Fundación
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Tucumán
Argentina

# Habitat use, coloration, and behavioral observations of fishes from mountainous headwaters of the northwestern La Plata basin

## Uso del hábitat, coloración, y observaciones del comportamiento de los peces de las cabeceras montañosas del Noroeste de cuenca del Plata

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➤ Ref. bibliográfica: Alonso, F.; Irigoyen, A. J.; Aguilera, G.; Terán, G. E.; Serra Alanís, W.; Méttola, V. E.; Waldbilllig, M. M.; Merlo, P. J.; Trobbiani, G.; Méndez López, A.; Avigliano, E.; Montes, M. M.; Pandolfi, M.; Mirande, J. M.; 2026. "Habitat use, coloration, and behavioral observations of fishes from mountainous headwaters of the northwestern La Plata basin". *Acta Zoológica Lilloana 70* (1): 413-450. DOI: https://doi.org/10.30550/j.azl/2380
➤ Recibido: 9 de marzo 2026 – Aceptado: 13 de abril 2026.
➤ URL de la revista: http://actazoologica.lillo.org.ar

## Abstract

We describe microhabitat use and provide observations on the coloration and behavior of fishes from the mountainous headwaters of the northwestern La Plata Basin, a highly endemic freshwater region of high conservation priority. Using underwater observations, video recordings, and in situ photography, we documented habitat associations, school formation, species interactions, and the potential ecological role of coloration patterns. In watercourses of the Bermejo and Juramento river basins we accumulated approximately 81.5 h of direct observations and 17 h 36 min of underwater video recordings. Fish assemblages showed marked habitat partitioning: benthic specialists (e.g., *Trichomycterus spegazzinii*, *Rineloricaria* spp., *Characidium* spp., and *Parodon carrikeri*) were concentrated in riffles and high-velocity microhabitats, whereas many characiforms were more frequent in pools and moderate-flow sections. Shallow vegetated secondary arms frequently functioned as nursery habitats by concentrating juveniles. Size-based altitudinal segregation observed in *Acrobrycon tarijae* (adults upstream; juveniles downstream) suggests a life cycle linked to elevation gradients and a possible reproductive migration. We also recorded notable behaviors, including aggressive mimicry in *Oligosarcus bolivianus*, contrasting predatory strategies of *Salminus brasiliensis*, and commensal feeding by small characiforms following substrate-disturbing individuals of *Steindachnerina brevipinna*. These results highlight the importance of structural habitat heterogeneity and the need for conservation measures to mitigate anthropogenic impacts such as river canalization.

**Keywords:** Coloration, Habitat partitioning, Interspecific schools, Altitudinal migration, Nursery areas.

## Resumen

Describimos el uso del microhábitat y presentamos observaciones sobre la coloración y el comportamiento de peces de las nacientes montañosas del noroeste de la cuenca del Plata, una región dulceacuícola altamente endémica y de alta prioridad para la conservación. Mediante observaciones subacuáticas, grabaciones de video y fotografía in situ documentamos asociaciones de hábitat, formación de cardúmenes, interacciones entre especies y el posible papel ecológico de los patrones de coloración. En cursos de agua de las cuencas de los ríos Bermejo y Juramento se acumularon aproximadamente 81,5 h de observaciones directas y 17 h 36 min de registros en video subacuático. Las asociaciones de peces mostraron una marcada partición del hábitat: especialistas bentónicos (por ejemplo, *Trichomycterus spegazzinii*, *Rineloricaria* spp., *Characidium* spp. y *Parodon carrikeri*) se concentraron en rápidos y microhábitats de alta velocidad de corriente, mientras que muchos caraciformes fueron más frecuentes en pozones y sectores de corriente moderada. Los brazos secundarios someros y vegetados funcionaron frecuentemente como áreas de cría al concentrar juveniles.

La segregación altitudinal por tamaño observada en *Acrobrycon tarijae* (adultos aguas arriba; juveniles aguas abajo) sugiere un ciclo de vida vinculado a gradientes altitudinales y una posible migración reproductiva. También se registraron comportamientos como mimetismo agresivo en *Oligosarcus bolivianus*, estrategias depredadoras contrastantes de *Salminus brasiliensis* y alimentación comensal de pequeños caraciformes siguiendo a *Steindachnerina brevipinna*. Estos resultados resaltan la importancia de la heterogeneidad estructural del hábitat y la necesidad de medidas de conservación frente a impactos antrópicos como la canalización de ríos.

**Palabras clave:** Coloración, partición del hábitat, cardúmenes interespecíficos, migración altitudinal, áreas de cría.

## INTRODUCTION

The northwestern section of the La Plata Basin, situated on the Atlantic slope of the Central Andes of South America, encompasses the headwaters of several major river systems, including the Bermejo, Pilcomayo, Salado, and Dulce rivers. In freshwater ecosystems, speciation is often driven by the interplay between geographic isolation and ecological differentiation, where environmental forcing factors such as hydrological variability, habitat heterogeneity, and physicochemical gradients promote divergent selection, phenotypic plasticity, and ultimately reproductive isolation. These processes can operate through both allopatric mechanisms, by limiting dispersal among basins or sub-basins, and ecological speciation, by favoring local adaptation to contrasting environmental conditions. In this context, a defining ecological characteristic of these rivers is their pronounced slope and summer rainy season, which triggers abrupt increases in discharge and turbidity. These hydrological fluctuations likely impose strong selective pressures on fish anatomy, ecology, and behavior, fostering ecological specialization and contributing to high levels of endemism in the region (Alonso et al., 2024, 2025; Aguilera et al., 2025).

Numerous studies have highlighted the remarkable fish diversity and endemism in this area (e.g., Calviño & Alonso, 2010; Casciotta & Almirón, 2004; Mirande et al., 2004, 2006, 2011, ; Aguilera et al., 2019; Alonso et al., 2024; Aguilera et al., 2025). Many species are classified in IUCN threatened categories, including Critically Endangered *Urkumayu gladysae* (Calviño & Alonso, 2010), *Urkumayu petracinii* (Calviño & Alonso, 2010), and *Jenynsia sulfurica* Aguilera et al., 2019; Endangered *Andromakhe latens* (Mirande et al., 2004), *Oligosarcus itau* Mirande, Aguilera & Azpelicueta, 2011, *Jenynsia maculata* (Regan, 1906), and *Jenynsia alternimaculata* (Fowler, 1940); and Vulnerable *Urkumayu micracanthus* (Regan, 1912), *Nantis indefessus* (Mirande, Aguilera & Azpelicueta, 2004), *Piabina thomasi* (Fowler, 1940), *Psalidodon chico* (Casciotta & Almirón, 2004), *Psalidodon endy* (Mirande, Aguilera & Azpelicueta, 2006), and *Microglanis nigrolineatus* Terán et al., 2016 (Alonso, 2022a–m).

Despite more than 20 years of research by our group on fish diversity in this region, detailed studies of habitat use and behavior remain scarce. Beyond a few anecdotal references (e.g., Aguilera et al., 2016; Alonso et al., 2016; Terán et al., 2019, 2024) and focused investigations on Corydoradinae (Alonso et al., 2018; Alonso et al., 2025), most species still lack field-based behavioral and ecological data.

Field observations of animal behavior and habitat use are fundamental for developing conservation policies, understanding evolutionary processes, and interpreting functional relationships among anatomy, ecology, and diversification. Yet such observations remain uncommon for freshwater fishes, and behavioral records are often absent or limited to anecdotal accounts even for widely used model species such as *Danio rerio* (Hamilton, 1822) (Engeszer et al., 2007). Natural history—the empirical foundation for generating biologically meaningful questions—has historically been undervalued within academia due to biases that favor other research agendas (Futuyma, 1998; Arnold, 2003; Dayton, 2003; Greene, 2005).

Interactions among behavior, habitat use, and coloration are particularly understudied in freshwater fishes. In Characiformes, aggressive mimicry has been documented, such as *Deuterodon heterostomus* (Eigenmann, 1911) mimicking *Psalidodon* species (Sazima, 1977) or *Salminus brasiliensis* (Cuvier, 1816) mimicking *Brycon hilarii* (Valenciennes, 1850) (Bessa et al., 2011). Müllerian co-mimicry has also been observed among Siluriformes, particularly within Corydoradinae (Alexandrou et al., 2011), including *Urkumayu micracanthus* and *Hoplisoma osvaldoi* (Alonso et al., 2024). In addition, coloration is central to taxonomic diagnosis, species identification, and the definition of species groups (e.g., Casciotta & Almirón, 2004; Lima & Sousa, 2009; Marinho et al., 2016; Vari et al., 2016; Soares, 2019; Alonso et al., 2018, 2023, 2024, 2025a–c), and some coloration traits show strong phylogenetic signal (e.g., Mirande, 2018; Alonso et al., 2023, 2024, 2025a).

This study investigates behavior, microhabitat use, and coloration of several endemic and threatened fish species inhabiting small rivers in the mountain region of the northwestern La Plata Basin. Through direct field observations, underwater recordings, and standardized in situ aquarium photography, we describe microhabitat associations, schooling behavior, and predator–prey interactions, and discuss their potential relationships with coloration patterns. By addressing these overlooked aspects of natural history, we aim to provide insights relevant to conservation and to establish a baseline for future ecological and evolutionary studies in this group.

## MATERIALS AND METHODS

### Study sites

Study sites within the Bermejo River Basin included (Fig. 1): (1) La Caldera River (-24.565711°, -65.391200°; 1484 m a.s.l.) and Lesser River (-24.682152°, -65.476162°; 762 m a.s.l.); (2) an unnamed stream along National Route 50 (-23.055411°, -64.343825°; 351 m a.s.l.); (3) Bermejo River (-23.192536°, -64.183119°; 306 m a.s.l.); (4) Parque Nacional El Rey, Popayán River (-24.730694°, -64.575028°; 786 m a.s.l.); (5) Geiser Mollinedo (-24.416999°, -63.990999°; 357 m a.s.l.); and (6) Laguna La Quinta (-23.884175°, -64.467428°; 627 m a.s.l.). Additional observations were conducted at (7) Aguas Calientes (-23.744398°, -64.519017°; 411 m a.s.l.). In the Juramento River Basin, the study site was (8) the Calchaquí River near Payogasta (-25.050156°, -66.105744°; 2433 m a.s.l.). Study sites are shown in Fig. 2.

Permits were granted by Administración de Parques Nacionales, Secretaría de Ambiente y Desarrollo Sustentable de Salta, and Ministerio de Ambiente de Jujuy. Fieldwork complied with the national ethics framework ("Comité Nacional de Ética en la Ciencia y la Tecnología" of Argentina) and with the National Research Council's Guide for the Care and Use of Laboratory Animals. All reasonable measures were taken to minimize stress, pain, and discomfort, and the study followed international standards of animal welfare and all applicable national regulations.

**Field observations and video recording.**— Observations were conducted both above water and underwater. For above-water observations, the observer remained stationary for at least five minutes before recording species presence and behavior within each microhabitat to minimize disturbance; observations were recorded in a field notebook. Observation duration varied depending on time available at each site.

Underwater observations were conducted using snorkeling equipment and two recording approaches. In the first approach, the observer performed slow upstream transects, pausing at each microhabitat (e.g., submerged logs, pools) to allow fish to acclimate before recording behavior using field notes and a video camera. In the second approach, a static camera was placed in selected microhabitats and left to record for 15 minutes. To reduce potential artifacts related to camera placement and retrieval, the first three minutes and the final minute of each recording were excluded from analysis.

In total, approximately 81.5 h of direct observations and 17 h 36 min of underwater video recordings were collected across all sites (Table 1). All underwater videos were recorded using a GoPro Hero3 Black Edition. These observations were complemented by fish sampling data from the same region (2015–2025) derived from several studies conducted by our team.

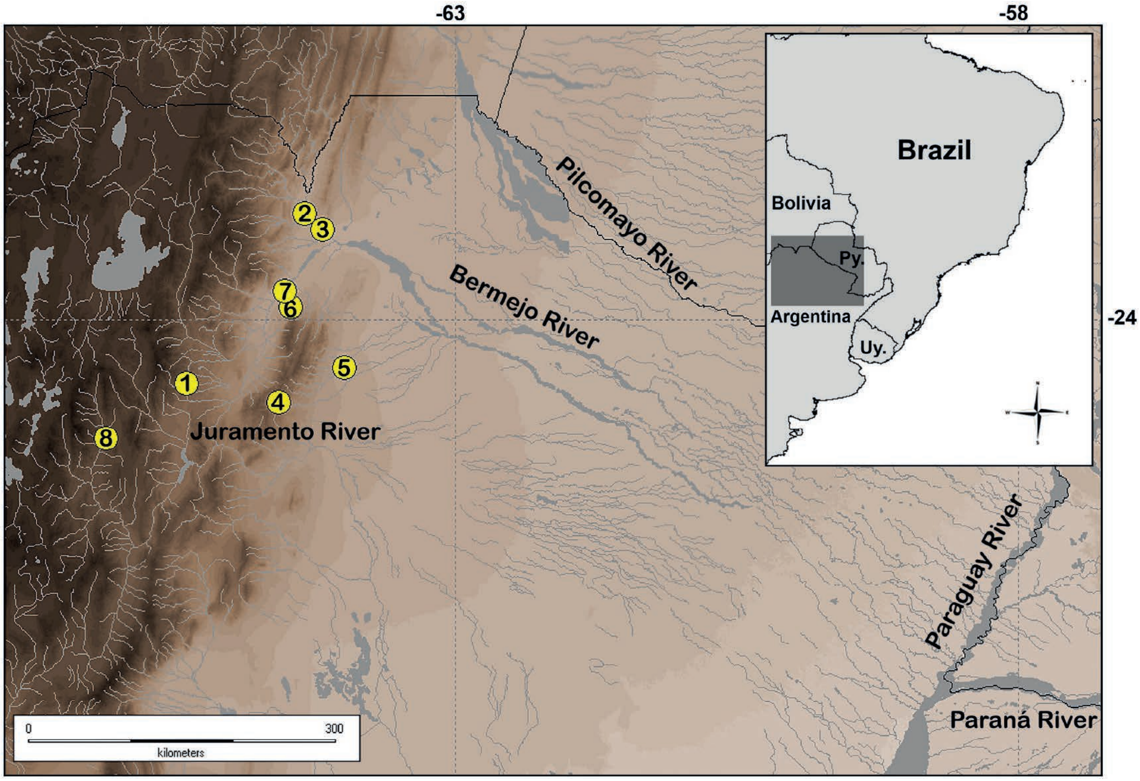

**Figure 1.** Geographic locations of study sites in the Andean region of the northwestern La Plata Basin: 1) La Caldera River (-24.565711°, -65.391200°, 1484 m asl) and Lesser River (-24.682152°, -65.476162°, 762 m asl), 2) an unnamed stream along National Route 50 (-23.055411°, -64.343825°, 351 m asl), 3) Bermejo River (-23.192536°, -64.183119°, 306 m asl), 4) Parque Nacional El Rey at Popayán River (-24.730694°, -64.575028°, 786 m asl), 5) Geiser Mollinedo (-24.416999°, -63.990999°, 357 m asl), and 6) Laguna La Quinta (-23.884175°, -64.467428°, 627 m asl). Additionally, observations were conducted at 7) Aguas Calientes (-23.744398°, -64.519017°, 411 m asl). In the Juramento River Basin, the study site was 8) the Calchaquí River near Payogasta (-25.050156°, -66.105744°, 2433 m asl).

**Figura 1.** Ubicación geográfica de los sitios de estudio en la región andina del noroeste de la cuenca del Plata: 1) río La Caldera (-24.565711°, -65.391200°, 1484 m s.n.m.) y río Lesser (-24.682152°, -65.476162°, 762 m s.n.m.), 2) arroyo sin nombre a lo largo de la Ruta Nacional 50 (-23.055411°, -64.343825°, 351 m s.n.m.), 3) río Bermejo (-23.192536°, -64.183119°, 306 m s.n.m.), 4) Parque Nacional El Rey en el río Popayán (-24.730694°, -64.575028°, 786 m s.n.m.), 5) géiser Mollinedo (-24.416999°, -63.990999°, 357 m s.n.m.), y 6) laguna La Quinta (-23.884175°, -64.467428°, 627 m s.n.m.). Adicionalmente, se realizaron observaciones en 7) Aguas Calientes (-23.744398°, -64.519017°, 411 m s.n.m.). En la cuenca del río Juramento, el sitio de estudio fue 8) el río Calchaquí cerca de Payogasta (-25.050156°, -66.105744°, 2433 m s.n.m.).

**Habitat use and behavioral observations.**— At each study site, habitat units were classified following Bisson et al. (1982), and fish species presence was recorded systematically. Video recordings were analyzed in the laboratory to document habitat characteristics (e.g., submerged wood, aquatic vegetation, substrate composition, turbulence) and to identify fish assemblages associated with each habitat type.

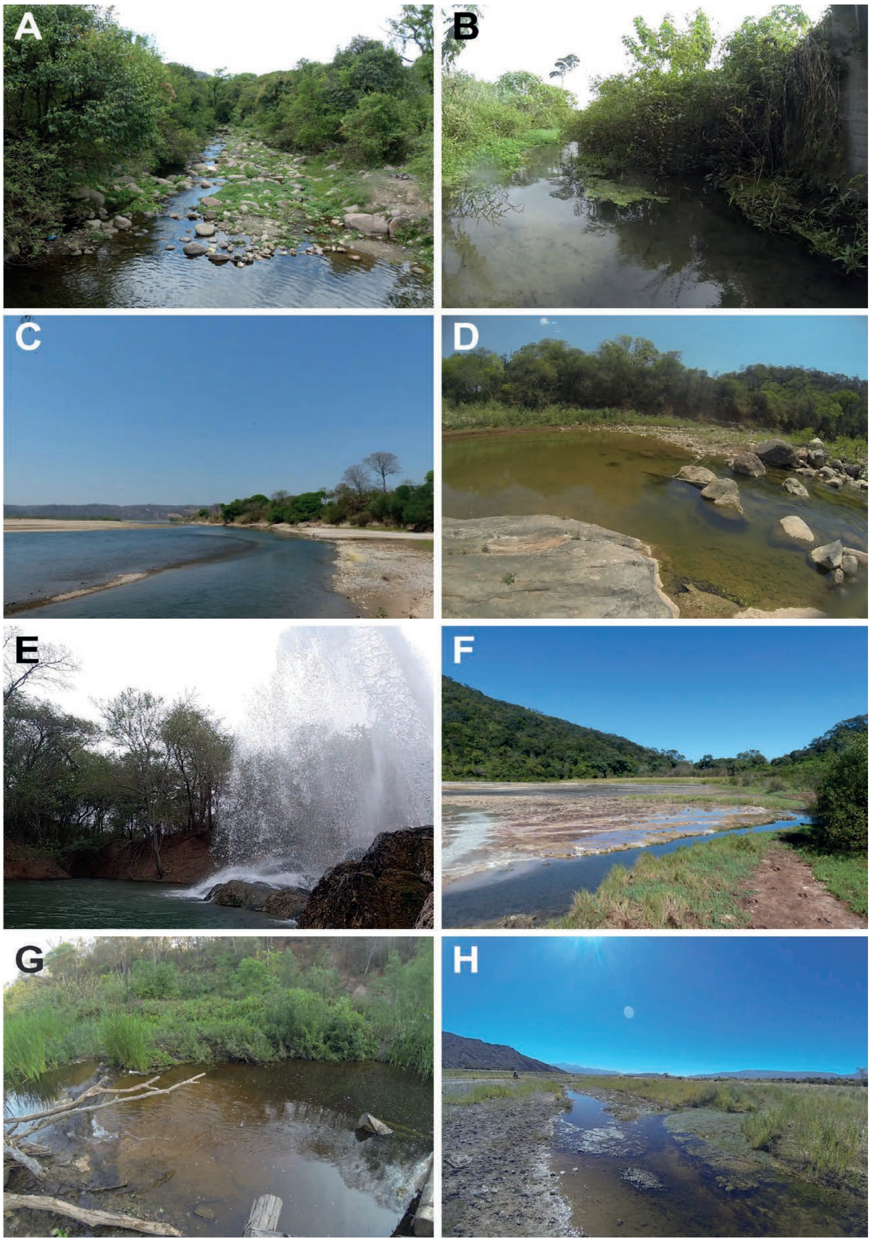

**Figure 2.** Study sites. Bermejo river basin: A) Stream in Lesser (-24.682152°, -65.476162°, 762 m asl), Salta province, B) an unnamed stream along National Route 50 (-23.055411°, -64.343825°, 351 m asl), C) Bermejo River (-23.192536°, -64.183119°, 306 m asl), D) Parque Nacional El Rey at Popayán River (-24.730694°, -64.575028°, 786 m asl), E) Geiser Mollinedo (-24.416999°, -63.990999°, 357 m asl), and F) Laguna La Quinta (-23.884175°, -64.467428°, 627 m asl), G) Aguas Calientes (-23.744398°, -64.519017°, 411 m asl); and in the Juramento River Basin: H) the Calchaquí River near Payogasta (-25.050156°, -66.105744°, 2433 m asl).

**Figura 2.** Sitios de estudio. Cuenca del río Bermejo: A) arroyo en el río Lesser (-24.682152°, -65.476162°, 762 m s.n.m.), provincia de Salta, B) arroyo sin nombre a lo largo de la Ruta Nacional 50 (-23.055411°, -64.343825°, 351 m s.n.m.), C) río Bermejo (-23.192536°, -64.183119°, 306 m s.n.m.), D) Parque Nacional El Rey en el río Popayán (-24.730694°, -64.575028°, 786 m s.n.m.), E) géiser Mollinedo (-24.416999°, -63.990999°, 357 m s.n.m.), F) laguna La Quinta (-23.884175°, -64.467428°, 627 m s.n.m.), G) Aguas Calientes (-23.744398°, -64.519017°, 411 m s.n.m.); y en la cuenca del río Juramento: H) río Calchaquí cerca de Payogasta (-25.050156°, -66.105744°, 2433 m s.n.m.).

**Table 1.** Survey effort across study sites used to assess fish behavior and habitat use, including external observations, dynamic underwater visual surveys, and stationary underwater surveys.

**Tabla 1.** Esfuerzo de muestreo en los diferentes sitios de estudio utilizado para evaluar el comportamiento y el uso del hábitat de los peces, incluyendo observaciones externas, censos visuales subacuáticos dinámicos y censos subacuáticos estacionarios.

| Site | External observations (hours) | Dynamic underwater surveys (hours) | Stationary Underwater surveys (N) |
|---|---|---|---|
| La Caldera River and Lesser River | 20 | 2.1 | 3 |
| Unnamed stream along National Route 50 | 1 | 1.2 | 1 |
| Bermejo River | 12 | 1.1 | 3 |
| Popayán River, Parque Nacional El Rey | 18 | 6.1 | 7 |
| Geiser Mollinedo | 1.5 | 0.6 | 2 |
| Laguna La Quinta | 4 | 0.9 | 1 |
| Aguas Calientes | 2 | 0.7 | 2 |
| Calchaquí River | 23 | 4.9 | 5 |
| **Total** | **81.5** | **17.6** | **24** |

Behavioral data were not collected using a standardized sampling protocol; however, conspicuous behaviors were recorded whenever observed (e.g., feeding, predation events, swimming mode, parental behaviors, schooling, heterospecific associations), based on above-water observations, underwater observations, and video recordings. These observations were recorded using an ad libitum approach to document the diversity of behaviors expressed across environments and conditions.

Coloration of species in relation to habitat use and schooling behavior

For standardized comparisons of live coloration, some individuals were photographed in situ using a specialized photographic aquarium and a Panasonic Lumix FZ200 camera. The aquarium included a movable glass plate that allowed gentle immobilization of specimens against the front panel to obtain high-quality photographs. We provide a comparative description of coloration patterns across species, emphasizing patterns recurrent in heterospecific assemblages and their relationship to habitat background.

In addition to sampling trips designed specifically for habitat-use and behavioral observations, standardized aquarium photography was carried out during other collection efforts (2005–2025). These additional campaigns provided complementary visual records of live specimens and facilitated comparisons of coloration patterns under controlled conditions. Specimens collected and/or observed during this study are listed in Supplementary File 1; additional examined voucher material is provided in Supplementary File 2. The images compiled here reflect this long-term effort and aim to ensure consistency in photographic documentation across species and sites.

# RESULTS

## Habitat description, use and behavioral observations

Habitat use is summarized in Table 2.

### Site 1: La Caldera River and Lesser River

(Videos available at: Riffles, Pools, Secondary arms).
The main habitat identified were 1) riffles, 2) pools, and 3) secondary arms.

**1. Riffles (Rapids)**.— Riffles were shallow (~20 cm), with high current velocity and a coarse substrate dominated by large stones. These habitats were dominated by adult *Trichomycterus spegazzinii* (Berg, 1897) (> 6 cm standard length, SL) and *Rineloricaria steinbachi* (Regan, 1906) (> 7 cm SL), typically positioned downstream of large stones, using them as current refuges.

**2. Pools (Cascades)**.— Pools alternating with riffles were deeper (40–70 cm), with intermediate current velocity and finer substrates interspersed with large stones. Adult *Psalidodon endy*, *Nantis indefessus*, and *Acrobrycon tarijae* (Fowler, 1940) were frequently recorded swimming against the current and feeding on suspended particles. Larger individuals and larger-bodied species tended to occur in deeper sections, often maintaining relatively stationary positions with subtle lateral adjustments. Bottom-associated species including *Characidium borellii* (Boulenger, 1895), *Jenynsia alternimaculata*, and *T. spegazzinii* also occurred in pools. Individuals of *J. alternimaculata* frequently formed monospecific schools near the bottom.

**3. Secondary Arms (Glide)**.— Secondary arms had low current velocity, very fine sediments, and abundant aquatic vegetation. These habitats included *Urkumayu micracanthus*, recorded filter-feeding on bottom sediments and often forming small aggregations (4–6 individuals). Juveniles of *J. alternimaculata*, *N. indefessus*, *P. endy*, and *T. spegazzinii* were concentrated in vegetated zones, apparently using them as refuge. In deeper portions of secondary arms, *Oligosarcus bolivianus* (Fowler, 1940) and *P. endy* were recorded associated with submerged vegetation.

### Site 2: unnamed stream along National Route 50

(Fig. 3, underwater video available at: *Video)*
The dominant habitat unit was a glide, which comprised three microhabitats: (1) vegetated margins with abundant submerged aquatic plants; (2) a central channel lacking aquatic plants; and (3) a sandy/fine-sediment bottom.

**Table 2.** Resume of habitat use.

**Tabla 2.** Resumen del uso del hábitat.

| Site | Habitat | Species Observed |
|---|---|---|
| La Caldera River and Lesser River | Riffles | *Trichomycterus spegazzinii, Rineloricaria steinbachi* |
| | Pools | *Psalidodon endy, Nantis indefessus, Acrobrycon tarijae, Characidium borellii, Jenynsia alternimaculata, Trichomycterus spegazzinii* |
| | Secondary Arms | *Urkumayu micracanthus, Jenynsia alternimaculata, Nantis indefessus, Psalidodon endy, Trichomycterus spegazzinii, Oligosarcus bolivianus* |
| Unnamed stream along National Route 50 | Margins with Aquatic Vegetation | *Moenkhausia bonita* |
| | Channel Without Plants | *Psalidodon chico, Psalidodon endy, Andromakhe latens, Piabina thomasi, Oligosarcus bolivianus* |
| | Bottom of Fine Sediments | *Rineloricaria catamarcensis, Steindachnerina brevipinna, Psalidodon* spp. |
| Bermejo River | Big Pool | *Astyanax abramis, Oligosarcus bolivianus, Steindachnerina brevipinna, Salminus brasiliensis* |
| | Riverbank Shallow Areas | *Piabina thomasi, Odontostilbe microcephala, Holoshesthes pequira, Characidium borellii* |
| Popayán River, Parque Nacional El Rey | High-velocity Microhabitats | *Parodon carrikeri, Characidium borellii, Characidium* sp. |
| | Deep Pools | *Prochilodus lineatus, Arhinolemur obtusidens, Salminus brasiliensis, Astyanax* spp. |
| | Fast-flowing Riffles | *Leporellus pictus, Parodon carrikeri, Astyanax* spp. |
| | Benthic Generalist | *Characidium borellii, Characidium* sp. |
| | Rheophilic Generalist | *Acrobrycon tarijae* |
| Geiser Mollinedo | Margins with Aquatic Vegetation | *Astyanax abramis* |
| | High-velocity Microhabitats | *Parodon carrikeri, Characidium borellii, Characidium* sp. |
| | Benthic Generalist | *Characidium borellii, Characidium* sp. |
| | Mixed-species Shoals | *Astyanax abramis, Parodon carrikeri, Characidium borellii, Characidium* sp. |
| Laguna La Quinta | Shallow Marginal Areas | *Jenynsia sulfurica* |
| | Deeper Downstream Zones | *Jenynsia sulfurica* |
| | Entrance Channels | *Jenynsia sulfurica* |
| Aguas Calientes | Underwater Caves | *Hypostomus boulengeri* |
| | Open Riverbed Territories | *Bujurquina vittata* |
| Calchaquí River | Riffles | *Trichomycterus spegazzinii, Rineloricaria steinbachi* |
| | Deep Pools | *Nantis indefessus, Psalidodon* aff. *eigenmanniorum, Bryconamericus rubropictus, Jenynsia maculata, Trichomycterus spegazzinii, Rineloricaria steinbachi* |
| | Vegetated Secondary Arms | *Jenynsia maculata, Nantis indefessus, Trichomycterus spegazzinii, Urkumayu gladysae, Heptapterus mustelinus* |

**Figure 3.** Left: mixed school in National Route 50 site of *Astyanax abramis* and *Steindachnerina brevipinna*, with a clear vertical stratification between species. Right: *Oligosarcus bolivianus* mixed in a school of *Astyanax abramis.*

**Figura 3.** Izquierda: cardumen mixto en el sitio de la Ruta Nacional 50 de *Astyanax abramis* y *Steindachnerina brevipinna*, con una clara estratificación vertical entre especies. Derecha: *Oligosarcus bolivianus* integrado en un cardumen de *Astyanax abramis*.

Recorded species included *Psalidodon chico*, *Andromakhe latens*, *Piabina thomasi*, *Moenkhausia bonita* Benine, Castro & Sabino, 2004, *O. bolivianus*, *Hoplias argentinensis*, *S. brevipinna* (Eigenmann & Eigenmann, 1889), *Rineloricaria catamarcensis* (Berg, 1895), and *Cichlasoma dimerus* (Heckel, 1840).

**1. Vegetated margins**.— Vegetated margins were dominated by schools of *M. bonita*, which did not integrate into mixed-species schools. Juveniles and smaller individuals were concentrated within vegetation in areas of reduced flow.

**2. Central channel**.— Mixed-species schools composed of *Astyanax abramis* and *S. brevipinna*, together with some *P. chico*, *P. endy*, *A. latens*, and *P. thomasi*, were recorded swimming in midwater. Individuals of *O. bolivianus* were occasionally recorded swimming adjacent to, or briefly integrating into, these schools. Larger fish were typically recorded in lower portions of the water column (Fig. 3).

**3. Sandy/fine-sediment bottom**.— This microhabitat was occupied by *R. catamarcensis* and *S. brevipinna*, which disturbed the substrate while feeding, displacing particles and exposing food items. Disturbed particles were subsequently exploited by smaller, occasionally commensal species (e.g., *Psalidodon* spp.).

### Site 3: Bermejo River

The main habitat units identified were pools and shallow riverbank habitats. (See Video)

**1. Pools (trench pools).**— A large central pool was ~4 m deep and included a vertical wall that was surveyed. Mixed-species schools swam at depths of 0.5–1.5 m and were dominated by *A. abramis*, *O. bolivianus*, and *S. brevipinna*, with the latter species typically occupying the lower portion of the aggregation. Below these schools, a single *S. brasiliensis* was recorded repeatedly targeting fish from below with rapid upward movements (Fig. 4).

**2. Shallow riverbank habitats (low-gradient riffles).**— Shallow riverbank habitats had low current velocity and substrates dominated by sand, pebbles, and gravel. These habitats supported smaller species, including *P. thomasi*, *Odontostilbe microcephala* (Eigenmann, 1907), and *Holoshesthes pequira* (Steindachner, 1882). These species formed mixed schools, feeding on the substrate and suspended particles while maintaining rheotactic orientation. Isolated *C. borellii* were recorded on the bottom, typically maintaining a rheotactic posture.

**Figure 4.** Predatory behavior of *Salminus brasiliensis* targeting mixed-species schools of *Astyanax* and *Steindachnerina*.

**Figura 4.** Comportamiento depredador de *Salminus brasiliensis* dirigido a cardúmenes multiespecíficos de *Astyanax* y *Steindachnerina*.

### Site 4. Popayán River, Parque Nacional El Rey

The main habitat associations were: (1) high-velocity microhabitats, (2) deep pools, and (3) fast-flowing riffles. Riverbed composition varied among habitats and included fine sediments, sand, and rocks. *Characidium* species were recorded across habitat types, from deep pools to riffles; individuals remained near the bottom and fed on the substrate, moving in short bursts.

**High-velocity microhabitats (Rapids)**.— High-velocity microhabitats occurred at waterfalls and at pool inlets, with fast current and substrates dominated by rocks and coarse sediments. *P. carrikeri* occurred in these microhabitats, swimming against the current and maintaining contact with the substrate using pectoral and pelvic fins. *Characidium* spp. also occurred in these habitats, remaining benthic and moving in short bursts. Schools of *P. carrikeri* fed on algae attached to rocks, whereas *Characidium* spp. fed on the bottom. Juvenile *A. tarijae* formed schools in very fast-flowing surface waters, swimming against the current and feeding on suspended particles.

**Deep pools (trench pools)**.— Deep pools (> 2 m) were common. *Prochilodus lineatus* (Valenciennes, 1837) and *Arhinolemur obtusidens* (Valenciennes, 1837) formed mixed schools, with *P. lineatus* notably more abundant. Both species scraped rocks, leaving visible mouth marks. *S. brasiliensis* was also present in deep pools, sometimes schooling and engaging in group hunting. Predation on *P. lineatus* and *M. obtusidens* was recorded, and one event involving *S. brasiliensis* attacking an *A. abramis* was documented. *A. abramis* formed large schools at midwater, maintaining rheotactic orientation and feeding on suspended particles. In one pool, a submerged tree was present and supported a large *A. abramis* school; *S. brasiliensis* individuals were also present. Juveniles of *P. lineatus*, *M. obtusidens*, and *S. brasiliensis* were not recorded in the sampled pools.

**Fast-flowing riffles (rapids)**.— Riffles had high water velocity and rocky substrates. *Leporellus pictus* (Kner, 1858) was recorded only on video, with four individuals swimming against the current alongside large *A. abramis* schools (Fig. 5). *Parodon carrikeri* was frequently recorded in riffles, exhibiting behavior similar to that observed in high-velocity microhabitats (Fig. 6): individuals swam against the current, adhered to the substrate, and grazed algae on rocks. Video documentation of this behavior is available at: https://youtu.be/st23RtMgCrM?si=QKW3GI-PerIqgt2m. *C. borellii* was also recorded.

**Figure 5.** *Leporellus pictus* video capture, observed in a very fast flowing riffle.

**Figura 5.** Captura de video de *Leporellus pictus*, observado en un rápido de velocidad de corriente alta.

**Figure 6.** A group of *Parodon carrikeri* swimming against the current, attached to the substrate and feeding on algae growing on a rock at the entrance of a pool. This area was characterized by very high flow velocity immediately downstream of a small cascade.

**Figura 6.** Grupo de *Parodon carrikeri* nadando contra la corriente, adheridos al sustrato y alimentándose de algas que crecen sobre una roca en la entrada de una poza. Esta área se caracterizaba por una velocidad de flujo muy alta inmediatamente aguas abajo de una pequeña cascada.

### Site 5. Geiser Mollinedo

(See https://youtu.be/vlwmfnLfWbw?si=tq3zVgO6DitNjdg)
Two habitat units were recognized: an impounded pool and plunge pools. The impounded pool occurred near the geyser, where crystal-clear water emerges from a thermal source as a 4–5 m water column; the substrate consisted of fine sediments. Water was impounded by logs and debris jams, forming a large pool (~20 m diameter), in which fish were associated primarily with vegetated margins. Downstream, four to five log/debris impoundments formed plunge pools (~1 m deep) characterized by high-velocity inflows, turbulence, and substrates composed of debris and gravel.

During disturbance caused by the observer, large mixed-species schools composed of *A. abramis*, *P. carrikeri*, and *Characidium* spp. formed. These schools showed consistent vertical structure: *A. abramis* led at the front and occupied higher positions in the water column, whereas *P. carrikeri* and *Characidium* spp. occupied lower positions. At times, *A. abramis* separated to form monospecific schools. On the bottom, *C. borellii* were conspicuous and maintained benthic positions (Fig. 7).

**Figure 7.** Video capture of a heterospecific school formed by *Astyanax abramis*, *Parodon carrikeri* and *Characidium* spp. where a clear stratification of the shoal can be seen. See https://youtu.be/3pZK6icVeRQ?si=Rqvu9as62cQpZ4x7

**Figura 7.** Captura de video de un cardumen heteroespecífico formado por *Astyanax abramis*, *Parodon carrikeri* y *Characidium* spp., donde se observa una clara estratificación del cardumen. Ver https://youtu.be/3pZK6icVeRQ?si=Rqvu9as62cQpZ4x7

**1. Vegetated margins**.— Along vegetated margins, schools of *A. abramis* frequently swam among aquatic plants.

**2. Rock-associated high-velocity microhabitats (Cascades)**.— *Parodon carrikeri* and *Characidium* spp. were closely associated with rocks beneath cascades and typically maintained rheotactic positions; *P. carrikeri* grazed algae on rock surfaces.

## Site 6. Laguna La Quinta

This habitat is characterized by elevated temperatures and high concentrations of dissolved sulfur and salts (Aguilera et al., 2019). Temperature decreases progressively from the upwelling source downstream, generating a gradient that permits fish occurrence. The only fish species recorded was *J. sulfurica*, a microendemic species restricted to this locality.

The main habitat units were: (1) glides, (2) shallow marginal areas, (3) deeper downstream zones, and (4) entrance channels.

**1. Shallow Marginal Areas (Glide)**.— The smallest juveniles occurred primarily in marginal areas with 2–3 cm water depth, particularly near entrance channels leading to larger habitat sections. These areas were slightly warmer due to proximity to springs.

**2. Deeper Downstream Zones (Backwater pools)**.— Larger *J. sulfurica* formed schools in cooler, stagnant water in shallow pools (~15 cm depth). Adults were also recorded at low density in draining arms with slow current.

**3. Entrance Channels**.— Smaller individuals formed schools in shallow areas near entrance channels connecting to larger habitat sections.

## Site 7. Aguas Calientes

The main habitat units were: (1) underwater caves and (2) open riverbed territories.

**1. Underwater caves**.— *Hypostomus boulengeri* (Eigenmann & Kennedy, 1903) occupied underwater caves excavated along earthen banks (See Video).

**2. Open riverbed territories**.— Numerous pairs of *Bujurquina vittata* (Heckel, 1840) were recorded, each accompanied by offspring. Pairs were spaced relatively evenly (~1 m apart) and showed aggressive displays toward neighboring pairs. Offspring remained close to the riverbed and followed parents closely. When perceiving threats, parents were recorded taking offspring into their mouths (See Video).

### Site 8. Calchaquí River

The main habitat units were: (1) riffles, (2) deep pools, and (3) vegetated secondary arms.

**1. Riffles**.— Riffles had high current velocity and coarse substrates, supporting adult *T. spegazzinii* (> 6 cm SL) and *R. steinbachi* (> 7 cm SL).

**2. Deep Pools**.— Deep pools had intermediate current velocity and finer substrates. A diverse adult assemblage occurred in these habitats, including *N. indefessus*, *Psalidodon* aff. *eigenmanniorum* (Cope, 1894), *Bryconamericus rubropictus* (Berg, 1901), *J. maculata*, *T. spegazzinii*, and *R. steinbachi* (Fig. 8).

**3. Vegetated Secondary Arms**.— Secondary arms were shallow, with low current velocity, fine sediments, and aquatic vegetation. These habitats included juveniles and adults of *J. maculata* and *N. indefessus*, sometimes forming mixed schools. *T. spegazzinii* occurred on the bottom, whereas *U. gladysae* and *Heptapterus mustelinus* (Valenciennes, 1835) were strongly associated with aquatic vegetation along margins.

**Figure 8.** Video capture of *Bryconamericus rubropictus* in its natural environment in the Calchaquí River, Payogasta, Northwestern Argentina.

**Figura 8.** Captura de video de *Bryconamericus rubropictus* en su ambiente natural en el río Calchaquí, Payogasta, noroeste de Argentina.

**Table 3.** Total number of species photographed at each site.

**Tabla 3.** Número total de especies fotografiadas en cada sitio.

| Study Site | Number of Species pictured |
|---|---|
| La Caldera River and Lesser River | 10 |
| Unnamed Stream (National Route 50) | 7 |
| Bermejo River | 6 |
| Popayán River (El Rey National Park) | 8 |
| Geiser Mollinedo | 5 |
| Laguna La Quinta | 1 |
| Aguas Calientes | 2 |
| Calchaquí River | 7 |
| **Total Photographed Species** | **46** |

## Coloration of species in relation to habitat use and schooling behavior

Various fish species were photographed across study sites (Tables 4–5; Figs. 9–13). Bottom-associated species such as *T. spegazzinii*, *R. steinbachi*, and *Urkumayu* spp. showed brown body coloration speckled with dark brown spots, particularly conspicuous in dorsal view. In contrast, midwater schooling species, including *A. tarijae*, *N. indefessus*, *Psalidodon* spp., and *A. latens*, shared broadly similar coloration: a silver to yellowish body, a prominent humeral spot, a dark gray mark at the base of the caudal peduncle, a brownish dorsal region, and reddish-yellow coloration on the anterior portion of the eye. Although *O. bolivianus* is not typically a schooling species, it was occasionally recorded associating with these schools.

Species inhabiting low-velocity bottoms of pools and secondary arms, such as *Jenynsia* spp., showed a brownish-gray body with small gray blotches. Other species, including *S. brevipinna* and *P. carrikeri*, exhibited a pale body with a distinct longitudinal stripe. These species were most often recorded in monospecific groups but occasionally integrated into mixed-species schools, typically occupying lower positions in the water column. While *S. brevipinna* facilitated commensal feeding through substrate disturbance, *P. carrikeri* was strongly associated with high-velocity microhabitats, maintaining rheotactic positions and often co-occurring with *Characidium* spp.

**Table 4.** General coloration patterns of observed species.

**Tabla 4.** Patrones generales de coloración de las especies observadas.

| Species | Coloration Pattern |
|---|---|
| *Trichomycterus spegazzinii*<br>*Rineloricaria steinbachi*<br>*Hypostomus* spp.<br>*Urkumayu* spp. | Brown body with dark brown spots, cryptic when viewed from above. |
| *Acrobrycon tarijae*<br>*Nantis indefessus*<br>*Psalidodon* spp.<br>*Astyanax* spp.<br>*Bryconamericus rubropictus*<br>*Piabina thomasi*<br>*Andromakhe latens*<br>*Oligosarcus bolivianus* | Silver-yellowish body, prominent humeral spot, dark caudal peduncle spot, brown dorsal region, reddish-yellow anterior eye portion. |
| *Jenynsia* spp. | Brownish-gray body with small gray blotches, inhabits bottoms of pools. |
| *Steindachnerina brevipinna* | Pale body with distinct longitudinal stripe, primarily in monospecific schools. |
| *Parodon carrikeri* | Pale body with distinct longitudinal stripe, grazes on algae. |
| *Characidium* spp. | Brownish-gray body with distinct longitudinal stripe and transverse bars, forming mixed shoals in lower water levels. |
| *Moenkhausia bonita* | Red caudal fin with black central portion, white-bordered black fin lobes, restricted to vegetated marginal zones. |

**Table 5.** Species associations to habitat type.

**Tabla 5.** Asociaciones de especies con el tipo de hábitat.

| Location | Habitat Type | Species in Mixed Schools |
|---|---|---|
| Unnamed stream along National Route 50 | Channel Without Aquatic Plants | *Psalidodon chico, Psalidodon endy, Andromakhe latens, Piabina thomasi, Oligosarcus bolivianus* |
| Bermejo River | Big Pool | *Astyanax abramis, Oligosarcus bolivianus, Steindachnerina brevipinna* |
| | Riverbank Shallow Areas | *Piabina thomasi, Odontostilbe microcephala, Holoshesthes pequira* |
| Popayán River, Parque Nacional El Rey | High-velocity Microhabitats | *Parodon carrikeri, Characidium* spp. |
| | Fast-flowing Riffles | *Leporellus pictus, Astyanax* spp. |
| Geiser Mollinedo | Mixed-species Shoals | *Astyanax abramis, Parodon carrikeri, Characidium* spp. |

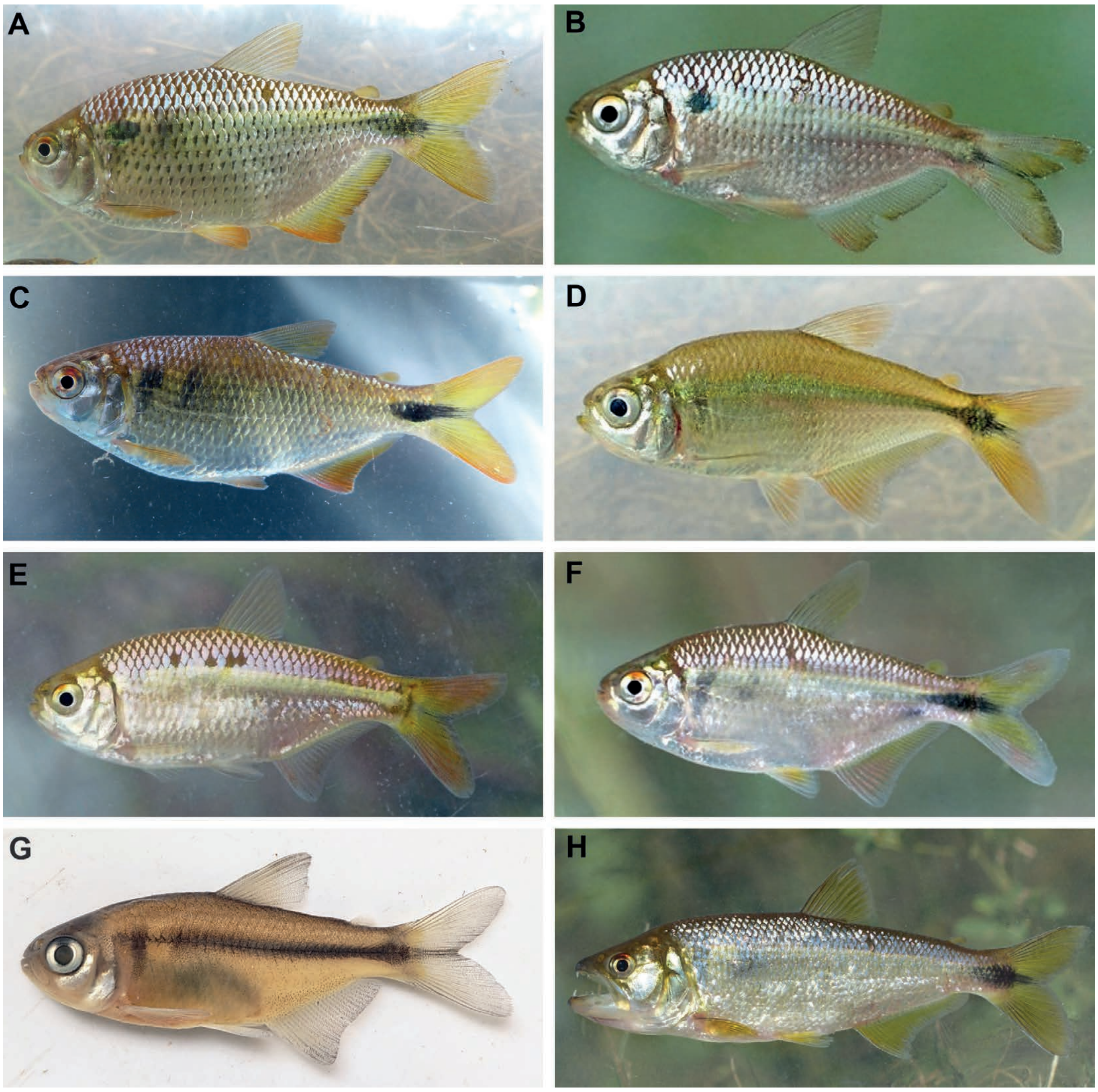

**Figure 9.** Species recorded: A) *Astyanax lacustris*, B) *Astyanax abramis*, C) *Astyanax lineatus*, D) *Andromakhe latens*, E) *Psalidodon endy*, F) *Psalidodon chico*, G) *Psalidodon* aff. *eigenmanniorum*, H) *Oligosarcus bolivianus*.

**Figura 9.** Especies registradas: A) *Astyanax lacustris*, B) *Astyanax abramis*, C) *Astyanax lineatus*, D) *Andromakhe latens*, E) *Psalidodon endy*, F) *Psalidodon chico*, G) *Psalidodon aff. eigenmanniorum*, H) *Oligosarcus bolivianus*.

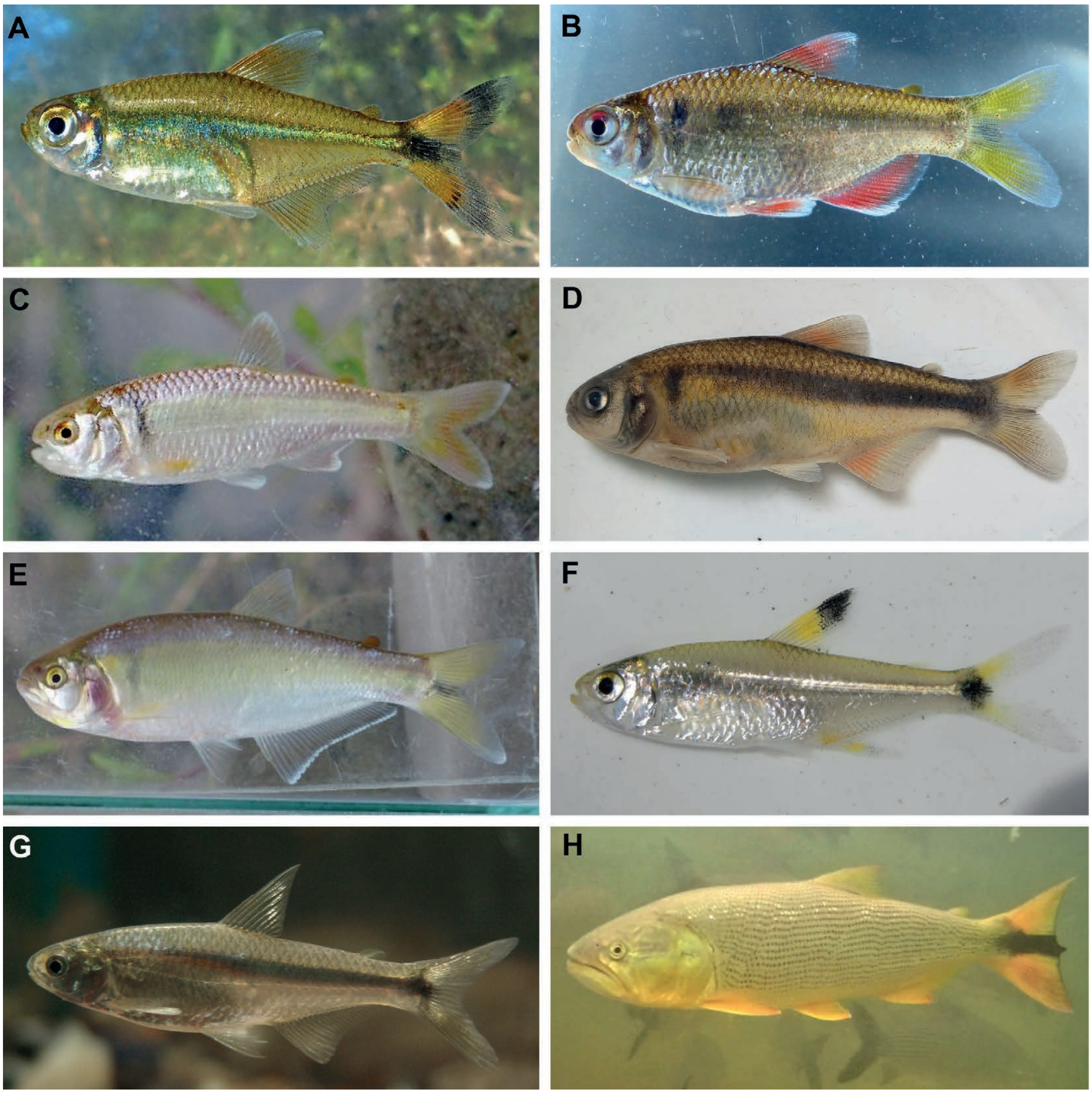

**Figure 10.** Species recorded: A) *Moenkhausia bonita*, B) *Piabina thomasi*, C) *Nantis indefessus*, D) *Bryconamericus rubropictus*, E) *Acrobrycon tarijae*, F) *Holoshesthes pequira*, G) *Odontostilbe microcephala*, H) *Salminus brasiliensis*.

**Figura 10.** Especies registradas: A) *Moenkhausia bonita*, B) *Piabina thomasi*, C) *Nantis indefessus*, D) *Bryconamericus rubropictus*, E) *Acrobrycon tarijae*, F) *Holoshesthes pequira*, G) *Odontostilbe microcephala*, H) *Salminus brasiliensis*.

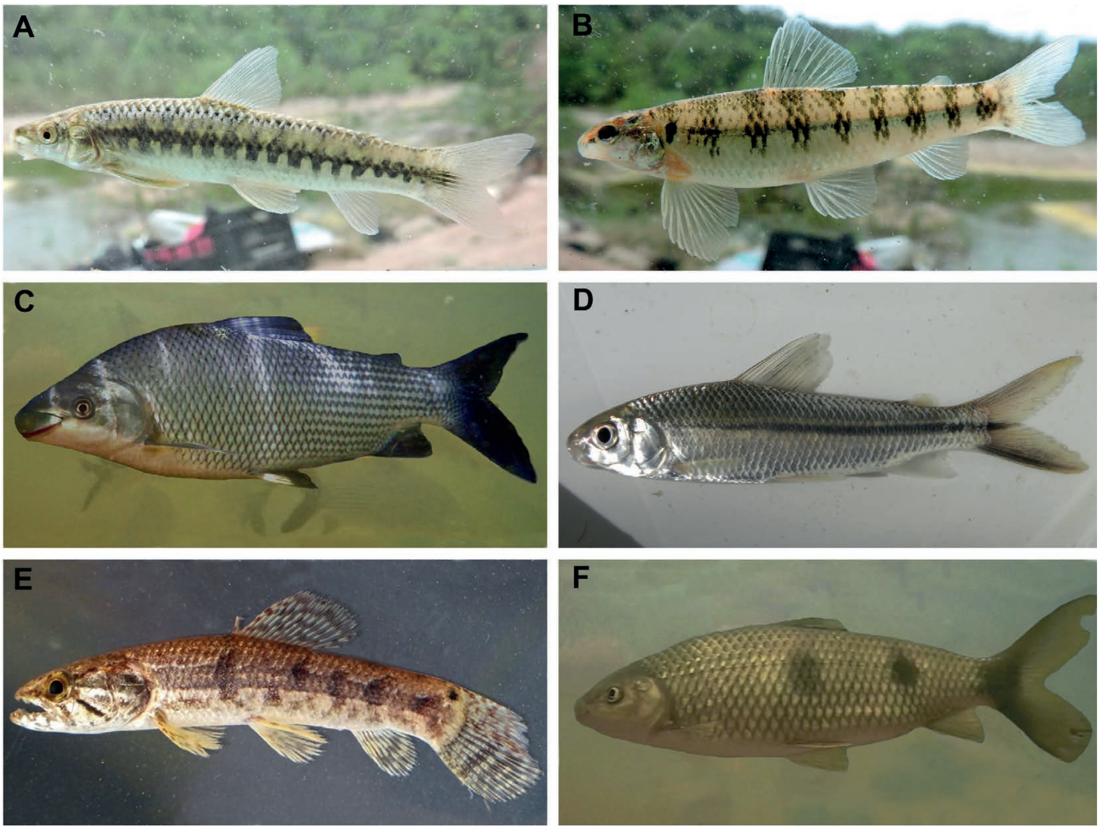

**Figure 11.** Species recorded: A) *Parodon carrikeri*, B) *Characidium borellii*, C) *Prochilodus lineatus*, D) *Steindachnerina brevipinna*, E) *Hoplias argentinensis*, F) *Arhinolemur obtusidens*.

**Figura 11.** Especies registradas: A) *Parodon carrikeri*, B) *Characidium borellii*, C) *Prochilodus lineatus*, D) *Steindachnerina brevipinna*, E) *Hoplias argentinensis*, F) *Arhinolemur obtusidens*.

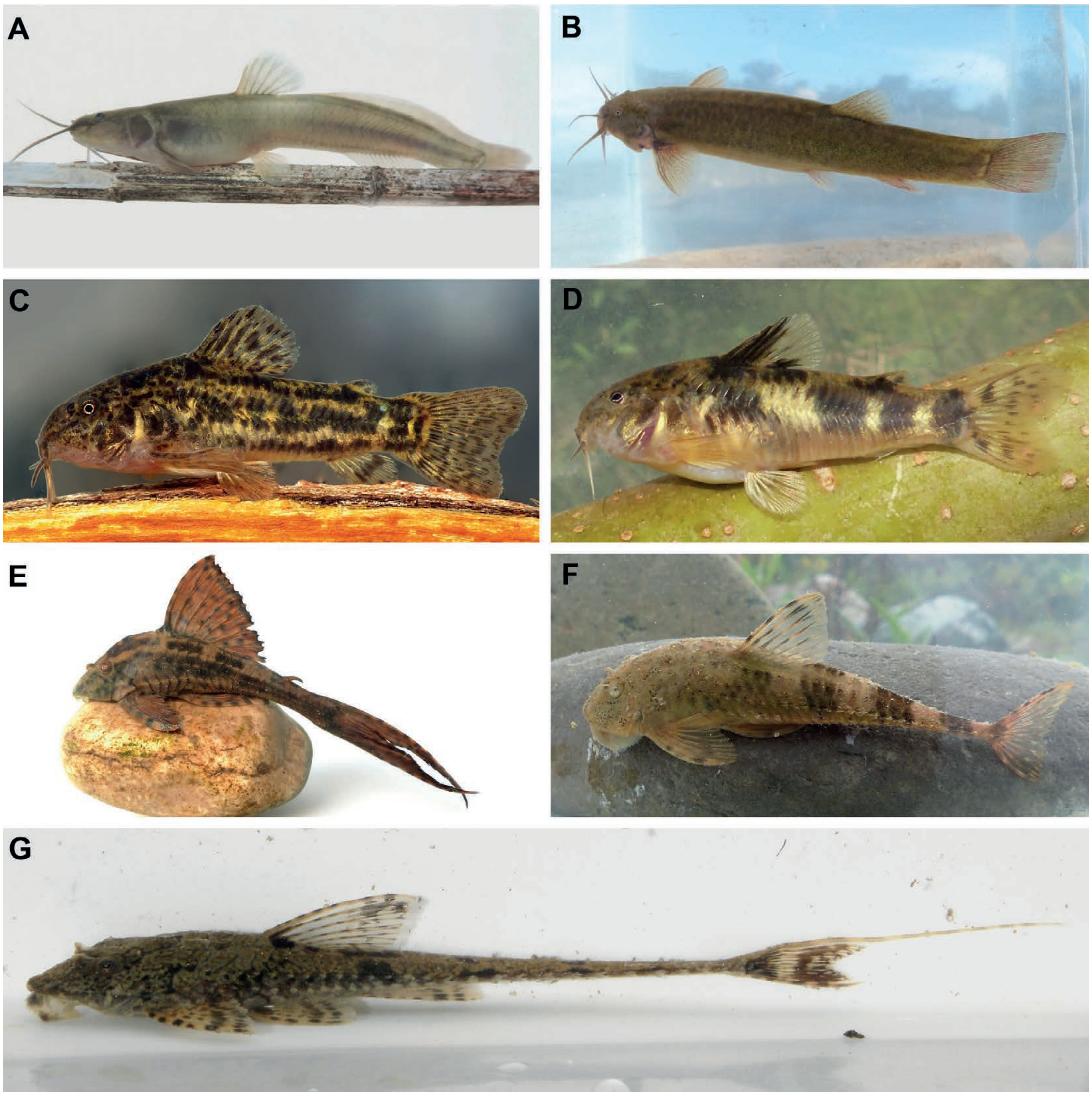

**Figure 12.** Species recorded: A) *Heptapterus mustelinus*; B) *Trichomycterus spegazzinii*, C) *Urkumayu gladysae*, D) *Urkumayu micracanthus*, E) *Hypostomus cochliodon*, F) *Rineloricaria steinbachi*, G) *Rineloricaria catamarcensis*.

**Figura 12.** Especies registradas: A) *Heptapterus mustelinus*, B) *Trichomycterus spegazzinii*, C) *Urkumayu gladysae*, D) *Urkumayu micracanthus*, E) *Hypostomus cochliodon*, F) *Rineloricaria steinbachi*, G) *Rineloricaria catamarcensis*.

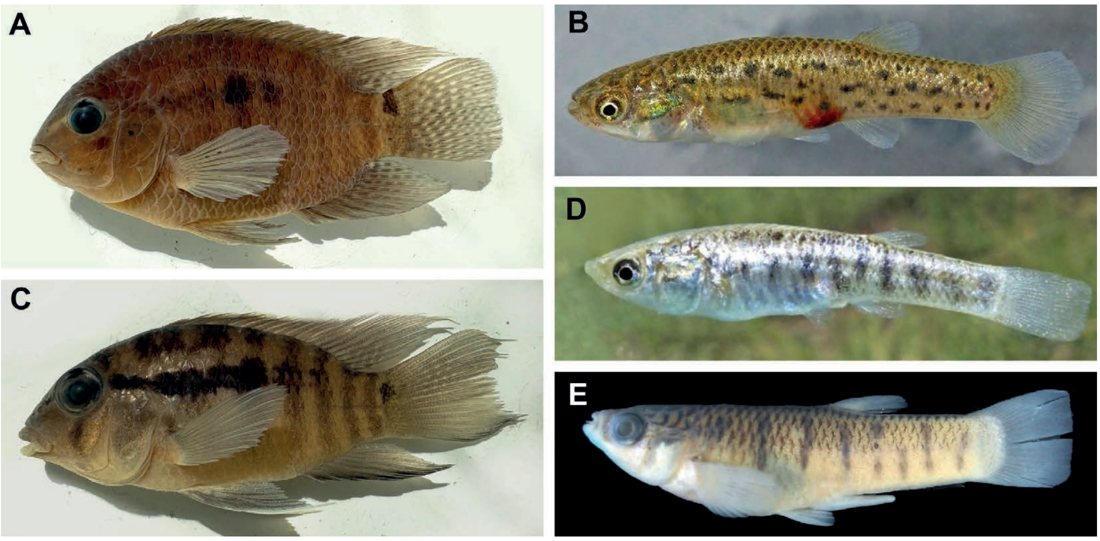

**Figure 13.** Species recorded: A) *Cichlasoma dimerus*, B) *Jenynsia maculata*, C) *Bujurquina vittata*, D) *Jenynsia sulfurica*, E) *Jenynsia alternimaculata*.

**Figura 13.** Especies registradas: A) *Cichlasoma dimerus*, B) *Jenynsia maculata*, C) *Bujurquina vittata*, D) *Jenynsia sulfurica*, E) *Jenynsia alternimaculata*.

## DISCUSSION

### Habitat heterogeneity and fish assemblage structure

Understanding how habitat heterogeneity structures fish assemblages is essential for interpreting ecological interactions and life-history strategies in Neotropical river systems. In the northwestern La Plata Basin, habitat diversity within river networks sustains a wide range of fish species because microhabitats differ in hydraulic conditions, trophic resources, and refuge availability. Here we identified several habitat types—riffles, deep pools, secondary arms, and vegetated zones—each supporting distinct species associations and ecological traits. Such structural diversity likely contributes to maintaining biodiversity by providing habitat templates for feeding, refuge, and potentially reproduction (Sabino et al., 2016; Ceneviva-Bastos & Casatti, 2014). Documenting habitat-associated assemblages therefore provides baseline ecological information relevant to management and conservation strategies in northwestern La Plata Basin rivers.

Similar relationships between habitat heterogeneity and assemblage structure have been documented in other Neotropical river systems, where hydraulic gradients, substrate composition, and vegetation structure influence species distributions and ecological interactions (e.g., Sabino & Castro, 1990; Winemiller et al., 2008; Casatti et al., 2012). In these environments, benthic specialists typically dominate fast-flowing riffles, whereas schooling characiforms often occupy pools and moderate-flow habitats where drifting resources are more accessible. The patterns documented here are consistent with these broader processes shaping stream assemblages in tropical and subtropical South America.

Riffles are dynamic habitats with strong currents and rocky substrates, where intense gas exchange occurs (Hynes, 1970; Allan, 1995). These conditions promote high food availability (e.g., algae and benthic macroinvertebrates) and provide shelter among substrate elements (Casatti & Castro, 2006). Riffles in our study were dominated by benthic species such as *T. spegazzinii* and *R. steinbachi*, whose morphology likely facilitates station-holding and exploitation of interstitial spaces. In contrast, midwater schooling species, including *A. tarijae* and *Psalidodon* spp., occurred primarily in moderate-flow habitats, where schooling may enhance predator avoidance while allowing exploitation of drifting resources. These patterns suggest that hydraulic gradients and substrate heterogeneity act as environmental filters that structure assemblages through species-specific functional traits.

Fishes may further exploit hydrodynamic heterogeneity through behavioral strategies that reduce locomotion costs and enable efficient use of hydraulic microhabitats. Behaviors such as rolling, surfing updrafts, substrate walking, drafting, hitchhiking, and the use of flow refuges can modify the relationship between swimming speed and energetic expenditure. These interactions among morphology, locomotor behavior, and local hydrodynamics highlight the importance of fine-scale hydraulic complexity for habitat selection and performance, and they underscore the need to maintain natural hydrodynamic conditions in restoration and conservation planning (Di Santo& Goerig, 2025).

Behavioral interactions also contribute to assemblage dynamics. Potential aggressive mimicry by *O. bolivianus* suggests that predators may exploit resemblance to smaller characiforms to approach prey within mixed-species schools, illustrating behavioral plasticity in structurally complex environments and emphasizing the role of species interactions in community structure.

Slow-flowing vegetated habitats appear to play a particularly important ecological role. These zones function as refuge for juveniles and likely operate as nursery habitats that support recruitment and population persistence. However, anthropogenic pressures are increasingly evident in the region. River channelization is widely used to reduce flood risk for nearby human populations, but it often simplifies channel structure and removes habitats such as vegetated secondary arms and deep pools (Agostinho, Gomes, Santos, Ortega, Pelicice, 2016; Winemiller et al., 2008). Loss of these habitats may disrupt recruitment, trophic interactions, and spatial connectivity within fish assemblages.

Preserving hydromorphological complexity is therefore essential for maintaining biodiversity and ecosystem function in northwestern La Plata Basin rivers. Conservation strategies should prioritize protection of habitat heterogeneity, restoration of degraded reaches, and maintenance of connectivity (Malmqvist & Rundle, 2002). Maintaining habitat diversity across elevational and hydromorphological gradients will likely be critical for conserving both ecological integrity and evolutionary potential of these assemblages.

## Morphofunctional adaptations and microhabitat partitioning

*Rineloricaria steinbachi* occurred predominantly in high-velocity riffles with coarse substrates, habitats also used by *T. spegazzinii*, although the latter also occurred at lower abundance in pools and secondary arms. Both species frequently positioned themselves downstream of large stones where current velocity is reduced. Their dorsoventrally depressed bodies likely reduce lift forces and facilitate station-holding in fast-flowing waters. By exploiting hydrodynamic refuges created by substrate complexity, these species may minimize energetic expenditure while maintaining access to benthic resources.

*Characidium* spp. and *P. carrikeri* occurred near the bottom in microhabitats with moderate to fast currents, particularly the latter, consistent with observations for *Characidium* reported by Sabino & Castro (1990). These fishes possess fusiform bodies with flattened ventral surfaces and use large, ventrally positioned pectoral and pelvic fins to maintain contact with the substrate. Similar morphofunctional adaptations have been documented for Corydoradinae species from the same region (Alonso et al., 2025).

Secondary arms concentrated juveniles of most species and likely function as nursery habitats; adults of *Urkumayu* spp., *Jenynsia* spp., and *N. indefessus* also occurred in these habitats, together with *Psalidodon* spp. and *O. bolivianus*, typically in deeper portions with submerged vegetation. Vegetation likely provides refuge that reduces predation risk and may limit competition with adults. Similar nursery functions have been documented in other Neotropical freshwater systems.

Partitioning was evident across the assemblage. *Urkumayu* spp. and *O. bolivianus* occurred in low-current pools of secondary arms in the Lesser River, with *O. bolivianus* tending to occupy deeper habitats and *U. micracanthus* occurring primarily within dense vegetation. In general, smaller individuals and species occurred in shallower vegetated habitats, whereas larger individuals were more frequent in deeper areas. The presence of fingerlings in shallow secondary arms supports a nursery role for these habitats, consistent with patterns reported for other Characiformes (Ceneviva-Bastos & Casatti, 2010).

Characiform species were most common in low to moderate current velocity habitats and were rarely recorded in riffles, where Trichomycteridae and Loricariidae were more abundant. Interestingly, relatively low-bodied species such as *N. indefessus*, *P. thomasi*, and *A. tarijae* occurred more frequently in faster and more turbulent habitats, whereas species such as *P. endy* and *P. chico* were more common in moderate-flow pools. Similar morphology–habitat associations have been documented in other fish groups, including cichlids (Feilich, 2016).

## Potential reproductive migration in *Acrobrycon tarijae*

Juveniles of *A. tarijae* were not recorded in higher portions of the Bermejo Basin but occurred in lower reaches (e.g., Parque Nacional El Rey and the Bermejo River). Adults were consistently recorded in upper reaches (~1400 m a.s.l.), whereas juveniles occurred between ~300 and 600 m a.s.l. This size-based altitudinal segregation suggests that *A. tarijae* may migrate along elevational gradients, with juveniles developing downstream before returning upstream for reproduction.

This pattern is consistent with longitudinal size structure in stream fishes sometimes referred to as the "larger fish upstream pattern" (LFUP) (Wolf, Marques, & Mazzoni, 2025). LFUP has been documented in multiple stream systems and may be influenced by distance upstream, food availability, and reproductive strategy, with pelagic or lithopelagic spawners often showing stronger associations with this pattern (Wolf et al., 2025).

Internal insemination reported for *Acrobrycon ipanquianus* (formerly treated as a senior synonym of *A. tarijae*; Weitzman et al., 2005; Briñoccoli et al., 2022) may facilitate reproduction in fast-flowing piedmont and mountain rivers, where fertilized eggs can be transported downstream. The exclusive capture of adult *A. tarijae* in pools of the La Caldera River further suggests that pools may function as aggregation sites during reproduction. Similar elevational migration patterns have been reported for other Andean characiforms, where elevational gradients may act as ecological corridors linking spawning habitats to juvenile development areas (Pouilly et al., 2006).

## Animal coloration and schooling behavior

Animal coloration has multiple ecological and evolutionary roles in fishes, including camouflage, communication, predator avoidance, and intraspecific recognition (Bates, 1863; Darwin, 1888; Cott, 1940; Kemp, 2015). In freshwater fishes, coloration can be dynamic and influenced by environmental conditions such as light availability, turbidity, and water depth (Akkaynak et al., 2011; Nilsson Sköld et al., 2013; Briñoccoli et al., 2018).

Males of several characiform species showed more intense coloration during the reproductive season than females. Many species displayed reddish coloration in the anal fin that appears linked to reproductive interactions. Because red wavelengths are rapidly absorbed in water, such coloration may function primarily as a short-range signal among conspecifics while remaining less detectable to predators at longer distances or greater depths.

Red anal-fin coloration was frequently associated with the presence of hooks on fin rays, particularly on the anterior portion of the anal fin as observed in *A. tarijae*. Histological studies in species such as *Knodus tanaothoros* and *Hyphessobrycon diancistrus* suggest that club cells in fin tissues may release pheromones during reproductive interactions (Weitzman et al., 2005). Similar structures have been reported in other Ostariophysi, including *Holopristis yinyang* (Lima & Sousa, 2009). Hooks on anal and pelvic fins may therefore represent a broader trait associated with reproductive interactions.

Coloration patterns such as longitudinal bands or asymmetric markings may facilitate recognition and coordinated movement during schooling. Markings near the caudal peduncle may provide directional cues that enhance group cohesion and movement efficiency. McRobert & Bradner (1998), for example, proposed that schooling fishes rely on visual coloration cues for conspecific recognition.

Similarity in coloration among species forming mixed-species schools may facilitate interspecific cohesion and enhance predator avoidance (McRobert & Bradner, 1998; Endler, 1993). These observations suggest that coloration patterns in these assemblages may reflect eco-evolutionary outcomes shaped by predation, reproductive signaling, and social interactions in multispecies communities.

*Moenkhausia bonita* did not participate in mixed-species schools and instead occurred in vegetated marginal habitats. This distribution may be related to its distinctive coloration, including a red caudal fin with a black central portion and black lobes bordered by white margins. Such conspicuous coloration may function in territoriality or mate attraction (Kemp et al., 2015; Endler, 1983) and/or reflect ecological specialization within vegetated habitats.

## Predatory strategies and trophic interactions

Potential aggressive mimicry by *O. bolivianus* was recorded when individuals associated with schools of *Astyanax* and *Psalidodon* in the upper Bermejo Basin. Resemblance in coloration may allow *O. bolivianus* to approach prey without detection, facilitating predation within these schools (Fig. 3). Comparable cases of aggressive mimicry have been documented in fishes in which predators exploit visual resemblance to increase hunting success (Sazima, 1977, 2002).

*Salminus brasiliensis* showed contrasting hunting strategies in the Popayán and Bermejo rivers (Fig. 4). In the clear waters of the Popayán River, individuals were recorded hunting cooperatively, coordinating attacks laterally. In the turbid waters of the Bermejo River, a solitary individual targeted mixed-species schools using upward ambush attacks. Reduced visibility in turbid water may favor ambush tactics by limiting prey detection of predators.

These observations align with previous work indicating that environmental conditions, particularly water clarity, influence predator–prey interactions and hunting strategies in aquatic ecosystems (Abrahams & Kattenfeld, 1997). Cooperative hunting may be favored in clear environments, whereas ambush predation may be more effective under low visibility. Together, these observations illustrate behavioral plasticity in *S. brasiliensis* and highlight the influence of local conditions on trophic interactions.

Feeding by *S. brevipinna* disturbed the substrate and exposed benthic particles subsequently consumed by follower species such as *Astyanax* and *Psalidodon*. Similar commensal feeding associations have been reported in fish assemblages from Mato Grosso, Brazil (Sabino et al., 2017).

## Methodological considerations

Visual sampling proved to be a valuable complement to capture-based methods. For example, although *S. brevipinna* and *Astyanax lacustris* were not captured at Site 3, both were recorded on video. Conversely, *C. dimerus* was captured with fishing gear but not detected during visual observations.

Visual surveys in Parque Nacional El Rey recorded *L. pictus* at the entrance of the pool "El Espumudo," a high-flow habitat ~1 m deep in which fish swam against the current near the bottom; this species was not captured by fishing methods. Conversely, fast-flow specialists such as *R. steinbachi* and *H. mustelinus* were not detected in visual surveys.

These results underscore the complementarity of multiple sampling approaches. Visual observations provide information on microhabitat use, orientation relative to current, vertical position in the water column, and schooling behavior that may not be obtainable from capture-based methods alone.

## Future perspectives

Our observations open avenues for investigating the ecological and evolutionary significance of coloration in freshwater fishes. Future studies combining pigment distribution, reflectance properties, and signal visibility with behavioral assays may clarify roles of coloration in dominance, territoriality, reproductive signaling, and mixed-species schooling (Kemp et al., 2015; Seehausen et al., 2008). Investigating intraspecific variation driven by environmental conditions or social context may further reveal plasticity and evolutionary responses to ecological pressures (McRobert & Bradner, 1998; Feilich, 2016). Integrating behavioral, ecological, and phylogenetic approaches will help clarify drivers of coloration patterns in these assemblages, with implications for conservation and management of freshwater biodiversity in the northwestern La Plata Basin.

### Integrative synthesis

Taken together, these results highlight how habitat structure, functional traits, and behavioral interactions jointly shape fish assemblages in Neotropical river systems. By documenting how species interact with hydraulic conditions, vegetation structure, and trophic dynamics across multiple habitats, this study contributes to evidence that habitat heterogeneity is a key driver of ecological organization and biodiversity in freshwater ecosystems of the La Plata Basin. These natural history observations also provide a foundation for interpreting ecological, anatomical, and evolutionary patterns in Neotropical fishes.

## ACKNOWLEDGMENTS

We extend our sincere gratitude to the Secretaría de Ambiente y Desarrollo Sustentable of Salta, Ministerio de Ambiente of Jujuy and Parques Nacionales de Argentina for granting the necessary permits to conduct fieldwork. We are deeply grateful to IBIGEO and Fundación Miguel Lillo for their continued institutional support throughout this study. Our heartfelt thanks go to the Killifish Foundation members and to Baltazar Bugeau, Leandro Antoniazzi, Virginia Martínez, Roberto Sanchez, Pedro Sanchez, Fernando Lobo and Julio Cruz for their unwavering assistance and encouragement.

This research was partially funded by the National Scientific and Technical Research Council of Argentina (CONICET) through PIBAA 2872021010 0128CO and by a grant from Fundación Williams, titled "Fondos complementarios para la investigación con impacto en el territorio argentino: Peces de la cuenca del Río Bermejo: biodiversidad, distribución espaciotemporal, efectos de las actividades antrópicas y conservación," awarded to F. Alonso. Additionally, we acknowledge support from the Agencia Nacional de Promoción de la Investigación, el Desarrollo Tecnológico y la Innovación (ANPCyT) through a grant to J.M. Mirande, and funding from FONCYT 2019-1004 awarded to G. Aguilera.

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## SUPPLEMENTARY MATERIAL

Table S1:
https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2380/2158

Table S2:
https://www.lillo.org.ar/journals/index.php/acta-zoologica-lilloana/article/view/2380/2159