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Parasites of *Anguilla anguilla* (L.) from three coastal lagoons of the River Ebro delta (Western Mediterranean)

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Abstract

Data are presented on the parasite fauna of the European eel, *Anguilla anguilla* (L.) in three coastal lagoons of the Ebro delta (NE Spain). Ten parasite species were found, four protozoans: *Eimeria anguillae*, *Trichodina pediculus*, *Myxidium giardi* and *Myxobolus* sp.; five helminths: *Pseudodactylogyrus anguillae*, *Deropristis inflata*, *Bothriocephalus claviceps*, *Proteocephalus macrocephalus*, *Anguillicola crassus* and one crustacean: *Ergasilus gibbus*. The highest prevalences were reported for *P. anguillae* 84.6%, *M. giardi* 44.4% and *A. crassus* 30.8%. Helminth communities were dominated by monogenean *P. anguillae*. These communities were compared with those from other European coastal lagoons.

Key words

Protozoan and metazoan parasites, fish, *Anguilla anguilla*, Ebro delta lagoons, Spain

Introduction

The Ebro delta (NW Spain) is the second largest wetland zone in Spain and is an ideal area for the practice of any type of marine aquaculture. Water reaching the delta through the Ebro is rich in nutrients, and water temperatures are high, this results in a high level of primary production and rapid growth rates (Castelló 1993). One of the species best suited to commercial farming is the European eel. In addition to being a species native to the region, its ability to live in seawater, brackish water or fresh water means that it is widely distributed in the Ebro delta (Demestre *et al*. 1977).

Although eels are widespread and abundant throughout the lagoons around the Mediterranean coasts, little is known about the parasite fauna of eels from saline lagoons (Køie 1988, Kennedy *et al*. 1997, Di Cave *et al*. 2001). Most studies of helminth communities of European eel have been carried out in freshwater locations (Seyda 1973, Moravec 1985, Kennedy 1990, Kažić *et al*. 1982, Schabuss *et al*. 1997, Sures *et al*. 1999).

The composition and structure of helminth parasite communities in eels have been studied by Seyda (1973), Kažić *et al*. (1982), Moravec (1985), Orecchia *et al*. (1987) and Køie (1988). Studies of helminth composition in relation to environmental salinity have also been performed (Seyda 1973, Køie 1988, Wierzbicka and Orecka-Grabda 1994). The most detailed analyses were carried out by Kennedy *et al*. (1997) and Di Cave *et al*. (2001) on eels from lagoons on the Tyrrhenian and Adriatic Sea coasts. Benajiba (1991) studied protozoan and metazoan parasites of eels in the coastal lagoon of Maugió (Languedoc, France).

Until now, there have been few reports on the parasites of European eels from the Ebro delta coastal lagoons (Gracia *et al*. 1995, Maíllo *et al*. 2001), these focused on faunistic aspects and do not provide sufficient data to undertake a comparative analysis of parasite communities. The present study was undertaken to fill this gap and forms part of an ongoing investigation into the parasites of Ebro delta eels. We present results on the composition and structure of parasite communities in eels from three brackish lagoons in the Ebro delta and compare it with data from other Mediterranean coastal lagoons.

Materials and methods

A total of 216 eels (25–57 cm length, mean length 34.15 ± 0.37 cm; 27.8–334.4 g weight, mean weight 75.48 ± 2.45 g)

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was examined between October 1999 and March 2000. Eels were sampled in 3 lagoons within the Ebro delta: Encanyissada – with a surface area of 5.5 km^2 this is the largest lagoon in the delta. Mean depth is 50 cm and the maximum is 1 m. Salinity ranges between 3 and 30‰. Tancada – this small lagoon has a surface area of 1.8 km^2. Mean depth is 37 cm and the maximum is 75 cm. The salinity ranges between 8 and 36‰. Canal Vell – it has a surface area of 4 km^2. Depth ranges from 30 to 50 cm and salinity varies between 8 and 30‰.

All three lagoons are shallow, connected to Mediterranean Sea by channels and they have some freshwater input that can be important. These lagoons were selected as being representative of the Spanish north-east coast lagoons. Eels were collected in eel traps once a month, brought back to the laboratory alive and then examined immediately for parasites. The skin surfaces and exterior cavities of the specimens were observed. Organs in the body cavity were examined for the presence of parasites visible to the naked eye. Organ samples were processed to detect small parasites and describe possible pathologies caused by them. Samples were embedded in paraffin and 4–5 µm sections were prepared. These were stained with haematoxylin-eosin for microscopic observation. Parasites were preserved in 4% formol. Various techniques were used for the identification of the different parasites found (Giemsa, haematoxylin, methylene blue and preparation in glycerinated gelatine). Some specimens were processed for scanning electron microscopy as in Gracia *et al*. (1997).

Prevalence and abundance were used according to Bush *et al*. (1997). Measures of metazoan species component community structure were species richness, Shannon-Wiener index and its evenness and Berger-Parker dominance index. Similarities were measured using the Sörensen's index. All indices were defined as in Magurran (1988) using natural logs (ln) where appropriate.

Results

Composition of the parasite community

Data on the composition of the helminth communities of the eels are summarized in Table I. Ten parasite species were identified. *Eimeria anguillae* oocysts were inserted between the enterocytes on the surface of the intestinal mucous membrane of the posterior segment of the intestine. No tissue reaction or pathogenic effects were observed in the area where the parasites were found. Prevalence range 0.0–32.2%. *Trichodina pediculus*, reported for the first time in the Ebro delta, causes irritation to the gills. It was disc-shaped and had an hourglass form when viewed laterally. This ciliate was only observed on several large eel specimens. *Myxidium giardi* forms oval plasmodia in the gills and, when infection is large, in the kidneys. Prevalence range: kidney 2.78–60.0%; gills 10.0–32.2%. *Myxobolus* sp. was found in kidney, but free spores of this parasite were in the kidney parenchyma. The spores were spherical, ovoid or ellipsoid (somewhat flattened parallel to the sutural plane, with two pyriform polar capsules below the sutural line). The mean length of the spores found was 7.53 ± 1.72 µm. The number of cysts was variable, but generally, they were numerous. Prevalence range 0.0–25.3%. We did not observe any type of inflammatory reaction in both myxosporidian species.

Myxidium giardi and *E. anguillae* are eel specialists, whereas *T. pediculus* is a generalist and the status of *Myxobolus* sp. remains unknown.

All metazoan species were eel specialists. *Pseudodactylogyrus anguillae* was attached to the gill lamellae. It has an elongated, dorsoventrally flattened body. Prevalence range 38.9–100%. High prevalences of this species were characteristics of all three lagoons. *Deropristis inflata* is a parasite with an elongated body, the anterior part of which is ventrally con-

Table I. Characteristics of the parasitic fauna of eels from the Ebro delta lagoons

		Lagoons					
		Encanyissada		Tancada		Canal Vell	
Lagoon area (km^2)		5.5		1.8		4.0	
Lagoon salinity range (‰)		3–30		8–36		8–30	
Parasites	Organ	%	A	%	A	%	A
Eimeria anguillae	I	9.06	N.D.	7.69	N.D.	0	N.D.
Myxidium giardi	G	15.86	N.D.	15.4	N.D.	25.00	N.D.
Myxidium giardi	K	44.38	N.D.	20.5	N.D.	2.78	N.D.
Myxobolus sp.	K	14.72	N.D.	7.69	N.D.	0	N.D.
Trichodina pediculus	G	0.02	N.D.	0	N.D.	0	N.D.
Pseudodactylogyrus anguillae	G	75.9	21.25 (29.93)	84.62	14.08 (19.69)	35.9	1.26 (3.55)
Deropristis inflata	I	14.9	0.57 (4.56)	41.03	2.31 (10.77)	23.1	1.13 (4.72)
Proteocephalus macrocephalus	I	2.1	0.03 (0.58)	0	0	5.1	0.23 (0.71)
Bothriocephalus claviceps	I	1.4	0.04 (1.41)	0	0	0	0
Anguillicola crassus	SB	21.3	1.23 (5.69)	25.64	1.97 (10.03)	30.8	0.72 (1.15)
Ergasilus gibbus	G	0	0	0	0	7.7	0.59 (8.33)

A – abundance (SD), G – gills, I – intestine, K – kidney, N.D. – number of parasites not determined, % – prevalence, SB – swimbladder.

cave and laterally widened. It has a spiny tegument and spines on the margin of the lateral expansions. Prevalence range 6.7–43.6%. Both trematode species were found in the gut. Cestodes, *Proteocephalus macrocephalus* and *Bothriocephalus claviceps*, were detected in gut. In some cases, as many as six specimens occurred in a single host. *P. macrocephalus* prevalence range 0.0–5.56%, *B. claviceps* prevalence range 0.0–9.6%. The dracunculoid nematode *A. crassus* is a haematophagous parasite that lives in the swimbladder. Specimens observed immediately after extraction present a dark colouring as a result of the blood ingested. L_3 stages were observed in the swimbladder wall, as well as adults and embryonated eggs in the swimbladder lumen. The presence of these parasites causes strong inflammatory reactions in the swimbladder, dilation of blood vessels, and fibrosis in the bladder, intestine and other organs. All stages prevalence range 6.7–46.1%. The ectoparasite species, *Ergasilus gibbus*, was found attached to the gill lamellae by their second antennae. Significant damage to the gills was observed, with serious haemorrhaging, inflammation and blood loss associated with the attachment and feeding of the parasite. Prevalence range 0.0–43.5%.

Most parasites found were freshwater species (*T. pediculus*, *E. anguillae*, *M. giardi*, *Myxobolus* sp., *P. anguillae*, *B. claviceps*, *P. macrocephalus* and *A. crassus*). The remaining species were marine or euryhaline species. *P. anguillae* and *A. crassus* are invader species, introduced to Europe from the Far East with Japanese eels (Køie 1988).

Characteristics of community structure

Diversity characteristics of the helminth component communities are summarized in Table II. Encanyissada and Canal Vell lagoons showed identical helminth species richness, whereas the Tancada lagoon species richness was lower. This lower species richness at Tancada is basically due to absence of Cestoda species in this lagoon. Diversity (Shannon-Wiener index) in the Canal Vell lagoon was higher than in Encanyissada or Tancada.

Nevertheless, it is worth emphasizing that diversity in Tancada almost reaches its theoretical maximum value, because all species present in the lagoon were showing a similar number of individuals. The evenness was nearly the same in Tancada and Canal Vell, offering high values that indicate a relatively similar abundance in all species present. In contrast, Encanyissada evenness is notably lower, and suggests very different species abundances. Evident differences in the Berger-Parker index were observed between the three lagoons. Encanyissada reaches its maximum (very close to 1), showing clear dominance on the part of a single species, whilst Canal Vell reaches its minimum, showing high evenness. The increase in the dominance index (Berger-Parker index) in Encanyissada lagoon is commensurate with the lower diversity index at that site and is clearly related to the lower prevalence and abundance values for four of the five species found there. However, in spite of the noteworthy differences in this index, the eel specific parasite *P. anguillae* is the dominant species in all three lagoons. Levels of total component similarity between the three lagoons on a qualitative basis (Sörensen's index) are high (0.67 Encanyissada vs. Tancada and Tancada vs. Canal Vell, 0.80 Tancada vs. Canal Vell) and have a similar order of magnitude, indicating considerable similarity. Much of the similarity is due to the presence of three species (*P. anguillae*, *D. inflata* and *A. crassus*) in all of the lagoons and to the domination of each by *P. anguillae*.

Table II. Characteristics of the helminth communities of eels of the three Ebro delta lagoons

	Lagoon		
	Encanyissada	Tancada	Canal Vell
No. of eels	141	39	36
No. of species	5	4	5
Shannon-Wiener index	0.98	1.13	1.41
Shannon-Wiener evenness	0.61	0.82	0.88
Berger-Parker index	0.92	0.75	0.32
Dominant species	*P.a.*	*P.a.*	*P.a.*

P.a. – Pseudodactylogyrus anguillae.

Discussion

Most studies on eel parasites dealt with metazoan parasites; protozoans have received comparatively little attention. In our study four protozoan species were recorded whereas Benajiba (1991) found in eels from coastal lagoons of Languedoc eight species.

The apicomplexan *E. anguillae* can be, in some cases, highly prevalent (32.2% in Encanyissada lagoon); equally high prevalences were described by Benajiba (1991) with 40.62% in the Mauguió lagoon.

Myxozoan *M. giardi* occurred in all the analyzed samples. The prevalence of *M. giardi* was high, reaching up to 60.0% in the kidney. This result is significantly higher than the 18.61% reported by Benajiba (1991). Since *M. giardi* do not seem to have preferences for a specific salinity (Køie 1988), absence of the annelid that acts as intermediate host in Languedoc lagoons could explain difference.

Helminth communities of eels in the studied Ebro delta lagoons, similarly to coastal Italian lagoons (Kennedy *et al*. 1997, Di Cave *et al*. 2001), comprise both freshwater and marine or euryhaline helminth species. Prevalence and abundance of the freshwater species in Ebro delta lagoons must relate to the individual conditions in the lagoon and especially to the water management regime and the extent of the freshwater input.

The monogenean *P. anguillae* is the most prevalent parasitic organism in Ebro delta lagoons (84.6% in Encanyissada lagoon). Kennedy *et al*. (1997) only described the presence of *P. anguillae* in the Burano lagoon with 33.7% prevalence,

whereas Di Cave *et al*. (2001) detected its presence in two Adriatic lagoons (Valle Figheri, 54.5% and Acquatina, 4.8%). In the coastal Pond of Biguglia (Corsica, France), *P. anguillae* prevalence was only 15% (Caillot *et al*. 1999). Differences in salinity levels could explain differences in prevalence.

Regarding two cestode species, *P. macrocephalus* and *B. claviceps*, the observed prevalences have been, in both cases, much lower than in the aforementioned Mediterranean coastal lagoons. Thus, *P. macrocephalus* shows 8.5% prevalence in the Burano lagoon (Kennedy *et al*. 1997), and 9.1% in Adriatic lagoons (Di Cave *et al*. 2001). The difference is very clear in *B. claviceps*, as Caillot *et al*. (1999) detected 20% prevalence in Corsica.

The prevalence range showed by the digenean *D. inflata* overlaps, to a large degree (Table I), with ranges reported in other studies; 32.1–44.7% in the Tyrrhenian (Kennedy *et al*. 1997); 19.0–93.9% in the Adriatic (Di Cave *et al*. 2001). However, it is much higher than the 1.3% found in Languedoc by Benajiba (1991). Absence of the intermediate host, mollusc *Hydrobia* sp. in Languedoc lagoons could explain differences, since *Hydrobia* sp. prefers unsalted waters (Altunel 1974).

The nematode *A. crassus* occurred in eels captured in all of the delta lagoons, but also in eels captured in marine areas of the delta (Gracia *et al*. 1995). That is to say that this parasite can survive with highly diverse levels of salinity, just as observed Kirk *et al*. (2000) under experimental conditions. *A. crassus*, which has a maximal prevalence of 43.5% (Maíllo *et al*. 2001), can lead to alterations in host physiological functions due to the large number of parasites that can affect the swimbladder (more than 15 in our case). *A. crassus* is one of the most frequently detected parasites in Mediterranean coastal lagoons, always showing relatively high prevalences (Benajiba 1991, Kennedy *et al*. 1997, Caillot *et al*. 1999).

The copepod *E. gibbus* was only present in Canal Vell lagoon (prevalence 7.7%). This species *E. gibbus*, leads to commercially significant problems in farmed fish and natural populations (Woo 1995).

Existing differences in eel parasitofauna from the Ebro delta compared with Italian lagoons could be due to salinity differences between the studied lagoons. Salinity in Italian lagoons range (15–42‰) in Adriatic (Di Cave *et al*. 2001) and (10–48‰) in Tyrrhenian (Kennedy *et al*. 1997), whereas in Ebro delta salinity range (3–36‰). Thus, Ebro delta lagoons can reach lower and higher salinity levels than Italian ones. Difference in frequency and volume of the freshwater inputs could also be important. These differences may be related to the greater or lesser abundance of intermediate hosts; many of the parasites studied have heteroxenous life cycles and require the presence of an intermediate host for their development.

Helminth communities in the Ebro delta lagoons were characterized by high prevalence of *P. anguillae*, *D. inflata* and *A. crassus* (Table I).

Specific richness range (4–5) was comparable with the Tyrrhenian lagoons (3–8) (Kennedy *et al*. 1997) and lower than in Adriatic lagoons (6–11) (Di Cave *et al*. 2001). In Ebro delta, Tancada lagoon was the most species poor, basically due to absence of cestode species in this lagoon. Composition and abundance of suitable intermediate hosts in a locality may contribute to the distribution and infection levels of cestodes (Nie and Kennedy 1991).

Values of Shannon-Wiener diversity index (0.98–1.41) were relatively high and showed a similar range to Italian lagoons (Tyrrhenian 0.86–1.34, Kennedy *et al*. 1997; Adriatic 0.85–1.45, Di Cave *et al*. 2001). In the Ebro delta diversity was higher in Canal Vell (1.41), that showed equal species richness that Encanyissada lagoon, due to the moderate abundance of *P. anguillae* in this lagoon, circumstances which substantially raise the Shannon-Wiener evenness index.

Helminth communities of eels in the Ebro delta comprise a higher rate of freshwater species (66.7%) than coastal Italian lagoons, both in Tyrrhenian Sea (50.0%) (Kennedy *et al*. 1997) and in Adriatic Sea (35.7%) (Di Cave *et al*. 2001). Lower salinity levels that Ebro delta lagoons can reach (Table I) could explain differences. Helminth communities in Ebro delta were characterized by high prevalences of parasites specific to eels – *P. anguillae*, *D. inflata* and *A. crassus*. Monogenean *P. anguillae* was the dominant species in all Ebro delta lagoons, contrasting with the aforementioned Italian lagoons, where digeneans dominated (*D. inflata* and *Bucephalus polymorphus*) (Kennedy *et al*. 1997, Di Cave *et al*. 2001), and with Languedoc where the nematode *A. crassus* was the dominant species (Benajiba 1991).

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