



RESEARCH ARTICLE

## Cestode Infection of the Native Brine Shrimp (*Artemia parthenogenetica*) in Çamaltı Saltpan (İzmir/Türkiye)

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*Flamingolepis liguloides*  
Live feeds  
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**Abstract:** *Artemia* sp. populations in saltworks throughout the world have been gaining importance due to their extensive use in aquaculture and their importance as the main prey organism for aquatic birds in hypersaline ecosystems. The genus *Artemia* sp. is also known as the intermediate host of some cestode species that are associated with flamingos. In this study, *Flamingolepis liguloides* parasitism was determined in *Artemia parthenogenetica* for the first time in Türkiye. Infected *A. parthenogenetica* was detected in İzmir Çamaltı saltpans between May-August 2018 and the parasite diagnosis was made. Parasites were detected near the abdomen, thorax and the intestinal tract of *A. parthenogenetica*. The prevalence of parasites was higher in adult *Artemia* (63.6%). The presence of *F. liguloides* in *A. parthenogenetica* was very high with a frequency of 72.2%. The most abundant and prevalent parasite infection was recorded in July which is the most suitable time of the year with respect to number of flamingos in the area. The results show the prevalence of this parasite infection in *A. parthenogenetica*, which may be important for both the local *Artemia* population in the area and the flamingos breeding in Çamaltı saltpans.

**Anahtar kelimeler:**

Akuakültür  
*Artemia parthenogenetica*  
Çamaltı tuzlası  
*Flamingolepis liguloides*  
Canlı yemler  
Parazitlik

### Çamaltı Tuzlası'nda (İzmir/Türkiye) Yerli Tuzla Karidesinde (*Artemia parthenogenetica*) Görülen Sestod Enfeksiyonu

**Öz:** Tuzlalardaki *Artemia* sp. popülasyonları, akuakültürde kullanımı ve çok tuzlu ekosistemlerde su kuşlarının besini olmasına göre önem kazanmaktadır. Ayrıca bu besin zincirinde *Artemia*, flamingo kuşları ile ilgili olarak bazı sestod türlerinde ara konakçısıdır. On iki ay süren bu çalışmada Türkiye'de ilk kez *Artemia parthenogenetica*'da *Flamingolepis liguloides* parazitliği saptanmıştır. Mayıs-Ağustos 2018 tarihleri arasında İzmir Çamaltı Tuzlasında enfekte olan *A. parthenogenetica* tespit edilmiş ve parazitin teşhisi konmuştur. Parazit, *A. parthenogenetica*'nın karın, göğüs kafesi ve bağırsak kanalı yakınında tespit edilmiştir. Parazitlerin yayılımı çoğunlukla erişkin bireylerde (%63,3) oranında daha yüksektir. Tuzla havuzlarında, *F. liguloides* varlığı %72,2 gibi oldukça yüksek bulunmuştur. En bol ve yaygın parazit enfeksiyonu, ekosistemdeki birey sayısı için yılın en uygun zamanı olan temmuz ayında kaydedilmiştir. Elde edilen sonuçlara göre, flamingo kuşlarının çok önemli bir göç noktası olan bu bölgede *A. parthenogenetica*'da bu parazit enfeksiyonunun yaygınlığı görülmektedir. *A. parthenogenetica*, bu sestodların parazitlediği ara konaktır. Bu sonuç Türkiye'deki birincil tespittir.

### Introduction

Many aquatic invertebrate taxa, such as crustacea, branchiopoda, ostracoda and copepoda, can adapt to extreme environmental conditions including high or low temperature and salinities, dry conditions, as well as predation and lack of food by forming dormant stages in dynamic environments such as estuaries and hypersaline ecosystems. (Hand et al., 2016). The genus *Artemia* sp. Leach, 1819 (Branchiopoda, Anostraca) are a complex of sibling species distributed in seven continents with the exception of Antarctica (Vanhaecke et al. 1987; Triantaphyllidis et al., 1998). *Artemia* also known as the brine shrimp is among the most intensely studied aquatic

organisms, due to its importance in aquaculture especially in the feeding of larval forms. The brine shrimp genus includes bisexual and parthenogenetic strains of different ploidy (Baxevanis et al., 2006; Gajardo, et al., 2002, 2012). Parthenogenetic *Artemia* populations are widely distributed except in the American continent. Bisexual and parthenogenetic *Artemia* species prefer different microhabitats and are usually temporally separated (Agh et al., 2007), although a handful of populations are mixed (Amat et al., 2007). From an ecological point of view, *Artemia* is a keystone taxon in hypersaline food webs due to its dominance; it is the main prey for aquatic birds

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(Sánchez et al., 2006), the intermediate host for many species of parasites (Georgiev et al., 2005; Rode, Landes, et al., 2013; Rode, Lievens, et al., 2013; Vasileva et al., 2009), and the main consumer of phytoplankton. *Artemia* sp. is particularly suitable for studying phase effects. For instance, it is a good tool for investigating how biological changes in a species translate into "cascading" effects throughout the population and the ecosystem. From this point of view, the first invasion of *Artemia* species by parasitic cestoda were reported from Tunisia (Heldt, 1926), followed by Spain, Italy (Amat et al., 1991a, b), France (Gabrion and MacDonald, 1980; Thiery et al., 1990), and many other countries (Di Cave et al., 1990; Di Cave and Mura, 1990; Mura, 1995). However, there are no data on the abundance of infested *Artemia parthenogenetica* by cestodes from Turkey. In this study, the cestode parasitism of *Flamingolepis liguloides* (Gervais, 1847) in *Artemia parthenogenetica* Barigozzi, 1974 was investigated in order to determine their abundance for the first time in Turkey. It is observed that flamingo populations in the Mediterranean region has increased due to the changing climatic conditions (BirdLife International, 2004) which may help spreading of cestode parasites. This study aimed to form a basis for further research to determine the distribution of this parasitism in Turkey, especially at the migration points of flamingos.

## Material and Methods

### Study area

The studied native *A. parthenogenetica* is from Çamaltı saltworks (38°30'12.73 "N, 26°54'12.94"E) (Figure 1). Five sampling stations with different salinities were selected and samples were collected for 12 months.

The study area is the Çamaltı salt pans (Figure 2) with an area of approximately 60 km<sup>2</sup> and a depth of 1-2,5 m. The area gradually overflows with seawater from April to September and continuously over the next few months.

The salinity of the water is high and varies between 40 and 320 ppt. Its avifauna is represented by 289 bird species (Figure 3) (Anonymous, 2019).

### Sampling

The *Artemia* samples were collected monthly from a depth of 0.5-2.5 m during January 2018 to December 2018 covering consecutive seasons: winter, spring, summer, and autumn. A 125 µm plankton net was used for collecting *Artemia* samples. All samples were fixed in 70% alcohol. The identification of parasites was performed according to Georgiev et al (2005). The mean number of parasites, prevalence of infection, standard deviation were calculated and a non-parametrical Z-test was performed to compare prevalences between adult and juveniles. The statistical analyses were performed with SPSS software.



**Figure 1.** The study area of the Çamaltı saltern ecosystem



**Figure 2.** The earthen ponds of Çamaltı saltworks

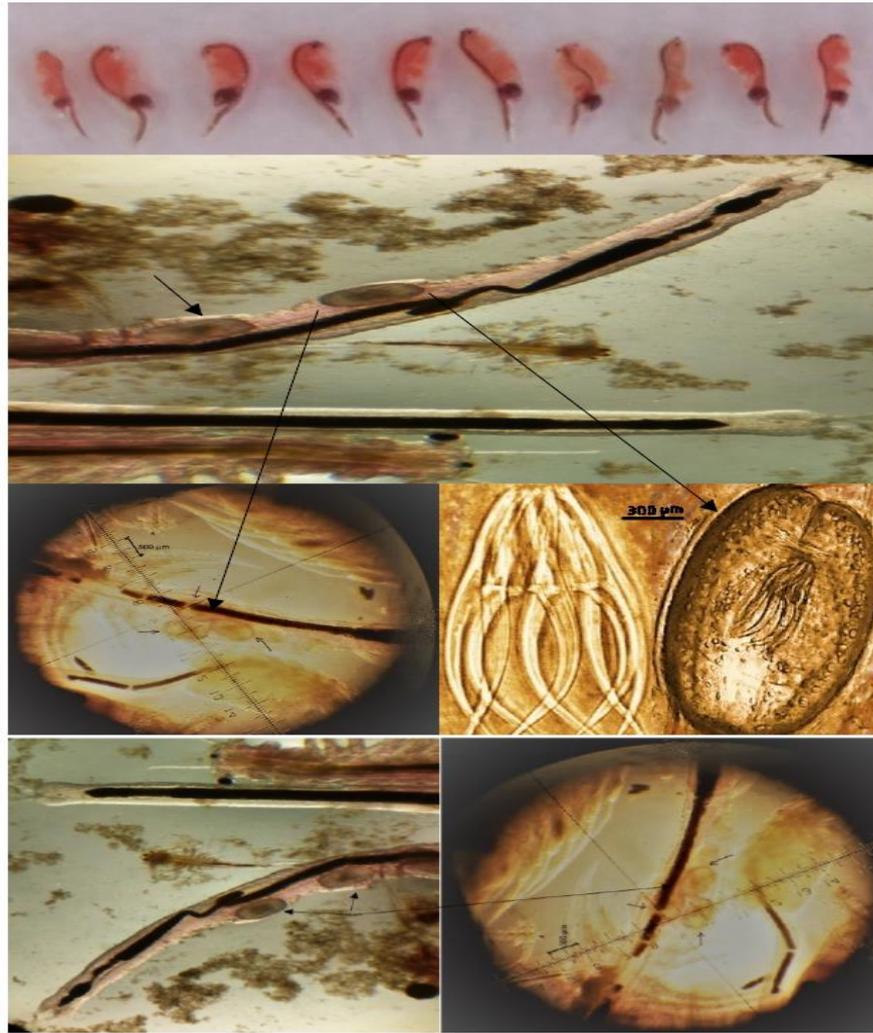


**Figure 3.** Flamingos in Çamaltı saltworks

## Results

In this study, *F. liguloides* parasitism in *A. parthenogenetica* was described for the first time. *F. liguloides* was the only cestode detected in all *A. parthenogenetica* individuals sampled. The parasites were mostly located in the thorax and abdomen, especially near the gut tract, (midgut and hindgut) of brine shrimps (Figure 4).

*F. liguloides* infection was recorded from both juvenile and adult *A. parthenogenetica* between May-August 2018. The overall infection rate was found very high, with a frequency of 72.2%. Among the 361 infected brine shrimps, 44.8% had only one cysticercoid. Only one individual had 7 cysticercoids. In total, 500 *A. parthenogenetica* were investigated and 139 individuals had no cysticercoid (27.8%) (Table 1).



**Figure 4.** Infected *A. parthenogenetica* individuals

The prevalences for adults and juveniles were found as 63.6% and 36.4%, respectively (Figure 5).

Monthly proportions of adult and juvenile *A. parthenogenetica* infected with *F. liguloides* were presented in Figure 6 and 7. A maximum of 7 and 4

cysticercoids were determined in adult and juvenile *Artemia*, respectively.

Monthly parasitic cestode intensity was relatively higher in adult *Artemia* (63.0% in May, 60.0% in June, 65.0% in July, 65.0% in August) (Table 2).

**Table 1.** Number of the prevalence of infected and uninfected *A. parthenogenetica*

Descriptions	Infected	Uninfected	Total
Number of <i>Artemia</i> samples	361	139	500
Prevalence of infection (%)	72.2	27.8	100
Minimum number of parasites	1	-	-
Maximum number of parasites	7	-	-

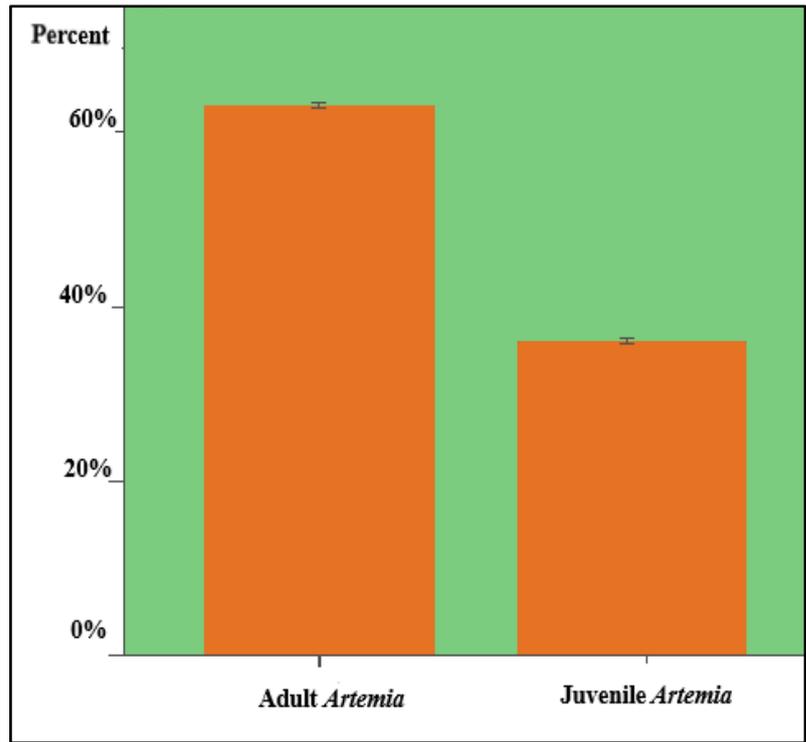


Figure 5. The percentage of *F. liguloides* in adult and juvenile individuals in the whole study

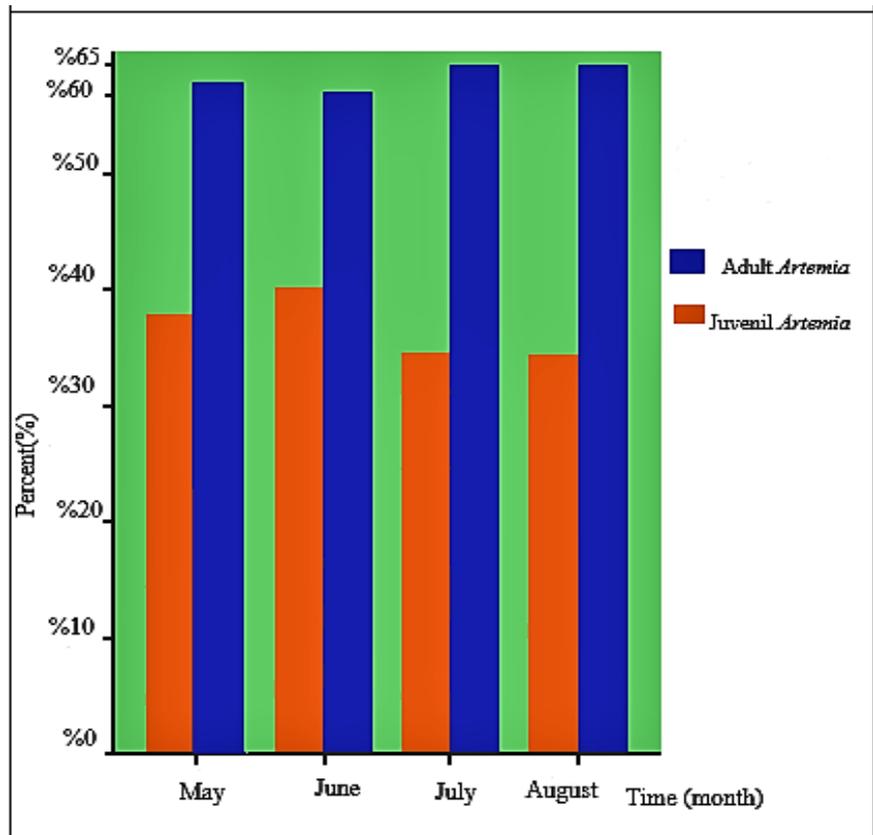
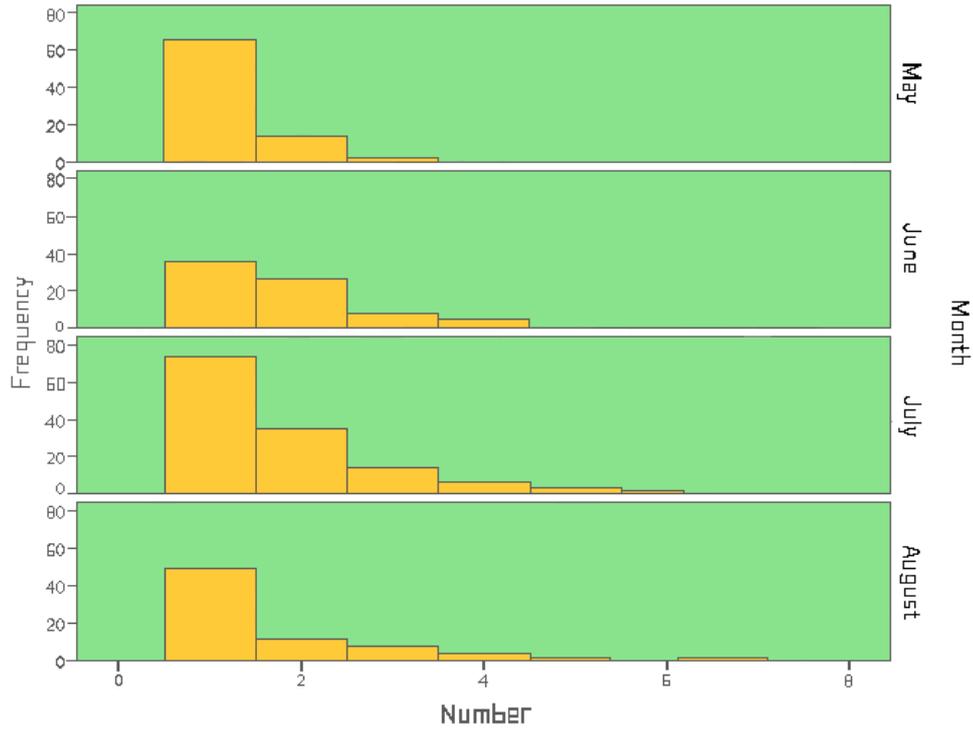


Figure 6. The monthly proportions of *A. parthenogenetica* infected with *F. liguloides* in adult and juvenile Artemia



**Figure 7.** Monthly variations in frequencies of *A. parthenogenetica* infected by *F. liguloides*

**Table 2.** Parasite infection Month / *A. parthenogenetica* cross-tabulation

Month	Specifications	<i>A. parthenogenetica</i>		Total
		Adult	Juvenile	
May	Count	63 <sub>a</sub>	37 <sub>a</sub>	100
	% within Month	63	37	100
	% within <i>A. parthenogenetica</i>	19.8	20.3	20
June	Count	60 <sub>a</sub>	40 <sub>a</sub>	100
	% within Month	60	40	100
	% within <i>A. parthenogenetica</i>	18.9	22	20
July	Count	130 <sub>a</sub>	70 <sub>a</sub>	200
	% within Month	65	35	100
	% within <i>A. parthenogenetica</i>	40.9	38.5	40
August	Count	65 <sub>a</sub>	35 <sub>a</sub>	100
	% within Month	65	35	100
	% within <i>A. parthenogenetica</i>	20.4	19.2	20
Total	Count	318	182	500
	% within Month	63.6	36.4	100
	% within <i>A. parthenogenetica</i>	100	100	100

Every subscript letter denotes an *A. parthenogenetica* subset category whose column proportions do not differ significantly from each other at the 0.05 level.

## Discussion

Determining the causative factors of an observed disease dynamic is often challenging, particularly with respect to seasonal and variable multi-host systems and other variable parameters. In this study, long-term field data was used to resolve the effects of seasonality and host specificity on prevalence in a simple host-parasite population. Uninfected parasites of *Artemia* individuals are found at the bottom of salt pans whereas those infected with parasites move towards the water surface and move horizontally (Fig. 8).

In the field, prevalence varied between 40 ppt and 320 ppt in all host and parasite combinations. *F. liguloides* is strongly seasonal, being highly prevalent in the summer and absent in the winter (Figure 5, 6, 7). Our findings revealed that cestod seasonality was mainly driven by the seasonality of the host *A. parthenogenetica*; normally *F. liguloides* is unable to persist in host community. As stated by the terminology proposed by Chervy (2002), the cysticercoids of *F. liguloides*, *F. flamingo*, *Wardium stellorae*, *Gynandrotaenia stammeri* belong to the group of the cercocysticercoids, while those of *Eurycestus avoceti*, *Anomotaenia tringae*, and *Anomotaenia* sp. are considered monocysticercoids. The cysticercoid of *Confluaria podicipina* is close to the modification termed "ramicysticercoid" but its cercomer is not branching. This proposes the necessity of further improvement of the terminology proposed by Chervy (2002). The following 14 cyclophyllidean cestode species were previously known to use brine shrimps of the genus *Artemia* sp. as an intermediate host in their life cycles: Hymenolepididae (11 species): *Confluaria podicipina* (Szymanski, 1905), *Fimbriarioides tadornae* (Maksimova, 1976), *F. liguloides* (Gervais, 1847), *F. caroli* (Parona, 1887), *F. flamingo* (Skrjabin, 1914), *F. tengizi* (Gvosdev and Maksimova, 1968), *Hymenolepis californicus* (Young 1952), *Wardium fusa* (Krabbe, 1869), *W. gvozdevi*, *Branchiopodaenia gvozdevi* (Maksimova, 1988) and *W. stellorae* (Deblock et

al., 1960). Dilepididae (2 species): *Eurycestus avoceti* (Gabrion and MacDonald 1980, Maksimova 1991, Robert and Gabrion 1991) and *Anomolepis averini* (Spasskii and Yurpalova, 1967). Progynotaeniidae (1 species): *Gynandrotaenia stammeri* (Fuhrman, 1936) (Gvozde and Maximov, 1968; Chervy, 2002; Vasileva et al., 2009; Amarouayache et al., 2009; Gajardo and Beardmore, 2012; Bray, 2014). *F. liguloides* cysticercoids in *Artemia* sp. were reported from the Mediterranean area by Robert and Gabrion in 1991 for the first time but this study is the first report from Turkiye. *Artemia* with its limited predator avoidance such as hypersaline tolerance and daily vertical migrations (Lenz, 1980; Forward and Hettler, 1992; Sanchez et al., 2007) are, in general, easy prey for aquatic birds, aquatic invertebrates and fishes alike. They are a major component of flamingo diet and have a crucial importance as a food source in saltwater habitats (Rolf, 2018). However, parasitic *Artemia* cestodes increase susceptibility of brine shrimps by reducing their fecundity (Cooper et al., 1984; Britton et al., 1986; Amat et al., 1991a; Verkuil et al., 2003; Sanchez et al., 2006; Sanchez et al., 2007). Overall, 15 cestode species use *Artemia* as an intermediate host which is linked by predation of avian hosts (Georgiev et al., 2005; 2007). The brine shrimp *Artemia* is a non-selective filter feeder (Provasoli and Shiraishi 1959; Dobbeleir et al., 1980; Sanchez et al., 2013), that feeds on microorganisms that are present in the water column including detritus (Savage and Knott, 1998; Sanchez et al., 2013). *Artemia* is infected with the cestode eggs called oncosphere (20 µm) when they ingest the eggs from the water column. The eggs, then, develop into cysticercoid (larva with scolex) in the hemocoel (Robert and Gabrion, 1991; Sanchez et al., 2007; Amarouayache et al., 2009). The parasitic cestodes transmission is completed when infected *Artemia* are ingested by aquatic birds (Sanchez et al., 2013). Once ingested, larval cestodes develop into mature worms in the digestive tract of flamingos and the eggs of the adult parasites are dispersed by defecation (Amarouayache et al., 2009).



**Figure 8.** *Artemia* individuals horizontally transmitted in saltern ponds

The salt pans of Çamaltı in İzmir, Turkey has become an important breeding site of flamingos (Figure 3) since 2000. In earlier studies, some biological characterization of this ecosystem and the native *A. parthenogenetica* were determined between January and December in the Çamaltı Region, İzmir which is the biggest marine coastal solar saltwork in Turkey (Koru, 2013; Koru and Deniz, 2017; Koru and Perçin, 2018). A breeding colony of flamingos can be observed in this area between March and August (Balkız et al., 2015). The parasitism of *Artemia* sp. by *F. liguloides* is probably related to the presence of flamingos. Similarly, Amarouayache et al (2009) observed cestode parasitism at the end of the winter (February-March) and in the spring (April-May) coinciding with the presence of flamingos in the study area in Algeria. In earlier studies the intensity of the parasitism was variable; less than 3 cysticercoids per individual in Algeria (Amarouayache et al., 2009), 13 in the populations of Spain (Georgiev et al., 2005) and about 9-11 in France (Thiéry et al., 1990). Sanchez et al (2013) reported multiple cysticercoid infections ranging between 2-4 and a maximum of 14 individuals. In our investigation, a maximum of 7 and 4 cysticercoids were determined in adult and juvenile *Artemia*, respectively (Figure 4). According to Thiéry et al., (1990), the accumulation of cysticercoids were associated with age and body size. It was reported that brine shrimps could digest 25-30 µm diameter particles at larval instar III-IV stages, and *F. liguloides* eggs with a diameter of 40-50 µm could only be ingested by older stages of brine shrimps (Dobbeleir et al., 1980; Mura 1995). This may explain the difference between the percentage of infected juvenile and adult *A. parthenogenetica* individuals. In this study, the location of the cysticercoids in *Artemia* was mostly in the thorax and abdomen regions (Figure 4). Similarly, the cysticercoids were reported in the thorax of *Artemia* juveniles, however, they were especially located in the abdomen of adults sampled from the saltworks of Sardinia (Mura, 1995). Thiéry et al., (1990) remarked that the location of the cysticercoids was related to the volume of the hemocoel and the dispersion was relevant to the allometric changes during the growth of *Artemia*. It is important to understand the effects of parasites in biological invasions and the interactions between predators and competitors (Combes, 1996; Torchin and Mitchell, 2004; Prenter et al., 2004; Cespedes et al., 2017). *A. parthenogenetica* spend most of the day in the 25% of the water column near the bottom and occupy the other 75% during the night (Britton et al., 1986; Sanchez et al., 2013) exhibiting strong negative phototaxis and positive diurnal geotaxis (Lenz 1980; Bradley and Forward, 1984; Sanchez et al., 2007). However, infection by cestodes changes the proportion of time that was spent at different depths (Sanchez et al., 2007; Sanchez et al., 2013). Cestode parasites increase buoyancy of infected *Artemia* and make them swim on the surface of the water facilitating predation by the water birds (Thomas et al., 1997; Helluy and Holmes 2005; Curio 1988; Amarouayache et al., 2009). Infected brine shrimps become photophilous and their surface-swimming behavior can be observed (Sanchez et al., 2007). Sanchez

et al (2007) studied the effects of cestode parasitism on the behaviors of *A. parthenogenetica* and reported that 86% of the uninfected *Artemia* showed positive geotaxis whereas 53% of infected *Artemia* showed surface-swimming behavior (negative geotaxis) (Figure 8). This behavior increases the likelihood of predation by the final avian host and makes parasite transmission easier (Sanchez et al., 2007).

In conclusion, in this study, *F. liguloides* infection of the native *A. parthenogenetica* which is the major food source of flamingos in Çamaltı saltworks was established. The prey-predator relationships and the ecological effects should be studied in order to understand the effects of *F. liguloides* parasitism in the region. More research is needed to understand the cycle of parasitism and interactions between the brine shrimp *Artemia* and flamingos in the Çamaltı region.

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### Conflict of Interest

The writer in this study declares that there is no relationship based on mutual interests.

### Author Contributions

The data collection, methodology, analysis, writing and arrangement of the study were carried out by Edis Koru.

### Ethics Approval

The material used in this article is invertebrate species therefore ethics committee approval is not required for this study.

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