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BRIEF COMMUNICATION

First record of intraspecific cleaning behavior by the threatened Leuciscidae *Squalius alburnoides* (Steindachner, 1866) at the Guadiana River basin (Portugal)

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Abstract

Squalius alburnoides (Steindachner, 1866) is an endemic threatened species from the Iberian Peninsula. Here, we report the first observations of intraspecific cleaning behavior in isolated summer pools in the Guadiana River Basin (Portugal). We found that focal *S. alburnoides* solicited cleaning by adopting an immobile tail-stand position known as "posing," which immediately signaled a response to a few conspecifics that approached and inspect them. Our study expands the list of cleanerfish species in freshwaters, giving emphasis to the importance of mutual positive behavior within an endangered species, particularly when facing seasonal disturbance.

KEYWORDS
cleaning behavior, endangered species, freshwater ecosystem, *Squalius alburnoides*

Teleost fish live in complex socio-ecological environments, exhibiting multiple behavioral interaction types that are crucial to understand community dynamics (Villegas-Ríos et al., 2022). Compared to marine environments, behavioral interactions (in situ and in controlled conditions) in freshwater systems are significantly understudied (Holomuzki et al., 2010), particularly those considered as "positive" (see Stachowicz, 2001). These positive interactions are described as encounters or associations between organisms that result in benefits (not necessarily equal for all involved) and share little to no costs (Stachowicz, 2001). These direct benefits include exchange of resources, provision of services, and amelioration of stressors, all of which provide a potential net increase in fitness. Cleaning interactions are defined as the removal of ectoparasites, bacteria, diseased and injured tissue, and unwanted food particles from cooperative host organisms known as clients by organisms known as cleaners (Feder, 1966). This is mutually beneficial because it provides an easy meal for the cleaner and a service for the one being cleaned. However, this definition is incomplete because it excludes the direct importance of being touched as means to decrease stress (Soares et al., 2011) and it also disregards the importance of communication as the engaging catalyst for the maintenance of these interactions (Vaughan et al., 2017).

More than 250 fish species are reported as cleaners in all marine realms, mostly from tropical and subtropical habitats (Vaughan et al., 2017; Quimbayo et al., 2021). However, reports of cleaning activities

in freshwater environments are relatively uncommon, thus far including cleaners of a few families such as Callichthyidae (Wickler, 1956), Cyprinidae and Percidae (Abel, 1971), Centrarchidae (Sulak, 1975), Cichlidae (Konings, 1989; Ward & Wyman, 1977), Doradidae (Carvalho et al., 2003), and even Serrasalmidae, the piranhas (Sazima & Machado, 1990; see Table S1). The marked lower frequency in these ecosystems could be the result of fewer observers (such as divers) (or even studies) in fresh water compared to marine environments (Carvalho, 2007).

Cleaning behavior does not solely refer to interspecific interactions as it also occurs between members of the same species (for a review see Vaughan et al., 2017). Here we report, for the first time, the occurrence of intraspecific cleaning behavior among the Iberian minnow, *Squalius alburnoides* (Steindachner, 1866), in one of the most

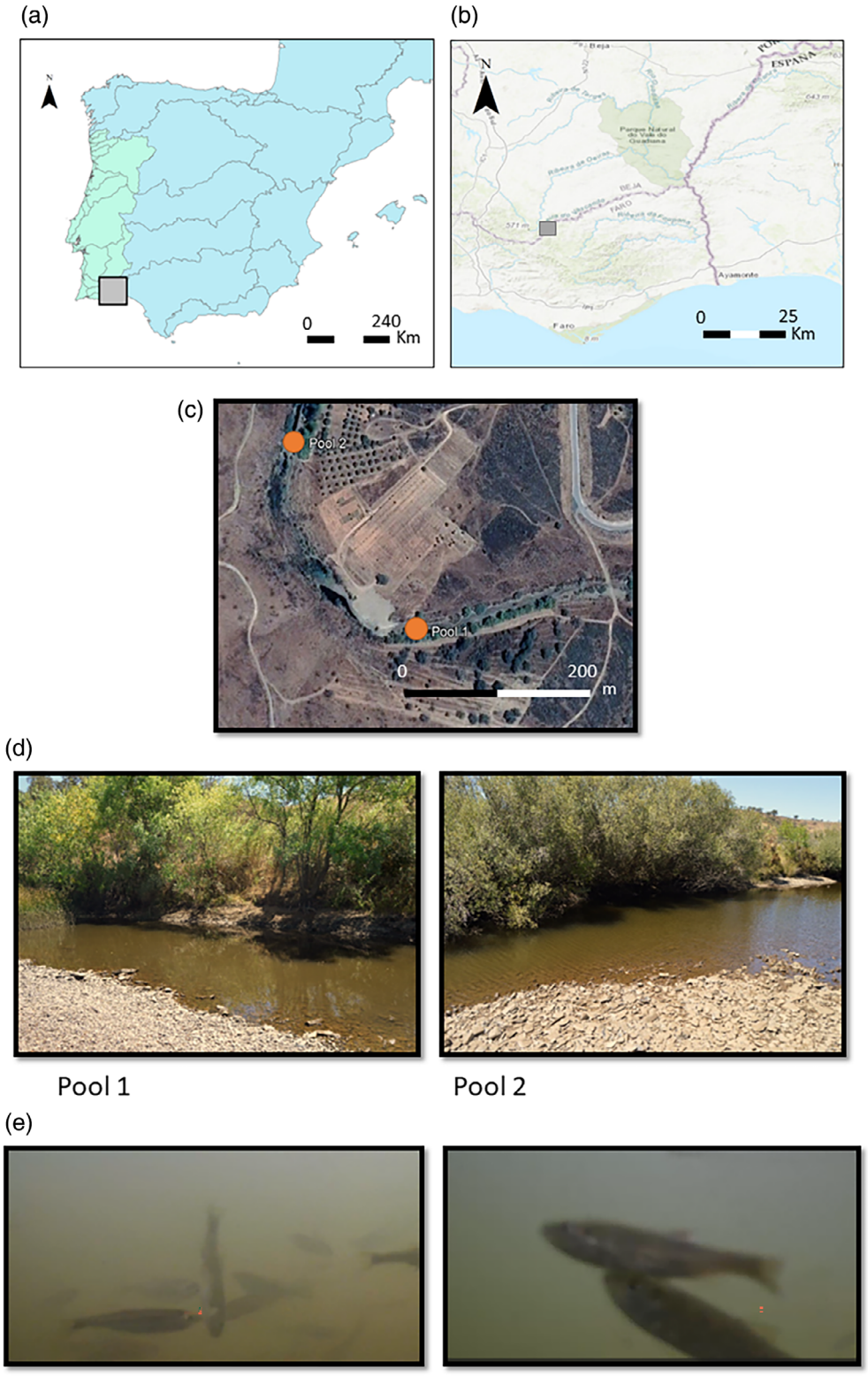

FIGURE 1 (a) Location of the Vascão river in the Iberian Peninsula. The study area in the upper part of the Vascão river is shown in (b) and the locations of the two isolated summer pools studied are shown in (c). (d) Images of each pool (left, Pool 1; right, Pool 2) and (e) images of cleaning events between *S. alburnoides* pairs.

pristine rivers of Portugal, the Vascão river. This is a unique Leuciscidae species regarding its evolution and reproduction, considered Vulnerable (A3ce) by the IUCN (Crivelli, 2006), inhabits the Atlantic drainage rivers of the Iberian Peninsula, and it is also known as the *Squalius alburnoides* complex (Collares-Pereira et al., 2021).

This species complex shows asexual reproduction mechanisms: gynogenesis, hybridogenesis, and meiotic hybridogenesis, in which individuals of other *Squalius* species act as sperm donors, namely *Squalius pyrenaicus* (in our study area, the Guadiana River basin, including the Vascão river) and *S. aradensis* in the southern river basins, and *Squalius carolitertii* in the northern basins (Alves et al., 2001; Sousa-Santos et al., 2006). The Iberian minnow *S. alburnoides* populations are composed by diploid ($2n = 50$), triploid ($3n = 75$) and rare tetraploid ($4n = 100$) males and females (Alves et al., 2001). Both hybrid females and males are fertile but there is also a nonhybrid all-male diploid lineage, which has allowed the preservation of the ancestral paternal genome and plays a significant role in the dynamics of the hybrid southern populations (Alves et al., 2002). Some studies suggest that the sexual tetraploid populations of *S. alburnoides* reduce competition with hybrid diploids and triploids by choosing different habitats (Cunha et al., 2009). However, all this may change when forced to be confined in isolated pools during the summer. All different *S. alburnoides* ploidy levels are generalist, opportunistic feeders, mainly preying on aquatic insects, with some terrestrial insects in summer and autumn (Collares-Pereira et al., 2021). Spatial segregation of feeding niches exists between the three forms and is particularly higher during dry summer periods when prey availability is lower, which may be a strategy for diminishing competition for food (Gomes-Ferreira et al., 2005).

Our study location, the Vascão River, is a Portuguese tributary of the Guadiana River, one of the last "wild rivers" in Europe, due to its biodiversity importance and pristine habitats. During the dry season (June–September), there is virtually no precipitation, flow stops, and the river is reduced to a series of isolated pools that function as refuges for aquatic organisms (Reis & Araujo, 2016). Fish assemblages in this river are highly pristine (Baduy et al., 2020), but invasive species may be present in the lower reaches. We studied two of these isolated summer pools (297 m apart distance) at the higher reaches of the river, where fish assemblages are still mostly composed of native species. The first and most upstream pool (Pool 1) had a 205 m^2 area and a maximum depth of 60 cm. The second pool was 348 m^2 area and approximately 1 m deep (Figure 1). On July 21, 2021, one device was deployed at each site and kept underwater for 7 days. These devices (Cam 1 and Cam 2) were developed to rest at the river bottom, coupled to an underwater camera 40 cm above the bottom of the pool, with a motion detection system. The cameras were able to record day and night, storing information for later analysis. Cam 1 at Pool 1 recorded 39 h, 33 min, and 35 s while Cam 2 at Pool 2 recorded 71 h, 29 min, and 32 s. Two sequences of cleaning interactions were observed between conspecifics of the *S. alburnoides*, one on Pool 1 and the second on Pool 2. On both occasions, the focal individuals solicited to be cleaned by adopting an immobile tail-stand position known as "posing," which immediately signaled a response to a few conspecifics that approached and inspected them. Posing is a species-specific posture that has been shown to increase the likelihood of being cleaned (Côté et al., 1998). The first occurrence (Pool 1) took 28 s, with the posing individual being in the middle of a shoal of *S. alburnoides*. The posing individual was approached and cleaned by two other conspecifics of similar size (~5 cm total length), each picking the its skin material (Figure 1 and Video S1). The cleaning interaction ended when the posing individual resumed its normal swimming behavior. The second cleaning occurrence (Pool 2) took about 125 s and included four cleaning bouts between the same pair of individuals (one cleaning 'cleaner' and the other being cleaned 'client'). It was not clear whether the posing solicitation occurred during the first interaction, which entailed one cleaning event for 2 s followed by a second event (19 s later) which included two cleaning bites for 3 s. Finally, after an interval of about 1 min and 12 s, the same inspected individual reinforced its willingness to continue to be cleaned by again adopting a tail-stand posture, which successfully solicited for a new inspection (one final event of skin picking, of about 2 s). Because this cleaning event occurred around the camera and not at the front as in Pool 1, it was the sequence of events between the same individuals and the occurrence of posing at the end that allowed us to understand the whole cleaning event. In this last sequence (Pool 2), the cleaner individual was larger (~7 cm total length) than the client (~5 cm total length) (see Video S1).

The ability of freshwater fishes to engage in cooperation and the importance of communication networks between individual fish in these aquatic environments is not well understood. The adaptive significance of this type of communication is that it allows decisions at both ends of the network (those wanting to be cleaned and those deciding to approach to inspect). Since this type of process was often assumed to exist solely in fully dedicated tropical cleaner fish, it further emphasizes the complexity of the *S. alburnoides* behavioral network and the pressing need for further studies.

This study expands the list of freshwater fish species that behave as cleaners and simultaneously benefit from these behaviors. It contributes to fill a gap on freshwater fish behavioral ecology knowledge, emphasizing the importance of mutual positive behavior within an endangered species, particularly when facing seasonal disturbance (Alvarez Cobelas et al., 2005). Indeed, Mediterranean climate conditions are experiencing substantial reductions in precipitation and water availability, with an increased likelihood of droughts (Kerr, 2005). The combination of long-term change, namely warmer average temperatures, and longer and more frequent droughts, exacerbated by pollution (Dassenakis et al., 1998; Hermoso et al., 2011) and invasive species (Clavero et al., 2004; García-Berthou et al., 2007), should increase the stress experienced by native endemic and more vulnerable species such as *S. alburnoides*. All these added climate-derived factors accumulating with the reduced space availability that occurs in summer pools may also favor parasite abundance and disease spread (Barber, 2007). In this context, cleaning interactions should increase the fitness of the individuals in this species by reducing parasitism levels and antagonistic/competitive events.

AUTHOR CONTRIBUTIONS

Conceptualization: Marta C. Soares, Filipe Banha; Methodology and field work: Filipe Banha, Marta C. Soares, José C. Alves, Sónia C. Cardoso, Pedro Fernandes, Video analysis: Pedro Fernandes, Marta C. Soares,

Filipe Banha, Writing – original draft: Marta C. Soares, FB.; Writing – review & editing: Marta C. Soares, Filipe Banha, Pedro M. Anastácio.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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