Conservation status and bio-ecology of *Brycon orbignyanus* (Characiformes: Bryconidae), an endemic fish species from the Paraná River basin (Brazil) threatened with extinction

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*Brycon orbignyanus* is an endemic species from La Plata basin whose stocks have been presenting significant reductions throughout the Paraná River. *Brycon orbignyanus* is categorized as an endangered species. This study evaluated aspects of the bio-ecology of this species that may be related to this threat, highlighting its distribution, abundance, and diet as well as the corresponding relationships between its recruitment and flood regimes. Data were obtained from different parts of the upper Paraná River (stretches free and regulated by dams) from 1986 to 2010 with more detailed data collected from the free remnant of this basin. The results indicate that no records for species exist at more than half of the sampling points located in dam-regulated sections of the Paraná River, whereas specimens were collected from 75% sites in the free plain remnant. We observed a remarkable effect of the hydrological regime on recruitment as well as distinct food demands during ontogenetic development, with adults almost exclusively consuming fruits and seeds, revealing that these individuals are supported by riparian vegetation. Thus, it is concluded that changes in the natural flood regime as well as riparian vegetation removal threaten *B. orbignyanus* populations in the Paraná River basin.

**Keywords:** Dam, Diet, Floodplain, Ontogeny, Recruitment.

Introduction

*Brycon orbignyanus* is an endemic species from La Plata basin whose stocks have been presenting significant reductions throughout the Paraná River. *Brycon orbignyanus* is categorized as an endangered species. This study evaluated aspects of the bio-ecology of this species that may be related to this threat, highlighting its distribution, abundance, and diet as well as the corresponding relationships between its recruitment and flood regimes. Data were obtained from different parts of the upper Paraná River (stretches free and regulated by dams) from 1986 to 2010 with more detailed data collected from the free remnant of this basin. The results indicate that no records for species exist at more than half of the sampling points located in dam-regulated sections of the Paraná River, whereas specimens were collected from 75% sites in the free plain remnant. We observed a remarkable effect of the hydrological regime on recruitment as well as distinct food demands during ontogenetic development, with adults almost exclusively consuming fruits and seeds, revealing that these individuals are supported by riparian vegetation. Thus, it is concluded that changes in the natural flood regime as well as riparian vegetation removal threaten *B. orbignyanus* populations in the Paraná River basin.

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their almost complete disappearance from upper and lower reaches of the Uruguay River (Agostinho et al., 2008; Lima, 2017; Oliveira et al., 2017).

The piracanjuba is currently on the official list of endangered species, listed as an endangered species (MMA, 2014). Various factors, including those related to riparian vegetation destruction, damming, pollution and the introduction of species, are considered to constitute the main threats to piracanjuba populations, with the former two having a particularly profound impact on the species (Agostinho et al., 2008). Riparian forest degradation is expected to negatively affect the piracanjuba’s diet, which largely consists of terrestrial food (Agostinho et al., 2008), while outflow dam control is a permanent threat to piracanjuba stocks since the reproduction and recruitment of this species depend on the occurrence of intense and prolonged flooding periods (Oliveira et al., 2015). Although intensely regulated by a cascade of upstream reservoirs, the flood pulse remains as the main force that shapes the structure and functioning of communities in the upper Paraná River floodplain (Agostinho et al., 2000). However, since the formation of the Porto Primavera reservoir at the end of 1998, flood attributes, such as their periods, intensity levels and durations, have been intensely altered (Agostinho et al., 2005). Under these conditions, an absence of regular flooding negatively affects the entire reproductive process of migratory species while the larvae of species that can reproduce under these conditions do not reach nearby lagoons, which serve as essential biotopes for growth and feeding in early development stages, due to a lack of connectivity between floodplain environments (Agostinho et al., 2005; Suzuki et al., 2009).

Actions aimed at the conservation of endangered species have already been implemented by the Brazilian government, including Portaria 445/2014, which prohibits fishing for endangered species. However, the measure is ineffective due to the limited number of inspections conducted by executive agencies (Ashikaga et al., 2015). Attempts made to protect the species are also evidenced by induced spawning, raising and reservoir stocking programs promoted by hydroelectric concessionaires (CEMIG-IESA; CESP; Itaipu Binacional; FURNAS; DUKE Energy International; AES Tietê) for 1986 to 2006 and on 40 reservoirs of this basin (Fig. 1). In the reservoirs, fish were collected with gillnets of different mesh sizes. Due to variations between reservoirs in terms of gillnet number (with mesh sizes of 3.0 to 16.0 cm between knots), we used presence and absence data to avoid sampling bias. Second, data on Brycon orbignyanus catches of the upper Paraná River floodplain (Paraná, Baía and Ivinhema River subsystems) for 1986 to 2010 (Fig. 1).

Fig. 1. The locations of reservoirs from which the Brycon orbignyanus occurrence was evaluated and sites sampled throughout the Upper Paraná River floodplain (the Paraná, Baía and Ivinhema River subsystems).
The upper Paraná River floodplain is positioned between the Porto Primavera Dam and the Itaipu reservoir (230 km), forming the most extensive lotic stretch of the upper Paraná River in Brazilian territory (Fig. 1). It is noteworthy that although heavily regulated by upstream reservoirs, the lowlands still present a flood pulse pattern (Fig. S1). Fish were sampled on monthly basis from October 1986 to September 1988 and from March 1992 through February 1993; every two months from March 1994 through February 1995; and every three months from 2000 to 2010. The sampling was carried out at 20 sampling stations located in floodplain lakes, channels, and river environments and distributed throughout the Paraná, Baía and Ivinhema River subsystems (Fig. 1). Gillnets of different mesh sizes were used to capture the fish. Fish were caught with a set of gillnets of different mesh sizes (with 3 to 16 cm of space between opposing nodes) that were submerged in water for 24 hours, and fish were removed in the morning, afternoon and evening.

After sampling, *B. orbignyanus* individuals were measured, weighed and eviscerated. Stomachs containing food were fixed in 4% formalin for diet analysis. *Brycon orbignyanus* abundance was expressed as CPUE (captures per unit of effort; individuals/1000 m² of gillnets over 24 hours) values transformed by log 10 (x + 1). Spatial (Paraná, Baía and Ivinhema River subsystems) and temporal (yearly) differences in CPUE values were tested through an analysis of variance (two-way ANOVA) after determining and applying assumptions for their application. Tukey’s post hoc test was used when significant differences were detected. The analysis of variance (two-way ANOVA) and Tukey tests were performed using Statistica 7.0 software (STATSOFT, 2005).

Paraná and Ivinhema River water level data were obtained through the Itaipu Binacional Hydrology Department. Flood regime attributes considered herein included interrupted (number of days the river exceeded water level thresholds during each hydrological cycle) and uninterrupted flood durations (days of continuous flooding). Flood durations were examined based on the recognized role of this variable in fish recruitment success (Gomes, Agostinho, 1997; Agostinho et al., 2004; Suzuki et al., 2009; Oliveira et al., 2015). For the Paraná River, the threshold hydrological level (river channel overflow) was measured as 4.5 m while values of above 2.75 m were considered for the Ivinhema River (Comunello et al., 2003).

Relationships between *B. orbignyanus* recruitment and the local flood regime were analyzed for the Paraná and Ivinhema Rivers. Recruitment was inferred based on young-of-year (YOY; < 23.0 cm, Oliveira et al., 2015) CPUE values for October 1986 to September 1988, March 1992 to February 1995 and January 2000 to December 2010. A covariance analysis (ANCOVA) was applied to evaluate the relationship between YOY abundance (CPUE; captures per unit of effort; individuals/1000 m² of gillnets over 24 hours) transformed by log 10 (x + 1) and flood duration (durations of interrupted and uninterrupted flooding). The slope models were adjusted separately when parallelism was not achieved (significant interactions). The YOY CPUE was applied as a dependent variable while the flood duration and rivers were used as co-variables and categorical variables, respectively. The covariance analysis (ANCOVA) was performed using the Statistica 7.0 program (STATSOFT, 2005). The *B. orbignyanus* diet was evaluated for specimens collected in 2010 from sampling stations distributed throughout the Paraná, Baía and Ivinhema River subsystems. The stomach contents of 150 individuals were examined under a stereoscopic microscope, identified and expressed as volumes (water displacement in a graduated cylinder; Hyslop, 1980). The identified items were grouped into the following food categories: fish, aquatic invertebrates (Crustacea, Diptera and Ephemeroptera), terrestrial invertebrates (Aranaeae, Coleoptera, Hemiptera, Homoptera, Isoptera, Lepidoptera and Orthoptera), higher aquatic plants, and higher terrestrial plants (leaves, flowers and seeds). To verify ontogenetic diet variations, individuals were grouped YOYs (n = 109; total length < 23.0 cm; Oliveira et al., 2015), juveniles (n = 28; 23.0 - 31.6 cm) or adults (n = 13; > 31.6 cm; Suzuki et al., 2004). Differences were tested through a Permutational Multivariate Variance Analysis (PERMANOVA) (Anderson et al., 2008). Post-hoc test pair-wise comparisons were drawn when significant dietary differences were detected. The PERMANOVA and Post-hoc tests were performed using PRIMER version 6.0 software. A significance of *P* < 0.05 was applied for all tests.

## Results

The surveys carried out from 1986 to 2006 in the upper Paraná River Basin reservoirs, during which at least one piracanjuba was observed in 16 of the 40 evaluated reservoirs (Tab. 1). It is noteworthy that piracanjuba occurred in only two of the 12 reservoirs evaluated in the Rio Grande Basin (the Agua Vermelha and Volta Grande reservoirs). The species was observed in eight of the 24 large reservoirs located in the other evaluated tributaries (Paranai, Tietê and Paranapanema) (Tab. 1). However, it was also found in the four main channels of the Paraná River reservoirs and in long upstream lotic stretches and lateral tributaries (Tab. 1).

Regarding the remaining free stretch of the Paraná River floodplain, the species was observed in 16 of the 20 sampled sites (Tab. 2, but was absent in five of the 17 sampling years (in 2000 and from 2002-2005) (Fig. 2). The largest abundance was observed between 1992 and 1993 and in 2010 (Fig. 2), when intense and prolonged floods occurred (Fig. S1). Our evaluation of species abundance between sites and years via a two-way ANOVA did not identify any interactions between the factors (*F* = 1.06, *P* = 0.38).
### Tab. 1. Presence of piracanjuba *Brycon orbignyanus* in reservoirs in the Upper Paraná River basin (+ = present; - = absent).
Source: Companhia Energética de São Paulo (CESP); Itaipu Binacional; Furnas Centrais Elétricas; Duke Energy Internacional; AES Tietê; Cemig-IESA.

| Reservoir                              | Basin | Status | Reservoir                              | Basin | Status |
|----------------------------------------|-------|--------|----------------------------------------|-------|--------|
| Água Vermelha                          | Grande| +      | Miranda                                | Paranaiba | +  |
| Caconde                                | Grande| -      | Nova Ponte                              | Paranaiba | +  |
| Camargos                               | Grande| -      | Paranoá                                | Paranaiba | -  |
| Estreito                               | Grande| -      | São Simão                               | Paranaiba | -  |
| Furnas                                 | Grande| -      | Canoas I                                | Paranaípanema | -  |
| Igaraipava                             | Grande| -      | Canoas II                               | Paranaípanema | -  |
| Jaguara                                | Grande| -      | Capivara                               | Paranaípanema | -  |
| Machado Mineiro                        | Grande| -      | Chavantes                              | Paranaípanema | -  |
| Marimbondo                             | Grande| -      | Jurumirim                               | Paranaípanema | +  |
| Mascarenhas de Moraes                  | Grande| -      | Rosana                                 | Paranaípanema | +  |
| Porto Colômbia                         | Grande| -      | Taquaruçu                               | Paranaípanema | +  |
| Volta Grande                           | Grande| +      | Bariri                                 | Tietê | -  |
| Ilha Solteira                          | Paraná | +      | Barra Bonita                           | Tietê | -  |
| Itapu                                  | Paraná | +      | Billings                               | Tietê | -  |
| Jupiá                                  | Paraná | +      | Guarapiranga                           | Tietê | -  |
| Porto Primavera                        | Paraná | +      | Ibitinga                               | Tietê | +  |
| Cachoeira Dourada                      | Paranaiba | -  | Ituporanga                             | Tietê | +  |
| Corumbá                                | Paranaiba | +  | Nova Avanhandava                        | Tietê | +  |
| Emborcação                             | Paranaiba | -  | Promissão                              | Tietê | -  |
| Itumbará                               | Paranaiba | -  | Três Irmãos                            | Tietê | +  |

### Tab. 2. Abundance (Capture per unit effort – CPUE) of *Brycon orbignyanus* caught in the Upper Paraná River floodplain in different years (1986-2010) and subsystems (Paraná, Baía and Ivinhema). 0 = Samplings occurred but no fish were caught. - = Sampling absent.

| Sites                     | Years | 1986 | 1987 | 1988 | 1992 | 1993 | 1994 | 2000 | 2001 | 2002 | 2003 | 2004 | 2005 | 2006 | 2007 | 2008 | 2009 | 2010 |
|---------------------------|-------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|------|
| Paraná Subsystem          | 0.026 | 0.26 | 0.768 | 3.12 | 0.98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 5.6 |
| Paraná River              | 0.026 | 0.26 | 0.768 | 2.95 | 0.98 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3.92 |
| Pau Véio Lake             | - | - | - | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.98 |
| Garças Lake               | - | - | - | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 1.96 |
| Cortado Channel           | - | - | 3.54 | 3.28 | 0.98 | 0 | 0 | - | - | - | - | - | - | - | - | - | 25.49 |
| Baía Subsystem            | 1.08 | 0.08 | 0.51 | 8.27 | 0.33 | 0.74 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.98 | 4.81 |
| Baía River                | 0 | 0 | 1.21 | 0.51 | 0.66 | 1.48 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0.98 | 0 |
| Curutuba Channel          | 0 | 0 | 0 | - | - | 0 | 0 | - | - | - | - | - | - | - | - | 7.84 |
| Fechada Lake              | 6.14 | 0.52 | 0.03 | - | - | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 6.86 |
| Guaranaí Lake             | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Pouso A Lake              | 0 | 0 | 0 | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 0 |
| Baia Channel              | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 0 |
| Gavião Lake               | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 5.88 |
| Onça Lake                 | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 7.84 |
| Ivinhema Subsystem        | 0.27 | 0.84 | 0.27 | 8.27 | 1.8 | 0.25 | 0 | 0.74 | 0 | 0 | 0 | 0 | 0.65 | 1.96 | 0.98 | 0.98 | 20.14 |
| Ivinhema River            | 0.79 | 1.53 | 0.35 | 7.87 | 0 | 0.49 | 0 | 0 | 0 | 0 | 0 | 0 | 0.98 | 4.9 | 0 | 0.98 | 30.39 |
| Ipoitã Channel            | 0 | 0.77 | 0.69 | - | - | 0 | 0 | - | - | - | - | - | - | - | - | 49.02 |
| Patos Lake                | 0 | 0.38 | 0 | 8.66 | 3.61 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2.94 |
| Ventura Lake              | - | - | - | - | - | 0 | 5.88 | 0 | 0 | 0 | 0 | 0 | 0.98 | 0.98 | 1.96 | 1.96 | 30.39 |
| Peroba Lake               | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 1.76 |
| Zé do Paco Lake           | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 17.65 |
| Raimundo Lake             | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 19.61 |
| Sumida Lake               | - | - | - | - | - | 0 | 0 | - | - | - | - | - | - | - | - | - | 7.84 |
0.37). Significant differences in \( B. orbignyanus \) abundance were found between the sites \( (F = 3.18, P = 0.04) \) and years \( (F = 13.85, P < 0.01) \). Abundance differed between the Ivinhema and Paraná Rivers \( (P = 0.04) \), and the species was more captured from the Ivinhema River (Fig. 2). No significant differences in abundance between the Paraná and Baía Rivers \( (P = 0.81) \) or between the Baía and Ivinhema Rivers \( (P = 0.16) \) were observed. A significant difference between abundance caught in 2010 and in the other evaluated years was detected \( (P < 0.01) \) with the highest piracanjuba abundance observed in 2010 (Fig. 2).

Regarding plain biotopes, the most widely sampled specimens were YOYs (69.8% of the catches) and juveniles, which together represented 93% of the catches, while adults were very rare even during the most favorable recruitment years (Tab. S1). The relationship between the main hydrological attributes and recruitment based on YOY abundance shows that recruitment was successful only in years with long flooding periods (Fig. 3a,b). Thus, relevant recruitment values were found only for years with flood durations of over 60 days in both Paraná (threshold \( = 4.5 \) m) and Ivinheima \( (2.75 \) m) (Fig. 3a; Fig. S1). The number of YOYs captured was also found to be relevant for less enduring but uninterrupted flooding events (Fig. 3b; Fig. S1). The results of the covariance analysis (ANCOVA) indicate that YOY CPUE was positively related to flood duration attributes for both rivers evaluated (Tab. 3).

The piracanjuba’s diet was found to be composed largely of fish and higher terrestrial plants (31.4%), juvenile mainly consumed higher terrestrial plants (62.4% - mainly \( Inga \) sp., \( Cecropia \) sp. and \( Mauritia \) sp. fruits and seeds) and fish to a lesser degree (17.1%), and adults almost exclusively consumed terrestrial higher plants (98.4% - mainly \( Inga \) sp., \( Cecropia \) sp. and \( Mauritia \) sp. fruits and seeds) (Fig. 4).

The PERMANOVA reveals significant ontogenetic dietary differences \( (\text{pseudo-}F = 7.20, P = 0.001) \) across the three piracanjuba development stages: YOYs and juveniles \( (t = 2.42, P = 0.022) \), juveniles and adults \( (t = 1.73, P = 0.025) \), and YOYs and adults \( (t = 3.13, P = 0.001) \). YOYs consumed larger amounts of fish (45.8% of their diet) and higher terrestrial plants (31.4%), juvenile mainly consumed higher terrestrial plants (62.4% - mainly \( Inga \) sp., \( Cecropia \) sp. and \( Mauritia \) sp. fruits and seeds) and fish to a lesser degree (17.1%), and adults almost exclusively consumed terrestrial higher plants (98.4% - mainly \( Inga \) sp., \( Cecropia \) sp. and \( Mauritia \) sp. fruits and seeds) (Fig. 4).
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Fig. 4. Ontogenetic variations in the diet of *Brycon orbignyanus* in the Upper Paraná River floodplain. FI = fish; AI = aquatic invertebrates; TI = terrestrial invertebrates; TP = terrestrial plants; AP = aquatic plants.

### Discussion

This study confirms that the pirançuba has almost disappeared from upper Paraná River tributaries with reservoir cascades (the Paranapanema, Tietê and Grande Rivers) and that its presence in some of these areas may be attributable to stocking programs (CESP, 2000). In the free stretches of this basin, the species is rare after prolonged periods of drought, making it abundant in years of flooding (Oliveira et al., 2015). The fact that the species is rarely found in reservoirs and that the success of its recruitment from dam-free areas depends on the occurrence of extraordinary flooding events (not regulated by upstream reservoirs) suggests that the interception of migratory routes and flood regulation through damming are the main causes of its nearly full disappearance from the upper Paraná River.

The disappearance of this species from upper reaches of the Paraná River Basin, including the Rio Grande, was recorded more than 40 years ago (Godoy, 1975). In upper reaches of the Uruguay River also transformed by damming, the species has been recorded as virtually extinct for 20 years (Zaniboni-Filho, 2012, 2013). However, the species continues to be found along Brazilian coastal basins (Gomiero et al., 2009; Vitorino-Jr et al., 2014) and *B. opalinus* (Cuvier, 1819) found along Brazilian coastal basins (Gomiero et al., 2008). These results, and the revision of the genus by Lima and colleagues (2017), highlight the importance of riparian vegetation for several *Brycon* species, and this peculiarity renders these species even more susceptible to changes in its biotopes.

The marked changes observed in *B. orbignyanus* diets throughout the life cycle may be a cause of notable declines in their population observed in the upper Paraná River floodplain as evidenced by the large number of juveniles found in years of heavy flooding and by the small number of adults observed in subsequent periods. Remarkable ontogenetic changes in the pirançuba’s diet, which is characterized by an extreme dependence on fruits and seeds during adulthood, render this species extremely vulnerable and especially in the Paraná River, where riparian vegetation is significantly altered in relation to its original phyto-physiognomy (Corradini et al., 2006). Native plants provide fruit for fish throughout the year. However, as riparian vegetation within the plain is under the influence of constant anthropogenic disturbances, such as periodic fires, partial removal and degradation by cattle trampling (Campos, Souza, 2002; Souza et al., 2004), vegetation succession processes lead to a predominance of early stage species, requiring more time for tree establishment (Campos, Souza, 2002; Pott et al., 2014) and, consequently,
greater diversity in the supply of fish food resources. In fact, fruits and seeds of pioneer species such as *Inga* and *Cecropia* are currently heavily consumed by piracanjuba. Floods readily provide terrestrial food resources to fish (Goulding et al., 1988; Walker et al., 2013; Quirino et al., 2017). Thus, the degradation of riparian vegetation coupled with decreased flood intensity levels and amplitudes especially affect species that depend on inputs of terrestrial food for the maintenance of species such as the piracanjuba.

In view of the obtained results, it has been evidenced herein that the preservation of riparian vegetation is essential for the maintenance of the piracanjuba population stratum, which feeds almost exclusively on fruits and seeds. In addition, as a species that migrates long distances (Oliveira et al., 2015), the piracanjuba may act as dispersing agent for seeds of riparian vegetation in the same way as suggested for other *Brycon* species (i.e., Goulding, 1980; Horn, 1997; Reys et al., 2009), facilitating the support and regeneration of riparian forests. In this case, the conservation of riparian vegetation is intrinsically related to the maintenance of the ichthyofaunistic biodiversity of the floodplain (Pusey, Arthington, 2003), and the maintenance of piracanjuba populations at the same time constitutes a facet of conservation strategies developed for this type of vegetation. Under this context, conservation units established in the dam-free segment of the Upper Paraná River play an essential role in biodiversity conservation (Agostinho et al., 2005). In fact, a marked increase in piracanjuba catches over the last years of the study period was observed and may be related to the creation and implementation of Parque Estadual do Rio Ivinhema (SEMA, 2001) with 73,345.15 hectares of extension (Decree 9.278, December 17, 1998). However, as is typically expected for large migrating populations, this species requires a wide area to complete its life cycle, exceeding the limits of this conservation unit.

*Brycon orbignyanus*’ biological and ecological characteristics allow for the use of this species as an indicator of aquatic ecosystem biotic integrity, given its high sensitivity to hydrological. Thus, conservation initiatives aimed at protecting the species’ habitats (maintenance and restoration) and restrictions on engineering projects that affect flood pulses may have direct effects on other species that share *B. orbignyanus* occurrence areas, indicating that conservation efforts have positive effects on other migratory species and on riparian vegetation conservation.

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