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Histological survey of symbionts and other conditions in razor clam *Ensis arcuatus* (Jeffreys, 1865) (Pharidae) of the coast of Galicia (NW Spain)

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ABSTRACT

Symbionts and abnormal conditions of razor clam *Ensis arcuatus* were surveyed in three commercially important natural beds of Galician estuaries (NW Spain). Samples of 15–20 *E. arcuatus* were collected every 2 months from January 2003 until July 2004 and processed for histological examination. Prokaryote-like colonies, renal coccidians, gregarines, *Trichodina* sp. ciliates, haplosporidian-like plasmodia, turbelaria, trematode metacercariae, cestode-like larvae and basophilic inclusion bodies were observed in razor clam tissues without causing host damage. Bucephalid digenean sporocysts and germinoma were seen in some samples causing moderate or severe damage to the host depending on the intensity of infection and both could be a cause for concern if prevalence reached epizootic levels in Galician *E. arcuatus* populations. None of the parasites detected is OIE notifiable and, in general, the commercially exploited beds studied seem to be devoid of serious pathogens.

1. Introduction

The razor clam, *Ensis arcuatus* (Jeffreys, 1865) (Pharidae) is a siphon feeder that lives in burrows in the substrate of the intertidal and subtidal zones from Norway to Spain, and along the British coast (Hayward and Ryland, 1998). In Spain it is found in the North littoral coast, especially in Galicia, where it is an important shellfish resource. Production in the last 2 years was approximately 408 tons, valued at 5.3 million euros (data from Plataforma Tecnolóxica da Pesca, Consellería de Pesca e Asuntos Marítimos). The most important natural beds, responsible for the majority of this production, are located in the SW coast of Galicia.

The expanding commercial harvest of this mollusc called for the development of management strategies based on a solid biological knowledge. Growth, reproductive cycle and biochemical composition of *E. arcuatus* have been studied (Robinson and Richardson, 1998; Darriba, 2001; Darriba et al., 2004, 2005). Nevertheless, prior to this paper, no comprehensive pathological study on this species had been completed, although studies documented the occurrence of *Nematopsis* sp., *Chlamydia*-like and trematode in *E. arcuatus* from Ireland (Fahy et al., 2002); and germinoma, *Nematopsis* sp. and trematode in *E. arcuatus* from Galicia (Darriba, 2001; Darriba et al., 2006). Previous studies reported in other species of the superfamilia Solenacea the occurrence of prokaryote as *Rickettsia* and *Chlamydia*-like organisms (Elston, 1986; Ceschia et al., 2001); protistan organisms as ciliates (Xu et al., 1999), gregarines (Bilei et al., 1997; Ceschia et al., 2001; Darriba, 2001), paramyxeans belonging to the genus *Marteilia* (Ceschia et al., 2001; López and Darriba, 2006; López-Flores et al., 2008), haplosporidians (Bilei et al., 1997; Ceschia et al., 2001) and metazoan organisms as larval stages of trematodes (Ceschia et al., 2001; Chai et al., 2001; Darriba, 2001).

The present study describes symbiotic organisms and other conditions affecting razor clams *E. arcuatus* from major populations of Galicia. This information should be useful for the management of this fishery resource.

2. Material and methods

2.1. Beds studied

This study was conducted in three *E. arcuatus* natural beds (Fig. 1): Rodas, San Martiño and Means. Rodas (42°13.5′N,

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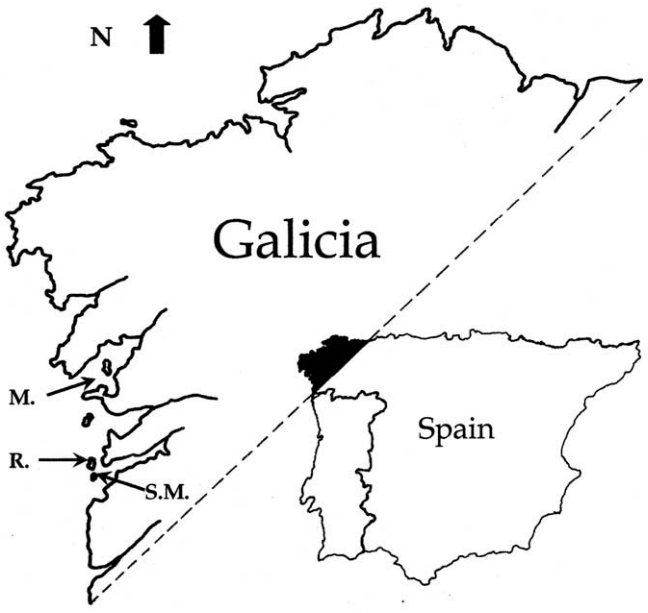

Fig. 1. Map of Galicia (NW of Spain) showing the location of *Ensis arcuatus* beds sampled. R. (Rodas) and S.M. (San Martiño) in Ría de Vigo and M. (Means) in Ría de Arousa.

8°54′W) and San Martiño (42°12.1′N, 8°54′W) are two beaches located in Rodas Island and San Martiño Island (Cíes Islands archipelago) in the outer of Ría de Vigo, approximately 2.5 km apart. From these two beaches important subtidal beds with an area of 228,800 and 117,800 m^2, respectively, are harvested. Means (42°30.3′N, 8°50′W) is an intertidal bed of 405,300 m^2, only being exposed during low water spring tides and located close to the mouth of the Umia River in the middle of Ría de Arousa.

2.2. Samples

Samples of 15–20 commercial razor clams (>100 mm in length) were collected, by diving, every 2 months, between January 2003 and July 2004, from Rodas and San Martiño and, by foot, from Means. The only deviation from this schedule occurred in Means during 2003, when logistical and technical problems delayed the March sampling until April, and in May when it was impossible to take samples.

2.3. Processing

Once in the laboratory, the valves were separated and gills, mantle and visceral mass were examined macroscopically for evidence of macroparasites, lesions, shell chambers, abnormal coloration and other malformations. Sometimes macroscopic examination of molluscs revealed heavy infections by trematode sporocysts and living sporocysts were dissected. Spontaneously shed cercariae were wet mounted and analysed under the light microscope. Pieces of digestive gland, gonad, kidney, foot, mantle lobes, labial palps and gills were taken from every sampled specimen, fixed in Davidson's solution (Shaw and Battle, 1957) and embedded in paraffin. Paraffin blocks were sectioned at 5 μm with a microtome. Tissue sections were deparaffinized, stained with Harris' hematoxylin and eosin and examined by light microscopy for symbiotic organisms and other conditions. The percentage of razor clams affected by each symbiont and condition was determined for each sample. Sites were statistically compared for some symbionts and conditions by calculating the overall prevalence for the whole study period and estimating a 95% confidence interval and a critical ratio (Z) test on the difference between two independent proportions given the proportion and sample size in each sample. These tests were performed using EpiCalc 2000 software Version 1.02 (Gilman and Myatt, 1998).

3. Results

A total of 456 razor clams were analysed in this study: 165 from Rodas, 156 from San Martiño and 135 from Means. Histological examination revealed the presence of various symbionts and other conditions affecting *E. arcuatus* (Tables 1 and 2).

3.1. Prokaryote

Rounded intracellular basophilic colonies, 9.27 ± 1.18 μm of length (mean ± SD, $n = 11$), of prokaryote-like organisms were detected at low prevalences in the natural beds of San Martiño and Rodas (Table 1). They were seen in digestive tubules (Fig. 2) with the exception of one case detected in connective tissue of the kidney. Intensity of infection was low (1–5 colonies per histological

Table 1
Prevalence (%) of prokaryote and protozoan symbionts of razor clams, *Ensis arcuatus*, from different locations. Ría de Vigo (R.V.): R, Rodas; S.M., San Martiño and Ría de Arousa (R.A.): M, Means.

Date		Prokaryote			Protozoa											
		Prokaryote-like colonies			Renal coccidia			Gregarine *Nematopsis* sp.			Haplosporidian-like			Ciliates *Trichodina* sp.		
		R.V.		R.A.	R.V.		R.A.	R.V.		R.A.	R.V.		R.A.	R.V.		R.A.
		R.	S.M.	M.	R.	S.M.	M.	R.	S.M.	M.	R.	S.M.	M.	R.	S.M.	M.
2003	January	0	0	0	17	0	0	90	89	31	0	6	0	0	0	0
	March	0	0	0[a]	8	17	17[a]	93	100	13[a]	0	13	0[a]	0	0	6[a]
	May	0	0	–	17	22	–	65	68	–	0	21	–	0	0	–
	July	0	0	0	27	43	0	60	93	40	0	0	0	0	0	47
	September	0	0	0	6	9	0	35	71	53	0	50	0	0	0	0
	November	0	0	0	14	29	0	40	100	29	0	87	0	0	0	7
2004	January	0	7	0	21	8	46	87	73	67	0	20	0	0	0	0
	March	0	7	0	20	7	64	47	100	64	0	20	0	0	0	0
	May	0	7	0	40	27	8	60	100	73	0	20	0	13	0	7
	July	7	0	0	33	25	0	73	100	80	0	60	0	0	0	20
Overall prevalence		0.61	1.82	0.00	19.55	18.66	16.50	64.85	89.10	49.63	0.00	28.85	0.00	1.21	0.00	9.63
95% confidence interval		0.03	0.47	0.07	13.39	12.65	10.18	56.99	82.87	40.96	0.06	22.02	0.07	0.21	0.06	5.43
		3.84	5.64	3.45	27.51	26.50	25.40	72.00	93.34	58.32	2.84	36.73	3.45	4.77	3.00	16.22

[a] Sampling date: April 15th.