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Biology of the vulnerable species *Diplomystes cuyanus* Ringuelet, 1965 (Siluriformes: Diplomystidae) in the arid west of Argentina: basis for its conservation

Biología de la especie vulnerable Diplomystes cuyanus Ringuelet, 1965 (Siluriformes: Diplomystidae) en el oeste árido argentino: bases para su conservación

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Abstract

The objective of this work was to analyze the trophic activity, sexual dimorphism, and reproductive state of *D. cuyanus* and to generate foundational information for the management and conservation of the species and its habitats. A total of 26 individuals were captured in the Los Patos river area over four years (2013-2017). Studies were conducted on the overall diet, differentiating by sex, season, and functional grouping in the ecosystem. Sexual dimorphism was evaluated using a set of 31 morphometric measurements, and reproductive parameters were determined using gonadosomatic values. *D. cuyanus* is identified as a generalist predator, with sexual and seasonal variations observed in its diet. Furthermore, this species exhibits sexual dimorphism. The spring season showed the highest reproductive activity.

Diplomystes cuyanus is classified as an endangered species in the province of San Juan, and available information is mostly limited to its distribution. The objective of this study was to analyze its feeding ecology, sexual dimorphism, and reproductive biology to generate essential information for the management and conservation of the species and its habitats. A total of 26 individuals were captured in the Los Patos River area over four years (2013–2017). Diet analyses were conducted, considering overall diet composition as well as differences by sex, season, and functional grouping within the ecosystem. Sexual dimorphism was assessed using 31 morphometric measurements, and reproductive parameters were determined based on gonadosomatic values. *D. cuyanus* was identified as a generalist predator, with significant sexual and seasonal variations in its diet. Additionally, the species exhibits clear sexual dimorphism. The highest reproductive activity was recorded during the spring season.

Resumen

Diplomystes cuyanus está clasificado como una especie en peligro de extinción en la provincia de San Juan, y la información disponible se limita principalmente a su distribución. El objetivo de este estudio fue analizar su ecología trófica, dimorfismo sexual y biología reproductiva para generar información esencial para la gestión y conservación de la especie y sus hábitats. Se capturaron un total de 26 individuos en el área del río Los Patos durante cuatro años (2013–2017). Se realizaron análisis de la dieta considerando la composición general, así como diferencias por sexo, estación y agrupación funcional dentro del ecosistema. El dimorfismo sexual se evaluó utilizando 31 mediciones morfométricas, y los parámetros reproductivos se determinaron a partir de valores gonadosomáticos. D. cuyanus fue identificado como un depredador generalista, con variaciones sexuales y estacionales significativas en su dieta. Además, la especie exhibe un claro dimorfismo sexual. La mayor actividad reproductiva se registró durante la primavera.

Keywords: reproduction, trophic ecology, sexual dimorphism, functional group, seasonal variation

Palabras clave: *reproducción, ecología trófica, dimorfismo sexual, grupo funcional, variación estacional*

Introduction

The biodiversity of freshwater ecosystems has diminished more rapidly than terrestrial or marine ecosystems over the last 30 years (Sala et al., 2000; Mclellan et al., 2014). Between 1970 and 2012, the Neotropical region experienced an 83% reduction in population, more marked than in the rest of the world, (Mclellan et al., 2014).

Changes in the aquatic environment affect fish at the physiological, behavioral, population dynamic and at the ecosystem level in productivity and/or trophic interactions (Rijnsdorp et al., 2009).

Temperature is an important factor that limits the range of distribution of fish on a large geographic scale, but also on a small scale within lakes and rivers (Shuter et al.,1980). It also models river flow, which is an index of ecological space and habitat heterogeneity for fish survival, growth and reproduction (Xenopoulos et al., 2005). Decreasing river flow has been one of the primary causes of species loss (Fischer and Kummer, 2000).

In addition, local changes driven by human population activity, such as overexploitation, the contamination of waters, modification of flows, habitat destruction or degradation and the invasion of exotic species, also affect fish populations (Revenga et al., 2005). In this context, arid regions are particularly vulnerable due to water scarcity and limited life in these extreme environments (Noy- Meir, 1973; Palma et al., 2013). Historically, ecological studies have focused mainly on birds and mammals, leaving aside reptiles and freshwater fish. However, the latter are excellent indicators of the ecology of aquatic habitats (Lorencio, 2000). Freshwater fish have a key role to play in trophic networks and in the functioning of ecosystems of rivers and lagoons due to their greater longevity, size and ability to move (García-Berthou *et al.*, 2015). Studies of biological parameters in fish are fundamental for understanding freshwater ecosystems (Dudgeon et al.,2006). Among them, the analysis of stomach content can be used to quantify a species trophic requirements and the characteristics of its habitat, its possible interaction with other species or age groups of the same species (competition and predation), energy consumed, and the establishment of the trophic framework which takes place in an ecosystem (Krebs, 1989).

While the study of sexual dimorphism is essential for understanding the ecology of behavior and life history of a species, it is also useful as a method for making morphological comparisons between populations (Kitano et al., 2007), which at the same time is related to reproductive and trophic aspects and habitat use, among others (Shine, 1989). And it is an important factor in population structure, primarily for the division of resources (Pianka, 2017).

Reproductive studies also allow for the interpretation of biological cycles, of utmost importance in ecology as these events sustain the survival of species (Lam, 1983).

The Dyplomystidae family is endemic to the Southern subregion of South America (Arratia, 1987), and is considered to be the most primitive of the Siluriformes order (Arratia, 1987; Sullivan et al., 2006) due to the existence of a functioning and dentiferous jaw (Ringuelet, 1965). It represents one of the first lines of division among the 43 recent families and fossils of this order (Lundberg and Baskin, 1969; Britz and et al., 2014). Unfortunately, the majority of species belonging to this family are in danger of extinction (Muñoz-Ramírez et al., 2020). In Argentina, the species *D. cuyanus* is found in the provinces of Río Negro (Azpelicueta, 1994), Buenos Aires (Cazorla, 1997), La Pampa (Atolaguirre, 2004), Mendoza and San Juan (Ringuelet, 1965; Arratia, 1983; Azpelicueta 1994; Acosta et al.,2016). In San Juan, it was cited in the Colorado river (Azpelicueta, 1994) without mention of reference material, corresponding to the northern limit of distribution in South America (Arratia, 1983), while Acosta et al. (2016) mentions its presence in the Los Patos river and possibly in the Castaño river. The *Diplomystes Cuyanus* (Ringuelet, 1965) species, belonging to the Dyplomystidae family, was categorized in Argentina by Sosa and Vallvé (1999) as relictual and by Chebez and Bertonatti (1994) as indeterminate, while López et al. (2003) categorized it as “rare” and in the province of San Juan it is classified as being a species in danger of extinction (Secretary of the Environment and Sustainable Development – Government of the province of San Juan, 2011). Nevertheless, these categorizations are frequently subjective as no method of analysis is mentioned nor is there biological information available in Argentina to make these status decisions.

The biological data known for the Dyplomystidae family comes primarily from studies carried out in Chile. These are strictly freshwater fish, benthic, nocturnal and carnivorous, their diet is based mainly on aquatic insects, both larva and adults (Arratia, 1983)

Regarding their use of microhabitats, they select sites according to ontogenetic development (Arratia, 1983). Despite their size, these fish present a great range of mobility in the fluvial system (Oyanedel et al.,2018) and as such, a high gene flow can be observed (Victoriano et al., 2012). As regards reproduction, Azpelicueta (1994) mentions that these fish have an uneven female gonad, while adult testicles have thick lobes (with no differentiation between front and back) occupying the greater part of the ventral cavity during maturation. With respect to sexual dimorphism, specific characteristics like the urogenital papilla can be used to differentiate sexes (Azpelicueta, 1994), as well as morphometric comparisons at the species level (Arratia, 2017).

The general objective of their study was to analyze the diet, sexual dimorphism and reproductive state of *D. cuyanus* in the Río de los Patos-Barreal-Calingasta area of San Juan, in order to generate information to be used for the management and conservation of the species and its habitats.

Methodology

Fieldwork

The biological study material belongs to the scientific collection of the Biology Department (Gabinete DIBIOVA, FCEFN, UNSJ) (UNSJ-P 621-646) and corresponds to the period between 2013 and 2017. A total of 26 individuals were captured, 22 in summer and 4 in spring. Capture efforts were seasonal; specimens were only collected during these seasons, as the fishing method used was only successful in these two periods.

Keeping in mind their conservation status, captures were done using passive methods with bottom lines. The individuals captured (Figure 1) were preserved according to the norms established by (Arámburu, 1996; Murrieta, 2019) to avoid the deterioration of tissue and digestion post-mortem.

Study Area

The Los Patos river is found in the town of Barreal at an altitude of approximately 2000 m a.s.l. in the Calingasta department in the southern part of the province of San Juan. Sampling took place in a section of river called "Tomas de Santa Marta" Latitude 31°44'37.39" and Longitude 69°32'36.11"O (Figure 2 A-B). The climate here is dry high mountain with annual precipitation less than 100 mm, average annual temperature 16.33 °C (climate-data.org), and soil on the banks of the river typical torrifluvents (Pozzo 1948). There are some dense patches of *Salix Humboldtiana* (Humboldt's willow), Tamarix ramosissima (tamarind) and some herbaceous plants such as *Tessaria absinthiodes* (bobo bird), *Cortaderia.* spp. (Pampa's grass), *Equisetum.* spp. (horse tail), among others.

The Los Patos river has marked seasonal characteristics with average annual flow of 24 m^3 / s at the Alvarez Condarco station and 49,92 m^3 / s at the Plateada station (Vich et al., 2016).

Labwork

Each individual was weighed on a digital Ohaus scale with 0.0001 g precision. Specimens were dissected and stomachs were removed through ventral dissection which was carried out in a Petri dish using a binocular lens (Arcano magnification 2x and 4x). The digestive tract was separated from the esophagus to the cloaca, later the stomach was separated from the intestine, the liver, the fatty tissue and the gonads. These were weighed and stored in containers for analysis. Subsequently, the gutted individual was weighed, along with the intestine and the empty stomach.

Trophic Biology

Identification of prey was carried out through macroscopic analysis of stomach and intestinal content using a macroscopic lens on a Petri dish on top of graph paper in order to obtain measurements of length and width. Classification was made to order level, utilizing Dominguez and Fernandez keys (2013) for identification. Volume of each prey item was calculated using Dunham's ellipsoid formula $V = 4/3 \pi \times (a/2) \times (b/2)^2$ (2013). Categorization

Figure 1. Photograph of an adult individual of the species *Diplomystes cuyanus* taken during fieldwork in the Los Patos River
Figura 1. Foto de un individuo de la especie Diplomystes cuyanus tomada durante trabajos de campo en el río los Patos

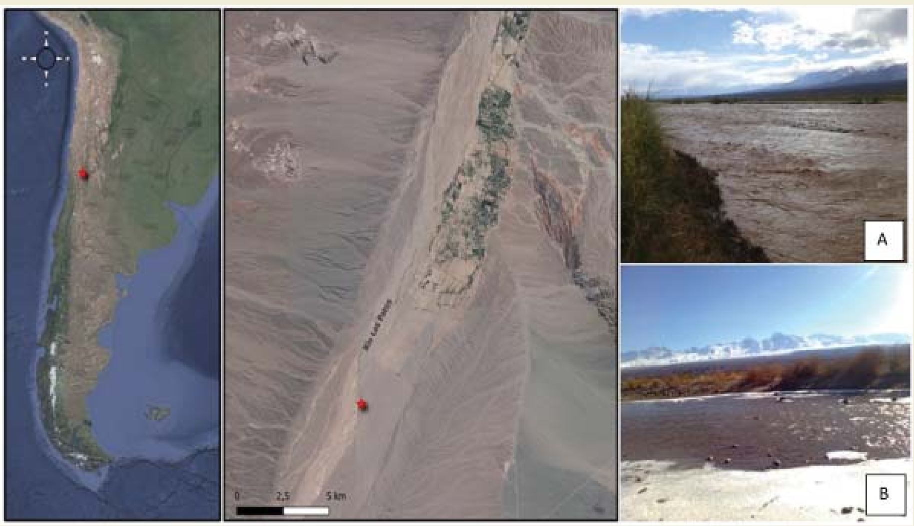

Figure 2. Map of Argentina zoomed in to the field site, situated along the "Los Patos river", with images of the site during the summer and winter seasons (labeled as 1A and 1B)
Figura 2. Mapa de Argentina con zoom en el sitio de trabajo, situado en el río Los Patos, con imágenes del sitio durante las estaciones de verano e invierno (identificadas como 1A y 1B)

of functioning guilds was carried out following Tomanova et al. (2006) characterization.

Sexual Dimorphism

Studies of sexual dimorphism were completed through morphometric analysis following techniques proposed by Strauss and Bookstein (1982), Barriga-Sosa et al., (2004), Barriga-Sosa and Battini., (2009) Paiva et al., (2015) and Arratia and Quezada-Romegialli., (2017). Of the literature cited, we compiled a set of 31 morphometric measurements, which were taken using digital caliper (K.L.D) with a resolution of 0.01 mm. Anteroposterior and left-right orientation were considered, respectively.

TL: Total length, SL: Standard length, SVL: Snout-vent length, PDL: Predorsal length, BDD: Body depth in dorsal fin, BDV: Body depth in ventral fin, LBA: Length of the base of the adipose fin, LULC: Length of the upper lobe of the caudal fin, BDA: Body depth in the anal fin, PVL: Pre-ventral length, PAL: Preanal length, VAD: Ventral anal distance, LBA: Length of the base of the adipose fin, LULC: Length of the upper lobe of the caudal fin, LA: Length of the adipose fin, PAL: Preanal length, VAD: Ventral anal distance, BDP: Body depth in the pectoral fin, HL: Head length, OL: Orbital length, ED: Depth of the eye, CL: Chin length, HW: Head width, MW: Mouth width, IOL: Interorbital length, MD: Depth of the mouth.

Reproductive Biology

The gonads were analyzed with a binocular lens (Arcano 2x and 4x), and states were determined as a function of degree of development and reproductive state, based on macroscopic characteristics of male and female gonads following the criteria of Vila et al. (1996).

In the case of males, mature testicles were considered to be those with a crème color and ample. When the testicles were seen to be strongly divided with longer rather than fatter front lobes, and the back lobes thinner and progressively longer, they were considered maturing testicles.

In the case of females, three fragments of the ovarian sac were taken and the diameter of the oocytes was measured. They were considered to be mature if 50% or more of the oocytes had a diameter of 2-3 mm and maturing if 50% or more of the oocytes had a diameter less than 2 mm.

Statistical Analysis

Central tendency statistics (mean, median, mode) and statistical dispersion (standard deviation and variance) were used to analyze the data obtained. Assumptions of normality and homogeneity of variance were tested to utilize parametric statistics. Populational variance was analyzed using the ANOVA test to determine morphometric differences associated with sex. Multivariate analyses were also carried out on diet differentiation according to sex. Data processing was carried out with the InfoStat program (Version 2020)

Data analysis (trophic biology)

The Index of Relative Importance (IRI) was calculated following Pinkas (1971), with the purpose of determining the contribution of prey to the diet. We used the following expression:

IRI = (%Ni+%Vi) x Fi, where: Ni = numerical percentage of i-degree food items; Vi = volumetric percentage of i-degree food items; Fi = frequency of occurrence of i-degee food items.

Diet hierarchy

The IRI value was applied identifying the highest value on the index and decreasing the remaining values percentage-wise from this value (Aun and Martori 1998, Villavicencio *et al*., 2005; García *et al*., 2017). As such, a scale of values was established: 100% - 75%: Fundamental, 75% - 50%: Secondary, 50% - 25%: Accessory and < 25%: Accidental. This index was applied classifying prey at order level and in functional ecosystem group categories.

Diversity and equity analysis

To determine trophic diversity (H´), the Shannon-Wiener (Magurran, 1988) index in Moreno (2001) was used. A Student's t-test was carried out using the Shannon – Wiener diversity index for each season (spring and summer). A Student's t-test was also used for the Shannon – Wiener diversity index according to sex, with a level of significance of 0.05.

To calculate trophic equity, Pielou's equity index (1975) in Moreno (2001) was used with the following equation:

$E=H/H'max$

Where Hmax=Ln S, with S being the total number of food categories.

This index measures the proportion of diversity observed as it relates to maximum diversity expected. Its value ranges from 0 to 1, a result of 1 corresponds to situations in which all species are equally abundant and a result of 0 situations in which abundance is heterogeneous (Moreno, 2001). This index was applied to seasonal and sexual differentiation of the diet's trophic components.

Dominance

Simpson's dominance index (1949) was applied in order to obtain the probability that two individuals belonging to *D. cuyanus* food items chosen by chance correspond to the same order, according to both sex and sampling season.

$\lambda= \Sigma(n^2/N^2) =\Sigma pi^2$

Where pi is abundance proportional to species i, which implies obtaining the number of individuals of species ni divided by the total number of individuals in the sample N.

Quantitative and qualitative similarity analysis

Morisita - Horn Magurran's Similarity index (1988) in Moreno (2001) was used for quantitative comparisons between the variables %N, %VOL, %FO and IRI. The index relates specific abundances to relative and total abundances, and is highly sensitive to the abundance of dominant species. With the objective of finding similarities between male and female diets:

$I\ M\text{-}H = 2\Sigma(a_{ni} \times b_{nj})/ (da+db)\ aNxbN$

Where a_{ni} = number of individuals from i-degree species at the site or sex A b_{nj} = number of individuals from j-degree species at the site or sex B $da = \Sigma ani^2 / aN^2$; $db = \Sigma bnj^2 / bN^2$

With the objective of detecting differences in diet among sexes, Jaccard's similarity index was calculated using qualitative data (richness of prey).

$Lj= c/ a+b-c$

Where a= number of prey present in category A (sex A); b= number of prey present in category B (sex B); c= number of prey present in both categories (sexes A and B).

Both indices vary between 0, when samples are totally different, and 1, when they are the same (Moreno, 2001).

Analysis of feeding strategies

A graphic analysis of feeding strategies was used, using data on stomach content as regards prey-specific abundance (Amundsen et al., 1996) modified from Costello's model (1990). Where the variable numerosity is replaced by another known as prey-specific abundance, greater sensitivity is present.

Multivariate analysis

A non-metric scaling test n- MDS was carried out using the Bray Curtis distance in order to obtain information about diet overlap between males and females.

Sexual Dimorphism Data Analysis

A simple linear regression was completed using each measurement as a response variable and taking total length as a predictor variable in order to establish linear relationships between the variables and total length. Subsequently, those dependent variables of size were analyzed with ANCOVA to identify the existence of some variation in morphometric measurements in relation to sex, removing the effect of total length. Fisher's LSD test was also carried out a posteriori.

Reproduction Data Analysis

To determine gonad maturity, both a qualitative and quantitative analysis of gonad state was done, taking into account Vila et al. (1996) criteria detailed previously. We also calculated the gonadosomatic index following the model proposed by Le Cren (1951), which is obtained through the percentage relationship between gonad weight and specimen weight. This index is used to determine the reproductive state of the population and to make comparisons between the different seasons. Its maximum value is considered to be indicative of the stage prior to reproduction and the subsequent decrease in average values is indicative of spawning having occurred (Rodríguez-Gutiérrez, 1992 in López-Hernández et al., 2018).

IGSi= (PGi / Pi) * 100

Where: IGSi = gonadosomatic index; PGi = Gonad weight (g); Pi = Specimen weight (g)

Sexual proportion was evaluated using Pearson's chi-squared test (X^2); potential fertility in mature females was determined (Number of mature oocytes) and the condition index or factor was evaluated (K = weight in grams over length in cm^3). This index is used to compare general condition or wellbeing of a fish or population, in which fish with greater weight at a determined length have a better condition (Froese *et al.*, 2011). This last analysis was used to compare between seasons (spring – summer) and its variation was analyzed according to the gonadosomatic index values obtained.

Results

Diplomystes cuyanus Trophic Ecology

Of a total of 26 stomachs, no empty stomachs were detected. 443 prey items were counted, divided into 12 categories.

Primary prey items in spring were Isopoda, Trichoptera and Coleoptera.

On the other hand, in summer Trichoptera and Isopoda were primarily recorded.

In the case of females, it was determined that the fundamental component was Isopoda, while Hymenoptera and Trichoptera. In males, the fundamental component was Trichoptera (Table 1)

Dominance, diversity and equity

Diplomystes cuyanus presented greater diversity in diet in spring (H´=2.09), and equity was also greater in spring (P = 0.815), though dominance was less (S= 0.14). Whereas in summer, diversity was lower (H´=1.38) as was equity (P= 0.540), but the dominance value was greater (S=0.363). There are significant differences with respect to the diversity index (Shannon – Wiener) between spring and summer (T= -3.5534, p=0.001).

As regards Simpson's index, a decimal difference was observed between males and females. Males presented higher values in Numerosity (M = 0.39; F= 0.32), IRI (M =0.268; F=0,2509) and Volume (M = 0.4875; F=0.3911). Males have a diet with a greater presence of dominant components than do females.

For both the diversity index (Shannon-Wiener) and the equity index (Pielou), values of Numerosity (Shannon- Wiener: M= 1.32; F=1.44; Pielou: (M=0.52; F=0.62) IRI (Shannon- Wiener: M=1.52; F=1.71; Pielou: M=0.59; F=0.74) and

Table 1. Trophic components recorded, overall hierarchy, spring, summer and sex hierarchy in accordance with the IRI values obtained
Tabla 1. Componentes tróficos registrados, jerarquía general, primavera, verano y jerarquía sexual de acuerdo con los valores IRI obtenidos

Prey items	Hierarchy Overall	Hierarchy Spring	Hierarchy Summer	Hierarchy Females	Hierarchy Males
Isopoda	Fundamental	Fundamental	Fundamental	Fundamental	Secondary
Trichoptera	Fundamental	Fundamental	Fundamental	Accessory	Fundamental
Coleoptera	Secondary	Fundamental	Secondary	Accidental	Secondary
Diptera	Accidental	Secondary	Accidental	Accidental	Accidental
Oligochaeta	Accidental	Accidental	Accidental	Accidental	Accidental
Hymenop-tera	Accidental	Accidental	Accidental	Accessory	Accidental
Araneae	Accidental	Accidental	Accidental	Accidental	Accidental
Ephemerop-tera	Accidental	Accidental	Accidental	Accidental	Accidental
Neuroptera	Accidental	Accidental	Accidental	Accidental	Accidental
Plecoptera	Accidental	Accidental	Accidental	Accidental	Accidental
Mollusca	Accidental	Accidental	Accidental	Accidental	Accidental
Odonata	Accidental	Accidental	Accidental	Accidental	Accidental

Volume (Shannon- Wiener: M=0.90; F=1.15; Pielou: M=0.50; F=0.55) did not show significant differences between males and females (T= -1.123, p=0.26).

The Morisita- Horn quantitative index gave high similarity in these three variables, in which the components of the male and female diet are similar (Numerosity = 0.93 IRI= 0.96 Volume= 0.62). Though the Jaccard index (qualitative index) gave a value of 0.53, indicating intermediate similarity between males and females.

In Figure 3, we observe that there is overlap in the majority of items consumed by *D. cuyanus* males and females, which agrees with the results obtained in the diversity, equity, dominance and similarity indices.

Feeding strategy

The prey consumed most frequently by the *D.* cuyanus catfish had specific prey abundance percentages less than 50%. The graphic reflects ample niche width due to the positioning of the points in the two lower quadrants. The Trichoptera, Isopoda, and Diptera prey items presented prey-specific abundance less than 30% and a frequency of occurrence greater than 50%. The other items are rare given that they are located below the diagonal of the lower left quadrant (Figure 4).

Trophic ecology of the species according to functional groups

Grouping of the *D. cuyanus* diet into functional guilds revealed primary prey items being terrestrial saprophagous, followed by collectors, which were

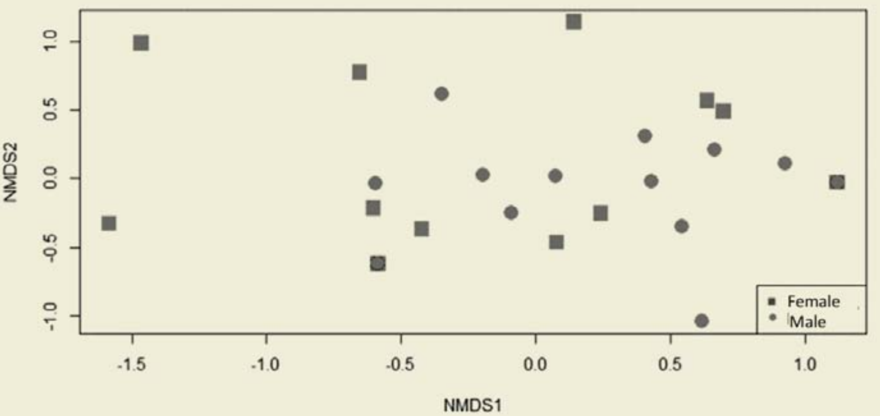

Figure 3. Graphic representation of a two-dimensional non-metric multidimensional scaling (NMDS) using the Bray-Curtis distance to illustrate the degree of similarity in diet composition between males and females of the species *Diplomystes cuyanus.* The proximity of the symbols indicates a greater degree of similarity, with a stress value of 0.127
Figura 3. Representación gráfica en dos dimensiones del análisis No métrico multidimensional (NMDS) usando la distancia d Bray-Curtis para ilustrar el grado de similaridad en la composición de la dieta entre masculinos y femeninos de D. cuyanus. La proximidad de los símbolos indica un mayor grado de similaridad, con valor de estrés de 0,127

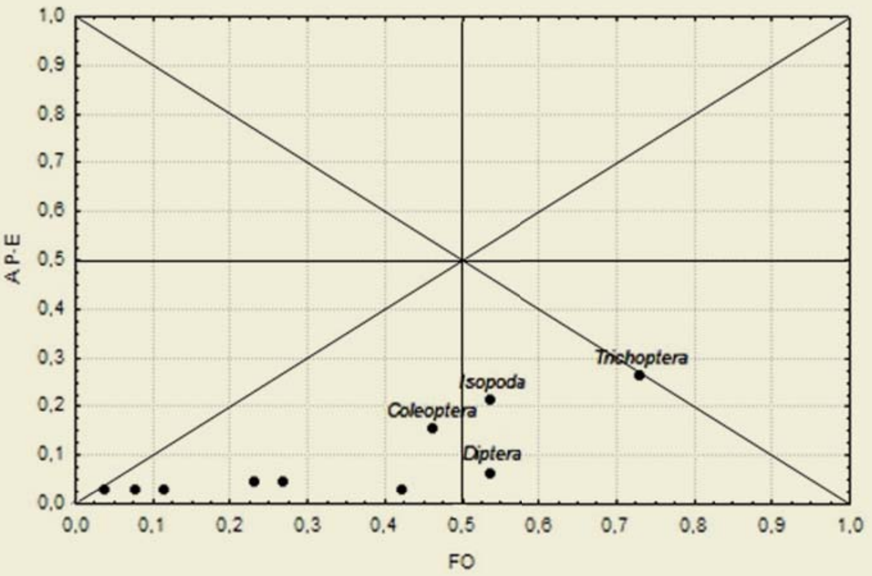

Figure 4. Diagram illustrating the feeding strategies (Amundsen et al., 1996) of the food components of *Diplomystes cuyanus*, showing prey-specific abundance (A-P-E) and frequency of occurrence (FO)

Figura 4. Diagrama ilustrando las estrategias alimentarias (Admundsen et al., 1996) de los alimentos componentes de D.cuyanus, mostrando la abundancia pre-específica (A-P-E) y la frecuencia de ocurrencia (FO)

considered fundamental food items (Table 2).

Sexual Dimorphism

A marked linear relationship was observed between the 30 measurements and total length. We found significant differences between males and females in four morphometric measurements, length of anal fin (LAa) (p=0.004), depth of caudal peduncle (PPC) (p=0.02), length of the base of the dorsal fin (LbAD) (p=0.03) and body depth in the pectoral fin (PCAP) (p= 0.009). Males had measurements different from and higher than those of females in PPC, LAa, and PCAP, while females had values different from and higher in LbAD (Figure 5).

Reproductive Biology

Sex ratio

The sex ratio was 1:1 ($X^2 = 0 < X^2_{1;\,0,05} = 3.84$), the test was not significant ($p > 0.05$) rejecting the alternative hypothesis ($H_{1=}\ \pi_{expected} \neq \pi_{observed}$); potential fertility in *D. Cuyanus* females (n = 3) resulted in an average value of 319 mature oocytes; with a range of (253 min – 401 max) and a standard deviation of 75.29.

Females

With a sample size of 13 females analyzed, three mature females were recorded (23% of the sample), for which the gonadosomatic index was its maximum and average size of the ovule

Table 2. Classification of prey items in functional groups taking into account frequency of occurrence, numerosity, volume and its hierarchy according to the IRI value

Tabla 2. Clasificación de los artículos de presa en grupos funcionales considerando la frecuencia de ocurrencia, numerosidad. Volumen y su jerarquía de acuerdo alos valores IRI

FUNCTIONAL GROUPS	%FO	%N	%Vol	Hierarchy
Terrestrial saprophagous	73	21.49	46.85	Fundamental
Collectors	81	19.76	30.47	Fundamental
Fragmentors	65	54.42	1.57	Secondary
Omnivores	31	2.25	10.89	Accidental
Terrestrial predators	8	0.35	9.26	Accidental
Predators	12	0.69	0.12	Accidental

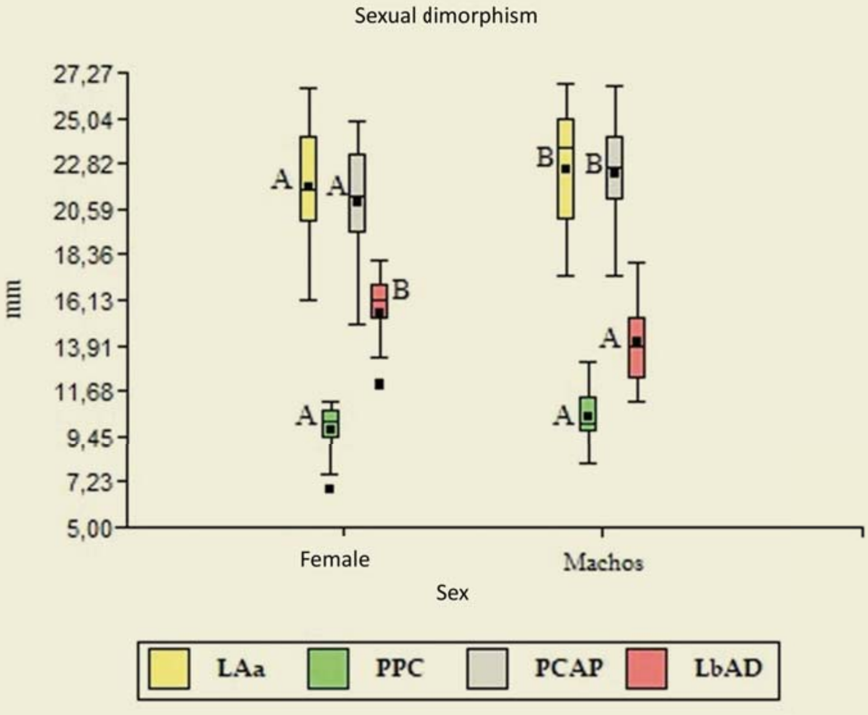

Figure 5. A box and whisker plot depicting the results of the ANCOVA analysis and the contrast with Fisher's LSD, using variables including (LAa) length of anal fin, (PPC) depth of caudal peduncle, (LbAD) length of the base of the dorsal fin, and (PCAP) body depth in the pectoral fin

Figura 5. Diagrama de caja mostrando los resultados del ANCOVA y el contraste con el test de Fisher LSD, usando las variables LAa: longituda anal, PPC: profundidad del pedúnculo caudal, LbAD: longitud de la base del dorsal y PCAP: profundidad del cuerpo en el extremo pectoral was greater than 2 mm. The remaining females were classified as immature (77% of the sample), with minimum values on the gonadosomatic index and oocyte size not exceeding 2 mm.

Mature females corresponded to the months of October 2014, December 2017 and January 2015. The minimum value of the gonadosomatic index was 0.35699607 and the maximum value 15,91983318, considering values greater than or equal to 15 as a mature state (Table 3), as well as when oocyte size was greater than 2 mm.

Males

Of 13 males studied, two presented gonad maturity (15% of the sample) while the rest (85%) were in an immature state (Table 3).

Comparison of the index or factor of condition (K) according to sex

The variable does not present normal distribution (p=0.31). It does not show significant statistical differences according to sex (KW, p=0.3113) and season (KW, p= 0.7223).

Discussion

Trophic Biology

Arratia (1987), in his study of the Diplomystidae family, determined that they are carnivorous fish, coinciding with that mentioned by Azpelicueta (1994) for the species *Diplomystes viedmensis* MacDonagh, 1931 (from the Ramos Mejía reservoir). These studies coincide with results obtained in this work for *D. cuyanus,* confirming that it is a carnivorous species.

Nevertheless, the specific content of the diet differs with respect to studies of other species. In a study of *Diplomystes spp* in Chile, it was shown that the most consumed items were Diptera and Plecoptera. Beltrán-Concha et al., 2012 also report the presence of Aeglidae in the diet, primarily in larger specimens. In this work, we did not identify the aforementioned items.

Azpelicueta (1994) reports for the *Diplomystes* spp diet (samples captured in rivers of the Mendoza province) the presence of Diptera pupae, different from what was observed for *D. cuyanus* which was relatively low. This item is a main component of the diet of other siluros such as *Hatcheria macraei* Girard 1855 (Ferriz, 2012; García *et al.* 2017), a species that coexists with *D. cuyanus* in the Los Patos river (Acosta *et al.*, 2010; Acosta *et al.*, 2013). As regards Odonata larvae, only one sample was recorded and it is not a fundamental item. However, in *Diplomystes spp* (Mendoza river), the presence of numerous Odonata nymphs was mentioned (Azpelicueta, 1994). Azpelicueta (1994) records the appearance of a mollusk shell, prey items also recorded in the diet of *Trichomycterus areolatus* Valenciennes, 1846 (Habit, 2005). In this work, only a single Mollusk was found.

The Isopoda item classified as fundamental prey in the *D. cuyanus* diet in this work does not appear in other works carried out on *Diplomystes spp*., being a distinctive characteristic of the *D. cuyanus* population in the Los Patos river. Azpelicueta (1994) mentions the appearance of crabs in stomachs of *D. cuyanus* in the San Juan river basin. Beltrán-Concha et al. (2012) report the presence of *Aegla* crabs in the diet of *Diplomystes* spp. captured in Chilean rivers. With the available information it

Table 3. Dates of capture, biometric values of male gonads and results of the gonadosomatic index in males and females
Tabla 3. Datos de captura, valores biométricos de gónadas masculinas y resultados del índice gonadosomático en individuos masculinos y femeninos

Date	Testicle volume (mm3)	Male G index	Date	Gonad weight	Female G index
Oct-14	7749.261879	3.54112633	Dec-17	6.871	15.9198332
Jan-16	7330.382858	2.78392155	Jan-15	5.2875	15.8886368
Jan-13	301.069296	0.45797204	Oct-14	6.346	15.6826887
Feb-13	703.7167544	0.42459398	Jan-15	0.8963	2.5383744
Jan-15	235.619449	0.41137909	Mar-15	0.7626	1.93372197
Feb-14	287.9793266	0.34735726	Jan-15	0.2287	0.77094219
Jan-15	452.3893421	0.32105245	Oct-14	0.0771	0.72104594
Jan-16	150.7964474	0.28109948	Mar-15	0.1494	0.54758973
Jan-15	222.5294796	0.27679533	Jan-15	0.1423	0.54606225
Jan-16	20.94395102	0.2644431	Feb-13	0.0831	0.51156406
Jan-15	287.9793266	0.25048946	Oct-14	0.1266	0.4977002
Jan-13	376.9911184	0.11716712	Feb-13	0.0435	0.42546948
Jan-15	31.41592654	0.0995665	Jan-15	0.1051	0.35699607

is possible to speculate that river crabs were historically fundamental in the diet of the *D. cuyanus* population in the Los Patos river.

As such, we can hypothesize about the existence of some factor that generated or generates pressure and decline in Aeglidae crab populations in the Los Patos river, and for this reason they are not recorded in the *D. cuyanus* diet. Aeglidae is a genus exclusive to South America and works show a measurable reduction in crab populations in some rivers (Bond-Buckup et al., 2007; Cumberlidge et al., 2009). It is possible that food type consumed by *D. cuyanus* has spatial variations, contrary to what Beltrán-Concha et al. (2012) present, who affirm that no modifications exist along the latitude gradient.

Seasonal variation in diet

Diet composition changes can be strongly influenced by seasonal variations in food resource availability (Xie et al., 2000). Currently, no literature is available addressing this topic in the species.

The spring season presents trophic components that are more diverse and equitable and less dominant than those of the summer season. This variation in diet could result from the low abundance of preferred food in certain seasons (Oliveros and Rossi, 1991). Salavert (2012) reported that Trichoptera abundance increases in rivers as daylight and temperatures rise, which could explain the high Trichoptera values recorded in this study during the summer months. Additionally, it is important to consider that this species tends to be a generalist (Figure 3).

The hydrological regime plays a key role in fish feeding habits, as reflected in the seasonal variability of their diet (Wootton, 1999). During the flooding season, inundated terrestrial vegetation provides an essential food source for fish (Junk, 1980), which explains the predominance of Isopoda as a dietary component due to its terrestrial origin. In contrast, during the low-water phase, food availability decreases significantly, potentially influencing the diet composition of fish (Goulding, 1980).

Although some changes in diet composition were observed in this study depending on the season, further research including more seasons and individuals is needed

Sexual variation in diet

No statistically significant differences were detected between sexes, however, it was observed that IRI values changed in hierarchy according to sex. In females, the fundamental item was Isopoda and Hymenoptera was accessory. On other hand, for males, Trichoptera larvae was the fundamental item and Isopoda was a secondary item. It is possible that females choose this item more frequently than males due to its size, it is larger compared to other items recorded. It is also observed that there is a division of resources where different resources are utilized by different individuals of a population (Pianka, 2017). It is likely that females find Isopoda to be nutritious and relatively easy to capture as these isopods are terrestrial components, as are components from the order Hymenoptera, possibly swept into the river during surges caused by melt or rain. This allows females to satisfy the great energy demands implied by the development of the ovarian sac, and presents a feeding behavior different from that of males.

As a hypothesis to prove, we suggest that feeding differences between males and females of this species are based on the use of space, these two groups (males and females) use different feeding sites. Currently there are no studies to verify this aspect of the *Diplomystes* diet, however, in his work Habit (2005) mentions the use of space by *Diplomystes nahuelbutaensis* and affirms that these fish as adults use deep wells with greater water flow, while youth use shallow banks with calmer waters.

In the graphic analysis of the *D. cuyanus* food strategy (Figure 4), items tend to concentrate in the two lower quadrants, as such being a generalist diet. Additionally, the fact that points are below the diagonal in the lower right quadrant indicate that individuals of the population participate or contribute quantitatively similar to the trophic niche, with no individualist specializations being recorded, that is, everyone eats a bit of everything (Encina, 2001). In the case of items in the lower left quadrant, these are rare trophic components as they present relatively low prey-specific abundance and frequency of occurrence, an analysis based on the work of Amundsen *et al.* (1996). The generalist diet of these fish may represent certain flexibility in the trophic interactions of competition. In their work on trophic interaction, García et al. (2017) mention that *Oncorhynchus mykiss* Walbaum, 1792 prefers terrestrial prey, for which it is important to note the hypothetical existence of intraspecies competition, comparing to the results obtained in this work where *D. cuyanus* is also a consumer of terrestrial prey.

Functional groups

Analysis of diet through functional groups placed *D. cuyanus* as a terrestrial consumer, that is, great part of its diet corresponds to terrestrial saprophagous and a significant aquatic component. This classification is in this sense novel, as its application lies in the importance of the aquatic macroinvertebrate communities present in river ecosystems. Macroinvertebrates are an important link between organic material resources, such as dead leaves, algae, and detritus, and consumers of higher levels of the trophic chain (Allan et al., 2021). Focusing on this aspect contributes to the how of nutrient circulation within the system (Yule, 1996), and in this sense involves functional groups in the *D. cuyanus* diet.

Sexual Dimorphism

Azpelicueta (1994) mentions a specific aspect of dimorphism referring to the urogenital papilla, tubular and very short in males and, though tubular and short in females, with a wider base. In this work, we were able to determine that *D. Cuyanus* males present in the Los Patos river have a longer anal fin than females. As a hypothesis, we establish that the anal fin is an instrument used by fish to disperse gametes (male) at the moment of external reproduction. In other siluros, as is the case of *Genidens genidens*, there are morphological differences associated with male functioning in reproductive activity and parental care, but variation is seen primarily in the head (Paiva et al., 2015). Other variables, such as body depth in the caudal peduncle and body depth in the pectoral fin, are statistically different between male and female populations, being more robust in males. A more robust body would serve as an instrument to compete for territory for mating, feeding, more turbulent microhabitat use, parental care and nest construction (Allendorf, 1987).

It is possible that the differences observed can be explained by eco-morphology, where an animal's shape is given in terms of niche use (Shine, 1989), as is differentiated use of space, time, temperature, and food, among others. This last provides support for the results in the trophic ecology section of this work, where males and females present differences in consumption of prey.

Reproductive Biology

The reproductive state of *D. cuyanus* females was variable, few mature females were found, 23% of the sample, of which two were found in summer and one in spring. According to Azpelicueta (1994), in studies carried out individuals of the same genus, mature females were found in the month of March (summer), but there are no reports of mature females in spring.

In terms of males, two mature individuals were found, one in spring and one in summer. No works dealing with the male state of sexual maturity exist for this species, but there are previous studies on other members of the family, as is the case of *Diplomystes nahuelbutaensis,* for which reproduction occurs over an extensive period during the summer and fall, coinciding with low water flow and high temperatures in the river (Vila et al., 1996). This event does not occur with the same climate characteristics in the Los Patos river.

In percentage values, the spring season saw 50% mature individuals (n=4) while in the summer season this value drops to 13% (n=22). We must clarify that this difference could be influenced by the reduced number of individuals captured in the spring. Nevertheless, the existence of cycles should not be ignored. Lundberg *et al.* (1996) carried out their work on *Diplomystes nahuelbutaensis* larvae from 13mm long and their samples were collected in the month of December, which lends a certain support for the result obtained in this work on *D. cuyanus* reproduction, where the season contributing the greatest percentage of mature individuals was spring. Therefore, reproduction in these siluros could be established in the months of October to December.

Sex ratio was balanced 1:1 and according to this ratio the species is in a state of equilibrium, that is, without aggregation of males or females. In other species, populations with greater numbers of females have been recorded, which would indicate that the population is experiencing a period of expansion (Lorencio, 2000). The opposite case would be a population reduction like what is happening with the trans-Andean member of the family; for *Diplomystes nahuelbutaensis* the proportion was 36.3% female, 56% male and 7% undetermined (Vila et al., 1996).

The fertility value was 319 oocytes per mature female, a value that is considered low. In their study of *D. nahuelbutaensis* Vila et al. (1996) also points out low fertility, with a maximum of 330 mature oocytes.

The condition factor (K) only showed variation with respect to the gonadosomatic index. This shows that fish going through a reproductive event present a certain deterioration of their general condition. In accordance with this idea, *Dyplomystes nahuelbutaensis* shows a decrease of the condition factor in individuals with mature gonads (Vila et al., 1996), similar to what Martínez et al. (2003) found in their reproductive studies.

Studies carried out on other species coincide with the fact that reproductive cycles are in harmony with environmental variables such as river flow, photoperiod, and concentration of nutrients dissolved, among others (Vila et al., 1996; Casatti 2003). On the other hand, rate of reproductive effectivity could be affected by physical-chemical variations in the water, for example a change in pH to values less than 5.5 completely hinders sperm mobility (Emri et al., 1998; Darszon et al., 1999).

Diplomystes cuyanus is confirmed to be a carnivorous species, with variations in specific diet components compared to other species within the Diplomystidae family. The presence of isopods in the diet of D. cuyanus is distinctive and may be linked to the local ecology of the Los Patos river. The diversity and dominance of trophic components in the diet vary between seasons, likely influenced by the availability of preferred food sources. Spring presents a more diverse diet compared to summer, with increased abundance of certain prey items like Trichoptera larvae.

While there were no statistically significant differences between sexes, differences in diet composition were observed. Females tend to consume more isopods, while males consume more Trichoptera larvae. This variation may be related to differences in resource utilization and energy demands associated with reproductive activities. *D. cuyanus* is classified as a terrestrial consumer with a significant aquatic component in its diet. This classification underscores the importance of aquatic macroinvertebrates in river ecosystems and highlights the role of D. cuyanus in nutrient circulation within the system.

Morphological differences, such as anal fin length and body depth, suggest potential differences in reproductive behavior and ecological niche utilization between males and females. These differences may contribute to variations in diet composition observed between sexes.

The reproductive state of D. cuyanus individuals varies seasonally, with a higher percentage of mature individuals observed in spring compared to summer. The species exhibits a balanced sex ratio, indicating equilibrium within the population. Fertility values are relatively low, and reproductive cycles may be influenced by environmental variables such as temperature, river flow and photoperiod.

The biological information obtained in this study, including details on feeding, reproductive seasons, and sex-related differences, is of great importance for implementing future management and conservation plans for the species and their environment.

It is important for governmental, business, and societal entities to take interest and truly understand the importance of these fragile aquatic ecosystems. Avoiding "unnecessary" anthropogenic disturbances such as fragmentation, pollution, and the introduction of exotic species is crucial.

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Voucher specimens

Biological study material belongs to the scientific collection of the Biology Department (Gabinete DIBIOVA, FCEFN, UNSJ) (UNSJ-P 621-646).

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