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Endoparasites of chub (*Leuciscus cephalus*) in two rivers of the Abruzzo region of Italy

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Summary

The relationship between communities of chub endoparasites (*Leuciscus cephalus*) fished in the Orta and Pescara Rivers in the Abruzzo region of Italy, and the quality of the water in which they are caught, were studied in surveys designed to evaluate the feed quality of fish in the inland waters of the Abruzzo. Samples were taken monthly from October 2000 to September 2001 in the Orta River (Buscesi District) and the Pescara River (near the Villareia bridge); a total of 86 chub were caught. During periods of low and moderate flow in both rivers, benthonic macro-invertebrates were sampled at the fish sampling sites to classify the water quality using the extended biotic index (EBI) method. The Orta River was moderately polluted and the Pescara River slightly more polluted than the Orta. A parasitological study of the fish was conducted using conventional methods. A morphological study of the parasites led to the identification of seven species of endoparasites. Five of these (*Allocreadium isoporum*, *Caryophyllaeus brachycollis*, *Caryophyllaeides fennica*, *Rhabdocona denudata* and *Pomphorhyncus laevis*) were found at both sampling sites, while *Acanthocephalus clavula* was found only in the Pescara River and *Neoechinorhynchus rutili* was found only in the Orta River.

The differences in epizootological indexes (prevalence, abundance and mean intensity) and the biotic indexes (Margalef, Shannon, Evenness, Simpson and Berger-Parker) suggested that the structure of the communities of chub endoparasites in the two rivers is a less sensitive biological indicator than benthic macroinvertebrates, because it does not detect small variations in water contamination.

Keywords

Benthic macroinvertebrates, Bioindicators, Chub, Fish, Italy, *Leuciscus cephalus*, Parasites, Pollution.

Introduction

Free-living organisms, such as benthic macroinvertebrates, have long been used as bioindicators of the quality of water and their use is validated by the literature (1, 11, 14, 18).

Due to the complexity of their biological cycles, parasites can be used as indicators of the health status of an ecosystem. Endoparasites are particularly interesting in this respect because the completion of their biological cycle requires interactions with several host vertebrates and invertebrates, and the effects on each of the hosts differ according to the pollution level of the habitat in question (13).

The diversity of fish parasite species is directly correlated to variations in the biodiversity of the

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surrounding environment. According to some authors, it can consequently be considered a highly sensitive indicator of alterations in polluted aquatic ecosystems (4, 5, 6, 7, 8, 10, 12, 13, 15, 17).

Some authors recently studied chub endoparasite communities in four rivers of northern Italy, all with varying degrees of pollution, and discovered a correlation between the degree of pollution and the parasites found in the species of fish examined (7). This finding is significant if the epizootiological and biotic indexes of unpolluted and heavily polluted rivers are compared. However, this correlation is not observed in environments with a moderate level of pollution (6, 7).

The purpose of this study was to investigate the species of chub endoparasites present and to establish whether any correlation exists between the structure of the endoparasite communities found in the species of fish examined and the different degrees of pollution of the two Abruzzo rivers.

Materials and methods

From October 2000 to September 2001 a total of 86 chub were sampled, 40 of which were caught in the Pescara River at the Villareia bridge and 46 in the Orta River in the Buscesi District (Fig. 1).

The fish species *Leuciscus cephalus* was selected due to its restricted home range and tolerance of chemical and physical pollution of the waters in which it lives (9).

The sampling sites were chosen on the basis of the presence of wild fish species and the different degrees of pollution of the water. Native chub are recorded on the fish map of the Pescara Province (19), both in the Orta River (class II of the extended biotic index [EBI]) and in the Pescara River (class III of the EBI).

The fish were caught using an electrostun device (model IG 200/2). The chub were sampled monthly for a year to study the existence of seasonal fluctuations in the number of parasites associated with their biological cycle and with the life-cycle of the fish.

For each specimen of *Leuciscus cephalus* examined, we measured the total length, standard length and weight to calculate the condition factor 'k' ($k=wt\ 10^5/l^3$, where 'wt' is the weight expressed in grams and 'l' is the standard length expressed in millimetres). This factor effectively determines the condition of the fish and consequently its nutritional status and the availability of feed in the environment. Therefore, it indicates whether the fish is likely to be infested with heteroxenous parasites through the ingestion of their intermediate hosts. The arithmetical means of factor k were also calculated for the fish caught in the two rivers, and subsequently compared using Student's *t* test to evaluate whether the difference in availability of feed may have significantly influenced the community of parasites studied.

The anatomopathological and parasitological examinations of the organs of the fish were performed using conventional methods. The parasites were identified on the basis of their morphological characteristics, with the aid of an

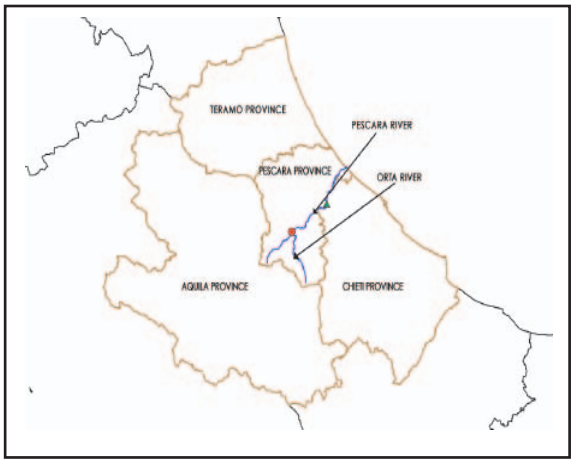

Figure 1
Map of sampling points in the rivers of the Abruzzo region

optical microscope with enlargements of 10x, 40x and 100x, equipped with a camera lucida. The prevalence, abundance and mean intensity indexes according to Bush *et al.* (2) were calculated for the parasites in the two rivers.

The prevalence and abundance of the individual parasites in the two rivers were statistically compared with Student's *t* test, and the mean intensities with the Mann-Whitney non-parametric test (3).

The differences between the two rivers were statistically evaluated with the Mann-Whitney non-parametric test in regard to prevalence, abundance and intensity values for the parasites as a whole (3).

A significance level of $p = 0.05$ was used for all statistical tests. Finally, the values of the Margalef (wealth of species), Shannon (diversity), Evenness (equal distribution), Simpson and Berger-Parker (dominance) biotic indexes were calculated to compare the structures of the two communities of endoparasites (16).

Benthic macroinvertebrates were also sampled during periods of low and moderate water flow at the fish sampling sites in the rivers to classify the quality of the waters according to the *Istituto per la Ricerca sulle Acque-Centro Nazionale Ricerche* (IRSA-CNR) EBI method so as to determine the different levels of pollution in the rivers (14).

Results

The parasitological examination of the stomach, intestine, liver and kidneys of the fish indicated the presence of seven species of intestinal parasites.

The following species of parasites were identified in the Pescara and Orta Rivers: *Allocreadium isoporum* (Fig. 2), *Caryophyllaeus brachycollis* (Fig. 3), *Caryophyllaeides fennica* (Fig. 4), *Rhabdocona denudata* (Fig. 5) and *Pomphorhyncus laevis* (Fig. 6). *Neoechinorhynchus rutili* (Fig. 7) was found only in the Orta River and *Acanthocephalus clavula* (Fig. 8) was found only in the Pescara River.

Figure 2
Allocreadium isoporum from the intestines of Leuciscus cephalus
(carmine red, 5x)

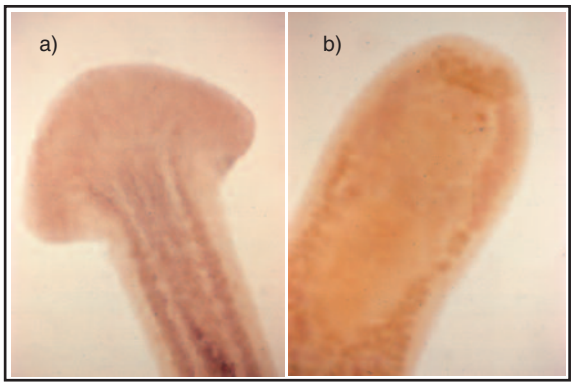

Figure 3
Caryophyllaeus brachycollis from the intestines of Leuciscus cephalus
a) Front extremity (lactophenol clarification, 5x)
b) Rear extremity (lactophenol clarification, 5x)

Upon analysis of the benthic macrovertebrates at the fish sampling sites, the Orta River proved to be moderately polluted (class II-III), and the Pescara River slightly more polluted (class III-IV).

Table I presents the benthic macroinvertebrates harvested at the fish sampling sites during periods

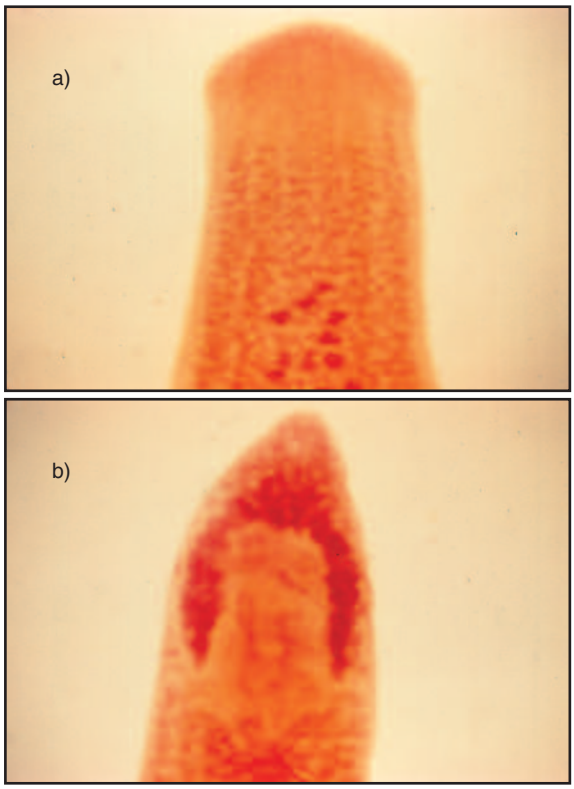

Figure 4
Caryophyllaeus fennica from the intestines of Leuciscus cephalus
a) Front extremity (lactophenol clarification, 5x)
b) Rear extremity (lactophenol clarification, 5x)

Figure 5
Front extremity of Rhabdocona denudata from the intestines of Leuciscus cephalus
(lactophenol clarification, 5x)

Figure 6
Front extremity of Pomphorhyncus laevis from the intestines of Leuciscus cephalus
(lactophenol clarification, 5x)

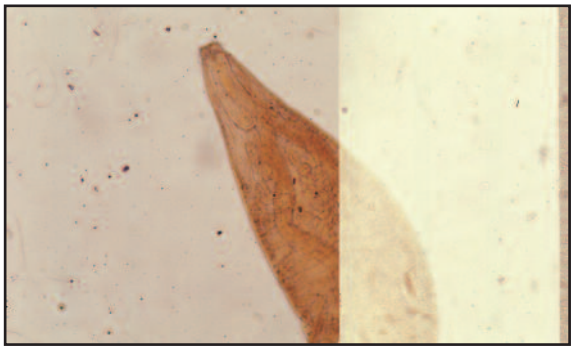

Figure 7
Front extremity of Neoechinorhynchus rutili from the intestines of Leuciscus cephalus
(lactophenol clarification, 5x)

Figure 8
Front extremity of Acanthocephalus clavula from the intestines of Leuciscus cephalus
(lactophenol clarification, 5x)

Table I
Benthic macroinvertebrates sampled during periods of low and moderate flow in the Pescara and Orta Rivers

Organism	Genus or family	Pescara (low water)	Orta (low water)	Pescara (moderate flow)	Orta (moderate flow)
Plecoptera	*Leuctra*	+	+	–	–
Ephemeroptera	*Baetis*	+	+	+	+
	Heptagenia	–	–	–	+
	Ecdyonurus	+	+	–	+
	Caenis	+	+	–	–
	Habrophlebia	–	+	–	–
	Ephemerella	–	+	–	–
Trichoptera	Hydropsychidae	–	+	+	+
	Hydroptilidae	–	+	–	–
	Philopotamidae	–	+	–	+
	Limnephilidae	–	+	–	–
	Polycentropodidae	–	+	–	–
	Rhyacophilidae	–	+	–	+
Coleoptera	Gyrinidae	–	–	–	+
	Elmidae	–	+	–	+
	Hydrophilidae	+	–	–	–
	Haliplidae	+	–	–	–
Odonata	*Colopterix*	–	–	–	+
	Onychogomphus	–	+	–	–
Diptera	Simuliidae	+	+	+	+
	Chironomidae	+	+	+	+
	Tipulidae	+	–	–	+
	Athericidae	–	–	–	+
Heteroptera		–	–	–	–
Crustacea	Asellidae	+	–	+	–
	Gammaridae	+	–	+	+
Gasteropoda	Acroloxidae	+	–	–	–
	Bithyniidae	+	–	–	–
	Ancylidae	+	+	–	–
	Lymnaeidae	–	+	–	–
Bivalvia	Pisidiidae	–	–	+	–
	Sphaeriidae	+	–	–	–
Tricladida	*Planaria*	–	–	+	–
	Dugesia	+	–	–	–
Hirudinea	*Dina*	+	–	+	–
Oligochaeta	Lumbricidae	+	+	+	–
	Tubificidae	–	–	+	–

– absence
+ presence

Table II
Standard length, weight and condition factor of chub sampled in the Pescara and Orta Rivers

Leuciscus cephalus	Pescara River	Orta River
Standard length (cm)		
Min-max	18-29	13.5-37.5
Mean	21.90	21.49
Standard deviation	3.06	5.56
Weight (g)		
Min-max	91-425.5	63-1 000
Mean	231.82	236.95
Standard deviation	119.07	213.47
Condition factor (k)		
Min-max	1.4-2.5	1.6-2.9
Mean	2.05	1.94
Standard deviation	0.29	0.24

of low and moderate flow.

The values of the standard length, weight, condition factor k of the fish, together with the mean values and standard variation are set out in Table II.

The arithmetical means of condition factor k do not exhibit statistically significant differences (t=–1.84; p=0.06), which means that trophic availability was the same for the fish sampled at both sites.

Table III shows the prevalence, abundance and mean intensity (with minimum and maximum values) of the parasite species at the two sampling sites. A statistical comparison of the individual parasite species found in the two rivers shows significant differences between the prevalence of *R. denudata* (t=–2.29; p=0.024), *N. rutili* (t=–2.84; p=0.007), *P. laevis* (t=2.97; p=0.004) and *A. clavula* (t=4.33; p=0.000), the abundance of *R. denudata* (t=–2.66; p=0.011) and *A. clavula* (t=2.83; p=0.007) and the mean intensity of *R. denudata* (Z = –2.55; p = 0.011). The epizootological indexes for the two rivers compared do not indicate any statistically significant differences (prevalence: Z = –0.26; p = 0.798; abundance: Z= –1.28; p = 0.201; mean intensity: Z = –1.41; p = 0.159).

The values of the Margalef (wealth of species), Shannon (diversity), Evenness (equal distribution), Simpson and Berger-Parker (dominance) biotic indexes are presented in Table IV.

The diversity values of parasitic species and the distribution of individuals between species increased in proportion to the degree of contamination of the waters, from the more polluted site (Pescara) to the less polluted site (Orta), which also presents lower dominance values and consequently greater species biodiversity (16). The values for wealth of species, highest in the more polluted river, were not correlated with pollution, however (D_{Mg} Pescara = 0.95; D_{Mg} Orta = 0.83).

Discussion

The results of this survey conducted in the Orta and Pescara Rivers demonstrate that the quality of the water deteriorated from 1998, date of the

Table III
Prevalence, abundance and mean intensity of endoparasites of chub sampled in the Pescara and Orta Rivers

Endoparasite	Pescara					Orta				
	Fish infested	Total parasites	P	A	Mean I (min-max)	Fish infested	Total parasites	P	A	Mean I (min-max)
Allocreadium isoporum	3	3	7.5	0.075	1 (1-1)	1	23	2.2	0.5	23 (23-23)
Caryophyllaeides fennica	1	8	2.5	0.2	8 (8-8)	3	14	6.52	0.3	4.7 (2-7)
Caryophyllaeus brachycollis	5	10	12.5	0.25	2 (1-3)	3	152	6.52	3.3	50.7(1-134)
Rhabdocona denudata	5	5	12.5	0.125	1 (1-1)	15	58	32.6	1.26	3.87 (1-14)
Pomphorhynchus laevis	28	131	70.0	3.2	4.7 (1-37)	18	139	36.9	3.0	8.1 (1-66)
Neoechinorhynchus rutili	0	0	0	0.0	0.0	7	31	15.2	0.7	4.4 (1-22)
Acantocephalus clavula	13	36	32.5	0.9	2.8 (1-7)	0	0	0.0	0.0	0.0

P prevalence
A abundance
Mean I mean intensity

last Pescara Province survey (19), to 2001, although a fairly large number of parasitic species with an indirect biological cycle were found in both rivers. The higher prevalence, abundance and mean intensity values for the nematode *R. denudata* found in the less contaminated Orta River are dependent on the greater presence of the intermediate host required to complete the biological cycle of the parasite.

Ephemeroptera larvae were present in abundance in the Orta River, but rare in the Pescara River. Unlike *R. denudata*, the prevalence of *P. laevis* was

Table IV
Biotic index values of the structure of parasite communities of chub in the Pescara and Orta Rivers

River	Margalef index D_{Mg} (wealth of species)	Shannon index H (diversity)	Evenness index E (equal distribution)	Simpson index D (dominance)	Berger Parker index D (dominance)
Pescara	0.95	1.02	0.57	0.50	0.68
Orta	0.83	1.48	0.82	0.27	0.36

greater at the more polluted site in the Pescara River where the intermediate host *Echinogammarus stammeri*, highly sensitive to pollution, was found in greater numbers than at the Orta site. The different distribution of the acanthocephalia *N. rutili* and *A. clavula* can be explained by the different situation in the areas of distribution of these species.

The EBI values for the parasite communities in the two rivers confirm the sensitivity and influence of water quality on the structure of the fish endoparasite communities.

The statistically insignificant differences in the epizootiological indexes and the biotic index values support the theory of Galli (6), according to whom fish parasite communities in rivers with a moderate degree of contamination show a weaker correlation with the degree of water contamination than those living in highly polluted rivers.

In view of the results obtained, it can be concluded that although fish parasite communities are influenced by water quality, they are less sensitive biological indicators of pollution than benthic macroinvertebrates because small variations in contamination have little effect on them and consequently do not allow rapid detection of increased deterioration of an aquatic ecosystem .

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