,b). Taken together, those findings suggest that larger fish individuals can be more effective seed dispersers than smaller ones (Anderson et al., 2011; Correa et al., 2015b; Galetti et al., 2008; Kubitzki and Ziburski, 1994). Our results offer an alternative scenario where seeds defecated by small fish were more likely to germinate and germinated faster.

Although we do not anticipate small individuals of *B. falcatus* to commonly consume fruits in natural settings (i.e., fruit consumption is opportunistic in young carnivorous fish), including both small juvenile and large adult individuals in our experiment was pivotal to allow us investigating ontogenetic effects of gut passage on seed germination. In our study, seeds passing through the digestive tracts of the smallest fish in our sample ($\leq$10 cm body length with $\approx$1:1 intestine length to body length ratio) had a higher probability of germination as well as faster germination speed, relative to seeds passing to larger fish. We believe that these patterns are driven by ontogenetic shifts in the diet of *Brycon* fishes, from carnivory as young to frugivory as adults. Although many species of vertebrates also undergo a dietary shift from carnivory to herbivory during ontogeny (White, 1993; Stevens and Hume, 1995), in fishes, this shift is accompanied by changes in gut morphology (i.e., greater intestine length to body length ratio; Montgomery, 1977; Benavides et al., 1994) and biochemistry (Moran and Clements, 2002; German, 2004). In the present study, larger *B. falcatus* have longer intestines, but the rate of increase is asymmetrical (e.g., fish between 10 and 20 cm have higher intestine length to body length ratio; Supplementary Information Fig. S2). Although we did not evaluate ontogenetic changes in gut biochemistry in our focal species, these changes do occur in *Brycon*. For example, *B. guatemalensis* undergoes ontogenetic changes in gut morphology (i.e., longer guts with fewer pyloric caeca) and

digestive enzyme activity as the fish switches from a predominantly insectivorous to a predominantly frugivorous/folivorous diet (Drewe et al., 2004). Overall, juvenile fish exhibited shorter guts, higher protease (i.e., pepsin and trypsin) activity, and lower amylase activity compared to larger ones (Drewe et al., 2004) which may explain why digestion of seeds by smaller *B. falcatus* in our study promoted germination and increased its speed. Protease enzymes likely drive chemical scarification of *F. gomelleira* seeds and, during the time that seeds remain in the intestine, a suite of such process likely occurs including the removal of germination inhibitors commonly present in pulp surrounding the seeds (Traveset et al., 2007 and references therein).

The enhanced seed germination rate and speed by small carnivorous fish, mediated by shorter intestine length, advance our mechanistic understanding of the ecological interaction between carnivorous animals and fruits. Carnivorous mammals (e.g., bears, marten, foxes, coyotes and raccoons) often consume fruits opportunistically and contribute seed dispersal services, particularly in temperate ecosystems (Koike et al., 2008). Large numbers of intact seeds are often retrieved from feces and passage through the digestive tract of carnivores can enhance seed germination rates (e.g., Rost et al., 2012). Because fruits and seeds have intermediate levels of fiber, seeds are likely to rapidly passed through the digestive tract of carnivorous animals undamaged as their digestive efficiency declines when consuming foods with high fiber content (e.g., black bear; Karasov and del Rio, 2007). Further research aimed to explore the effects of seed passage through carnivorous animals will contribute knowledge on the qualitative component of seed dispersal effectiveness (Schupp et al., 2010).

### 4.3. Significance

Our feeding experiment advances our mechanistic understanding of the effects of seed passage through the digestive tract of animals. We showed that fig seeds retained their viability after passing through the fish's gut with germination probability and speed being affected by intestine length, and probably all biochemical changes discussed above. Thus, our study not only provides evidence to demonstrate that *B. falcatus* can contribute to seed dispersal of *F. gomelleira* (and likely other riparian plants) within the Amazon River Basin but it also shows that these effects vary across life stages. As such, future feeding trials should account for intra-population variability by including animals with a broad range of body sizes, particularly when the focal species reaches a large body size. In addition, our findings highlight the potential role of juvenile fish as seed dispersers of small-seeded plant species. For instance, both juvenile and adult *C. macropomum*, the largest scaled frugivorous fish in Amazonia, are effective at dispersing the tiny seeds of *Cecropia* (Urticaceae) trees, a group of pioneer species abundant in Amazonian floodplains (Luque and Pinilla, 2019; Anderson et al., 2009). Given that the historic overexploitation of *C. macropomum* caused a demographic shift to smaller fish (Campos et al., 2015), seed dispersal by juvenile fish can play an important role in facilitating succession in floodplain forests affected by deforestation and fires (Luque and Pinilla, 2019; López-Bao et al., 2015; Rost et al., 2012). Faster germination and early seedling emergence can be detrimental to plants distributed at the interior of seasonally inundated forests (Correa et al., 2007). However, by accelerating germination speed, juvenile fish can give competitive advantages and enhance the fitness (Verdu and Traveset, 2005) of pioneer species, such as *Cecropia*, which colonize river and floodplain shores that become exposed faster than the forest interior as the water level recedes.

### Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

## Acknowledgements

## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.actao.2020.103628.

## References

Abadi, A., Saadat, S., Yavari, P., Bajdik, C., Jalili, P., 2011. Comparison of Aalen's additive and Cox proportional hazards models for breast cancer survival: analysis of population-based data from British Columbia, Canada. Asian Pac. J. Cancer Prev. APJCP 12, 3113–3116.

Adams, S.B., Hamel, P.B., Connor, K., Burke, B., Gardiner, E.S., Sage, D., 2007. Potential roles of fish, birds and water in the marsh privet (A*cuminate forestiera*) seed dispersal. SE. Nat. 6, 669–682. https://doi.org/10.1656/1528-7092.(2007).6 [669: PROFBA].2.0.CO; 2.

Agresti, A., 1990. Categorical Data Analysis. John Wiley, New York, USA. https://doi.org/10.1002/sim.4780111313.

Anderson, J.T., Nuttle, T., Saldaña Rojas, Pendergast, T.H., Flecker, A.S., 2011. Extremely long-distance seed dispersal by an overfished Amazonian frugivore. Proc. Roy. Soc.B Biol. Sci. 278 (1723), 3329–3335.

Anderson, J.T., Saldaña Rojas, J., Flecker, A.S., 2009. High-quality seed dispersal by fruit-eating fishes in Amazonian floodplain habitats. Oecologia 161, 279–290. https://doi.org/10.1007/s00442-009-1371-4.

Banack, S.A., Horn, M.H., Gawlicka, A., 2002. Disperser-vs. Establishment-limited distribution of a riparian fig tree (*Ficus insipida*) in a Costa Rica tropical rain forest. Biotropica 34, 232–243. https://doi.org/10.1646/0006-3606(2002)034[0232: DVELDO]2.0.CO;2.

Bates, D., Maechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. J. Stat. Software 67, 1–48. https://doi.org/10.18637/jss.v067.i01.

Benavides, A.G., Cancino, J.M., Ojeda, F.P., 1994. Ontogenic changes in gut dimensions and macroalgal digestibility in the marine herbivorous fish, *Aplodactylus punctatus*. Funct. Ecol. 8, 46–51. https://doi.org/10.2307/2390110.

Bleher, B., Potgieter, C.J., Johnson, D.N., Bohnng-Gaese, K., 2003. The importance of figs for frugivores in a South African coastal forest. J. Trop. Ecol. 19, 375–386. https://doi.org/10.1017/S0266467403003420.

Boedeltje, G., Spanings, T., Flik, G., Pollux, B.J.A., Sibbing, F.A., Verberk, W.C.E.P., 2015. Effects of seed traits on the potential for seed dispersal by fish with contrasting modes of feeding. Freshw. Biol. 60, 944–959. https://doi.org/10.1111/fwb.12550.

Boedeltje, G., Jongejans, E., Spanings, T., Verberk, W.C.E.P., 2016. Effect of gut passage in fish on the germination speed of aquatic and riparian plants. Aquat. Bot. 132, 12–16. https://doi.org/10.1016/j.aquabot.2016.03.004.

Cameron, C., Trivedi, P., 1998. Models for Count Data. Oxford University Press, Oxford, UK.

Campos, C.P., Costa Sousa, R.G., Catarino, M.F., de Albuquerque Costa, G., Freitas, C.E.C., 2015. Population dynamics and stock assessment of *Colossoma macropomum* caught in the Manacapuru Lake system (Amazon Basin, Brazil). Fish. Manag. Ecol. 22, 400–406. https://doi.org/10.1111/fme.12139.

Correa, S.B., Winemiller, K., Lopez-Fernandez, H., Galetti, M., 2007. Evolutionary perspectives on seed consumption and dispersal by fishes. Bioscience 57, 748–756. https://doi.org/10.1641/B570907.

Correa, S.B., Winemiller, K.O., 2014. Niche partitioning among frugivorous fishes in response to fluctuating resources in the Amazonian floodplain forest. Ecology 95, 210–224. https://doi.org/10.1890/13-0393.1.

Correa, S.B., Costa-Pereira, R., Fleming, T., Goulding, M., Anderson, J., 2015a. Neotropical fruit-fish interactions: eco-evolutionary dynamics and conservation. Biol. Rev. 90, 1263–1278. https://doi.org/10.1111/brv.12153.

Correa, S.B., Araujo, J.K., Penha, J.M.F., da Cunha, C.N., Stevenson, P.R., Anderson, J.T., 2015 b. Overfishing disrupts an ancient mutualism between frugivorous fishes and plants in Neotropical wetlands. Biol. Conserv. 191, 159–167. https://doi.org/10.1016/j.biocon.2015.06.019.

Corner, E.J.H., 1965. Check-list of *Ficus* in Asia and Australasia with keys to identification. Gardens Bull. Singapore 21, 1–186.

Drewe, K.E., Horn, M.H., Dickson, K.A., Gawlicka, A., 2004. Insectivore to frugivore: ontogenetic changes in gut morphology and digestive enzyme activity in the characid fish *Brycon guatemalensis* from Costa Rica rain forest streams. J. Fish. Biol. 64, 890–902. https://doi.org/10.1111/j.1095-8649.2004.0357.x.

Fedriani, J.M., Delibes, M., 2009. Functional diversity in fruit-frugivore interactions: a field experiment with Mediterranean mammals. Ecography 32, 983–992.

Galetti, M., Donatti, C.I., Pizo, M.A., Giacomini, H.C., 2008. Big fish are the best: seed dispersal of *Bactris glaucescens* by the pacu fish (*Piaractus mesopotamicus*) in the Pantanal, Brazil. Biotropica 40, 386–389. https://doi.org/10.1111/j.1744-7429.2007.00378.x.

German, D.P., 2004. Digestive enzyme activities in herbivorous and carnivorous prickleback fishes (Teleostei: Stichaeidae): ontogenetic, dietary, and phylogenetic effects. Physiol. Biochem. Zool. 77, 789–804. https://doi.org/10.1086/422228.

Gomiero, L.M., Manzatto, A.G., Braga, F.M.S., 2008. The role of riverine forests for food for *Brycon opalinus* in the Serra do Mar, southeast Brazil. Braz. J. Biol. 68, 321–328. https://doi.org/10.1590/S1519-69842008000200013.

Goulding, M., 1980. The Fishes and the Forest: Explorations in Amazonia Natural History. University of California Press, Berkeley and Los Angeles, CA.

Herrera, C.M., Pellmyr, O., 2002. Plant-animal Interactions: an Evolutionary Approach. Blackwell Publishing, Oxford, UK.

Horn, M., 1997. Evidence for dispersal of fig seeds by the fruit-eating characid fish *Brycon guatemalensis* Regan in a Costa Rican tropical rain forest. Oecologia 109, 259–264. https://doi.org/10.1007/s004420050081.

Horn, M., Correa, B.B., Parolin, P., Pollux, B.J.A., Anderson, J.T., Lucas, C., Peter, Widmann, Tjiu, Albertus, Galetti, Mauro, Goulding, M., 2011. Seed dispersal by fishes in tropical and temperate fresh waters: the growing evidence. Acta Oecol. 37, 1–17. https://doi.org/10.1007/s004420050081.

Jordano, P., 2017. What is long-distance dispersal? And a taxonomy of dispersal events. J. Ecol. 105, 75–84. https://doi.org/10.1111/1365-2745.12690.

Karasov, W.H., del Rio, C.M., 2007. Physiological Ecology: How Animals Process Energy, Nutrients, and Toxins. Princeton University Press.

Koike, S., Morimoto, H., Goto, Y., Kozakai, C., Yamazaki, K., 2008. Frugivory of carnivores and seed dispersal of fleshy fruits in cool-temperate deciduous forests. J. For. Res. 13, 215–222. https://doi.org/10.1007/s10310-008-0069-5.

Kubitzki, K., Ziburski, A., 1994. Seed dispersal in flood-plain forests of Amazonia. Biotropica 26, 30–43. https://doi.org/10.2307/2389108.

Larsen, H., Burns, K.C., 2012. Seed dispersal effectiveness increases with body size in New Zealand alpine scree weta (*Deinacrida connectens*). Austral Ecol. 37, 800–806. https://doi.org/10.1111/j.1442-9993.2011.02340.x.

Lima, F.C.T., 2017. A revision of the cis-andean species of the genus *Brycon* Müller & Troschel (Characiformes: Characidae). Zootaxa 4222. https://doi.org/10.11646/zootaxa.4222.1.1, 001-189.

Lin, D.Y., Ying, Z., 1997. Additive hazards regression models for survival data. In: Proceedings of the First Seattle Symposium in Biostatistics. Springer, New York, NY, pp. 185–198. https://doi.org/10.1007/978-1-4684-6316-3_10.

López-Bao, J.V., González-Varo, J.P., Guitián, J., 2015. Mutualistic relationships under landscape change: carnivorous mammals and plants after 30 years of land abandonment. Basic Appl. Ecol. 16, 152–161. https://doi.org/10.1016/j.baae.2014.12.001.

Luque, F.J., Pinilla, G.A., 2019. Juveniles of the overexploited Amazonian fish *Colossoma macropomum* (Characiformes: Serrasalmidae) as potential seed dispersers of *Cecropia* spp. Rev. Biol. Trop. 67, 654–666.

Martin, T.G., Wintle, B.A., Rhodes, J.R., Kuhnert, P.M., Field, S.A., Low-Choy, S.J., Tyre, A.J., Possingham, H.P., 2005. Zero tolerance ecology: improving ecological inference by modelling the source of zero observations. Ecol. Lett. 8, 1235–1246. https://doi.org/10.1111/j.1461-0248.2005.00826.x.

Matos, L.S., Silva, J.O.S., Andrade, P.S.M., Carvalho, L.N., 2016. Diet of characin, *Brycon falcatus* (Muller and Troschel, 1844) in the Amazon basin: a case study on an attractant for fish in the Teles Pires River. J. Appl. Ichthyol. 32, 1080–1085. https://doi.org/10.1111/jai.13108.

Montgomery, W.L., 1977. Diet and gut morphology in fishes, with special reference to the monkeyface prickleback, *Cebidichthys violaceus* (Stichaeidae: Blennioidei). Copeia 1, 178–182.

Moran, D., Clements, K.D., 2002. Diet and endogenous carbohydrates in the temperate marine herbivorous fish *Kyphosus sydneyanus* (Perciformes: *Kyphosidae*). J. Fish. Biol. 60, 1190–1203. https://doi.org/10.1086/jfbi.2002.1936.

Neiffer, D.L., Stamper, M.A., 2009. Fish Sedation, Anesthesia, Analgesia, and Euthanasia: Considerations, methods, and Types of drugs. ILAR J. 50, 343–360. https://doi.org/10.1590/1519-6984.15015.

Pollux, B.J., 2017. Consistent individual differences in seed disperser quality in a seed-eating fish. Oecologia 183, 81–91. https://doi.org/10.1007/s00442-016-3749-4.

Pollux, B.J.A., 2011. The experimental study of seed dispersal by fish (ichthyochory). Freshw. Biol. 56 https://doi.org/10.1111/j.1365-2427.2010.02493.x, 197-21.

Pollux, B.J.A., De Jong, M., Steegh, A., Ouborg, N.J., Van Groenendael, J.M., Klaassen, M., 2006. The effect of seed morphology on the potential dispersal of aquatic macrophytes by the common carp (*Cyprinus carpio*). Freshw. Biol. 51, 2063–2071. https://doi.org/10.1111/j.1365-2427.2006.01637.x.

Pollux, B.J.A., Luteijn, A., Van Groenendael, J.M., Ouborg, N.J., 2009. Gene flow and genetic structure of the aquatic macrophyte *Sparganium emersum* in a linear unidirectional river. Freshw. Biol. 54, 64–76. https://doi.org/10.1111/j.1365-2427.2008.02100.x.

Reys, P., Sabino, J., Galetti, M., 2008. Frugivory by the fish *Brycon hilarii* (Characidae) in western Brazil. Acta Oecol. 35, 136–141. https://doi.org/10.1016/j.actao.2008.09.007.

Rost, J., Pons, P., Bas, J.M., 2012. Seed dispersal by carnivorous mammals into burnt forests: an opportunity for non-indigenous and cultivated plant species. Basic Appl. Ecol. 13 (7), 623–630. https://doi.org/10.1016/j.baae.2012.08.016.

Shanahan, M., SO, S., Gompton, S.G., Gorlett, R., 2001. Fig-eating by vertebrate frugivores: a global review. Biol. Rev. 76, 529–572. https://doi.org/10.1017/s1464793101005760.

Schupp, E.W., Jordano, P., Gómez, J.M., 2010. Seed dispersal effectiveness revisited: a conceptual review. New Phytol. 188, 333–353. https://doi.org/10.1111/j.1469-8137.2010.03402.x.

Souza-Stevaux, M.C., Negrelle, R.R.B., Citadini-Zanete, V., 1994. Seed dispersal by the fish *Pterodoras granulosus* in the Paraná River Basin, Brazil. J. Trop. Ecol. 10, 621–626. https://doi.org/10.1017/S0266467400008294.

Stevens, E.D., Hume, I.D., 1995. Comparative Physiology of the Vertebrate Digestive System, 2nd. Cambridge University Press, New York, USA.

Therneau, T., 2014. A Package for Survival Analysis in S. R Package Version 2, 37-7.

Traveset, A., Verdú, M., 2002. A meta-analysis of the effect of gut treatment on seed germination. In: J Levey, D., Silva, W.R., Galetti, M. (Eds.), Seed Dispersal and Frugivory: Ecology, Evolution and Conservation. CABI Publishing, Wallingford, UK, pp. 339–350.

Traveset, A., Robertson, A.W., Rodríguez-Pérez, J., 2007. A review on the role of endozoochory on seed germination. In: Dennis, A.J., Green, R.J., Schupp, E.W., Westcott, D.A. (Eds.), Seed Dispersal: Theory and its Application in a Changing World. CABI Publishing, Wallingford, UK, pp. 78–103.

Traveset, A., Rodríguez-Pérez, J., Pías, B., 2008. Seed trait changes in dispersers' guts and consequences for germination and seedling growth. Ecology 89, 95–106.

Tsuji, Y., Campos-Arceiz, A., Prasad, S., Kitamura, S., McConkey, K.R., 2020. Intraspecific differences in seed dispersal caused by differences in social rank and mediated by food availability. Sci. Rep. 10, 1–9. https://doi.org/10.1038/s41598-020-58381-0.

Van Leeuwen, C.H.A., Beukeboom, R., Nolet, B.A., Bakker, E.S., Pollux, B.J.A., 2016. Locomotion during digestion changes current estimates of seed dispersal kernels by fish. Funct. Ecol. 30, 215–225. https://doi.org/10.1111/1365-2435.12485.

Verdú, M., Traveset, A., 2005. Early emergence enhances plant fitness: a phylogenetically controlled meta-analysis. Ecology 86, 1385–1394. https://doi.org/10.1890/04-1647.

Weiss, B.A., Zuanon, J.A.S., Piedade, M.T.F., 2016. Viability of seeds consumed by fishes in a Lowland forest in the Brazilian Central Amazon. Trop. Conserv. Sci. 1–10 https://doi.org/10.1177/1940082916676129.

Wood, S.N., 2011. Fast stable restricted maximum likelihood and marginal likelihood estimation of semiparametric generalized linear models. J. Roy. Stat. Soc. 73, 3–36. https://doi.org/10.1111/j.1467-9868.2010.00749.x.

White, T.C.R., 1993. The Inadequate Environment: Nitrogen and the Abundance of Animals. Springer-Verlag, Berlin.

Yee, T.W., Mitchell, N.D., 1991. Generalized additive models in plant ecology. J. Veg. Sci. 2, 587–602. https://doi.org/10.2307/3236170.

Yule, T.S., Severo-Neto, F., Tinti-Pereira, A.P., da Costa, C., L de B, L., 2016. Freshwater sardines of the Pantanal delay seed germination in a floodplain tree species. Wetlands 36, 195–199. https://doi.org/10.1007/s13157-015-0723-6.

Zwolak, R., 2018. How intraspecific variation in seed-dispersing animals matters for plants. Biol. Rev. 93, 897–913. https://doi.org/10.1111/brv.12377.