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# Acanthocephalan parasites reflect ecological status of freshwater ecosystem

[FANTON](#) Hadrien<sup>1</sup>, [FRANQUET](#) Evelyne<sup>1</sup>, [LOGEZ](#) Maxime<sup>2</sup>, [CAVALLI](#) Laurent<sup>1</sup> & [KALDONSKI](#) Nicolas<sup>1</sup>

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<sup>1</sup>Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France.

<sup>2</sup>INRAE, Aix Marseille Univ, RECOVER, Aix-en-Provence, France

<sup>3</sup>INRAE, UR RiverLy, F-69625 Villeurbanne Cedex, France

**Author for correspondence:** Hadrien Fanton, E-mail: [hadrien.fanton@imbe.fr](mailto:hadrien.fanton@imbe.fr)

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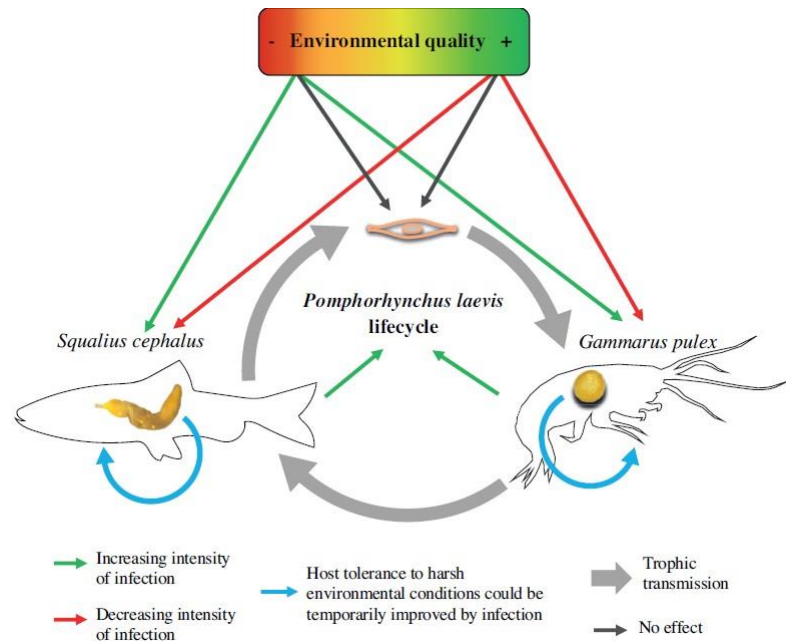
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## Highlights

- We link bacteriological, physicochemical and biological water parameters on acanthocephalans, and their intermediate and definitive hosts
- High *P. laevis* intensity of infection was correlated to high *G. pulex* intermediate host abundance and high *S. cephalus* definitive host occurrence in the stream (i.e. *G. pulex* and *S. cephalus*).
- Good ecological status assessed by the IBD, IPR and IBGN indexes are not favorable to *P. laevis* and *P. tereticollis* infection.
- High intensities of infection of *P. laevis* and *P. tereticollis* at cystacanth stage in *G. pulex*, and high prevalence of infected *G. pulex* would be indicative of strong bacteriological concentration.

## Graphical Abstract



## Abstract

Acanthocephalans' position in food webs, in close interaction with free-living species, could provide valuable information about freshwater ecosystem health through the viability of the parasites' host populations. We explored *Pomphorhynchus laevis* cystacanths' and adults' intensities of infection, and the prevalence of infected hosts respectively in their *Gammarus pulex* intermediate hosts and *Squalius cephalus* definitive hosts in a Mediterranean river. First, we analysed the relationship between *P. laevis* intensity of infection, its two hosts populations and the other acanthocephalan species found (*Pomphorhynchus tereticollis* and *Polymorphus minutus*). Second, we characterised the influence of bacteriological, physicochemical and biological water parameters on these acanthocephalans, and their intermediate and definitive hosts. This research highlights that *P. laevis* infection was closely related to their two preferential hosts population in the river. Moreover, *P. laevis* intensity of infection was positively correlated with organic pollution in the river but negatively correlated with biodiversity and with ecological indexes of quality. *Pomphorhynchus laevis* could thus benefit from moderate freshwater pollution, which promotes their tolerant intermediate and definitive hosts.

**Keywords:** anthropogenic disturbances; host-parasite interactions; *Pomphorhynchus* sp.; *Polymorphus minutus*; *Gammarus pulex*; *Squalius cephalus*

## Introduction

Freshwater ecosystems are among the most endangered and vulnerable, under constant and growing anthropogenic pressures (Walsh et al., 2005; Harper et al., 2021). These sensitive ecosystems suffer greatly from chemical and bacteriological degradation induced by anthropic pollution of watersheds (Allan and Flecker, 1993; Kaushal et al., 2017; Winton et al., 2020). Better understanding how environmental perturbations might impact freshwater biodiversity has become urgent. It is not only the visible free-living aquatic fauna, but also their hidden parasites, that are likely to see their ecology and biology modified (Marcogliese, 2001; Gomez and Nichols, 2013; Carlson et al., 2017).

The more complex the parasite's lifecycle, the more likely it is to be influenced by environmental shifts (Marcogliese, 2005). All species of acanthocephalan have the same fundamental lifecycle, their development and reproduction requiring two different hosts: an arthropod intermediate host and a vertebrate definitive host. To enhance trophic transmission between their two hosts, numerous freshwater acanthocephalans are able to manipulate the behaviour of their intermediate hosts and increase their predation risk by final hosts (Lagrué et al. 2007; Bakker, Frommen and Thunken 2017). The acanthocephalan's particular position within the food web in freshwater ecosystems requires complex biological interactions and depends on the viability of intermediate and definitive host populations (Galli et al., 1998; Hatcher et al., 2012). At their different developmental stages, acanthocephalan endoparasites are impacted either directly by their environment or indirectly through effects on their hosts (Galli et al., 2001; Hatcher et al., 2012; Fanton et al., 2021). Therefore, environmental perturbations affecting either their free-living stages or their hosts will also lead to physiological, ecological and behavioural changes for acanthocephalans (Marcogliese, 2008; Labaude et al., 2017; Sanchez-Thirion et al., 2019). For example, acanthocephalan parasites could be affected by increases in water temperature which could induce a shorter lifespan and faster growth, with a modification in their manipulation capacities (Marcogliese, 2008; Labaude et al. 2017). While some acanthocephalan species will adapt to these conditions, many others will not be able to (Marcogliese, 2001). Because of their contribution to productivity and stability of ecosystems through their biodiversity, biomass and services, parasite biodiversity loss would lead to considerable unpredictable consequences from individual to ecosystem scale (Wood and Johnson 2015; Strona 2015).

There is growing interest in the effects of pollution on freshwater parasites (Lafferty 1997; Marcogliese 2005; Sures et al., 2017; Grabner and Sures 2020; Fanton et al., 2020; Duarte et al., 2020). Acanthocephalans could provide a clear picture of ecosystem composition by reflecting the presence and the viability of their intermediate, definitive and paratenic host populations (Marcogliese and Cone 1997). In a review, Lafferty (1997) pointed out that acanthocephalan parasites are negatively impacted by sewage sludge, industrial effluents, crude oil and heavy metal organic pollution. Another study on the acanthocephalan *Pandosentis* aff. *iracundus* shows a negative correlation between parasite abundance and concentrations of fecal coliforms and ammonia (Lacerda et al., 2018). Contrastingly, the meta-analysis of Blonar et al. (2009) on studies of the effects of pollution (pesticides, polycyclic aromatic hydrocarbons, polychlorinated biphenyls and heavy metals) on acanthocephalans found no significant effect of pollution on acanthocephalan species. Indeed, some acanthocephalan populations may decrease when faced with a perturbation, while others may take advantage of water pollution. Moreover,

acanthocephalans were not only affected by direct impacts of pollution, but were also impacted by the combined risk of these environmental changes upon their hosts (Carlson et al. 2017). In a polluted environment, the occurrence of acanthocephalans may be largely driven by the sensitivity of their respective hosts (Galli et al., 2001). Given this varying response among parasites, more information is needed regarding the ecology, physiology and varying pollution- sensitivity of acanthocephalan parasites and their associated hosts.

*Pomphorhynchus laevis* is a freshwater acanthocephalan found in both polluted and unpolluted ecosystems (Galli et al., 1998; Nachev and Sures 2009). Analysing the distribution of the acanthocephalan parasites *P. laevis* in *Squalius cephalus* on four rivers with different pollution levels, Galli et al., (1998) observed that populations of *P. laevis* were limited to unpolluted or slightly polluted rivers. By contrast, Nachev and Sures (2009) analysed parasite communities and found that *P. laevis* was the dominant species in the most polluted sampling sites. These two contrasting findings indicate that *P. laevis* populations are found under heterogeneous environmental quality, and therefore that acanthocephalan distribution may depend on complex interactions between their hosts and their ecosystem quality.

This paper investigates how acanthocephalan parasites are influenced by their hosts and the quality of their freshwater environment. We focus on the hosts-parasite complex formed by *P. laevis*, its *G. pulex* intermediate host and its *S. cephalus* definitive host. We studied *P. laevis* population hosted by *G. pulex*, and *S. cephalus* at 6 different stations of a Mediterranean river. We analysed biological, bacteriological and physicochemical parameters of each station to determine water quality. We hypothesised that acanthocephalan mean intensity of infection at each stage could reveal environmental shifts in the river. We also expected *P. laevis* mean intensity of infection to be correlated with hosts' distribution in the stream and indicative of environmental status, this could enable us to determine how the distribution of acanthocephalan parasites at each stage of their lifecycle is related to environmental quality.

## Materials and methods

### The *Pomphorhynchus laevis* lifecycle

We focused on the hosts-parasite complex *P. laevis*-*G. pulex*-*S. cephalus*. The *P. laevis* lifecycle is heteroxenous and requires two hosts: a gammarid intermediate host for its growth (e.g. *G. pulex*), and a definitive fish host for its reproduction (e.g. *S. cephalus*). After reaching sexual maturity and breeding within the digestive tract of their definitive host, *P. laevis* eggs (acanthor) are released into the stream via the feces of the fish definitive host. When gammarids involuntarily ingests acanthors, the parasite grows in the general cavity of the gammarid until it becomes an acanthella, then an infective stage called "cystacanth". At this stage, *P. laevis* is able to manipulate the behaviour of its intermediate host to enhance trophic transmission toward its *S. cephalus* definitive host (Kaldonski et al., 2007; Lagrue et al., 2007; Perrot-Minnot et al., 2016). The cycle is completed when the infected amphipod is consumed by a fish definitive host like *S. cephalus*.

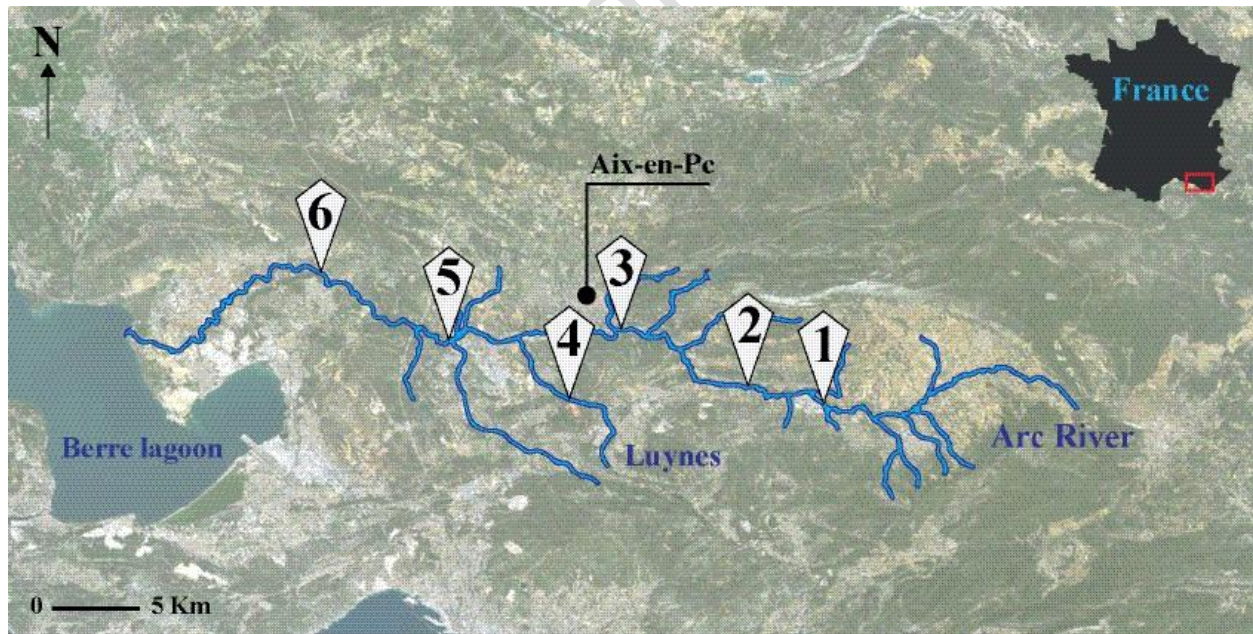
## Ethical note

*Pomphorhynchus laevis*, *S. cephalus* and *G. pulex* are common species in Mediterranean rivers. Sampling permits were provided by the Direction Départementale des Territoires et de la Mer (DDTM) des Bouches-du-Rhône, for the purpose of Hadrien Fanton's PhD work. Individuals' origin, sampling and killing are described below.

## Sampling site

*Gammarus pulex* and *S. cephalus* were collected during 2018 from the Arc River and Luynes tributary (Bouches-du-Rhône, Southern France, Fig. 1). The Arc River, flowing along a linear 85 km with a 780 km<sup>2</sup> watershed area, has been particularly influenced by anthropogenic pressures like agriculture, land clearing or industrial pollution. The main perturbations in the Arc River involve high bacteriological concentrations due to water treatment plants, and phosphorus and nitrogen inputs due to industrial and agricultural waste. Water temperature in the Arc River ranges between 4 °C in winter and 27 °C during the warmer periods of summer (Maasri et al., 2008). Average discharge is 1 to 3 m<sup>3</sup>/s depending on season, with alternating periods of summer drought and winter flooding.

Six sampling stations were selected over upstream and downstream locations, based on the monitoring stations used by the Syndicat d'Aménagement du Bassin de l'Arc (SABA), and the Agence de l'Eau Rhône Méditerranée Corse (AE RM&C), and Eau France (Fig. 1, Tab. 1).



**Fig. 1.** Location of the 6 stations studied in the Arc River and the Luynes tributary. Station 1 = Rousset; Station 2 = Chateauneuf-le-Rouge; Station 3 = Pont des Trois Sautets; Station 4 = Luynes; Station 5 = St-Pons; Station 6 = Moulin du Pont. The Arc River flows into the Berre Lagoon (France).

**Table 1.** Names and locations of the 6 stations sampled in the Arc River and the Luynes tributary.

Station	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
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Name	Rousset-le-Rouge	Châteauneuf	Pont des Trois Sautets	Luynes	St-Pons	Moulin du Pont
Longitude (X)	43.471951	43.480079	43.511258	43.471297	43.505889	43.541269
Latitude (Y)	5.617146	5.565648	5.473530	5.443511	5.347853	5.258331

## Environmental parameters

Environmental parameters (physical, chemical and bacteriological data) on the Arc River and Luynes tributary were obtained from the freely available database Nâiades ([Eau France](#)), and also provided by AE RM&C and SABA. From the environmental parameters available for each season of 2018, data for 17/04/2018, 25/06/2018, 04/09/2018 and 20/11/2018 were selected for the 6 stations. A total of 24 environmental parameters were studied: dissolved organic carbon (DOC); biochemical oxygen demand (BOD); Kjeldhal nitrogen (NKj); total phosphorus (P<sub>tot</sub>); phosphate (PO<sub>4</sub>); potassium (K); ammonium (NH<sub>4</sub>); chloride (Cl); conductivity (C); calcium (Ca); magnesium (Mg); total suspended matter (TSM); oxygen saturation level (O<sub>2</sub>%); dissolved oxygen (O<sub>2</sub>); nitrate (NO<sub>3</sub>); nitrite (NO<sub>2</sub>); runoff (Runoff); sulfate (S); total coliforms (Total coliforms); *Enterococcus* spp. (*Enterococcus* spp.); *Escherichia coli* (*E. coli*); temperature (T°C); pH (pH); sodium (Sodium). For the definition of the ecological status of a river, or assessment of the quality elements, the Water Framework Directive (WFD) provides assessment tables that classify each quality element through qualitative assessment (high, good, moderate, poor and bad). The classification specified in the WFD is used below to define the ecological status of Arc River and Luynes tributary (European Commission, 2000; Carballo et al., 2009).

## Biological parameters

Fifteen biological parameters were studied. Data on four parameters were provided by the SABA database: the Shannon index of benthic macroinvertebrates and three biotic indexes: (1) IBGN (french macroinvertebrate index; Archimbault and Dumont, 2010), a macroinvertebrate-based index; (2) IBD (french diatom index; Prygiel and Coste, 2000) based on diatom communities; (3) IPR (french fish index; Oberdorff et al., 2002) based on freshwater fish assemblage structure. Each index defined the ecological status of the river through qualitative assessment (very good, good, moderate, bad and very bad). Data on the remaining parameters came from our sampling campaigns: six from *G. pulex* sampling, 3 from *S. cephalus* sampling, 2 from fish community sampling.

### *Gammarus pulex* sampling

*Gammarus pulex* individuals were collected from the Arc River in each season during 2018. Sampling campaigns were conducted on 18/05/2018, 05/07/2018, 04/10/2018 and 06/12/2018. Two different methods were used to sample benthic gammarids living in the substrate and margins of the river:

1. Surber sampling: three gravelly and rocky locations were chosen for each station and used for all campaigns. A surber (25x25 cm, mesh size 500 µm) was used at all three locations to sample benthic fauna. All samples were placed in ethanol (90 %) directly after capture.
2. Handnet sampling: three locations with roots and vegetation were chosen in the margin of the stream for each station and used for all campaigns. A handnet was used at all three locations

to sample margin fauna. All *G. pulex* sampled were placed in ethanol (90 %) directly after capture.

Back in the laboratory, gammarid individuals were measured (length of the fourth coxal plate) with a SMZ1500 Nikon binocular lens (Mitsubishi, Tokyo, Japan) linked to an R1 Nikon camera (Mitsubishi, Tokyo, Japan) connected to a computer with NIS-Br software. All *G. pulex* were measured and dissected to identify, if they were infected by acanthocephalan, and therefore to identify the acanthocephalan species at cystacanth stages. Developmental stage and species were determined based on morphological identification following Perrot-Minnot (2004). However, *G. pulex* harboring acanthella stages were not include in the dataset due to the difficulty to identify at species level and early acanthella stage. These sampling campaigns enabled us to calculate 6 biological parameters: the mean intensity of infection of *P. minutus*, *P. tereticollis* and *P. laevis* at their cystacanth stage (average number of individuals of a parasite species among *G. pulex* infected host (Bush et al. 1997)), the prevalence of infected *G. pulex* (%), *G. pulex* abundance and *G. pulex* size.

### *Squalius cephalus* sampling

*Squalius cephalus* were collected from the Arc River during 2018 in sampling campaigns conducted on 27/04/2018, 25/07/2018, 02/10/2018 and 10/12/2018. Conventional electrofishing gear (EFKO FEG 3000) was used, according to the multipass method (Peterson et al., 2004). The length of the sampling reach was restricted to 100 m at each station. *Squalius cephalus* juveniles under 10 cm and adults over 30 cm were not selected, as their diet does not favour gammarid consumption (Keith et al., 2011). At each station, the first 10 *S. cephalus* found measuring between 10 and 30 cm were collected and were killed immediately after sampling. Back in the laboratory, *S. cephalus* individuals were weighed, measured (fork length) and dissected. The intestine (pylore to anus) was removed, measured and opened longitudinally to identify the number of adult acanthocephalans. Acanthocephalans attached to the intestinal wall were numbered, then removed and stored in ethanol (98%). The taxonomic revision of *P. laevis* by Perrot-Minnot (2004) revealed a confusion between *P. laevis* and *P. tereticollis*, suggesting the need for genetic identification of these two parasites at mature stages. Here, all adult acanthocephalans found in the *S. cephalus* intestinal wall are reported at the genus level. Our *S. cephalus* sampling campaigns enabled us to calculate 3 biological parameters: *S. cephalus* size, the mean intensity of infection of adult acanthocephalans (*Pomphorhynchus* sp.) among *S. cephalus* infected host (Bush et al. 1997) and the prevalence of infected *S. cephalus* (%).

### *Fish community sampling*

To analyse the fish community of the Arc River and the Luynes tributary, additional electrofishing was conducted on 14/06/2018 for stations 1, 2 and 3 and on 19/06/2018 for stations 4, 5 and 6, using the multipass method (Peterson et al., 2004). As with the *S. cephalus* sampling, fish community sampling reach length was restricted to 100 m at each station. The fish were captured, identified and measured before release. The data collected enabled us to calculate two biological parameters: fish Shannon diversity index and *S. cephalus* frequency of occurrence in the 6 stations sampled.



### *Statistical analysis*

A principal component analysis (PCA) based on correlation matrix was performed to characterise the relationships between the 15 biological parameters for the 6 stations over the entire sampling period. Biotic indexes (IBD, IPR, IBGN), Shannon indexes of benthic invertebrates and fish and frequency of occurrence of *S. cephalus* were added to the PCA as supplementary variables because of the low number of values available for 2018 at the 6 stations. Pearson correlations for biological variables were tested. A second PCA (also on correlation matrix) was performed to characterise the relationships between the 24 environmental parameters for the 6 stations over the entire sampling period. Finally, co-inertia analysis (CIA) between the two PCAs was performed to analyse the potential link between Arc River biological and environmental parameters (Dolédéc and Chessel, 1994; Dray et al., 2003). The strength of the link between the two tables was measured through the *RV* coefficient (Escoufier, 1973), which extends the Pearson correlation coefficient to two tables, and its associated permutation test.

Statistical analyses were conducted using R 3.5.0 software (R Core Team, 2018, A Language and Environment for Statistical Computing), and the additional libraries “*FactoMineR*” (Lê et al., 2008), “*factoextra*” (Kassambara and Mundt, 2017), and “*ade4*” (Dray and Dufour, 2007).

## **Results**

Over the course of the *G. pulex* sampling campaigns, 8439 *G. pulex* were captured in the substrate and margins of the river and dissected. The highest abundances of *G. pulex* were found at stations 2 and 5 (Tab. 2).

1 115 (13.2 %) *G. pulex* were infected by an acanthocephalan parasite, including 2 095 cystacanths of three species: 1 730 *P. laevis*, 233 *P. tereticollis*, and 43 *P. minutus*. Interestingly, we found in station 4 more than fifty percent of the *P. laevis* and *P. tereticollis* individuals. Mean intensity of infection of *P. laevis* cystacanths were highest at stations 4 and 5 (Tab. 2). Mean intensity of infection of *P. tereticollis* cystacanths was also highest at station 4. No *Polymorphus minutus* were found at station 4, and their intensity of infection never exceeds one individual per infected host (Tab. 2).

The *S. cephalus* sampling campaigns led to capture and dissection of 240 *S. cephalus*, of which 192 (80%) were infected by an adult acanthocephalan parasite. 2185 adult acanthocephalans were found in *S. cephalus* intestines. The highest mean intensity of infection of adult acanthocephalans was found at station 5 (Tab. 2).

For the fish community, 768 fish were sampled, including 15 different species. Station 1 and 2 exhibited the highest diversity of fish, but the highest frequency of occurrence and the highest prevalence of infected of *S. cephalus* were found at station 4 (Tab. 2).

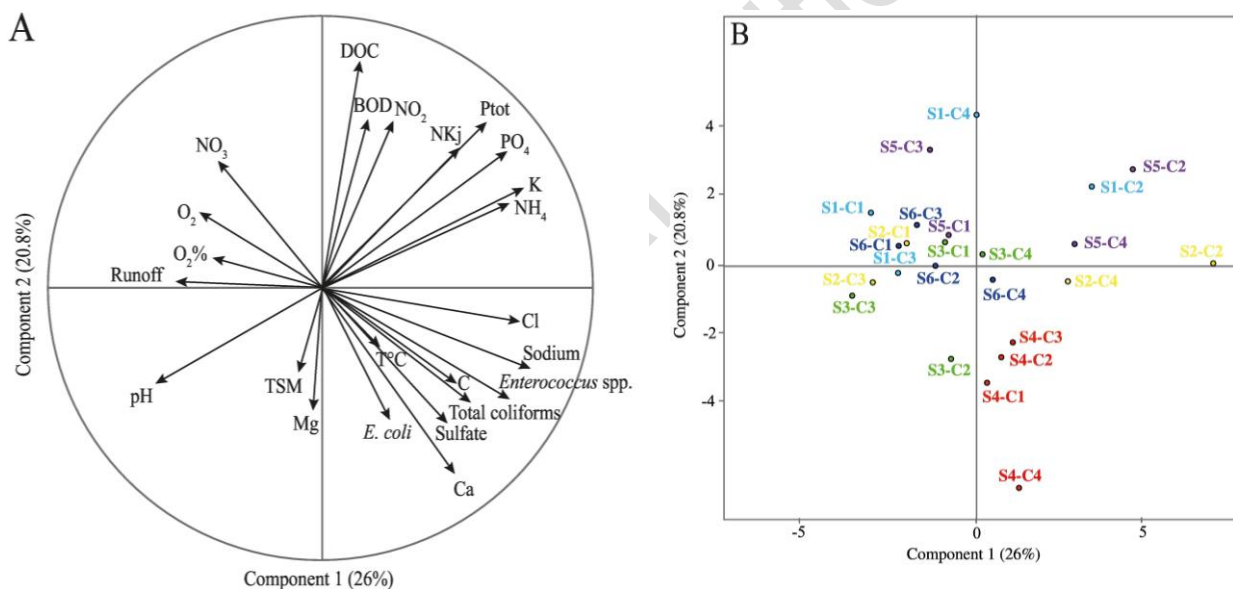
Heterogeneous bacteriological, physico-chemical and biological characteristics were found in the 6 stations. Moreover, their ecological status, as assessed by the three biotic indexes, also differed considerably (Tab. 2).

**Table 2.** Relevant biological and physico-chemical characteristics of the 6 stations studied. *P. laevis* = *P. laevis* cystacanth mean of intensity of infection in *G. pulex*; *P. tereticollis* = *P. tereticollis* cystacanth mean of intensity of infection in *G. pulex*; *P. minutus* = *P. minutus* cystacanth mean of intensity of infection in *G. pulex*; Ac. Adults = Adult acanthocephalan mean of intensity of infection in *S. cephalus*; *G. pulex* = mean of *G. pulex* abundance; Infected *G. pulex* = prevalence of infected *G. pulex*; H' Invertebrates = Shannon indexes of benthic macroinvertebrates; *S. cephalus* % = *S. cephalus* frequency of occurrence in fish community; Infected *S. cephalus* = prevalence of infected *S. cephalus*; H' Fish = Shannon indexes of fish community; Total coliforms = Total coliforms concentrations; BOD = Biochemical oxygen demand; NO<sub>3</sub> = Nitrate; O<sub>2</sub>% = Oxygen saturation level; C = conductivity. IBD = French diatom index; IBGN = French macroinvertebrate index; IPR = French fish index. For the ecological indexes, colours indicate ecological status (Blue: very good, green: good, yellow: average, orange: bad, red: very bad, sensu water framework directive (European Commission, 2000)).

	Station 1	Station 2	Station 3	Station 4	Station 5	Station 6
<i>P. laevis</i> (±SD)	1.3 ±0.35	1.02 ±0.74	1.15 ±0.2	1.82 ±0.44	1.47 ±0.46	1.39 ±1.09
<i>P. tereticollis</i> (±SD)	1 ±0	1.2 ±0.35	1.2 ±0.4	2.21 ±2.1	1.17 ±0.27	1 ±0
<i>P. minutus</i> (±SD)	1 ±0	1 ±0	1 ±0	-	1 ±0	1 ±0
Ac. adults (±SD)	4.43 ±3.91	6.33 ±0.63	14.57 ±5.6	9.2 ±2.98	24.74 ±8.19	3.48 ±2.71
<i>G. pulex</i> (±SD)	230.25 ±52.2	475.5 ±157.4	271.25 ±46.6	398.75 ±79.7	496.75 ±109.8	237.25 ±37.2
Infected <i>G. pulex</i> (%)	12.9	6.99	10.5	49.5	19.1	3.1
H' Invertebrate	1.84	2.08	2.35	1.77	1.56	3.12
<i>S. cephalus</i> (%)	33.25	33.90	6.90	96.40	40	39
Infected <i>S. cephalus</i> (%)	70	70	75	100	97.5	67.5
H' Fish	1.90	1.92	1.21	0.19	1.38	1.26
Total coliforms (CFU/100mL)	7507 ±3130	28822 ±17810	5317 ±853	36000 ±3500	40050 ±5517	11450 ±3334
BOD (mg/L)	1.33 ±0.28	1.58 ±0.37	1.45 ±0.53	0.8 ±0.17	3.1 ±1.1	1.78 ±0.51
NO <sub>3</sub> (mg/L)	12.5 ±0.64	8.03 ±1.48	7.2 ±0.66	6.73 ±0.62	8.6 ±0.57	8.3 ±0.82
O <sub>2</sub> %	97.25 ±5.9	95.25 ±5.5	94.25 ±2.89	91.9 ±2.81	88 ±3.24	102.75 ±2.92
C (µS/cm)	845 ±22.5	989 ±80.5	856 ±34.4	949 ±47.1	810 ±58.6	855 ±39
IBD	14.3	15	13.2	12.8	11.8	13.5
IBGN	12,0	13.7	14.8	9.2	11.6	13.7
IPR	32.7	11.4	6.4	44.5	11.7	8.8

## PCA with environmental parameters

The first two axes of the environmental PCA explained 46.8 % of the total inertia, distinguishing three groups of parameters. First, bacteriological parameters were correlated both with each other and with ionic water components like conductivity, chlorure, sodium, sulfate and calcium. The second group was driven by organic pollution, and revealed a link between NO<sub>2</sub>, COD, BOD, AzoteK, Ptot, PO<sub>4</sub>, K, NH<sub>4</sub>. The third group linked NO<sub>3</sub>, Runoff, O<sub>2</sub> and O<sub>2</sub>% parameters indicating a gradient of oxygenation. Station 4 appeared to have the highest bacteriological and conductivity levels. Station 2 showed the most unstable water quality, with high bacteriological and organic pollution during campaigns 2 and 4 as well as high oxygen concentrations and good ecological indexes during campaigns 1 and 3 (Tab. 2 and Fig. 2). Stations 3 and 6 appeared to have the highest oxygen levels and the lowest pollution, mainly due to inflow from three tributaries in station 3 and high runoff combined with the proximity of a waterfall in station 6. Despite the spatial heterogeneity between stations, we did not find an upstream-downstream gradient with the environmental parameters which highlight that each station was influenced by specific perturbations.

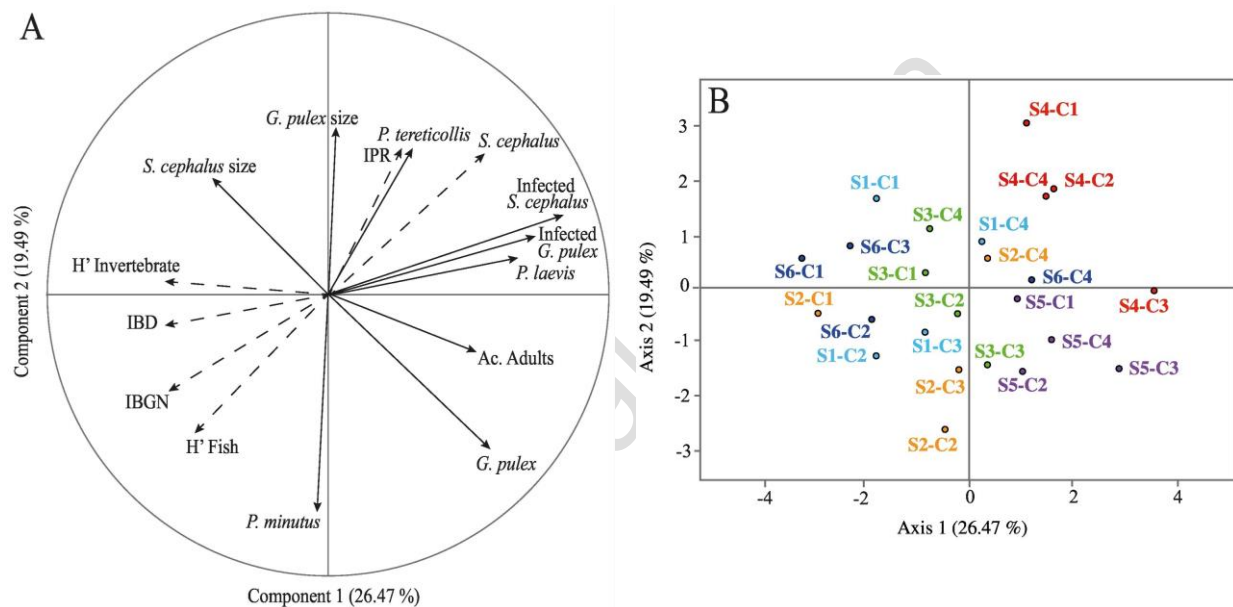


**Fig. 2.** A) Principal component analysis (PCA) showing the link between environmental parameters during 2018 on the Arc River. DOC = Dissolved organic carbon; BOD = Biochemical oxygen demand; NKj = Kjeldhal nitrogen; Ptot = Total phosphorus; PO<sub>4</sub> = Phosphate; K = Potassium; NH<sub>4</sub> = Ammonium; Cl = Chlorure; C = Conductivity; Ca = Calcium; Mg = Magnesium; TSM = Total suspended matter; O<sub>2</sub>% = Oxygen saturation level; O<sub>2</sub> = Dissolved oxygen; NO<sub>3</sub> = Nitrate; NO<sub>2</sub> = Nitrite; Runoff; S = Sulfate; Total coliforms; *Enterococcus spp.*; *Escherichia coli*; T°C = Temperature; pH; Sodium. B) Projection of the stations studied at each campaign in the environmental PCA (S1=Station 1; S2=Station 2; S3=Station 3; S4=Station 4; S5=Station 5; S6=Station 6. C1=Spring campaign; C2=Summer campaign; C3=Autumn campaign; C4=Winter campaign).

## PCA with biological parameters

The first two PCA axes explained 45.96 % of the total inertia of the biological table. This PCA highlights a positive correlation between the mean intensity of infection of the two cystacanth

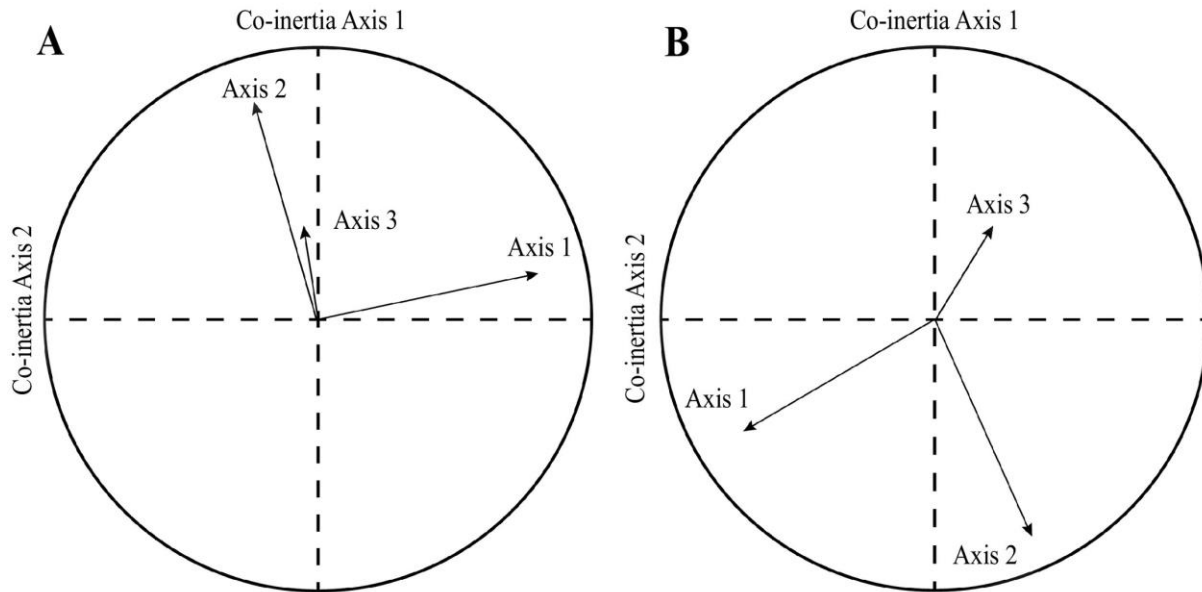
species *P. laevis* and *P. tereticollis* (Fig. 3A). Positive correlation was also observed between the intensity of infection of *P. laevis* and the prevalence of its intermediate and definitive host (Fig. 3A). The two life stages of *Pomphorhynchus* species, cystacanths and adults, were negatively correlated (opposed on the first axis) to the biotic indices evaluating the ecological status (IBD and IBGN) and negatively correlated to the diversity indices (H' Fish, H' Invertebrate). On contrary, *P. minutus* intensity of infection, was not correlated to the intensity of infection of the two other cystacanths species (Fig. 3A). Projections for stations 4 were driven by the highest intensity of infection of cystacanths *Pomphorhynchus* sp., and the highest prevalence of infected hosts (Tab. 2 and Fig. 3). Projections for stations 5 were driven by the highest intensity of infection of adults *Pomphorhynchus* sp. and *G. pulex* abundances. By contrast, station 6 was driven by high biodiversity and good ecological indexes, and therefore low acanthocephalan intensity of infection (Tab. 2 and Fig. 3).



**Fig. 3.** (A) Correlation circle with the first two components of the PCA on the biological parameters of the Arc River and the Luyne tributary (2018). *P. minutus* = mean intensity of infection of *P. minutus*; *P. tereticollis* = mean intensity of infection of *P. tereticollis*; *P. laevis* = mean intensity of infection of *P. laevis*; *G. pulex* = abundance of *G. pulex*; *G. pulex* size = mean size of *G. pulex*; *S. cephalus* = *S. cephalus* frequency of occurrence in fish community; Infected *G. pulex* = prevalence of infected *G. pulex*; Infected *S. cephalus* = prevalence of infected *S. cephalus*; H' Fish = Shannon index of fish; H' macroinvertebrate = Shannon index of benthic invertebrates; Ac. Adults = mean intensity of infection of adult acanthocephalans; IBD = French diatom index; IBGN = French macroinvertebrate index; IPR = French fish index. Dashed arrows represent supplementary variables. (B) Projection of the stations studied at each campaign in the biological PCA (S1=Station 1; S2=Station 2; S3=Station 3; S4=Station 4; S5=Station 5; S6=Station 6. C1=Spring campaign; C2=Summer campaign; C3=Autumn campaign; C4=Winter campaign).

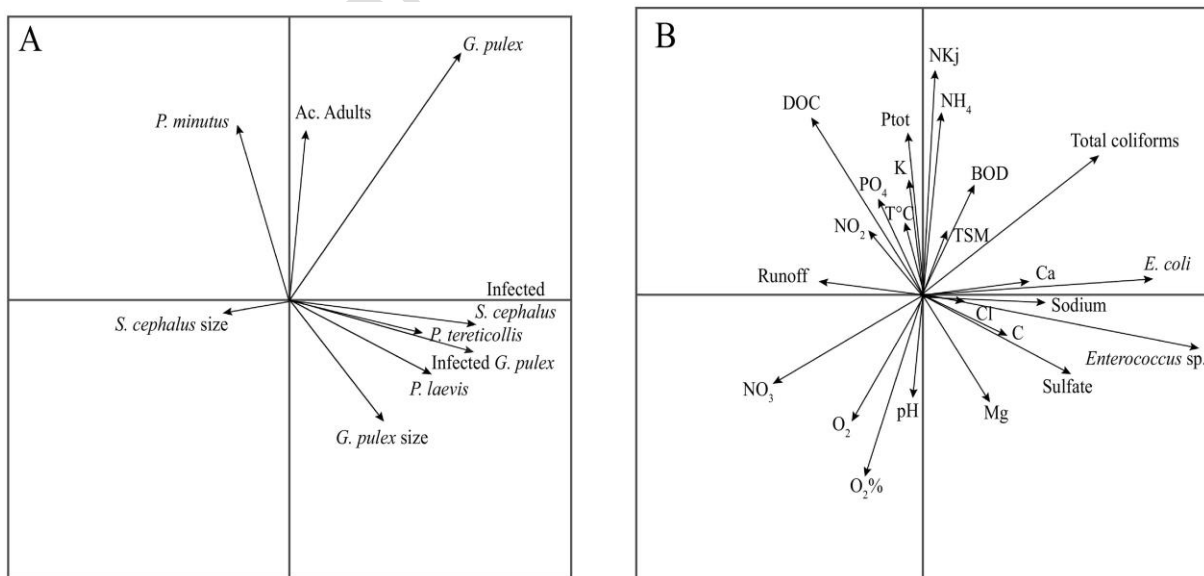
## Co-inertia analysis

The co-structure described by co-inertia axis 1 is not far from the structures of the two datasets (biological and environmental) described by axes 1 in each separate analysis (Fig. 4). The overall similarity in structure of the datasets results in an RV coefficient of 0.349 ( $P < 0.05$ ,  $N = 999$ ), indicating a significant co-structure between the datasets (Robert and Escouffier, 1976).



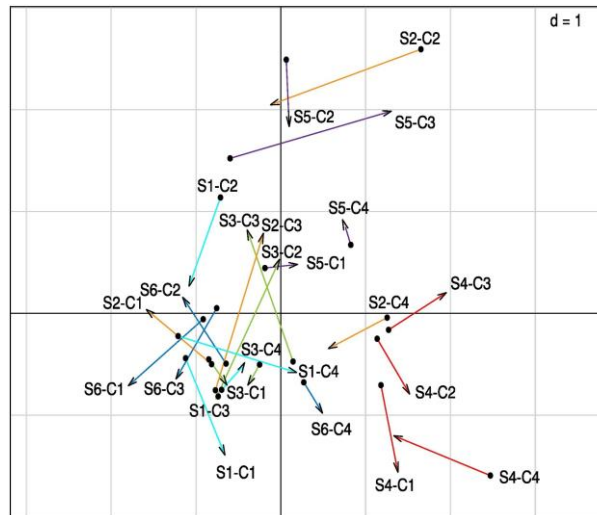
**Fig. 4.** Relationship between PCA and co-inertia analysis. Arrows indicate axis of PCA. Labels above arrows show PCA axis number. (A) Components of the biological PCA projected on co-inertia axes. (B) Components of the environmental PCA projected on co-inertia axes.

*Pomphorhynchus* sp. intensity of infection at cystacanth stages were positively correlated with high *Enterococcus* spp. and *E. coli* concentrations (Fig. 5). *Pomphorhynchus laevis* and *P. tereticollis* intensities of infection were negatively correlated with runoff and  $\text{NO}_3^-$ . Whereas adults *Pomphorhynchus* sp. and cystacanth *Polymorphus minutus* intensities of infection were correlated with  $\text{NKj}$ ,  $\text{Ptot}$ ,  $\text{NH}_4^+$  and DOC concentrations. Prevalences of infected *S. cephalus* and infected *G. pulex* were correlated with *P. laevis* and *P. tereticollis* intensities of infection at cystacanth stages. The highest abundance of *G. pulex* was found to be positively correlated with total coliforms pollution (Fig. 5).



**Fig. 5.** Co-inertia analysis between biological PCA and environmental PCA. (A) Components of the biological PCA projected on co-inertia axes. (B) Components of the environmental PCA projected on co-inertia axes.

There was a good convergence between the environmental and biological datasets for stations 1, 4 and 6 (Fig. 6). The generally small size of the arrows and their similar location in the factorial plan indicate a good fit between the two datasets for these stations (Fig. 6). However, the two datasets did not clearly converge for stations 2, 3 and 5 at sampling campaign 3, and for station 2 and 3 at sampling campaigns 2. These different locations in the factorial plan point to inconsistencies between the biological and the environmental datasets (Fig. 6).



**Fig. 6.** Comparison of sampled stations using co-inertia analysis between biological PCA and environmental PCA. Dots and arrows represent the projected co-ordinates of each dataset, where the length of the arrow is proportional to the divergence between biological and environmental datasets. S1=Station1; S2=Station2; S3=Station3; S4=Station4; S5=Station5; S6=Station6. C1=Spring campaign; C2=Summer campaign; C3=Autumn campaign; C4=Winter campaign.

## Discussion

This study found that acanthocephalan distribution varied widely at different locations in the river Arc which can be attributed to the local ecological conditions. Cystacanths found in *G. pulex* hosts were dominated by the genus *Pomphorhynchus*, and especially by *P. laevis* representing more than eighty percent of the acanthocephalan community in this host. *Pomphorhynchus laevis* and *P. tereticollis* intensities of infection were positively correlated with the prevalence of infected *G. pulex* and *S. cephalus*. These two parasite species have a similar lifecycle and a niche overlap, using gammarids as intermediate host and fish as definitive host. Although *P. tereticollis* is more specific to benthic fish (e.g. *Barbus barbus*) and *P. laevis* more specific to benthopelagic fish (e.g. *S. cephalus*, Perrot-Minnot et al., 2019). Cystacanths intensities of infection of these two parasites were the highest in station 4 in which *S. cephalus* represent 96.40% of the fish community. When *B. barbus* was not current in the stream *P. tereticollis* could use the same definitive host as *P. laevis* and achieve their lifecycle in *S. cephalus*.

In the biological PCA, a high abundance of *G. pulex*, high occurrence of *S. cephalus* and intensities of infection of *Pomphorhynchus* species were positively correlated to the second axis. These results highlight that the intensity of infection of *P. laevis* cystacanths could be linked to

availability of these intermediate and definitive hosts. The *Polymorphus minutus* lifecycle differs from the two other acanthocephalans in needing a bird definitive host. Few individuals of this acanthocephalan species were found in the Arc River, however high *P. minutus* intensity of infection was also correlated with high *G. pulex* abundances. Intensities of infection of all three acanthocephalan species found in the Arc River and Luynes tributary were therefore correlated with their intermediate host's abundance or their definitive host occurrence in the river. This is consistent with Lagrue and Poulin (2015), who found that a dense and stable host population meant more dense and stable parasite populations. Both the continuous presence of *G. pulex* and its high density allow acanthocephalans to find a great number of suitable intermediate hosts. Sures and Streit (2001) found that eel acanthocephalan *Paratenuisentis ambiguus* was related to the abundance of its intermediate crustacean host *Corophium curvispinum*. Evidence from field studies revealed that acanthocephalans were largely dependent on benthic invertebrate fauna, themselves directly dependent on water quality and benthic habitats (Galli et al., 1998; Sures and Streit 2001).

*Pomphorhynchus laevis* and *P. tereticollis* intensities of infection at cystacanth and adult stages were negatively correlated with IBGN (french macroinvertebrate index), IBD (french diatom index), Shannon index of fish population, Shannon diversity of benthic invertebrates, and positively correlated with IPR (the high index values indicating poor water quality). These three ecological indexes indicate the ecological status of each station sampled. The densities of their *G. pulex* intermediate and *S. cephalus* definitive host are impacted in the same way, decreasing when water quality or benthic invertebrates and fish biodiversity improves. Indeed, high invertebrate or fish biodiversity could increase competition and hence decrease the density of gammarid or *S. cephalus* hosts for *Pomphorhynchus* sp. acanthocephalans. Then, *Pomphorhynchus laevis* abundance could be affected by fish population diversity, becoming less abundant when the fish population is not dominated by potential hosts like *S. cephalus*. Our results are also in agreement with Chapman et al., (2015), who found that parasite diversity increased while abundance decreased within healthy ecosystems. For example, increased abundance of Helminth parasites, *Posthodiplostomum* spp. and *Actinocleidus* sp. indicated poor environmental health (Chapman et al., 2015). In the Arc River, *Pomphorhynchus* sp. distribution was linked to the ecological indexes of water quality. The negative correlation between ecological indexes and *Pomphorhynchus* species population in the biological PCA was confirmed by the co-inertia analysis. Interestingly, our co-inertia analysis demonstrated that both the prevalence of infected hosts and *Pomphorhynchus* intensities of infection at cystacanth stage of development were linked to higher bacteriological pollution. The highest *Pomphorhynchus* intensities of infection were found at station 4, the station most affected by bacteriological pollution. *Polymorphus minutus* intensity of infection did not react in the same way, and was correlated with high concentrations of phosphate and nitrogen, with the highest abundance of *P. minutus* found at station 5. This is possibly linked to the station's harbouring water birds, because there could be high spatial variation in the distribution of these parasites depending on where water birds are located along the river (Latham and Poulin 2003). The co-inertia analysis also demonstrated that to *Pomphorhynchus* sp intensities of infection at cystacanth stages in *G. pulex* was not correlated to *Pomphorhynchus* sp. intensity of infection at adult stage in *S. cephalus*, but correlated to the prevalence of infected *S. cephalus*. In this river, more parasites in intermediate host does not always go hand in hand with more parasite in the definitive host. This could be the consequence of the availability of *S. cephalus* in the fish community. For example, in station 4, the occurrence of *S. cephalus* was very high and even if there is more cystacanth in *G. pulex*, the elevated number of potential definitive host could induce a decrease of the intensity of infection.

Eutrophication, pollution and fragmentation have been identified as factors influencing overall parasite species composition in fish (Valtonen et al., 1997). Our results contrast with Kussat's (1969) finding that catfish were more heavily infected by acanthocephalans upstream from effluent than downstream. Our results also differ from Galli et al. (1998, 2001), who examined *S. cephalus* parasites and found that the distribution of *P. laevis* was limited to unpolluted and slightly polluted sites. These authors suggested that differences in acanthocephalan occurrence related to levels of pollution could be attributed mainly to the sensitivity of their respective intermediate hosts. In fact, in these two studies, the *P. laevis* intermediate host *Echinogammarus stammeri* was affected by pollution and was absent from the two most polluted sites (Galli et al., 2001). However, our results are consistent with Valtonen et al. (1997), who also correlated the occurrence of parasite species in fish with the abundance of intermediate hosts. Examining lakes differing in trophic status and degree of pollution, they found that the acanthocephalan *Acanthocephalus lucii* showed the highest prevalence in perch (*Perca fluviatilis*) from a eutrophic and polluted lake. Our results are also in line with the findings of Nachev and Sures (2009), that the combined effect of metallic pollution and high nutrient concentrations increases *P. laevis* occurrences (Nachev and Sures 2009). These authors hypothesised that metal contamination fosters parasite occurrence by decreasing hosts' immune defences. In the Arc River and the Luynes tributary, organic and bacterial pollution could have had a dual impact, both increasing hosts' abundances by reducing biodiversity and disturbing hosts' immune systems in such a way as to promote *P. laevis* occurrence.

Sures and Streit (2001) revealed that the composition of fish helminth communities was mainly dependent on benthic macroinvertebrates, themselves directly dependent on ecosystem health. Given this interdependency, and because the complex lifecycle of acanthocephalans requires specific hosts, coextinction models predict high vulnerability for these species. However, Strona et al. (2013) demonstrated that complex lifecycle parasites like acanthocephalans have generally tolerant hosts with low extinction vulnerability. Even in a polluted environment, acanthocephalan parasites could prosper with their tolerant hosts. Abundances of *P. laevis* and *P. tereticollis* could be promoted by bacteriological or organic pollution in the Arc River that could foster increased abundances of their gammarid intermediate hosts and *S. cephalus* definitive hosts.

Following the "increased host abilities" of Médoc and Beisel (2008), acanthocephalan parasite could improve its survival until transmission through increasing the ability of its intermediate host. Additionally, infection by acanthocephalan parasites could be temporarily beneficial to their hosts in a polluted environment (Fanton et al., 2020; Molbert et al., 2020). *Pomphorhynchus laevis* is able both to tolerate very high concentrations of pollutants and to bioaccumulate high pollutant concentrations in freshwater ecosystems (Sures et al., 2017). For example, *S. cephalus* individuals infected by *P. laevis* were shown to exhibit lower oxidative damage compared to uninfected ones under polluted conditions (Molbert et al., 2020). Moreover, in a heavily polluted environment, *P. laevis* still manipulates the behaviour of its intermediate host (Fanton et al., 2020). During infection, acanthocephalans could slightly promote their definitive and, to a lesser extent their intermediate hosts' tolerance to polluted conditions. This relationship could be beneficial to acanthocephalans, increasing their chances of completing their lifecycles.



Finally, our results provide strong evidence that the distribution of freshwater acanthocephalans in their hosts is strongly influenced by freshwater ecological status. *Pomphorhynchus laevis*, *P. tereticollis* and *P. minutus* can survive in both unpolluted and polluted environments. Indeed, their intensities of infection and the prevalence of infected hosts varied widely according to the pollution levels at the sampled stations. High pollutant concentrations, and especially high bacteriological concentrations, provide conditions favouring increased of *P. laevis* and *P. tereticollis* intensities of infection. Contrastingly, the combined effects of high macroinvertebrate diversity, high fish diversity and good ecological indexes reduce the abundances of *Pomphorhynchus* species at their different lifecycle stages. The irregular occurrence of the three acanthocephalan species as driven by environmental quality is inseparably linked to the varying densities of their hosts, themselves dependent on ecosystem health.

## Conclusion

Intensity of infection of *P. laevis* is strongly related to their intermediate and definitive hosts availability and the local water quality. Distribution of *P. laevis* individuals in the Arc River and Luynes tributary would be first influenced by the density and viability of *G. pulex* intermediate host and definitive *S. cephalus* populations, themselves influenced by variable freshwater environmental conditions. Good ecological status assessed by the IBD, IPR and IBGN indexes were not advantageous for the proliferation of the two *Pomphorhynchus* species. Conversely *P. tereticollis* and *P. laevis* intensities of infection were positively correlated with high bacteriological pollution. Therefore, high intensities of infection of *P. laevis* and *P. tereticollis* cystacanths associated with high prevalence of infected intermediate and definitive hosts would be indicative of strong bacteriological pollution. *Pomphorhynchus laevis* species could thus benefit from this moderate freshwater pollution, which could promote the availability their tolerant intermediate and definitive hosts. Nevertheless, some aspects of acanthocephalan ecology require more attention, and others Mediterranean rivers or others polluted sites should be investigated to clarify the link between acanthocephalan species and the ecological status of freshwater ecosystems.

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## Author contributions statement

**Hadrien Fanton:** Conceptualization; Data curation; Formal analysis; Data curation; Investigation; Methodology; Writing original draft.

**Evelyne Franquet:** Writing review & editing.

**Maxime Logez:** Formal analysis; Writing review & editing.

**Laurent Cavalli:** Methodology; Writing review & editing.

**Nicolas Kaldonski:** Conceptualization; Investigation; Methodology; Writing review & editing.

### Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Hadrien FANTON reports financial support was provided by Ministère de la recherche et de l'enseignement supérieur. Hadrien FANTON reports a relationship with Labex DRIIHM that includes: funding grants.

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