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SHORT COMMUNICATION

Diet and reproduction of the fish *Joturus pichardi* (Mugiliformes: Mugilidae) in the Pacuare River basin, Costa Rica

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ABSTRACT: Introduction: *Joturus pichardi* is an important migratory species that inhabits the rivers of the Atlantic zone of Costa Rica and has been little studied. **Objective:** To describe the diet and reproduction of *J. pichardi* and its relationship with environmental factors in the Pacuare River basin. **Methods:** Sixteen sampling sites were selected along the main channel or Pacuare River. The samplings were performed from October 2015 to October 2016. The gonads, stomach, and intestines of seven individuals of *J. pichardi* were analyzed. The relationship of *J. pichardi* between the physicochemical variables of the water and the structure of the habitat was analyzed. **Results:** Twenty individuals were collected at an elevation of 225-235masl. The occurrence of *J. pichardi* was related to sites with higher river velocity, high values of dissolved oxygen, rock substrate and forest land use. All the individuals analyzed had visceral fat and endoparasites in the stomach. Bryophytes, ferns, macroinvertebrates, and detritus were identified as part of the diet of *J. pichardi*. According to the analysis of gonads in adults, the individuals were identified in the gonadal state types I, II and V. **Conclusions:** Our results suggest *J. pichardi* is an omnivorous species associated with sites with rocks, rapids, well-oxygenated waters, and forest land use. Also, the analysis of gonads coincides with the known reproduction period of the species. Finally, individuals of *Neoechinorhynchus* spp. are recorded for the first time in stomachs of *J. pichardi* in Costa Rica.

Key words: Freshwater fish; Central America; Diadromous fishes, Migration, Endoparasite.

RESUMEN: "Alimentación y reproducción del pez *Joturus pichardi* (Mugiliformes: Mugilidae) en la cuenca del Río Pacuare, Costa Rica"**. Introducción:** *Joturus pichardi* es una importante especie migratoria que habita en los ríos de la zona Atlántica de Costa Rica y ha sido poco estudiada. **Objetivo:** Describir la dieta y reproducción de *J. pichardi* y su relación con factores ambientales en la cuenca del Río Pacuare**. Métodos:** Se seleccionaron 16 sitios de muestreo a lo largo del canal principal. Los muestreos se realizaron de octubre de 2015 a octubre de 2016. Se analizaron gónadas, estómago e intestinos de siete individuos de *J. pichardi*. Se analizó la relación de *J. pichardi* entre las variables fisicoquímicas del agua y la estructura del hábitat. **Resultados:** Veinte individuos fueron recolectados a una elevación de 225-235msnm. La presencia de *J. pichardi* se relacionó con sitios con mayor velocidad del río, altos valores de oxígeno disuelto, sustrato de roca y uso de suelo forestal. Todos los individuos analizados tenían grasa visceral y endoparásitos en el estómago. Se identificaron briófitos, helechos, macroinvertebrados y detritos como parte de la dieta de *J. pichardi*. Según el análisis de las gónadas en adultos, los individuos fueron identificados en el estado gonadal tipos I, II y V. **Conclusiones:** Nuestros resultados sugieren que *J. pichardi* es una especie omnívora asociada con sitios con rocas, rápidos, aguas bien oxigenadas y uso de suelo forestal. Además, el análisis de gónadas coincide con el período de reproducción conocido de la especie. Finalmente, individuos de *Neoechinorhynchus* spp. se registran por primera vez en estómagos de *J. pichardi* en Costa Rica.

Palabras claves: Peces de agua dulce; Centro América; Peces diádromos, Migración, Endoparásito.

Costa Rica has an important richness of freshwater fish diversity within the Central American region (Bussing, 2002). Currently, 253 freshwater fish have been reported for the country (Angulo, Garita-Alvarado, Bussing, & López, 2013; Álvarez, Herrerea, & Angulo, 2018). Of these species, according to their tolerance to salinity, 63% are peripheral, 24% are secondary freshwater and 13% are primary freshwater (Angulo et al., 2013). The dominance of peripheral species in freshwater ecosystems in Costa Rica is also a proven fact in other Central American countries (e.g. Greenfield & Thomerson, 1997; Matamoros, Schaefer, & Kreiser, 2009; McMahan et al., 2013). This dynamics of colonization of freshwater by peripheral fish species have been studied on many occasions, mainly in those species that show migrations with reproductive purposes (e.g. Myers, 1949; McDowall, 1988; McDowall, 2007; McDowall, 2008).

In the Central American region, migratory freshwater fishes are known mainly within the families Mugilidae, Eleotridae and Gobiidae (Matamoros et al., 2009; Lorion, Kennedy, & Braatne, 2011; Angulo et al., 2013; McMahan et al., 2013), and there are some efforts to understand the migratory behavior, aspects of distribution, diet and reproduction of some them, mainly species as *Joturus pichardi* and *Dajaus monticola* (Mugilidae) and some species of the genus *Sicydium* (Gobiidae) (Bussing & López, 1977; Cruz, 1987; Cruz, 1989; Bussing, 2002; Lyons, 2005; Ribeiro & Villalobos, 2010; Eslava & Díaz, 2011; Lorion et al., 2011; Barboza & Villalobos, 2018; González-Murcia & Álvarez, 2018), however, there are still gaps in information. In addition to this, there are currently conservation difficulties for this group due to development of hydroelectric plants (Anderson, Freeman, & Pringle, 2006; Liermann, Nilsson, Robertson, & Ng, 2012), fragmentation and alteration of habitat, and different environmental problems that threatens the conservation of freshwater ecosystems and fish populations (McDowall, 1992, 1999; Hardesty-Moore et al., 2018).

Joturus pichardi, known as *"bobo fish"* in Costa Rica, is a peripheral species distributed from the Mexican Caribbean to Colombia, including some rivers of the Island of Cuba (Cruz, 1987; Cruz, 1989; Bussing, 2002; Eslava & Díaz, 2011). In Costa Rica, this species inhabits the rivers of the Atlantic zone of the country (Bussing, 2002; Angulo et al., 2013). According to some authors, *J. pichardi* is cataloged as a catadromous species due to its migrations to the sea to complete its reproductive cycle (Bussing, 2002). In Costa Rica, *J. pichardi* presents high demand for consumption by artisanal fishermen and local communities (Angulo, Naranjo-Elizondo, Rojas, & Ley-López, 2017; Valencia, Davidson-Hunt, & Berkes, 2019), in some cases, they are captured with prohibited fishing gear such as gillnets or dynamite (Bussing, 1975; Bussing, 2002). This species can reach about 70cm in length (Bussing, 2002; Angulo et al., 2017), making it an attractive fish because of its large size and meat quality (Eslava, 2009). In Costa Rica, despite the fact that *J. pichardi* is a species of great local interest and that has interesting migratory patterns, there are very few studies that address the current status of the populations (e.g. Bussing & López, 1977; Bussing, 2002; Lorion et al., 2011; Barboza & Villalobos, 2018).

Between 2015 and 2016 the *Asociación Costarricense de Acuarismo para la Conservación de los Ecosistemas Dulceacuícola*s (ACACED) developed a monitoring program on the Pacuare River, in the central Caribbean of Costa Rica, with the purpose of providing relevant information to take actions in favor of the conservation of this species whose results are herein provided. The main goals of this study were a) describe some biological aspects of the diet and reproduction of *J. pichardi* and b) describe the relationship of the species with habitat structure variables and physicochemical factors of the water.

MATERIALS AND METHODS

Study area and sampling sites: The Pacuare River basin has an area of 914km^2 with a length of 134,39km and an average rainfall of 2 000 to 5 000mmy^{-1} (Barboza & Villalobos, 2018). The basin has a maximum elevation of approximately 3 000masl. The Pacuare River starts in the Talamanca Mountain Range and then crosses a large part of the Caribbean side of Costa Rica, until its mouth in the vicinity of Siquirres, Province of Limón. It has important tributaries such as the Siquirres, Surú, Peje and Piedras Blancas rivers (Rojas, 2011). The samplings were done once per month from October 2015 to October 2016. Sixteen sites were selected and distributed in three zones (Fig. 1 in Digital Appendix): four sites in the zone with the lowest elevation (0 to 16masl), eight sites in the intermediate zone (225-235masl) and four sites in areas with higher elevation (622-624masl). The sites correspond to the sectors of Paso Marcos, Turrialba (9°48’43,36”N; 83°30’2,14”W); Bajos del Tigre, Siquirres (9°59’52,02”N; 83°32’31,68”W); and Siquirres, Limón (10°12’43,27”N; 83°19’10,68”W). To carry out the samplings, the support of owners adjoining the river was requested, as well as authorization from Cabécar indigenous leaders and research permits provided by the *Área de Conservación La Amistad Caribe* (ACLAC-PIME-R-043-2015).

Fishing gear and preservation: For the capture of individuals, cast-nets fish of 3m of coverage and 4cm mesh size were used. The sampling was made in transects of 100 linear meters along the river. Each transect was traversed in the form of a "*zig-zag*" in the opposite direction to the current of the river and each throwing of cast-net was made every three meters approximately three times. Sampling was standardized to a total of 300 effective throws along each transect. The captured fish were identified following taxonomic keys (Bussing, 2002; Angulo et al., 2017) and some morphological measurements (Standard Length, Total Length, Height) were taken. Seven specimens were sacrificed and stored in 70% alcohol for analysis in the laboratory, where the gonads, stomach, and intestines were extracted and analyzed. The rest of the captured specimens were released in the river. The gonads were analyzed qualitatively using the methodology proposed by Nikolsky (1963) to determine the state of maturation. The intestines and stomachs were analyzed in the Laboratory of Parasitology and Parasitic Diseases of the School of Veterinary Medicine of the Universidad Nacional de Costa Rica.

Environmental variables: The physicochemical parameters of the water as pH, temperature, salinity, conductivity, and dissolved oxygen were measured in the field using Hanna HI9813-5N and Hanna HI9147-04 equipment. Each measurement was made three times along each transect. In addition, some habitat structure variables, such as the presence of rapids (%), river width (m), canopy cover (%) and substrate type (%; rock, silt, sand, and leaf litter) were measured. For the measurement of substrate coverage, variables were visually measured in $1m^2$ at three points across the width of the sampling transect; the canopy cover was estimated according to the percentage of shade that generated the vegetation next to the river in each sampling site. The presence of rapids was estimated according to the number of rapids present over the length of the river, minimum values correspond to little or no presence of rapids on the sampling transect. The river width values were measured with a digital distance-meter. In addition, land-use variables were measured on each sampling point using Geographic Information Systems (GIS). The land use variables were stratified in four main variables; forest, urban area, crops, and grassland (paddocks). For this, a diameter of 250m (~5ha) was measured around each of the sampling sites. The physicochemical variables of the water and habitat structure were measured in each of the field visits, while the spatial variables of land use were measured only once.

Statistical analysis: A Principal Components Analysis (PCA) was carried out for the description of each of the sampling sites. For the analysis, the values of each variable were standardized to reduce the effect of the units of measurement of each variable and average data of each one of the measured variables were used. A Spearman Correlation Analysis was performed to visualize the significant contribution of each variable within one of the components of the PCA analysis (≥ ± 0,7; p=0,05). All the statistical analyses were performed with the InfoStat software (Di Rienzo et al., 2011).

Ethical, conflict of interest and financial statements: The authors declare that they have fully complied with all pertinent ethical and legal requirements, both during the study and in the production of the manuscript; that there are no conflicts of interest of any kind; that all financial sources are fully and clearly stated in the acknowledgements section; and that they fully agree with the final edited version of the article. A signed document has been filed in the journal archives.

RESULTS

A total of 20 individuals of *J. pichardi* were collected between April and October 2016 in the intermediate zone of the river in Bajos del Tigre (225-235masl) (Fig. 2 in Digital Appendix). The largest size obtained was 33,5cm and the smallest of 20,5cm, while the weight obtained from the individuals ranged from 150g to 600g. With respect to the state of gonadal maturation, three individuals captured in April, May and July presented gonadal state type I (TL; 33,5cm, 31cm, and 27cm respectively), followed by three individuals captured in June, August and September that presented gonadal state type II (TL; 32cm, 33cm, and 30cm respectively), while a 35cm (TL) female captured in October was in gonadal state type V. The analysis of the intestines and stomachs in seven individuals evidenced the presence of parasites and visceral fat. According to laboratory identification (code PA-216-16), the parasite was identified as *Neoechinorhynchus* spp. Regarding the diet in seven individuals, it was determined that it is constituted mainly by detritus, bryophytes, ferns, and macroinvertebrates within the groups of Trichoptera, Lepidoptera, Diptera, and Plecoptera (see more details at Table 1 in Digital Appendix).

The Principal Component Analysis (PCA) explained 75,5% of the variation of the data. The PC1 component explained 55,2% of the variation of the data with the variables of river width, elevation, temperature, conductivity, rock cover, leaf litter, sand, silt and canopy cover. While the PC2 component explained 20,3% of the variation of the data with the variables of dissolved oxygen and grassland. The sites with the lowest elevation were characterized by having higher values of temperature, greater values of river width, higher salinity, higher pH, greater coverage of substrate of silt, sand and leaf litter and were associated with sites with a greater presence of crops and greater canopy coverage. In the case of the intermediate zones, they were characterized by their greater association with rapids, higher values of dissolved oxygen, rock substrate and land use of the forest. Whereas the areas with higher elevation were characterized by their association with areas of land use of grassland and urban areas (see more details at Fig. 3 and Table 2 in Digital Appendix).

DISCUSSION

The results obtained describe some important aspects of diet and reproduction of *J. pichardi*. Our habitat structure data coincide with other authors about the preference for sites with higher water velocity, high values of dissolved oxygen and are related to sites with greater forest cover (Bussing & López, 1977; Cruz, 1987; Bussing, 2002; Eslava & Díaz, 2011; Lorion et al., 2011; Barboza & Villalobos, 2018). This relationship could condition the presence of this species within the altitudinal gradient of the river, so a change within the flow or drastic modifications of land use can generate a negative effect within the populations. Our data are also consistent with other authors about the habitat preference in sites with higher elevation, although abundances could are greater in intermediate elevation areas within the rivers (Lorion et al., 2011; Barboza & Villalobos, 2018). It is probably that in higher places not present favorable conditions for the presence of this species in the case of Pacuare river. Despite a low abundance of *J. pichardi* was evident in our samples, similar studies in Costa Rica with other capture techniques do not show high values of abundance (e.g. Lorion et al., 2011; Barboza & Villalobos, 2018). Therefore, the low abundance of records in our study may be due to this trend and not to a sampling bias. It is likely that populations are being impacted by different factors that were not considered in this study as aspects of overfishing, contamination, natural or anthropic alteration of habitat.

Our data record an average size (TL) of approximately 28,4cm, these data are similar to the results obtained by Barboza and Villalobos (2018) for the same river. However, there are records that this species can reach about 70cm (Bussing, 2002; Angulo et al., 2017). Some authors suggest that this fish is consumed by local fisheries and indigenous communities in Costa Rica (Lorion et al., 2011; Angulo et al., 2017; Valencia et al., 2019). Personal interviews with artisanal fishermen and local indigenous affirm that *J. pichardi* fishing has decreased in recent years, they also affirm that there is the use of illegal fishing gear in low areas of rivers for their capture and commercialization. It is likely that overfishing is affecting the development of populations, decreasing their abundance and fish with large sizes along the river. Also, we observed individuals in a mature state in the month of October, coinciding with other authors about the period of reproduction (Cruz, 1987; Cruz, 1989; Eslava & Díaz, 2011; Martínez-Moreno, Palomares-García, & Falcón-Rodríguez, 2015). However, we recorded the development of gonads in small individuals, possibly this species could be accelerating the reproductive period as a measure of adaptation to current changes or environmental pressures in the area.

Our results about the diet of *J. pichardi* coincide with some descriptions made by other authors for this species (Cruz, 1987; Bussing, 2002; Correa Polo, Eslava Eljaiek, Martínez, & Narváez Barandica, 2012; Barboza & Villalobos, 2018). We agree that this species can feed mainly on plants and different groups of insects as part of their diet. In addition, within the stomach analysis, the presence of endoparasites of the genus *Neoechinorhynchus* was recorded for the first time in all the individuals evaluated. It is likely that this is the first documented record for Costa Rica for this species, although endoparasites of this genus have been documented in others fish species in the Central American region (Pinacho-Pinacho, Sereno-Uribe, Pérez-Ponce de León, & Garcia-Varela, 2015; Pinacho-Pinacho, Sereno-Uribe, García-Varela, & de León, 2018). Due to the absence of monitoring data on the populations of *J. pichardi*, it is difficult to sustain the hypothesis of the decline of these populations. We recognize difficulties in making solid conclusions, for which we suggested to increase the studies that consider the use of different techniques and methodology that allow obtaining scientific evidence about these populations of *J. pichardi*. However, we consider it is essential to make a connection between the negative factors already known that decline the population of bobo fish and that affect the reproductive aspects and development of this species. Therefore, we suggest considering this information within local strategies for the management and conservation of Costa Rican wildlife.

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