BRINE SHRIMP ARTEMIA PARASITIZED BY FLAMINGOLEPIS LIGULOIDES (CESTODA, HYMENOLEPIDIDAE) CYSTICERCOIDS IN SPANISH MEDITERRANEAN SALTERNS Quantitative aspects

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BRINE SHRIMP *ARTEMIA* PARASITIZED BY *FLAMINGOLEPIS LIGULOIDES* (CESTODA, HYMENOLEPIDIDAE) CYSTICERCOIDS IN SPANISH MEDITERRANEAN SALTERNS

Quantitative aspects

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ARTEMIA
FLAMINGOLEPIS LIGULOIDES
CESTODA
CYSTICERCOIDS
IMPLANTATION
EFFECTS INDUCED IN THE HOST

ABSTRACT – This is the first report on the presence in Spanish Mediterranean salterns of wild brine shrimp *Artemia* populations with individuals bearing an hemocoele parasitism caused by *Flamingolepis liguloides* (syn : *F. dolguschini*) cysticercoids (Cestoda, Hymenolepididae). A close survey of these populations in a saltern at Santa Pola (Alicante, Spain) was performed for three years. Data obtained allow us to quantify the prevalence of this parasitism, establishing a mean intensity of about 1.26 cysticercoids per parasitized specimen. Intensities for the presence of one or more cysticercoids (6 maximum) per parasitized *Artemia* individual mathematically fit a negative binomial distribution. Selective implantation of cysticercoids in several anatomical brine shrimp areas and a drastic effect on reproductive capability were verified.

ARTEMIA
FLAMINGOLEPIS DOLGUSCHINI
CESTODA
CYSTICERCOIDES
IMPLANTATION
EFFECTS INDUITS SUR L'HOTE

RÉSUMÉ – La présence d'une population d'*Artemia* (Crustacé Anostracé) parasitée par des cysticercoïdes d'un Hymenolepididae *Flamingolepis liguloides* (syn : *F. dolguschini*) est signalée pour la première fois dans les salins du littoral méditerranéen espagnol. L'infestation parasitaire a été suivie pendant trois années dans les salines de Santa Pola (Alicante, Espagne). Les résultats obtenus ont permis d'évaluer la prévalence de ce parasitisme qui présente une intensité moyenne de 1,26 cysticercoïdes par *Artemia* et des intensités d'infestation d'un ou plus cysticercoïdes (6 au maximum) par Crustacé. La distribution de ces intensités permet un ajustement à une loi binomiale négative. La localisation sélective des cysticercoïdes dans le corps des individus parasités, ainsi qu'une réduction sensible de la capacité reproductive de l'hôte sont observées.

INTRODUCTION

The great availability of literature on the brine shrimp *Artemia* (Branchiopoda, Anostraca) mainly arises from the important role that this crustacean has achieved in mariculture. However, a recent bibliography (McCourt and Lavens, 1986) contains relatively few references on parasitism involving *Artemia* as intermediate or final host.

The first available references on cestode parasitism in *Artemia* (Heldt, 1926; Young, 1952) described the presence of cysticercoids and larvae of cestodes (Hymenolepididae) in wild populations from Tunisia and California, respectively. Maksimova (1973, 1974, 1976) and Jarecka (1984) stated the important role of several branchiopods (Artemia, Branchinella, Branchinecta) as intermediate hosts in the propagation of early forms of hymenolepidids until their final hosts, usually water birds like gulls, flamingos, avocets and grebes.

Codreanu and Codreanu-Balcescu (1978) reported wild *Artemia* populations parasitized in the lake Tekirghiol (Rumania), while Gabrion and McDonald (1980) and Gabrion *et al.* (1982) showed the presence of cysticercoids from different cestode species parasitizing the brine shrimp at Salin de Giraud, south of Etang du Vaccarès, in the Camargue (France).

During an ecological research program on the *Artemia* populations from La Mata lagoon (Torrevieja, Alicante), the presence of cysticercoids in the hemocoele of wild brine shrimp was observed for the first time in two different salinas of the Spanish Mediterranean shore : La Mata lagoon and Bonmati saltern (Santa Pola, Alicante). Later, this parasitism was found in Cotorrillos saltern (Mar Menor, Murcia) and also reported in a saltern from the southwest Spanish shore at Huelva (Martinez, 1989).

These findings justify the study of their incidence on the autochthonous *Artemia* populations, as well as the influence of the cysticercoids in several aspects of the lifespan of parasitized brine shrimp specimens.

MATERIALS AND METHODS

Our study was concentrated in the Bonmati saltern, because it appeared to have the highest prevalence of infected *Artemia* (parasitism observed 18 times from 29 May 1985 to 23 October 1987 versus only 4 times for the La Mata lagoon and one time for the Cotorrillos saltern). This saltern, exploited following the Mediterranean solar salt way (Amat, 1988), fills an old salt marsh south and southwest of Santa Pola, detached from the sea by a barrier beach (Orti Cabo *et al.* 1984). Sampling was accomplished usually in two ponds (evaporators) connected to the brine circuit lying beside « La Pedrera » pump station, between the national road Alicante-Cartagena (N-332) and the shore line. Because of the diverse brine management types common to this kind of saltworkds, it was unusual to find the same salinity and brine shrimp populations in both ponds, even though sometimes brine flowed between them. Samples were taken from the pond holding denser populations. A 300 µm mesh hand net was used to scoop *Artemia* from the bank lying between the ponds, after recording the temperature and salinity.

Living samples were placed in plastic carboys filled with dilute original brine (1/3 to 1/2) in order to assure specimens survival during transfer to the laboratory. Before their examination, samples were sieved through a 0.5-1 mm mesh net in order to discard nauplii and early metanauplii, in which cysticercoids never appeared. The remaining specimens were narcotized with a few drops of chloroformed water (Lochhead and Lochhead, 1941; Amat 1980 a). A first selection was performed in order to separate parasitized from unparasitized shrimp, which allowed quantification of the percentage of infected shrimp (prevalence). A second selection among parasitized brine shrimp removed males and classified females as zygogenetic or parthenogenetic (Amat, 1983; 1985). These females were sized and classified according to their developmental stage (late metanauplii, juveniles and adults) and reproductive level : immature juvenile, active ovulating, full ovisac (oviparous or ovoviviparous offspring) and empty ovisac. Finally the cysticercoids present in every shrimp were counted and localization in the host was identified : head, thorax, ovisac (or penis) and abdomen.

A subsample of parasitized specimens from the 4 December 1986 sample was dissected in order to isolate 53 cysticercoids. They were fixed in 4 % formalin for later taxonomic identification according to morphometric criteria (Maksimova, 1974; Gabrion and McDonald, 1980).

RESULTS

All the measurements given here are in micrometres. The cysticercoids show ovoid shape (Fig. 1 and 2A) attaining a length or main axis of 488.20 to 434.20 and a width or minor axis of 314.33 to 281.13. They are provided with a cercomer of a length about 800. The tegument is 13.50 thick, inside of which there is the scolex measuring 329.90 to 316.90 per 255.90 to 243.80, furnished with four oval suckers 163.40 to 152.30 length and 96.90 width. The rostellum sac attains 304.70 to 280.30 per 134 to 121.90 with the retracted rostellum inside, endowed with a circular strong musculature and a crown of 8 hooks measuring 185 to 174.50, curved and sharp in the distal tip about 110 length. All these characters agree

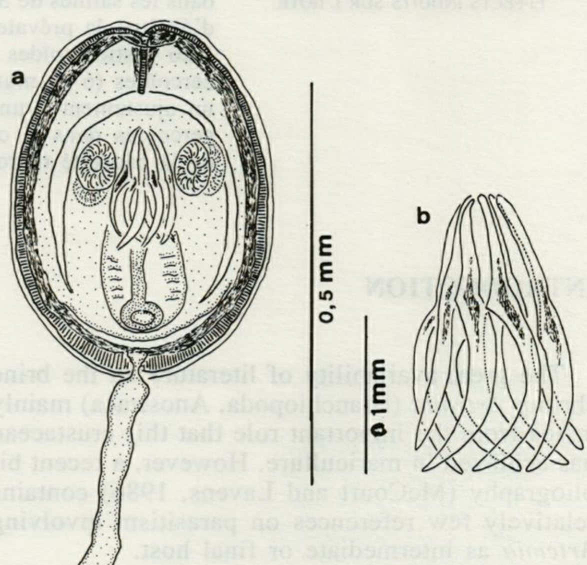

Fig. 1. – *Flamingolepis liguloides* (Gervais 1874) Spasski and Spasskaya 1957. a, cysticercoid; b, arrangement of rostellum hooks.

Fig. 2. – A : Cysticercoids appearance after dissection (50 x); B : Parasitized brine shrimp *Artemia* (10 x) displaying diverse cysticercoid implantation intensities.

with *Flamingolepis liguloides* (Gervais, 1874) Spasski and Spasskaya, 1954. Syn : *F. dolguschini* Gvosdev and Maksimova, 1968.

The numerical data obtained after the detailed survey of samples provided clear information on the parasitism prevalence in the brine shrimp populations and an accurate view of the cysticercoids localization inside the brine shrimp anatomy, as well as its biological implications. Therefore they allow us to describe several relationships worthy of discussion.

The data collected during three years are summarized in Table I. The total number of brine shrimp examined on each sampling date (A) divided into the number of parasitized specimens (B) allow us to calculate the prevalence (C) and to quantify the total number of cysticercoids observed (D). The ratio D/B defines the mean intensity of parasitism (E). From the totals (A) and (C) are to be excluded the data obtained after the 25 June 1985 and 25 June 1986 samples because a certain mortality hindered a suitable evaluation of the relationships explained above; instead, it was possible to select several parasitized specimens worth to provide fitting data about the predominant location of cysticercoids in them. Table I also gives brine temperature and salinity data for each sampling date.

Table I. – Cysticercoids distribution in the different *Artemia* samples studied.

	1985			1986										1987						
	05	06	10	01	02	05	06	07	08	09	10	12	12	01	03	05	06	07	10	
Sample	29	25	02	16	20	22	25	23	13	25	23	04	04	21	06	21	23	15	23	Total
Parameters																				
(A)	126	?	327	204	53	300	?	565	2000	966	256	86	45	1353	1445	1015	1860	37	1366	12004(*)
(B)	54	65	18	108	16	42	48	26	37	87	42	60	40	5	20	39	20	1	41	769
(C)	42.8	?	5.5	52.9	30.2	14	?	4.6	1.85	9	16.4	69.8	88.9	0.4	1.4	3.8	1.1	2.7	3	5.46(*)
(D)	63	82	21	146	19	46	62	29	38	112	46	99	81	5	21	39	20	1	42	972
(E)	1.16	1.26	1.16	1.35	1.18	1.09	1.29	1.11	1.02	1.28	1.09	1.65	2.02	1	1.05	1	1	1	1.02	1.26
S ^{o}/oo	127		119	60	132	130	142	102	128	132	92	90	90	105	110	100	196	108	134	
T ^{o}C	23		26	10	20	22	28	28	34	24	22	18	18	9.50	20	24	29.50	34.80	23	
												(N)	(R)							

(*) Without 06.25.85 and 06.25.86 samples

(A) : Number of Artemia specimens examined per sample
(B) : Number of Artemia specimens bearing parasites
(C) : Prevalence
(D) : Number of cysticercoids per sample
(E) : Mean Intensity = (D) / (B)
(N) : Normal females, (R) : Red females

Table II. – A, Intensity of cysticercoids (M = observed, N = calculated); B, anatomical implantation according to the size of parasitized specimens; C, density and localization in the anatomy of parasitized specimens. Total (B) = Number of specimens. Total (D) = Number of cysticercoids (see Table I).

	1985			1986										1987						TOTAL					
	05	06	10	01	02	05	06	07	08	09	10	12	12	01	03	05	06	07	10						
Number of cysticercoids	29	25	02	16	20	22	25	23	13	25	23	04	04	21	06	21	23	15	23	(M)	%	(N)	%		
1	47	54	15	75	13	38	36	24	36	67	38	31	16	5	19	39	20	1	40	614	79.84	618	80.20		
2	6	8	3	28	3	4	11	1	1	16	4	22	12		1				1	121	15.73	116	15.00		
3		1		5				1		3		4	9							23	3.00	27	3.50		
4	1	1					1			1		3	2							9	1.17	7	0.91		
5		1																		1	0.13	2	0.26		
IIA 6													1							1	0.13	1	0.13		
Artemia size																				Nº	%	HE	TH	OV	AB
4 – 5 mm						1				2	2			3					4	12	1.55	–	9	2	1
5 – 6	2					3			1	6		2		1					7	22	2.87	–	16	2	4
6 – 7	6		2						6	4	4	1			1	5			11	40	5.22	–	27	5	8
7 – 8	8		1		3	4		1	4	6	7	1			1	10	1		9	56	7.31	–	30	6	20
8 – 9	6	6	5	2	1	8		3	3	10	9	11			10	12	1		1	88	11.50	6	37	11	34
9 – 10	6	7	2	3	7	7	2	5	5	19	11	30	14	1	5	2	8	1	4	139	18.15	3	43	14	79
10 – 11	7	18	6	22	3	12	3	3	3	23	4	12	6		3	2	8		4	139	18.15	4	33	10	92
11 – 12	5	22	2	57		5	9	1	10	15	5	2	9			4	2		1	149	19.45	4	29	4	112
12 – 13	10	7		18	1	1	31		1	2			10			4				85	11.10	–	17	3	65
13 – 14	2	4		6		1	3	4	4			1	1							26	3.13	–	6	1	19
14 – 15	1	1			1			6												9	1.05	–	–	1	8
15 – 16								2												2	0.26	–	1	–	1
16 – 17	1							1												2	0.26	–	–	2	–
IIB Total (B)	54	65	18	108	16	42	48	26	37	87	42	60	40	5	20	39	20	1	41	769	100	17	248	61	443
HEAD	2	1	1	2			2	1	1	6	1	2	1		1				1	22	2.26				
THORAX	31	21	10	26	17	20	16	10	8	27	23	13	14	1	15	9	5	1	25	292	30.05				
OVISAC	10	16	1	2	2	3		3	4	4	6	13	3	2	2	1			7	79	8.12				
ABDOMEN	20	44	9	116		23	44	15	25	75	16	71	63	2	3	29	15		9	579	59.57				
IIC Total (D)	63	82	21	146	19	46	62	29	38	112	46	99	81	5	21	39	20	1	42	Nº	%				

The intensity of cysticercoids per parasitized specimen (Table IIA) indicates that its highest value is for the presence of only one cysticercoid, with a spectacular drop for intensities about 5-6 cysticercoids per specimen. These data are best fit to a negative binomial distribution with the equation :

$$P(x) = 1 + \frac{\mu^{-k}}{k} \; \frac{(k+x+1)\,!}{x\,!\,(k-1)\,!} \; \frac{\mu^{x}}{\mu + k}$$

where k = distribution parameter and P (x) = probability for the existence of x specimens in a simple unity (Elliot, 1983). Once the parameters are computed, the results of the theoretical distribution are calculated by means of :

$$\hat{k} = -\frac{\overline{x}^{\,2}}{S^2 - \overline{x}^{\,2}} = 0.1717$$

$$\hat{\mu} = \overline{x} = 0.081$$

for $\overline{x}$ = sample mean and S^2 = sample variance. The values calculated can be checked with those observed (Table II A). Location of cysticercoids in the parasitized *Artemia* specimens was primarily in the abdomen (60 %) and thorax (30 %), with much smaller percentages observed in the ovisac (8 %) and head (2 %) (Table II C and Fig. 3).

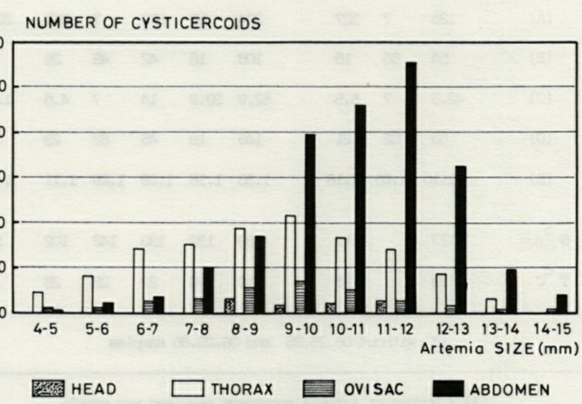

Fig. 3. – Quantitative distribution and location of cysticercoids, according to size to the parasitized *Artemia* specimens.

Table III. – Effect of cysticercoids presence on the reproductive capacity of *Artemia* specimens.

	1985			1986										1987							
	05	06	10	01	02	05	06	07	08	09	10	12	12	01	03	05	06	07	10		
	29	25	02	16	20	22	25	23	13	25	23	04	04	21	06	21	23	15	23	№	%
IMMATURE	31	21	18		13	18		12	17	54	33	31	22	4	14	29	12		31	359	47.30
OVULATING	3			4							1									8	1
OVATE	1	3		78	3				2	3	4	14			2	1		1	1	113	15
EMPTY UTERUS	19	41		26		20	48	14	18	30	4	15	18			9	8		9	280	36.70
						3 ♂♂									4 ♂♂						

The majority of specimens containing cysticercoids were in the 9-12 mm size range (56 %) with lesser intensities in the 4-9 mm range and 12-17 mm range (Table II B). Cysticercoids were predominant in the thoraxes of specimens in the 4-9 mm range, but were predominant in the abdomen of specimens larger than 9 mm (Fig. 3) About 84 % of the parasitized shrimps were either immature or had an empty ovisac (Table III). The immaturity was not a question of early age or development, given that the immature parasitized specimens achieved bigger sizes than those not parasitized displaying normal breeding activity.

DISCUSSION AND CONCLUSIONS

Most samples showed parasitized shrimps displaying noteworthy characteristics compared to unparasitized ones : reddish colour, bigger size, weak ovarian activity, scarce oocytes in the ovaries as well as embryos or cysts in the ovisac, reduced offspring, etc. Usually red parasitized shrimps did not display reproductive vigor after attaining sizes in which unparasitized ones achieved full broad pouch. The biggest parasitized red females usually displayed a broad but empty ovisac. This circumstance alerted us to pay special attention to the two appearances among parasitized specimens, normal (N) and red (R), and they will be subject of different results and arguments in this research.

It should be noted that during this research period there were occasions in which *Artemia* populations were absent from the sampled ponds, while sometimes, with dense populations present, there were no infected specimens at all. These situations are not displayed in the reported data.

The prevalence value for parasitized specimens, 5.46 %, was slightly higher than the 4.25 % found by Gabrion *et al.* (1982) for *F. liguloides* and *Artemia* from La Camargue (France), but lower than 6.10 % determined by Maksimova (1973) in the lake Tengiz (URSS). The latter author reported different values, about 0.033 % until 10-12 %, according to sampling places, changeable presence and density of water birds (feeding areas, nesting areas) or different species of cestode, etc, always in *Artemia*, so the diverse prevalence values in the various studies are probably not significantly different.

The mean intensity achieved here was 1.26 cysticercoids per *Artemia*, with a maximum of 6 once only. Gabrion *et al.* (1982) found similar values, a mean of 1.20 and also a maximum of 8 once. Maksimova (1973) reported intensities between 1 and 4. Gabrion *et al.* (1982) suspected that brine shrimps bearing 5 or more cysticercoids could be committed to high mortality. This is possible, but our preliminary results, arising from living wild populations kept in the laboratory, do not show mortalities specially bound to cysticercoids presence, regardless of their density (Amat *et al.*, in press).

A turnover phenomenon among populations of different *Artemia* strains, common in all the Mediterranean salterns in which there is not an exclusive brine shrimp species or strain (Amat, 1981, 1983, 1985) was also evident in this saltern. The surveyed samples showed the presence of diploid zygogenetical populations, as well as diploid and tetraploid parthenogenetical ones (Amat, 1980 a, 1980 b, 1985). Perhaps this fact could mask any kind of cyclic cysticercoid emergence related to the different number of *Artemia* generations developed along the year (Gabrion *et al.*, 1982). The population turnover among different brine shrimp strains happens as an answer to changing climatic factors, mainly the temperature. Although it is sometimes possible to find pure populations for any one of those strains, wide overlaping periods among them are common. Concurrent zygogenetical and parthenogenetical diploid populations are present during the early months of the year (males reported in 22 June 1986 and 6 March 1987 samples) and parthenogenetic diploid and tetraploid during summer and autumn.

High prevalences and mean intensities found in 16 January 1986 and 4 December 1986 samples deserve special attention mainly because they arise from periods in which salinities and temperatures were lower than usual. More data will be required to determine wether low temperature and salinity increase the incidence of parasitism, either directly or through a relationship with the cestode life cycle.

The plentiful parasitosis which appeared in the 4 December 1986 sample provided a large availability of both normal (N) and red (R) *Artemia* specimens. Parasitism prevalence was 69.80 % and 88.90 % for (N) and (R), respectively, the highest mean intensities here reported, even significantly higher for red specimens (2.02) than normal ones (1.65).

The means of introduction of the cestode egg from the aquatic medium to the brine shrimp is not known. If entrance is via the alimentary tract, then the egg is probably not bigger than 50-60 µm, the maximum particle size that *Artemia* telopodites can filter properly.

There is also a lack of information on any special ability period for the egg entrance and implanting according to the crustacean metamorphic growth. From data in Table II B it is possible to see that cysticercoids were not found in *Artemia* specimens smaller than 4-5 mm size, when they pass the metanaupliar stage to become juvenile. Sizes endowed with higher parasitism intensity lay between 8 and 12 mm, namely 67 % of observed parasitized specimens. Wild *Artemia* populations usually living in strong brines seldom provide individuals bigger than 13 mm total length, except for the tetraploid parthenogenetical strain. Mueller (1963) and Pearre (1976) showed that the presence of parasitism is related to an increase in the observed average size of individuals in the population.

Once inside the brine shrimp body, the cestode egg can settle in any of several anatomical areas, with densities about 30 % in the thorax, specially in the base of the phyllopods, and 59 % in the abdominal segments, commonly between the 4 th and 6 th. We suspect that the change in predominant location of cysticercoids with increasing size of brine shrimp is related to different rates of development of different anatomical areas. Metanauplii and juveniles achieve more thoracic development (specially the thoracopods or limbs, essential for food filtering, respiration and motion) earlier than the abdomen, which becomes more conspicuous in favor of reproductive activity later (testis and ovaries development). Cysticercoid implantation is more often in several thoracic areas of the shrimp anatomy until sizes are about 8-9 mm. From then on (Fig. 3) higher densities are found in the abdomen.

The presence of parasites can produce severe changes in the reproductive capabilities, including total inhibition of their intermediate or final host (Maema, 1986; Pearre, 1976). The parasite can settle in the host gonad itself inducing severe atrophy (Baudoin, 1975). Sometimes it can disturb normal gonadal activity through the emission of substances toxic or alien for the host, or by means of anomalies caused in the host hormonal systems (Hurd and Arma, 1987) that can induce sex changes, hermaphroditism, or the reproduction of the parasite's offspring instead of the host's (Van Duijn, 1973; Nappi, 1973; Noble and Noble 1982; Post, 1983).

There is no explanation available as yet for the unusual event arising from the 16 January 1986 sample in which 72 % of the parasitized females displayed normal aspect and breeding activity. Perhaps this could be linked to the parasite biological cycle through a great abundance of parasite eggs in the brines, promoting a strong infection pressure in the *Artemia* population. Another explanation could be a specificity for parasitism on one of the *Artemia* strains present in this saltern along the year, e.g. the diploid parthenogenetic one, the most common.

Finally, it is possible to venture an explanation according to the behavior of the final host, which is unknown but is most likely one of the water bird species living there (Charadriformes, Anseriformes, Lariformes) that feed largely on *Artemia*. It is widely demonstrated that intermediate hosts can develop abnormal or exciting behaviour, and shape or colour (Margalef, 1974; Moore, 1983, 1984; Helluy, 1983, 1984; Brown and Thompson, 1986), in order to attract the attention and the attainment of the suitable target host, a critical step in the life-cycle of parasites. These mechanisms (favorisation) increase the chances of being recruited by potential hosts for the infectious stages (Combes, 1980). Most samples surveyed here contained a lot of parasitized *Artemia* females displaying red colour. A close observation of these red parasitized shrimps showed a lot of minute refringent droplets spread inside the whole body, specially in the thoracopods. The droplets (presumably of lipidic nature, Goodwin, 1960) arrangement excludes an hemoglobin coloration origin, as is usual in *Artemia* hemolymph, mainly when living in strong brines with low oxygen content. This red colour makes brine shrimp very conspicuous in or near the brine surface. This behaviour could be helpful to the fulfillment of the parasite life-cycle. That sample displaying a great number of parasitized *Artemia* females endowed with normal shape and colour could be the consequence of a strong predatory pressure of birds on previously existing red specimens.

We have described here the prevalences and intensities of *F. liguloides* parasitism on brine shrimp in one saltern. Further research is necessary to document physiological, biochemical and behavioral changes in parasitized individuals, to determine whether parasitism differentially affects different *Artemia* strains in this salina, and to elucidate the life cycle of the parasite and *Artemia*'s role in it.

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