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Aggregata (Protozoa: Apicomplexa) infection in the common octopus *Octopus vulgaris* from the West Mediterranean Sea: The infection rates and possible effect of faunistic, environmental and ecological factors

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ABSTRACT

Prevalence and distribution of the coccidian parasite *Aggregata octopiana* (Protozoa: Apicomplexa) in common octopus (*Octopus vulgaris*) in the Mediterranean Spanish coasts were studied. A total of 114 octopuses were sampled from 30 geographic sectors by trawl fleet, and whitish macroscopic oocysts typical of *A. octopiana* infection were recorded in 96% of octopuses in the digestive tract and mainly in intestine and spiral caecum. The univariate analysis showed that lesion extension varied according to specific octopus, environmental and faunistic variables. A subsequent multivariable analysis indicated that the risk of macroscopic lesions in the caecum was greater in males compared to females, in octopuses living in deeper compared to shallower waters and in hauls where the crustacean *Pagurus excavatus* was present. The study provides further evidence of the abundance of *A. octopiana* in octopus ecosystems urging for further studies to evaluate its health impact. The combined abundance of infected octopuses and *P. excavatus* merits attention.

1. Introduction

Cephalopods are of increasing economic importance as evidenced by the rapid rise in their global landings over recent decades (ICES, 2011). Among them, the common octopus, *Octopus vulgaris* Cuvier 1797, is the most fished octopus species in the world. While worldwide the greatest octopus fishery takes places in the Saharan Bank (Northwest coast of Africa), other larger fisheries exist along the European Atlantic coast and the Mediterranean Sea, as well as in the waters of Japan and Venezuela (Guerra, 1997). In the Spanish Mediterranean Sea, *O. vulgaris* is the most landed cephalopod species, averaging around 4000 t per year between 2004 and 2011 (Ministerio de Agricultura Alimentación y Medio Ambiente, 2013). They are mainly fished by trawlers, but also captured by clay pots, trammel nets and other fishing gears (Tsangridis et al., 2002). Species in the Mediterranean Sea show a narrow depth distribution and most of them are found in the top 100 m; below 200 m, catches are scarce (Belcari et al., 2002). As other cephalopod species, the common octopus plays an important role in the food webs of marine ecosystems. In spite of its importance, little is known about protozoan diseases affecting octopus populations in their natural environment.

The occurrence of a given marine parasite in any cephalopod host species will depend on the presence of a suitable definitive host, a suitable intermediate host (IH) and complex abiotic and biotic factors hierarchically arrayed and dynamically interactive (González et al., 2003). One of these cephalopod parasites, the coccidian genus *Aggregata* (Protozoa: Apicomplexa), is an intracellular parasite with a two-host life cycle, transmitted through the food-web. Gamogony and sporogony mostly occur in the digestive tracts of a wide diversity of cephalopods, the definitive host, whereas merogony occurs in the digestive tract of crustaceans, the IH (Levine, 1988). Infection by parasites of this genus has been considered the most important infectious agent in wild and cultured cephalopod stocks from Spanish Atlantic waters (Estévez et al., 1996). It is responsible for a malabsorption syndrome (Gestal et al., 2002a), making the host more vulnerable to other biotic and abiotic stressors (Gestal et al., 2007). Coccidian infection has also been reported in reared *O. vulgaris* from Mediterranean Sea (Licciardo et al., 2005; Mladineo and Bocina, 2007; Mladineo and Jozić, 2005; Peñalver et al., 2008). Nevertheless, data from its prevalence and distribution in Mediterranean Sea as well as factors associated to this parasitosis are scarce. So, the aims of this work were to study the prevalence and distribution of *Aggregata octopiana* in common octopus from the Spanish Mediterranean coasts and, to identify abiotic and biotic factors related to infection.

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2. Material and methods

2.1. Study area and sample collection

This study was carried out within the framework of the Mediterranean International Trawl Survey (MEDITS financed by DG MARE and UE members Council Regulation (EC) No. 199/2008) and was conducted along the Spanish Mediterranean coast by the Spanish Institute of Oceanography (IEO). A total of 114 *O. vulgaris* were randomly taken in May 2011 from 30 geographic sectors (Fig. 1 and Table 1) with a bottom trawl (model GOC-73) with a 4 m vertical opening and a 20 mm cod end mesh size. Haul duration was 30 min and further information on the sampling design and on the characteristics of the gear is available in the MEDITS-Handbook (2012).

The following parameters were obtained from each octopus: dorsal mantle length (DML) to the nearest mm, body weight (BW) to the nearest 0.1 g, body condition score, sex and maturity stage. Body condition score (K) was calculated following Fulton's index as K = BW / DML3 × 100 (Ricker, 1975). Maturity stage was determined according to the three-stage maturity scale described by Sánchez and Obarti (1993) which includes (I) immature (ovary whitish, very small and with no signs of granulation in females; spermatophoric organ transparent or whitish in males), (II) maturing (ovary yellowish with a granular structure; spermatophoric organ with white streaks of sperm) and (III) mature (ovary very large with plenty of eggs; spermatophoric sac with spermatophores).

2.2. Analysis of coccidial infection in octopuses

Mesenteries of digestive gland and gonad, gills and mantle musculature were visually inspected to detect extra-intestinal macroscopic whitish oocysts. Intestine samples of 8 octopuses were fixed in 10% buffered formalin. Octopus gastrointestinal organs were dissected on board and stored at −20 °C.

Table 1
Salinity, temperature and depth of the geographical sectors of octopus capture.

Geographical sector	Salinity	Temperature	Depth
1	37,838	16,176	46
2	38,038	13,009	75
3	38,084	12,920	154
4	37,997	13,578	60
5	38,041	13,033	88
6	38,022	13,082	113
7	37,940	14,267	55
8	37,972	13,701	66
9	38,121	13,008	128
10	38,027	12,922	110
11	38,024	12,953	88
12	37,991	13,161	85
13	38,049	13,017	115
14	38,061	13,048	115
15	37,935	14,460	58
16	38,065	13,016	80
17	37,868	15,618	46
18	38,032	12,981	75
19	38,047	13,106	82
20	38,046	13,043	95
21	38,066	13,225	92
22	37,899	15,536	36
23	38,010	13,232	77
24	38,001	13,931	68
25	37,949	15,332	57
26	37,872	16,467	41
27	38,059	13,447	143
28	38,061	13,450	101
29	38,044	13,467	77
30	37,948	16,705	59

To estimate infection intensity, the presence of macroscopic whitish oocysts in the oesophagus, crop, digestive gland, caecum and intestine was semiquantitatively evaluated on a 6-degree scale with grade 0 corresponding to absence of oocysts and grade 6 to heavy infection

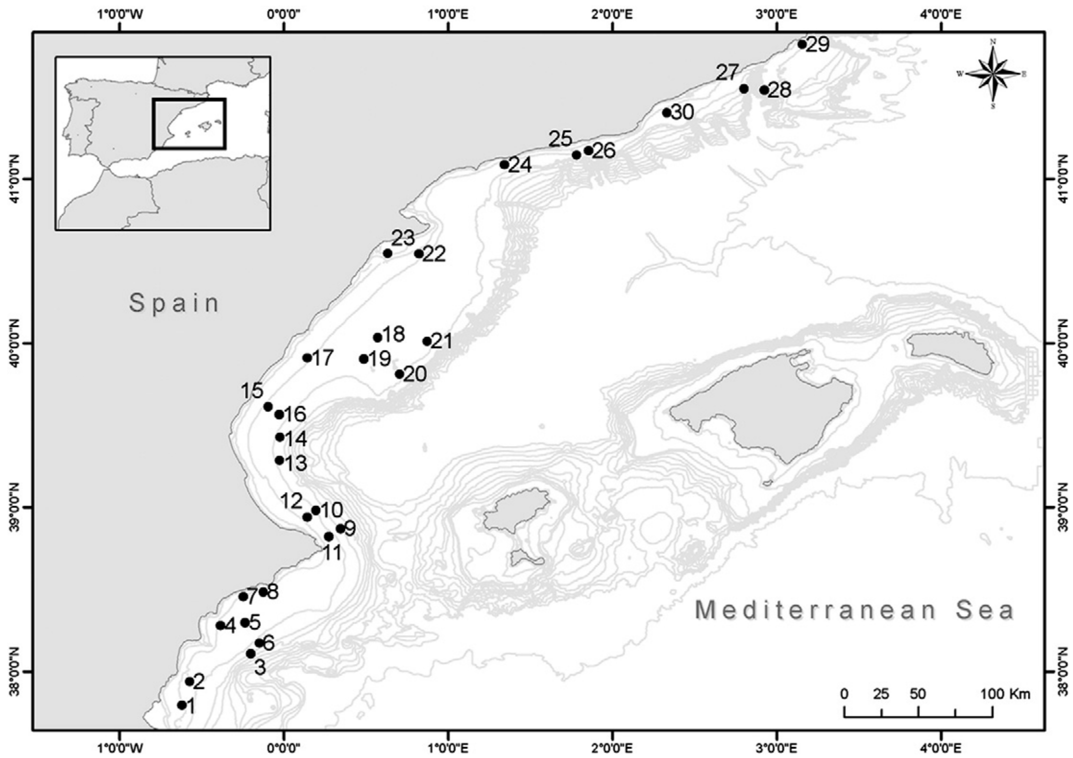

Fig. 1. Map of the studied area (Northwest Mediterranean) showing the position of each geographic sector.

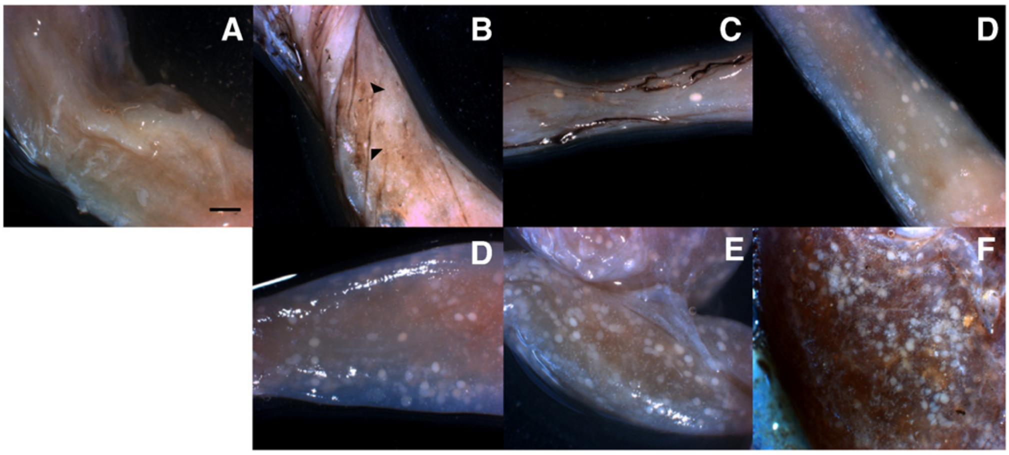

Fig. 2. Intestine of *Octopus vulgaris* showing different *Aggregata octopiana* macroscopic lesions according to a 6-degree scale with grade 0 corresponding to absence of oocysts and grade 6 to heavy infection (A = grade 0, absence, B = grade 1, scarce, C = grade 2, slight D = grade 3, moderate, E = grade 4, abundant, F = grade 5, very abundant, G = grade 6, massive). Arrowheads point isolated cysts.

(0 = absence, 1 = scarce, 2 = slight 3 = moderate, 4 =abundant, 5 = very abundant, 6 = massive) (Fig. 2). Additionally, to correlate the semiquantitative scale with a sporocyst number, the caecum of some octopuses was homogenized in an electric tissue grinder (IKA-UltraTurrax T-25) following standard protocol described by Gestal et al. (1999). The number of *Aggregata sporocysts* infecting the caecum was estimated in a Neubauer counting chamber. The number of sporocysts was expressed as the number of sporocysts/g of caecum (S/G) (Table 2).

Moreover, fixed intestine samples were processed for histological analysis briefly; they were dehydrated in a graded ethanol series, embedded in paraffin and sectioned using a microtome. Sections were stained with hematoxylin–eosin following standard procedures.

2.3. Faunistic assemblages

The species composition of the different taxocoenosis (molluscs, crustaceans and fish) was studied by analysing abundance and biomass data coming from the MEDITS survey (GSA06). Raw catch data (biomass and number of specimens collected of each haul) were converted into abundance (number of specimens/30 min of haul) and biomass indices (kg/30 min of haul). Given the characteristics of the nets used, sampling was adequate for *Decapoda* and *Stomatopoda* species and not for recovering crustacean *phyla* typically with small-sized species, such as *Euphausiacea*, *Isopoda* and *Mysidacea*.

2.4. Statistical analysis

Non-parametric ANOVA (Kruskal–Wallis) was used to compare the mean *A. octopiana* macroscopic lesion score in different organs according to categorised octopus, environmental and faunistic explanatory variables (Table 3) and to compare the hauls' mean number of crustacean species (those present in at least four hauls) according to macroscopic lesions' presence or absence in octopuses (Table 4). Octopus variables included gender, BW, DML, maturity and condition. Environmental variables were depth, longitude, latitude, temperature and salinity of the capture geographical sector. Faunistic variables studied were mollusc, crustacean and fish species diversity, abundance and biomass in the haul.

Logistic regression analysis was then used to further investigate the relationship between the presence/absence of *A. octopiana* macroscopic lesions in the caecum (outcome variable) and crustacean species presence or absence, adjusted for other octopus and environmental explanatory variables. Univariate chi-squared tests were initially used to select explanatory variables significantly associated with the caecum lesions' presence as candidates for inclusion in the logistic regression analysis. Following a backward-elimination strategy, the final logistic model included only those variables significantly associated with the outcome (Kleinbaum et al., 1998). The maximum likelihood estimation method and significance were taken for $p < 0.05$ (two sided). All analyses were performed using the software EpiInfo 2000 (CDC, Atlanta).

Table 2
Number of sporocysts per gramme of caecum tissue (S/G) in according with the 6-degree macroscopic scale.

Grade of the macroscopic scale	N	S/G $\times$ 10^6 ($\pm$SD)
0	10	0.111 ($\pm$0.08)
1	6	0.144 ($\pm$0.024)
2	10	0.327 ($\pm$0.083)
3	10	0.638 ($\pm$0.227)
4	3	1.399 ($\pm$0.468)
5	–	–
6	–	–

SD = standard deviation.

3. Results

3.1. Coccidial infection stages, localisation and lesions

Histological examination of infected tissues allowed identifying different life cycle phases of *Aggregata* including gamogony and sporogony. Parasites were most frequently found in the submucosa and muscularis and less common in the serosa (Fig. 3). Infected host cells were hypertrophic and in heavy infections, most infected host tissue was replaced by parasites, there was loss of intestinal epithelium and destruction of the tissue organ architecture. Oocysts contained up to eight sporozoites, as described previously for *A. octopiana* (Estévez et al., 1996; Hochberg, 1990).

3.2. Macroscopic cysts' prevalence and mean (range) lesions' scores

A large number of white macroscopic cysts, indicative of *A. octopiana* infection, were found by direct observation in 96% (109/114) of the examined octopuses (Fig. 2). The highest percentage of cysts was found in intestine (92%) and spiral caecum (81%)

Table 3
Relationship between infection intensity and biological parameters of octopus, environmental and faunistic variables.

Variable and range	No. of octopuses	Macroscopic cyst mean score					
		Oesophagus	Crop	Digestive gland	Intestine	Spiral caecum	Mean
Sex							
Male	62–66	0.55	2.62[b]	0.17	2.78	2.2[b]	1.68
Female	40–43	0.5	1.72	0.15	2.75	1.66	1.42
Maturity stage (MS)							
MS$_1$: Stage I	57–60	0.51	1.76[b]	0.22	2.61	1.85	1.41[b]
MS$_2$: Stages II + III	45–50	0.56	2.86	0.08	2.96	2.14	1.76
Body weight (BW)							
BW$_1$: 117–551 g	33–37	0.7	2.75	0.27	2.76	1.81	1.67
BW$_2$: 553–902 g	36–38	0.6	2.16	0.16	2.78	2.08	1.56
BW$_3$: 933–6700 g	33–36	0.27	1.83	0.03	2.75	2.06	1.47
Dorsal mantle length (DML)							
DML$_1$: 52–105 cm	42–43	0.64	2.24	0.21	2.71	2.07	1.57
DML$_2$: 107–158 cm	32–33	0.29	2.59	0.17	2.81	2.16	1.73
DML$_3$: 168–215 cm	35–36	0.83	1.94	0.09	2.77	1.72	1.41
Body condition (K)							
K$_1$: 0–0.066	51–55	0.53	2.51	0.15	2.67	1.96	1.61
K$_2$: 0.067–0.203	50–54	0.5	1.96	0.17	2.79	2.07	1.52
Capture depth							
Depth$_1$: 36–79 m	57–61	0.35	1.72	0.12	2.24	1.49	1.22
Depth$_2$: 80–158 m	50–54	0.71	2.78[b]	0.2	3.25[a]	2.48[a]	1.99[a]
Latitude (Lat)							
Lat$_1$: 37°47′61″–38°52′15″	48–55	0.59	2.82	0.12	2.98	1.95	1.73
Lat$_2$: 38°56′38″–41°49′15″	57–55	0.46	1.64[a]	0.19	2.47	1.95	1.36[b]
Longitude (Long)							
Long$_1$: +01°20′55″–+0°52′31″	52–59	0.46	2.66	0.09	2.93[b]	2	1.69
Long$_2$: −00°01′46″ – −00°37′39″	53–54	0.57	1.7[b]	0.22	2.48	1.89	1.37
Water temperature							
T$^a_1$: 12.9–13.22 °C	50–54	0.5	2.34	0.14	3.26	2.41	1.77
T$^a_2$: 13.23°–16.71C	51–54	0.49	1.93	0.19	2.11[a]	1.41[a]	1.26[a]
Salinity (S)							
S$_1$: 37.838–37.997	53–56	0.43	1.89	0.09	2.31	1.61	1.3
S$_2$: 38.001–38.121	48–52	0.56	2.4	0.24[a]	3.1[a]	2.27[a]	1.75[a]
Fish diversity/haul (FD)							
FD$_1$: 12–21 species	59–61	0.52	1.91	0.15	2.60	1.97	1.46
FD$_2$: 23–34 species	50–52	0.51	2.56	0.16	2.86	1.92	1.63
Fish abundance/haul (FA)							
FA$_1$: 138–547 specimens	45–46	0.56	2.60	0.24	2.80	2.22	1.71
FA$_2$: 606–1580 specimens	24–25	0.52	2.0	0.04	3.3	2.16	1.64
FA$_3$: 2409–6492 specimens	40–42	0.46	1.90	0.13	2.20[a]	1.52[b]	1.28[b]
Fish biomase/haul (FB)							
FB$_1$: 9–23 Kg	38–41	0.68	2.80	0.18	3.0	2.30	1.82
FB$_2$: 27–83 Kg	26–27	0.46	1.70	0.19	2.30	1.40	1.24
FB$_3$: 100–613 Kg	41–45	0.39	2.0	0.12	2.70	2.0[a]	1.46[b]
Crustacean diversity/haul (CD)							
CD$_1$: 0–5 species	72–75	0.56	2.2	0.19	2.61	1.95	1.52
CD$_2$: 6–11 species	33–38	0.42	2.21	0.09	2.86	1.95	1.57
Crustacean abundance/haul (CA)							
CA$_1$: 0–6 specimens	39–41	0.69	2.49	0.25	2.34	1.68	1.52
CA$_2$: 8–27 specimens	30–32	0.3	1.39	0.07	3	2.41	1.47
CA$_3$: 68–133 specimens	32–35	0.44	2.37	0.15	2.82	1.77	1.55
CA$_4$: 1093 specimens	4–5	1	3.8	0	3.2	2.4[b]	2.06
Crustacean biomase/haul (CB)							
CB$_1$: 0–0.1 kg	36–38	0.53	2.21	0.14	2.71	1.97	1.55
CB$_2$: 0.14–0.54 kg	42–45	0.45	1.75	0.16	2.73	2.02	1.45
CB$_3$: 1.60–4.26 kg	27–30	0.59	2.87	0.18	2.69	1.8	1.66
Mollusca diversity/haul (MD)							
MD$_1$: 4–7 species	55–58	0.62	2.10	0.21	2.70	1.98	1.54
MD$_2$: 8–12 species	50–55	0.40	2.30	0.10	2.80	1.89	1.54
Mollusca abundance/haul (MA)							
MA$_1$: 14–49 specimens	36–37	0.58	1.97	0.14	2.49	1.76	1.39
MA$_2$: 54–95 specimens	24–26	0.54	2.0	0.13	2.77	1.96	1.55
MA$_3$: 140–224 specimens	45–50	0.44	2.49	0.19	2.85	2.08	1.64
Mollusca biomase/haul (MB)							
MB$_1$: 4–16 kg	40–44	0.53	2.48	0.17	2.77	2.16	1.67
MB$_2$: 17–22 kg	29–30	0.72	2.50	0.24	3.0	2.27	1.78
MB$_3$: 34–189 kg	36–39	0.33	1.66	0.08	2.41	1.46[a]	1.21[b]

[a] Statistically significant $p < 0.01$.
[b] Statistically significant $p < 0.05$.

while lighter infections were observed in crop (55%), oesophagus (24%) and digestive gland (8%) ($p < 0.05$). No macroscopic cysts were observed outside the digestive tract. The mean (range) lesion scores in affected organs varied and was 2.2 (1–4) in the oesophagus, 4.5 (1–6) in the crop, 1.9 (1–3) in the stomach, 3.0 (1–6) in the intestine and 2.4 (1–4) in the caecum (Table 3).

Table 4
Number of captures of the most frequent crustacean species according to the presence/absence of lesions.

Crustacean species	No. of hauls[c]	Macroscopic cysts	Oesophagus	Crop	Digestive gland	Intestine	Spiral caecum	Mean
			N: 80 (A), 25 (P)	N: 50 (A), 62 (P)	N: 100 (A), 9 (P)	N: 9 (A), 102 (P)	N: 22 (A), 91 (P)	N: 5 (A), 109 (P)
Liocarcinus depurator	14	A	7.34	4.94	8.33	0.33	1.05	0.6
		P	11.72	11.15	8	9.06	10.12[a]	8.63
Dardanus arrosor	13	A	2.73	3.32	2.62	4.78	4.73	1.4
		P	2.2	2.18	3	2.43	2.17[a]	2.75
Macropodia longipes	11	A	6.6	5.98	6.67	0	2.73	0
		P	3.52	6.37	0.22[b]	6.37[b]	6.99[a]	6.38
Pagurus excavatus	8	A	0.33	0.3	0.34	0	0.091	0
		P	0.2	0.34	0	0.34	0.374[b]	0.33
Squilla mantis	6	A	0.15	0.18	0.15	0	0	0
		P	0.16	0.15	0.11	0.18	0.2[b]	0.17
Macropipus tuberculatus	6	A	1.91	1.14	2.02	0.33	0.32	0.6
		P	2.32	3.21	1.44	2.48	2.74	2.32
Pontonia flavomaculata	4	A	0.14	0.14	0.13	0	0.045	0
		P	0.12	0.13	0.22	0.15	0.15	0.138
Pagurus cuanensis	4	A	0.26	0.22	0.27	0	0.05	0
		P	0.2	0.32	0	0.3	0.33	0.28

N: number of octopus, A: absent, P: present.
[a] Statistically significant $p < 0.01$.
[b] Statistically significant $p < 0.05$.
[c] Number of hauls where species were found (of a total of 30 hauls).

3.3. Relationship between prevalence and lesion intensity and biological and environmental variables

In the univariate analysis mean lesion scores varied significantly according to levels of explanatory variables (Table 3). They were significantly greater in males compared to females, in maturating and mature octopuses rather than in immature ones, in octopuses living in depths >80 m compared to animals in shallower waters, in those in western compared to eastern longitudes, in those in southern compared to northern latitudes, as well as for octopuses captured in waters with a higher salinity and lower temperature ($p < 0.05$) (Table 3). Moreover, mean lesion scores were negatively associated to fish abundance, and mollusca biomase and were greatest in hauls with lowest fish biomase and with higher crustacean abundance ($p > 0.05$). Mean lesions scores were not associated to the octopus' body condition, weight or dorsal mantle length ($p > 0.05$).

3.4. Association of crustacean species with A. octopiana infection

The number of vertebrata, invertebrata and algae species in the hauls was 189, including 77, 34 and 32 fish, crustacean and mollusc species, respectively. Among the Crustaceans, the most common species was the portunid crab *Liocarcinus depurator*, present in 14 hauls, followed by the hermit crab *Dardanus arrosor*, the spider crab *Macropodia longipes* and the hermit crab *Pagurus excavatus*, found in 13, 11 and 8 hauls, respectively (Table 4). Haul mean (range) biomass was 67 (9–613) kg of fish, 21 (4–189) kg of molluscs and 0.5 (0–4.5) kg of crustaceans.

In the univariate analysis, the mean number of *L. depurator*, *M. longipes*, *Macropipus tuberculatus*, *P. excavatus* and "*Squilla mantis*" captures was positively associated with lesions in caecum and/or intestine (Table 4). In contrast, the mean number of the hermit crab *D. arrosor* was negatively associated with lesions in caecum ($p < 0.05$) (Table 4).

Logistic regression analysis indicated that the macroscopic lesions in the caecum were only significantly associated with the presence of *P. excavatus* or *L. depurator* as well as with gender (males had greater risk than females) and haul depths (risk was greater at increasing depths). However, when both crustacean species were included in the model only the presence of *P. excavatus* was significantly associated with caecum lesions ($p < 0.05$) (Data not shown).

4. Discussion

In this study, high *A. octopiana* prevalence (96%) was detected in agreement with data reported by other authors in wild octopuses in Atlantic waters (Pascual et al., 1996). This coccidian mainly infects areas of octopus digestive tract lacking cuticle, such as the caecum and intestine (Gestal, 2000), being the caecum as the preferred site of infection (Estévez et al., 1996). Octopus crop and digestive gland possess a hard cuticle (Boucaud-Camou and Boucher-Rodoni, 1983) which was suggested to impair the settlement of the merozoites

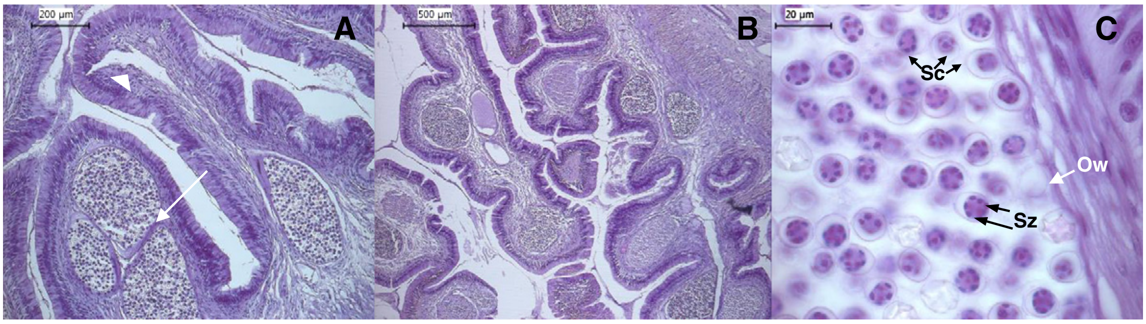

Fig. 3. Histological sections of *Octopus vulgaris* intestines infected by *Aggregata octopiana*. A) Distension (arrow) of infected tissue and destruction of the tissue organ (arrowhead). B) The majority of heavily infected area is replaced by parasites. C) Oocyst containing mature sporocysts (Sc) with sporozoites (Sz).Ow: oocyst wall.

(Sardella and Ré, 1990). In *O. vulgaris* massive infections, cysts may be observed in various organs outside the digestive tract (Gestal, 2000; Mladineo and Jozić, 2005; Pascual et al., 2006). In the present study, prevalence and infection intensity were highest in the intestine although cyst distribution in this organ was not uniform but patchy. In contrast, infection intensity in caecum was slightly lower than in the intestine but cysts were uniformly distributed.

Although *A. octopiana* has been reported to lead to poorer body condition (Gestal et al., 2007), this was not detected in the present work. Nevertheless, a decreased in K-Fulton body condition index was observed in heavily infected octopus (Gestal et al., 2002a) whereas infections in the present study were possibly less severe as no extra-gastrointestinal cysts were detected and only a small number of octopus showed macroscopic lesions in stomach or oesophagus.

Most of the octopuses sampled were immature and maturating specimens, whereas only one octopus was mature. Similar results were observed by Belcari et al. (2002) suggesting that this is probably a consequence of various factors: a) the time of the year when samplings were carried out, when largest octopuses progressively disappear from trawling grounds, mainly as consequence of migration to rocky bottoms and coastal habitats for spawning; b) the type of trawl nets used which were unsuitable to capture large, mature individuals. Despite that, statistically significant differences in infection intensity were observed in the univariate analysis in relation to maturation stage as well as gender. These could be due to sex specific differences in feeding ecology. Male and female octopuses have a similar diet (Guerra, 1978; Sánchez and Obarti, 1993). However, *O. vulgaris* may change their feeding behaviour when they become mature and reproduce (González et al., 2011; Wodinsky, 1978). Males were more abundant in deeper depths and females predominated in shallow waters, probably due to the fact that females had moved to shallow zones of spawning as it has been previously described (González et al., 2011; Mangold-Wirz, 1963). Increased feeding among mature males captured in deep waters could lead to a greater risk of infection in this group.

The univariate analysis revealed a significant relationship between the infection intensity and some environmental factors such as water temperature and salinity. Infection intensity was highest in octopuses living at lower water temperatures and higher water salinities. Nevertheless, when modelled together with haul depths these variables were no longer associated to infection and were excluded from the model. Clearly haul depths, water temperature and salinity are related to each other so, at deeper depths salinity is increased while temperature decreases (Vargas-Yánez et al., 2010). Our results agree with González et al. (2003) that showed that depth is an important determinant of *A. octopiana* infection intensity in octopuses. The significantly higher intensity of infection observed in octopus fished in deeper depths may be related to higher availability of crustaceans that serve as IH for this species. In the present study and in those conducted by Abelló et al. (2002) throughout the entire Spanish Mediterranean coast, crustaceans are most abundant in deep waters. Moreover, in the Iberian Peninsula Continental shelf bottoms *L. depurator* and *D. arrosor* were also found to be the predominant species (Abelló et al., 2002).

Natural and experimental infections have concluded that there is a high specificity of *Aggregata* parasites regarding the definitive but not in the intermediary host (Dobell, 1925; Gestal et al., 2002b; Léger and Duboscq, 1906, 1908; Porchet-Henneré and Richard, 1971a, 1971b). The prawn *Palaemons elegans* is the only known IH of *A. octopiana* (Arias et al., 1998) however, other species of the *Aggregata* genus have a wide IH variety (Dobell, 1925; Léger and Duboscq, 1906, 1908; Porchet-Henneré and Richard, 1971a, 1971b). The univariate analysis performed in this study revealed a significant relationship between *A. octopiana* and various crabs (*L. depurator*, *M. longipes*, *M. tuberculatus*, and *P. excavatus*) and the shrimp *S. mantis*, drawing attention to the possible role of some of these species as IH for *A. octopiana*. In the logistic regression model however, only the presence of *P. excavatus* or *L. depurator* were significantly associated with macroscopic lesions in the caecum. *L. depurator* and *Pagurus* sp., have been previously described as part of wild *O. vulgaris* diet from Mediterranean Spanish coasts (Quetglas et al., 1998; Sánchez and Obarti, 1993). Additionally both crustacean species have been used in aquaculture as live food for *O. vulgaris* feeding (Villanueva, 1994; Villanueva et al., 1996).

Cage fattening of octopuses captured as subadults in the wild is commercially important in a growing number of Mediterranean countries (Iglesias et al., 2000). *A. octopiana* massive superinfection can represent a threat for experimental or commercial octopus rearing. In fact, mortality by *A. octopiana* infection in reared octopus has been previously reported by Mladineo and Jozić (2005). In order to attenuate or completely prevent the emergence of *Aggregata* sp. in reared octopus, feeding without crustaceans has been suggested (Mladineo and Jozić, 2005). Nevertheless, various formulated diets for feeding subadult cephalopods were inferior to natural diets (Cerezo Valverde et al., 2008; Querol et al., 2012a, 2012b). Octopus natural diet is based on a wide variety of prey, including crustaceans (63–80%), fish and other molluscs (Guerra, 1978; Nigmatullin et al., 1976; Smith, 2003). In laboratory conditions, given the choice between live crustaceans (mostly crabs), bivalves, gastropods and fish, the common octopus always prefers crabs, and ignores the other species. If crabs are not offered, octopuses also accept fish and molluscs, but only after days or weeks of no eating (Mangold, 1983). According to Iglesias et al. (2007) a diet made up of enriched Artemia and shrimps or selected crabs is currently the most appropriate to achieve better survival and growth indexes under culture conditions. In fact, the use of crabs determined a higher food intake, an advantageous conversion index, and more efficient feeding behavioural traits (Cagnetta and Sublimi, 2000).

The faunistic approach conducted in the present work is useful to identify potential crustacean candidate species to include or exclude in the cage-reared octopus diet. For example, the negative association between the hermit crab *D. arrosor* and coccidian infection in the univariate analysis may suggest that this species does not play a part in *A. octopiana* life cycle and could be used for feeding reared *O. vulgaris*. This crab, previously used for octopus feeding (Villanueva, 1994), is an abundant species without commercial interest and is usually discarded by fishermen (Baro et al., 2010; Castriota et al., 2004). Notwithstanding, this remains speculative and other studies including experimental infections need to be performed to confirm the species implication in the parasites' life cycle.

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