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Parasitological Evaluation of *Diplodus Annularis* (Linnaeus, 1758) Fish from Libyan Seawater

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Abstract

Diplodus annularis are common public seafood in Libya. Parasites like crustaceans are dangerous threats to fish species because they can lead to less quality seafood production besides the shortcuts of the fish life cycle that significantly affects marine life's biodiversity. So, it was recommended to follow up the health status of fish by identifying and counting different parasites spread in Libyan seawater, especially around Tripoli. This research aims to evaluate the common parasites of *D. annularis*. About ~ 3300 g of fish samples (60 individual units) were examined for the presence of helminthes endoparasites, copepods, branchiura, and isopoda. Results evaluated in terms of prevalence percentage with different ranges of length and weight of fish samples in addition to maturity. Results prove that branchiura (*Argulus purpureus*) and isopoda (*Ceratothoa oestroides*) are the common parasites that lived on *D. annularis* with the highest percentage of infestation ~ 10.34 and 4.35 % for (12-14) and (14-16) cm length respectively. The sex of a *D. annularis* species was confirmed by examining the genital papilla located behind the anus and finding a balance between the numbers of males and females, but male are more infested than female samples. A correlation between length, maturity and infection was theoretically confirmed by applying a mathematical exponential model to male and female and hence probability function (Ø) of maturity and parasitological infection of *D. annularis* can be expected within a particular length range.

Keywords: *Diplodus annularis*, Sparidae Family, Helminth, Copepods, Branchiura, Isopoda, Crustacean, Parasitology, *Ceratothoa Oestroides*, *Argulus Purpureus*

1. Introduction

Sparidae including *D. annularis* (Linnaeus, 1758) is a very large family widely distributed mainly in the Atlantic, Indian and Pacific Oceans [1]. About 22 species of Sparidae belonging to 10 genera are found in the Mediterranean Sea [2] including the Libyan seawater. Other resources estimated that the number of species of the Sparidae family is 162 with 38 genera [3].

D. annularis can be infested by so many parasites like helminthes, Copepods, Branchiura, or Isopoda. Parasitic order, Isopoda belongs to the subphylum Crustacea, phylum Arthropoda, with more than 10,300 species found in the oceans and supports free-living parasites that infested fish. Isopods are typically marine parasites that inhabit the warmer seas [4], having a small body of 0.5 to 3.0 cm in length. Isopods parasites act as ectoparasites, while, for example, Cryptoniscoidea was reported as an endoparasite of crustacean hosts [5]. Parasitic isopods become harm to the host in different ways e.g., larva stage feed in high-speed rate and easily kill the host through the tissue damage e. g. the adult Ceratothoa oestroidea can slow-down the growth and inhibit reproduction of the fish host. In the mouth, they can change the oral structures and may completely replace the tongue [4]. It was found that isopod parasites suck the blood of their hosts, and the fish becomes weak due to deficiency of oxygen and nutrients and hence, the weak fish is more vulnerable to various fatal diseases [6]. Other fish parasites, the order Arguloida belongs to the subphylum Crustacea, phylum Arthropoda, and subclass Branchiura. There are approximately 130 species of Branchiura subclass that are described as ectoparasites. Branchiuran crustaceans can cause several undesired issues to the fish host e.g., itching, visible parasites are seen as little green specks that move around the fish in some sensitive regions such as eyes and gills. Recently, several descriptions of new species of isopods parasites were done in detail [7-9]. Branchiura are fully described and reviewed in Iran [10], Nigeria [11], and The Slovak Republic [12]. Due to shortage of resources of parasites that infested fish in the Libyan seawater, so the present study aims to identify and evaluate the prevalence of different parasites like helminth, copepods, branchiura, and isopoda on *D. annularis* fish around Tripoli city. Finally, isolated parasites were identified and classified according to the normal taxonomy system for the animal kingdom and the available literature.

2. Material and methods

Fish Samples: In May 2021, about ~ 3300 g of *D. annularis*, fig 1 was obtained by purchasing from the local fish market at Tripoli seaport, Tripoli, Libya (32º 53' 13.95" N, 13º 11' 28.82" E) [13]. Samples stored into an ice box and dispatched to the zoology laboratory for experimental investigations. Samples were classified according to their weight, length, maturity, and the presence of parasites. All fish samples were preserved in a solution of 70% ethanol (C_2H_5OH) in water which is traditionally used as a sterilizing concentration [14, 15]. 10 % formalin (HCOOH) was used for long-term sample preservation. The length of each individual fish was measured in centimeters (cm) using a flexible tape measure and was taken from mouth to tail. The weight in grams (g) of fish samples was measured using a digital electronic balance of maximum capacity 250 g with two fractional digits (0.01). Sex of *D. annularis* was determined macroscopically by examining the genital papilla located behind the anus. Placing a drop of methylene blue dye on the genital region can highlight the papilla and its opening. Finally, fish samples were dissected not only to confirm the sex, but also to isolate parasites inside the body. Stock fish samples contain three species: *Diplodus annularis*, *Sardinella aurita*, and *Boops boops* with percentage of ~ 94.55, 2.42, and 3.03 % (W %) respectively. Characteristics of stock fish samples were summarized in table (1). Investigations were done only for *D. annularis*.

Kingdom: Animalia
Phylum: Chordata
Class: Actinopterygii
Order: Perciformes
Family: Sparidae
Genus: *Diplodus*
Species: *Diplodus annularis*

Fig 1: *Diplodus annularis* fish and its classification

Table 1: Characteristics of stock fish samples

Libyan common name*	Scientific name [16]	English name [16]	Weight		Number		Average weight per unit
			(g)	(%)	Units	(%)	
Sabaris (Investigated)	*Diplodus annularis*	Annular Seabream	3120	94.55	60	89.55	~ 52 g
Sardine (Excluded)	*Sardinella aurita*	Round sardinella	80	2.42	3	4.48	~ 27 g
Bogue (Excluded)	*Boops boops*	Bogue	100	3.03	4	5.97	~ 25 g
Total			3300	100	67	100	------

***** The local name

Parasites Examination: To examine the presence of parasites inside fish, a longitudinal incision is made starting from the gill to the end of the abdominal cavity. For every individual fish, all body parts, skin, mouth, gills, fins, nasal cavity and abdominal region were accurately examined. Gills and guts were placed in a small portion of seawater in the Petri dish and carefully examined for the presence of endoparasites. Nomenclature, taxonomy, and shapes of parasites were taken into consideration. Found parasites were carefully isolated, collected then immersed in 70 % ethanol solution inside a 10 mL translucent penicillin bottle, afterwards cleared for about ≈ 2 h in lactic acid solution before being observed under a ×50 binuclear microscopes.

Prevalence of parasites on *Diplodus annularis:* Prevalence (*P*) of parasite inside and outside the fish samples have been confirmed and calculated using the general formula [17]:

$$P\ (\%) = \frac{n}{N} \times 100$$

Where n is the number of infected fish and N is the total number of fish examined. Prevalence (*P*) of parasites fauna on *D. annularis* was calculated for different four groups of length and different four groups of weight as-well-as for male, female, and immature fish samples.

3. Results and discussion

Density of Parasites

Density of Parasites has been estimated for four types of parasites on six organs of *D. annularis* as shown in table (2). Results show that no copepods in examined organs for all samples of *D. annularis* whereas one fish sample was infected by one unit of helminth or branchiura at least. Parasitic helminth was observed lying along fins in one sample and parasitic branchiura was observed as a small spot on the skin of head. It was found that at least one *D. annularis* sample was infected by two or more units of small isopoda in the mouth of fish. Results emphasize that the mouth is the favorite organ for isopoda, skin is the convenient place for branchiura, and fins are suitable for the helminth to lie on. Nose, gills, and abdominal cavity are free from all types of examined parasites.

Table 2: Density of parasites in different organs of *D. annularis*

Examined Organ	Density of Parasites			
	Helminth	Copepods	Branchiura	Isopoda
Skin	Nil	Nil	+	Nil
Mouth	Nil	Nil	Nil	++
Nose	Nil	Nil	Nil	Nil
Gills	Nil	Nil	Nil	Nil
Fins	+	Nil	Nil	Nil
Abdominal region	Nil	Nil	Nil	Nil

(+) At least one fish sample infected by one unit of parasite.
(++) At least one fish sample infected by 2 or more unites of parasite.

Prevalence of parasites

Prevalence of parasites (*P*, %) has been calculated for all categories mentioned in table 3. Obtained results show prevalence (*P*) of four different parasites on 60 samples of *D. annularis* fish. It was noticed that only one fish sample was infected by helminth which represents about ~ 1.7 % of all *D. annularis* samples. Also, no copepods were observed on the host samples. This result may be attributed to the clean and healthy marine environment around Tripoli city. Apparently, *D. annularis* from the Libyan seawater can be considered free from parasites, especially helminth and copepods. Additionally, this result is a positive or a negative phenomenon according to the point of view, the positive side means a healthy growth of fish in a good quality environment. On the other hand, absence of parasites in marine environments can be considered as a negative phenomenon due to the fact that the role of parasites in ecosystem functioning has been considered trivial because a cursory examination reveals that their relative biomass is low compared with that of other trophic groups. However, there is increasing evidence that parasite-mediated effects could be significant: they shape host population dynamics, alter interspecific competition, influence energy flow and appear to be important drivers of biodiversity [18, 19]. According to the last fact, the number of animals increases in an environment as the biodiversity increases and *vice versa*. We can say that density-dependent transmission of parasites and host specificity can generate a causal link between parasitism and biodiversity. There is evidence that generalist parasites can reduce biodiversity through the process of apparent competition, but specialist parasites can act to increase biodiversity [20]. Disappearance of some parasites such as helminth and copepods from the Libyan environment may be attributed to the low level of biodiversity within the sampling period. Accordingly, it was highly recommended to consume the Libyan *D. annularis* fish without fearing the presence of noticed worms.

Table 3: Prevalence of parasites of *D. annularis* based on length, weight, and maturity

Parameter		Found (%)	Infected (%)	Prevalence of Parasites inside mouth			
				Helminth	Copepods	Branchiura	Isopoda
Length, cm	12-14	48.33	10.34	Nil	Nil	+	++
	14-16	38.33	4.35	Nil	Nil	+	+
	16-18	10.01	0.00	Nil	Nil	Nil	Nil
	Above 18	3.33	0.00	Nil	Nil	Nil	Nil
Weight, g	27-47	46.67	10.71	Nil	Nil	+	++
	47-67	38.33	4.35	Nil	Nil	+	+
	67-87	8.33	0.00	Nil	Nil	Nil	Nil
	87-107	6.67	0.00	Nil	Nil	Nil	Nil
Maturity	Male	45.00	11.11	Nil	Nil	+	++
	Female	46.67	3.57	Nil	Nil	+	+
	Immature	8.33	0.00	Nil	Nil	Nil	Nil

From table (3), the only parasites found on *D. annularis* are isopoda and branchiura. Generally, the number of *D. annularis* samples infected by isopoda is greater than that infected by branchiura and some fish samples can be described as doubly infected with two types of parasites, whereas some others can be described as a single infected with one type of parasites. As a function of length of sample, prevalence of parasites on *D. annularis* was inversely proportional with the length of the fish, and this means that the long fish has the low percent of prevalence and vice versa. *D. annularis* with 12-14 cm length was infected by more than 10.3 % while samples of 14-16 cm length were infected by more than 4.3 %. The above result was in agreement with the data published for Antalya Gulf, Turkey [21], the prevalence of isopoda (*Anilocra physodes*) is 4.76 on *D. annularis*. There are no countable infectious fish with length above 16 cm. It concluded that the calculated infected to not-infected ratio of a *D. annularis* is about ~ 1 to 5 around the marine environment of Tripoli city and this ratio of infection is acceptable worldwide. According to the weight of investigated fish samples, the percent of prevalence (%) has the following sequence:

Samples with (27-47 g) > Samples with (47-67 g) > Samples above 67g

The average length of the fish samples is 16 cm with mean prevalence (%) ≈ 3.6725 % and the average weight of the fish samples is 67 g with mean prevalence (%) ≈ 3.7650 % and therefore the two results are nearly the same, so for simplicity it was concluded that prevalence (%) of parasites on fish, can be calculated by either using length or weight as a practical function. Authors suggest generalizing this extrapolated fact on the upcoming marine animal environmental research to facilitate the research tools or to prevent repeating techniques that can goes-up to the same results.

Table 3 shows that male and female fish are found in a near similar ratio and equal to 45 and 46.67 % (1:1.04) respectively. This equality ratio may reflect a type of biological and ecological balance between male and female of *D. annularis* in the Libyan seawater that can be attributed to the use of non-selective fishing tools. This result is in contrast with the data published in the literature [22], where the coastal waters of the Canary Islands have the ratio (1:0.79) in favor of males. As shown in table (2), male were infected more than females by more than 3 times, this may be due to the high biological activities of male. In order to determine the length-maturity correlation and hence parasitic infection, a mathematical relationship [23] between the length class and probability function of mature fish (male and female) in each class of length was employed using the following equation:

$$Probability\ Function\ (\phi) = \frac{1}{[1 + e^{-a\,(Tl - TL_{50})}]}$$

This relationship is described by an exponential function, where, (ϕ) is the probability of finding of mature individuals; TL, average length corresponding to specific range of length, a, the constant of the exponential function (a=0.694 for male and a= 0.568 for female); and TL_{50} the average length of ~ 50 % mature individuals. From table (2), $TL_{50} \approx TL_{48.33}$ = 13 cm and the estimated values of the probability function (ϕ) for male and female of *D. annularis* were calculated with four different ranges of length. Results of the probability function (ϕ) were presented in Fig 2, from which it was noticed that maturity of fish was affected greatly by the length and as the length increased from 12 to 20 cm, the probability of finding a mature individual increased from 0.5 to near unity. This length-maturity and infection relationship is correct for either male or female, especially at the started and ended points of the curve. The infection region is concentrated with the probability of 0.5 maturities within the first length class (12-14 cm) and as the probability of maturities increases to unity; the infection rate reaches to a minimum value.

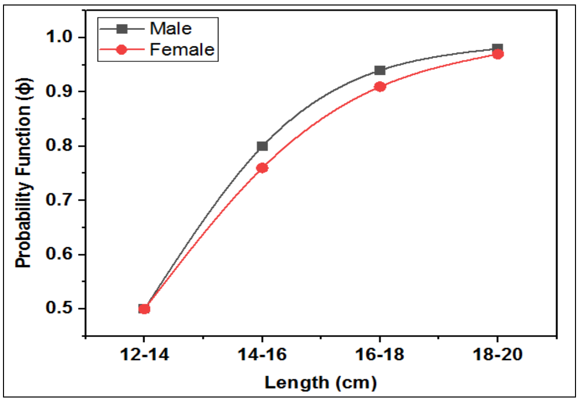


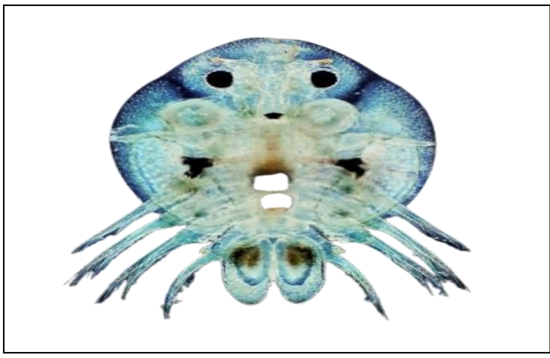
Fig 2: Length and maturity correlation as a probability function

Parasites: taxonomy and specifications

The only two parasites found on *D. annularis* have been classified as shown in Fig (3 & 4), from which the parasitic isopod species is *Ceratothoa oestroides* and branchiura species is *Argulus purpureus*. The two species share their kingdom, phylum, and subphylum. *Ceratothoa oestroides* is a crustacean isopod ectoparasite of marine fish that infect the mouth of the host [24]. They cause various issues to the host including damage of the tongue, stopping or decreasing the growth rate, weight loss [25]. It was recorded that *Ceratothoa oestroides* was found in Sparidae, Carangidae, Clupeidae, Maenidae, Scorpenidae, and Mugilidae. Embryos of *Ceratothoa oestroides* grow in the female, and the new generation develops through different stages until they are changed to a free-swimming parasite, ready to infest the hosts. *Ceratothoa oestroides* was considered the most dangerous parasite in Mediterranean seawater [26-28].

Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Malacostraca
Order: Isopoda
Family: Cymothoidae
Genus: *Ceratothoa*
Species: *Ceratothoa oestroides*

Fig 3: Isopoda and its classification [31]



Kingdom: Animalia
Phylum: Arthropoda
Subphylum: Crustacea
Class: Ichthyostraca
Subclass: Branchiura
Order: Arguloida
Family: Argulidae
Genus: Argulus
Species: Argulus purpureus

Fig 4: Branchiura and its classification [32]

Fish lice or *Argulus purpureus* belongs to Argulidae family, has different sizes from a few millimeters to 30 mm long, and females mostly bigger than males. *Argulus purpureus* have a flattened oval body and acts as ectoparasites mostly on the fish host [29]. *Argulus purpureus* as an adapted parasite has compound modified eyes and the mouth was developed to form a hook to suck the blood of the host. The body of *Argulus purpureus* has four pairs of thoracic appendages used to swim when it becomes free in the sea [30].

4. Conclusion

The present study was conducted on 60 units of *D. annularis* obtained from the marine coast of Tripoli, Libya to evaluate the parasitic status of this region. Branchiurars (fish louse) and isopods are the only two types of crustacean that are isolated, identified and recorded as a common parasite of Libyan *D. annularis* species. No parasites were recorded in the nose, gills, and abdominal cavity of fish. It was found that the short length and the less weight of fish samples have the highest percentage of prevalence (~ 10.3 %) with maturity probability of 0.5. Accordingly, it was highly recommended that to consume the Libyan *D. annularis* species without fearing the presence of helminth, copepods, branchiura, or isopoda.

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