Ectoparasites of native cyprinid *Barbus haasi*: first record of *Trichodina acuta* and *Trichodina fultoni* in Iberian catchments

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Abstract

Parasitological studies of wild freshwater fish in Iberia are needed to provide baseline data for management strategies, but the conservation status of many species means application of non-lethal sampling procedures is mandatory. A survey of Iberian redfin barbel (*Barbus haasi*) from five Mediterranean streams in north-eastern of Spain using mucus scrapings revealed the presence of *Trichodina acuta, T. fultoni* and *Gyrodactylus* spp. with prevalences of 35-60%, 8%, 2-50% and densities of 54-197, 42-89, 2-50 parasites/mm^2 on fish skin, respectively. Biometrics of the trichodinid species are provided, and we discuss the potential application of trichodinids as eutrophication bioindicators in these rivers.

Introduction

Mediterranean rivers have suffered a long history of anthropogenic disturbances resulting in declining fish populations (Smith and Darwall, 2005). This situation is of particular conservation concern in areas of low species richness and high degree of endemicity (Doadrio, 2001; Maceda-Veiga, 2013). The status of the Iberian ichthyofauna is typified by the redfin barbel (*Barbus haasi*), a sedentary benthopelagic cyprinid, which is endemic to the mid and upstream reaches of rivers in north-eastern Spain (Doadrio, 2001). The geographical range of this cyprinid has been reduced by 54% over the last 50 years, and it is now included in Annex V of the European Union Directive and is classified as vulnerable in the International Union for Conservation of Nature (IUCN) freshwater fish list (Doadrio, 2001; Smith and Darwall, 2005; Maceda-Veiga et al., 2010). Similar to other freshwater fish species, the main threats to *B. haasi* are habitat degradation, including water pollution events, and the introduction of exotic species due to angling and aquaculture practices (García-Berthou et al., 2007; Maceda-Veiga, 2013). Pathogens also present a major problem for fish populations,

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but there are only two known parasitological surveys of wild, native, strictly freshwater species in north-eastern Spain (Lacasa-Millán, 1993; Maceda-Veiga et al., 2009).

More parasitological studies of wild freshwater fish in Iberia are needed to provide baseline data for management strategies, but the conservation status of many species means application of non-lethal sampling procedures is mandatory. In this regard, mucus scrapings have been successfully employed for detecting common ectocommensals (e.g. trichodinids) and ectoparasites (e.g. monogeneans) in wild and culture fish (Lom and Dykova, 1992; Bakke et al., 2007). The detection of trichodinids might also be useful for water quality monitoring in this region as several studies have targeted trichodinids as potential indicators of organic pollution in marine systems (Palm and Dobberstein, 1999; Ogut and Palm, 2005), lakes (Valtonen et al., 1997) and UK rivers (Yeomans, 2000). In the current study, we use mucus skin scrapings to assess the ectocommensals on *B. haasi*.

Material and methods

Sampling was performed as part of a larger monitoring study organised by the Biodiversity Databank of Catalonia (Font et al., 2013) to describe animal and plant diversity of Moià (Catalonia, NE of Spain). Small streams in the region have monospecific fish communities naturally shaped by a typical Mediterranean hydrological cycle in which floods mainly occur in autumn and droughts in summer (Gasith and Resh, 1999). Surveys were conducted in five streams (Marfà, Fàbrega, Gavarresa, Oló and Vallvidrera) in Llobregat basin in June 2012. Low flow conditions increased electrofishing efficiency and maximized the chance of finding infected fish (Maceda-Veiga et al., 2009). The rivers were classified as baseline (E1 and E2) and polluted (E3, E4 and E5) according to information provided by Catalan Water Agency (http://aca-web.gencat.cat/aca/) and forest Guards. Habitat features and water quality at each sampling site was assessed during the current study as detailed in Table 1.

Fish were captured with a portable electro-fishing unit, identified to species level, counted and then catch per unit of effort (CPUE) was calculated based on the time spent electrofishing and the surface area of river surveyed. Each fish was then anaesthetised with 0.02% MS 222 (Sigma Aldrich®), weighed (W, g) and measured (fork length, FL, mm). A visual gross examination of fish revealed no evidence of external pathology. Fulton condition factor was calculated ($CF = W \cdot FL^{-3} 10^5$) as a general fish health indicator (e.g. Maceda-Veiga et al., 2009). Mucus scrapings collected in the field from the fish skin were distributed using a clean slide on the surface of another slide, air-dried and dyed using Klein's stain (Klein, 1958). Total number of parasites was recorded in 30 fields of view per slide per fish using an Olympus CH2 microscope at x400 magnification. Trichodinids were identified based on morphological traits following Lom and Hoffman (1964), Lom et al. (1970) and Basson et al. (2010). Complete biometrics of 30 specimens per species (when available) individually photographed are provided in Table 2. Parasite load was expressed as a relative density in relation to approximate host skin area examined (individuals per cm^2 of skin). Approximate skin area examined was calculated based on the width of the slide used for scrapings and the length of fish measured from behind the operculum to the tip of the caudal fin. Using non-parametric statistics as data lacked

Table 1. Environmental variables recorded at reference (E1 and E2) and polluted (E3, E4 and E5) sites in the Llobregat catchment, NE Spain. Letters (a,b,c) grouped sites with similar hidromorphological features at $P<0.05$.

	E1	**E2**	**E3**	**E4**	**E5**
Stream name	Gavarresa	Fàbrega	Marfà	Oló	Vallvidrera
Width (m)	6.40±2.38[a]	4.18±0.82[a]	10.77±6.25[b]	2.13±0.66[c]	4.18±0.85[a]
Depth (cm)	25.40±4.43[a]	9.08±7.63[b]	17.33±10.18[a]	12.00±4.04[b]	9.00±4.35[b]
Water flow (m/s)	0.9±0.1[a]	0.75±0.02[a]	0.02±0.01[b]	0.50±0.25[a]	0.08±0.03[c]
Temperature (ºC)	19.8	18.3	20.1	21.2	18.5
Conductivity (µS/cm)	710	611	972	2700	1243
Dissolved oxygen (mg/l)	7.36	7.79	7.21	7.81	7.21
Degree of silting (%)	45	10	80	45	80
Ammonia (mg/l)	<0.1	<0.1	<0.1	<0.1	<0.1
Nitrites (mg/l)	0.05	0.02	0.07	0.02	0.05
Nitrate (mg/l)	10	5.50	30	2.50	12
Phosphates (mg/l)	0.02	0.02	0.70	0.07	0.30
General hardness (ºdH)	22	25	25	19	27

normality and homogeneity of variances, parasite density was compared on fish from baseline and polluted sites using Kruskal-Wallis and Mann-Whitney *U* tests for general and pair-wise comparisons, respectively. Spearman rho coefficient explored pair-wise relationships between environmental variables, fish size and parasite density. All analyses were carried out with the stats package in R (R Development Core Team, 2012).

Results and discussion

Of the 147 Iberian redfin barbel (*Barbus haasi*) examined, we detected the presence of trichodinids and *Gyrodactylus* spp. in 17.7% (26) and 12.2% (18) of mucus scrapings examined, respectively. Two species of trichodinid were identified based on morphology: *Trichodina acuta* (N=129) and a large species *T. fultoni* (N=3) (see Table 2; Figure 1). The only two previous records of trichodinids identified to species level in Iberian catchments are *T. pediculus* and *T. jadranica* from European eels (*Anguilla anguilla*) in the Ebro Delta (Maíllo et al., 2005) and Rivers Ulla and Tea in Galicia (north-western Spain) (Aguilar et al., 2005), respectively. As trichodinid species have low host specificity (Lom and Dykova, 1992; Abowei et al., 2011), it is likely that they will be detected on other fish species in Iberia. The poor preservation of *Gyrodactylus* in the current study prevented reliable identification, but no other parasites were detected.

Although only a few sites were examined in the current study, our observations show the same trend as in previous reports with trichodinid occurrence possibly linked to eutrophication (Yeomans, 2000; Ogut and Palm, 2005; Vidal-Martínez et al., 2010). The highest prevalence

Table 2. Biometric data (mean ± SD, and minimum-maximum range) of *Trichodina acuta* and *Trichodina fultoni* specimens recorded in the current study and from previously published studies.

Species	***T. acuta***	***T. acuta* (n=34)**	***T. fultoni***	***T. fultoni* (n=3)**
Comparative studies	Lom, 1970 and Basson, 2010	Present study	Lom and Hoffman, 1964	Present study
Biometric measurements (μm)	Range (min-max)	mean±SD (min-max)	Range (min-max)	mean±SD (min-max)
Body diameter	59-78	57.88±3.49 (48.99-4.79)	91-102	84.85±6.09 (80.72-91.85)
Adhesive disc diameter	42-63	48.54±3.7 (39.49-53.74)	75-90	70.28±3.93 (67.88-74.82)
Border membrane width	3.5-6	4.81±0.23 (4.13-5.09)	5-7	6.02±0.24 (5.74-6.17)
Denticle ring diameter	23-36	29.81±3.19 (22.46-36.2)	47-58	44.23±2.35 (42.87-46.95)
Denticles number	18-22	20.44±0.83(19-23)	25-31	26.66±1.53 (25-28)
Denticle length	10-11	8.32±0.7 (6.73-9.31)	nd	11.49±0.28 (11.26-11.81)
Blade length	4.5-6	5.02±0.57 (4.04-7.15)	6.5	7.4±0.55 (6.94-8.01)
Thorn length	nd	6.3±1.03 (2.09-7.71)	5.5	7.81±0.08 (7.71-7.86)
Width of the central part	3-4.5	3.58±0.56 (2.04-4.79)	4	5.18±0.08 (5.1-5.27)
Radial-pin per denticle	8-13	9.06±0.80 (8-11)	12-14	13.33±0.58 (13-14)

nd – no data available

and densities (individuals/cm^2) of *T. acuta* were observed at sites E3 and E5 (M-W, $P<0.001$), and the prevalence and densities of *T. acuta* were also positively related to nitrate and phosphate concentrations, and degree of siltation (all cases $r>0.80$, $P<0.03$) (Table 3). Trichodinids were not detected at polluted site E4, possibly because factors other than siltation or poor water quality determine trichodinid occurrence, such as the higher density of gyrodactylids on fish from this site (M-W, all $P<0.001$) (Table 3). As predicted (Madsen et al., 2000), trichodinid density was higher on larger fish (length, $r=0.35$, $P<0.01$; weight, $r=0.35$, $P<0.01$).

In conclusion, we detected two trichodinid

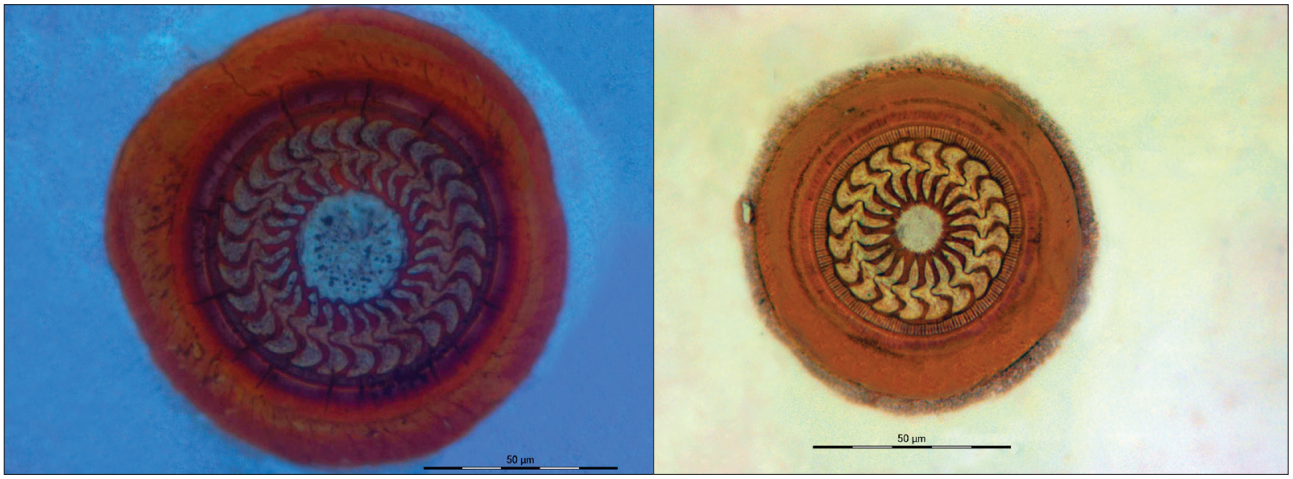

Figure 1. Photomicrographs of *Trichodina acuta* (A) and *Trichodina fultoni* (B) from the Iberian redfin barbel (*Barbus haasi*).

Table 3. Mean and standard deviation of the fish size (fork length and weight) and condition factor (CF), and parasite load and fish CPUE at reference (E1 and E2) and polluted (E3, E4 and E5) sites in the Llobregat catchment, NE Spain. Letters (a,b,c) grouped sites considered homogeneous at $P<0.05$.

	E1	**E2**	**E3**	**E4**	**E5**
Samples (N)	30	30	25	30	30
CPUE	5.21	18.96	2.32	49.06	14.37
LF (mm)	101.48±30.47^a	72.98±22.44^b	128.08±24.53^a	101.38±12.36^a	107.61±30.05^a
W (g)	22.67±21.1^a	8.22±7.75^b	36.52±19.76^a	14.41±5.70^b	20.27±17.94^a
CF	0.02±0.01^a	0.02±0.002^a	0.02±0.001^a	0.01±0.00^b	0.01±0.00^b
Trichodina acuta					
Prevalence (%)	0	0	60	0	35
Mean density (ind/cm^2)	0	0	198±76^a	0	54±10^b
Trichodina fultoni					
Prevalence (%)	0	0	8	0	0
Mean density (ind/cm^2)	0	0	7±8	0	0
Gyrodactylus spp.					
Prevalence (%)	9	0	8	50	3
Mean density (ind/cm^2)	7±4^a	0	7±4^a	50±6^b	2±4^a

species and gyrodactylids on this endangered fish species. Trichodinids are often considered harmless to the host but the pathology associated with gyrodactylids is widely recognised (Bakke et al., 2007). It is therefore necessary to reinforce the policy of equipment disinfection when moving fish or sampling tools between rivers.

Acknowledgments

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