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Original article

Prevalence of Some Parasitic Infestations in *Lagocephalus sceleratus* in Eastern Libya

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ABSTRACT

Background and aims*. Lagocephalus sceleratus (L. sceleratus) has been listed as one of the 100 "worst invasive fish species" of the Mediterranean Sea. This study was carried out to investigate the prevalence of parasitic infection in L. sceleratus.* ***Methods****. During this study, one hundred and ten fish were caught in different locations in eastern Libya from March to May 2022. We examined all fish after dissecting them using gross pathology and direct examination using light microscope. We tracked the prevalence of parasites depending on the region, gender, age, and the affected organs.* ***Results****. The result showed that the overall parasitic prevalence was 12(10.91%) Cryptobia iubilans, 3(2.73%), Haplosplanchnida sp. 3(2.73%), and Anisakis sp. (L3) 6(5.45%). Regarding the sexes, the study showed that the prevalence among males and females were 10.71% (6/56) and 11.11% (6/54) respectively. Also, it was noticed that the highest rate of infection with parasites was in the area of Susah 50% (2/4) followed by Talamitha 38.9% (7/18), then Khalij-Al Bambah 18.8%, (3/16), with no infection recorded in Ain El Ghazala. In this study, a number of parasites, two of which termed "Haplosplanchnida" and "Cryptobia iubilans" were found in the intestine, and this is in according to our knowledge is the first study published on the parasitic infection of L. sceleratus in Libya. It has been unknown whether these parasites can cause a disease in L. sceleratus or not, and that needs to be further investigated.* ***Conclusion****. L. sceleratus is a new host for some indigenous parasites, providing an additional niche for the success and increase of local populations of these parasites.*

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INTRODUCTION

Overfishing, pollution, and invasive species are currently endangering the world's biodiversity [1]. With roughly 800 marine non-indigenous species currently present, The Mediterranean Sea is the world's most invaded sea as well as a hotspot for biodiversity [2-4]. 500 of these invasive fish are Lessepsian species that arrived from the Red Sea after the Suez Canal was built [5,6].

Invasive species represent an interesting topic, and have given rise to concerns and debates among the scientific community with respect to the impact and threats that they pose, especially to native ecosystems and their biodiversity,

and the methods to control them [7, 8]. By changing community structure and function, ecosystem processes, and ecosystem services, marine invasive species can represent serious risks to biodiversity and have long-lasting ecological and economic effects [9]. The opening of the Suez channel that has led to the entrance of numerous exotic marine animal species (the so called "Lessepsian" species) to the Mediterranean Sea [10]. This high number of exotic species recorded in the Mediterranean Sea has also been attributed to the expansion of aquaculture activities [11].
Worldwide, there are 197 species of pufferfish, with 112 of them being marine, 48 brackish, and 37 freshwater species [12].
According to previous study [13], *L. sceleratus* is among the top 100 "worst invasive fish species" in the Mediterranean Sea. *L. sceleratus* is one of the largest members of Tetraodontidae family [14, 15], a pest for fisheries and a threat for native biodiversity and human health [16, 17]. It is now found in abundant populations along the coasts of many countries, Lebanon, Turkey (Mediterranean and Aegean coasts), Greece (Aegean and Ionian coasts) and Cyprus [16-18]. Moreover, *L. sceleratus* has quickly spread towards the west, along the shores of Egypt and Libya, as reported by [19, 20].
The genus *Lagocephalus* comprises four species, three of which are exotic and inhabit the coastal waters of the Mediterranean Sea.
So far, *L. sceleratus* represents a common fish species in the coastal regions of the eastern Mediterranean from north to south, with rapid expansion westward [17,21-26]. This fish belongs to the family Tetraodontidae (puffer fish), which comprises poisonous species to humans if consumed. The poisonous toxin is named tetrodotoxin TTX [27]. Numerous studies have supported the idea that TTX in pufferfishes is accumulated through the food chain (exogenous origin hypothesis) as opposed to being produced by endosymbiotic or parasitic bacteria inside the pufferfish body (endogenous origin hypothesis), despite the fact that the origin of TTX in pufferfishes is still largely unknown [28,29]. Consumption of these fishes has led to numerous cases of TTX poisoning in areas where they are consumed [30]. Most of the research on the puffer fish has focused on human health [27,31,32], hence, the impact of parasites on such invasive species is unpredictable [33].
For ectoparasites on fish, the Lessepsian movement of parasites was first noted in 1971–1972. On rabbitfish (Siganidae), both monogeneans [34-36].
Four digenean species have been documented [37,38], and three myxozoan parasite species have so far made their way from the Red Sea and established themselves [39].
The blue-spotted cornetfish, *Fistularia commersonii* Rüppell, 1838, another Lessepsian migrating fish, is parasitized by *Allolepidapedon fistulariae* Yamaguti, 1940, and *Neoallolepidapedon hawaiiense* Yamaguti, 1965, according to the most current studies.
Heterobothrium victorwepeneri sp., the first species of its genus to be recorded and named from South Africa and from the tetraodontid Amblyrhynchotes honckenii, was found on the gills of evileye pufferfish Amblyrhynchotes honckenii with a prevalence of 100% and mean intensity of 23 (4-72) [40]. Fish in various parts of the world are frequently affected by parasitic diseases, with a significant impact in tropical regions [41]. The rationale behind selecting this subject is the importance of fish wealth in Libya and the threat posed by the Pufferfish to this wealth, as well as, the lack of sufficient studies on the diseases that can affect this fish.
This study aimed to study the prevalence of fish parasites that infect *L. sceleratus* in eastern Libya.

METHODS

Study design and setting

The study was carried out in eastern Libya, from Ain-El Ghazal Lagoon, Khalij-Al Bambah, Susah, and Talamitha (see figure 1).

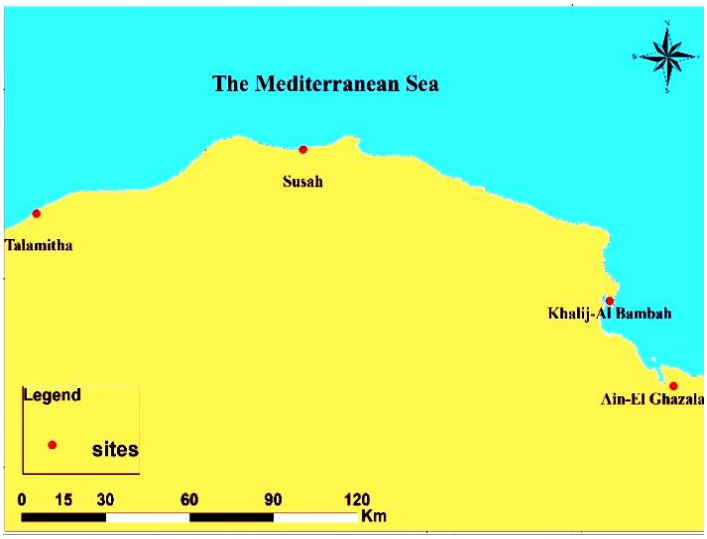

Figure 1. Locations of L. sceleratus sampling in eastern Libya.

Data collection procedure

Sampling

One hundred and ten samples of *L. sceleratus* were collected. All samples were collected by local fishermen, commercial ships, fishing nets, and fishing lines. Some of them were obtained by deep-sea dredgers. during the spring season at a depth of 0.5-70 m. Once the fish were caught, they were immediately placed in the ice box and transferred to the laboratory of the Veterinary Pathology in Omar Al-Mukhtar University.

Fish were individually weighed using a normal calibrated balance, then the lengths were measured by standard ruler. Determination of sex of each individual was performed by macroscopic examination of the gonads.

Parasitic examinations

Fish were examined according to the method described by direct examination [42]. No dyes were used and the imaging was done using A digital camera of a type Leica, of German origin. It is available at Omar Al-Mukhtar University/ College of Veterinary Medicine/ Department of Pathology and Clinical pathology / Pathology Laboratory.

Gross pathology was applied for external and internal organs, followed by washing the internal organs with an isotonic saline solution (NaCl 0.9%) several times to remove any attached mucous. After that, smears from different organs were microscopically examined under the light microscope.

Each and every organism discovered during the necropsy was preserved in 5% glycerin in 70% ethanol, then left to dry for 30 minutes on a hot plate at 30 to 50 °C.

Nematodes have been identified as stage 3 larvae of *Anisakis sp*. type I according to [43, 44]. One of the most significant zoonotic nematodes affecting marine fishes globally is Anisakis [45]. According to [46, 47], this family of nematodes often referred to as anisakids belongs to the Ascaridioidea superfamily, family anisakidae, and Raphid-ascarididae. Trematodes have been identified as *Haplosplanchnida sp*. according to [48]. which are belonging to Digeneans, these parasites range in size from microscopic species that are under 250 m to giants, with the majority measuring between 0.5 mm and 5 mm. Their morphology is typically inconspicuous, and they have an oral sucker that opens into the gut and a ventral sucker that is only used for attachment.

Protozoa they were tentatively identified as *Cryptobia iubilans* (order Kinetoplastida, family Bodonidae) only C. *iubilans*, one of the seven Cryptobia species connected to fish's digestive tract, is known to be pathogenic and to function as a parasite. based on [48].

Statistical analysis

The data was analyzed using the Statistical Package for Social Sciences (SPSS) version 26. And graphically, Microsoft Office Excel 2019 was used to represent the data. The prevalence was calculated by dividing the number of fish over all the examined fish.

RESULTS

The results showed that out of the total examined *L. sceleratus*, 12 (10.91%) were found infested with parasites. Among them, 6 (5.45%) were found infested with nematode genera (*Anisakis* sp. (L3)). These larvae were found freely on the skin and liver, as well as in the bile duct (see Figure 2a). Additionally, 3 (2.73%) fish were infested with trematode genera (*Haplosplanchnida* sp.) observed in the digestive tract (gut) (see Figure 2b). Furthermore, 3(2.73%) fish were found infested with protozoa (*Cryptobia iubilans*) in the lumen and within the intestine (see Figure 2c).
The fish's weight varied from 0.5 to 5.1 kg, and the body length ranged from 35.5 to 72 cm.

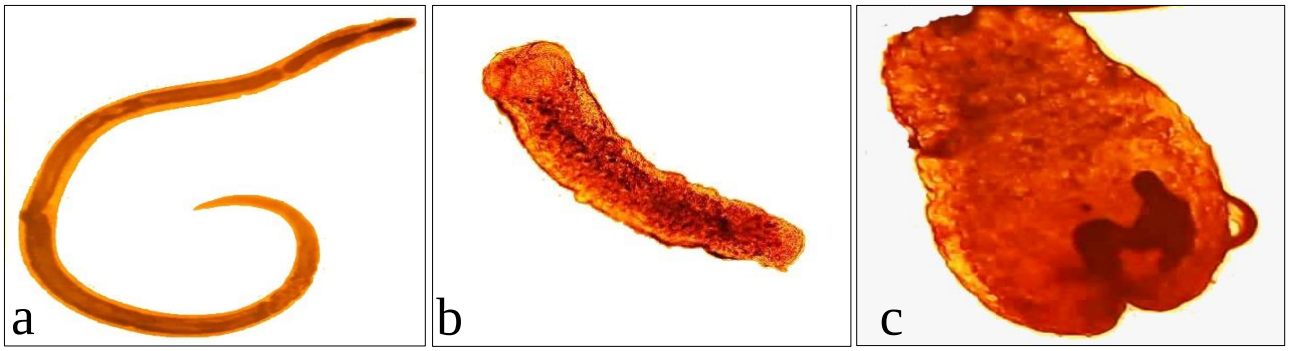

Figure 2. Light microscopy images show the prevalence of different types of parasites in various organs of L. sceleratus (x40)

The number of infected fish varies by location as shown below (Fig. 3). Ain El Ghazala Infection Ratio (0%), Talamitha (58.3%), Susah (16.7%), Khalij- Al Bambah (25.0%). A significant correlation was observed between the infection rate and regions, as indicated by a P-value of 0.000.

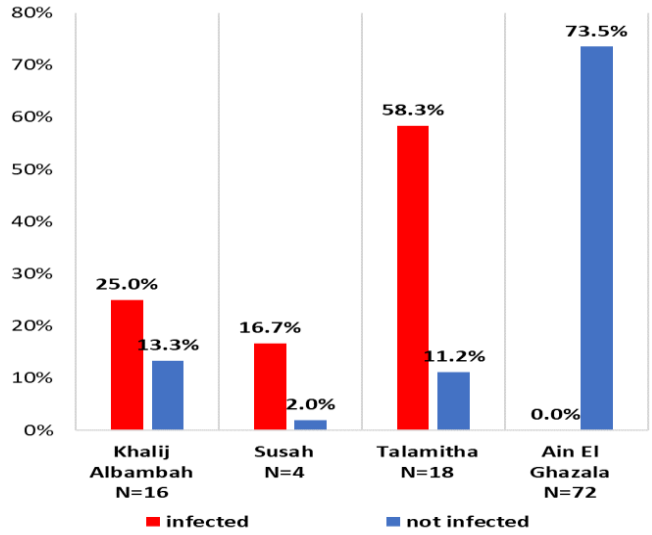

Figure 3. The prevalence of parasites in different regions.

Our research revealed that the rate of infection increased with fish length (Figure 4). The correlation was observed between the infection rate and the length of the fish, as indicated by a P-value of 0.000. Hence, there are significant differences.

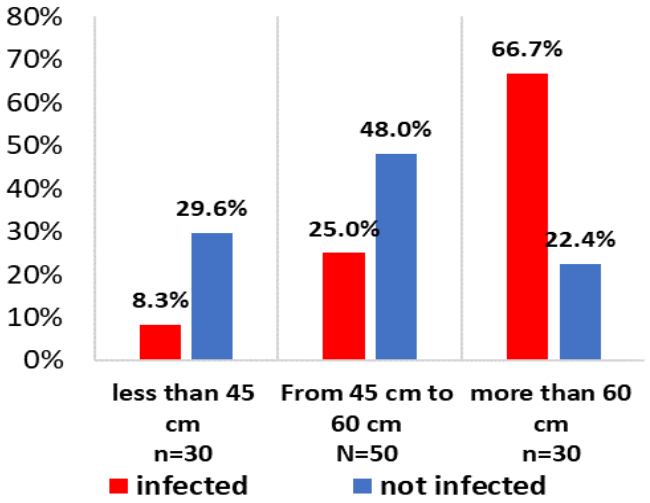

Figure 4. The prevalence of parasites according to the length of fish

In terms of weight, our study found that the infection rate increased with the fish's weight (Figure 5). A P-value of 0.000 indicates that a link between the infection rate and fish weight was found.

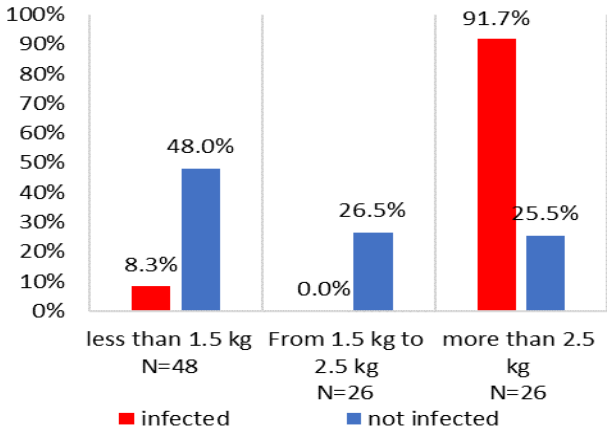

Figure 5. The prevalence according to the weight.

No relationship was found between infection rate and sex (P-value = 0.947). The prevalence of infection among males and females was similar. Also, no relationship was found between infection rate and infected organs (P-value = 0.000).

DISCUSSION

According to our search in the literatures, no scientific research has been published so far on parasitic infestation in *L. sceleratus in* Libya. In our study, 12 out of 110 specimens of *L. sceleratus* caught in eastern Libya were found to be infected with parasites. Two of these parasites identified as indigenous species in the Mediterranean Sea with low host specificity. These parasites are *Haplosplanchnida sp.*, *Cryptobia iubilans, Anisakis sp. (L3)* and they are already reported [43,44,48].

To the best of our knowledge, we describe here cases of infestation of the *L. sceleratus* with the *Haplosplanchnida sp.*, *Cryptobia iubilans* for the first time in Libya. The high prevalence of parasitic infestation in *L. sceleratus* in the Talamitha region was clear and significant, which makes us wonder about the reasons for this, and since we could not identify a specific reason, we discovered a study whose results (67.5%) are comparable to ours (38.8%) and which attributes this high incidence of the sort of sand found in the area around Brazil's Ubatuba Beach [49]. Additionally, the prevalence and variety of parasites in water can serve as a marker for its quality, as polluted waters tend to foster greater numbers and types of parasites [50,51]. Higher water temperatures and humidity may make it more likely for some parasitic species to spread, which could have an impact on how parasites spread in water as a result of climate change. A few parasite species may be more likely to spread depending on the kind and quality of water sources.

Our study indicated differences in the parasites hosted by *L. sceleratus* in different geographical locations in eastern Libya. But interestingly, no parasitic infestations were found in the 72 specimens collected from Ain El Ghazala. Perhaps because the fish in this area are obtained from distant depths, and therefore factors such as pressure, pollution levels, and harsh environmental conditions may play a role in this result.

During its early life stages, *L. sceleratus* feeds on various invertebrates, whereas *Sepia officinalis* and Octopus vulgaris were the main species that are found in the diet of larger fish [17]. These diet preferences may explain the low mean intensity of infestation of *L. sceleratus* by these parasites, as these parasites have not been described in Sepia and Octopus [17].

When females and males were examined, only mathematically, the rate of infection in males is higher than that in females, while the percentage of infection was equal, and there were no significant differences. This may be because parasite infestations were not affected by sex of fish [52], and our results were agreed with previous studies [53]. Because all of the aforementioned parasites transmit through food, the high prevalence of infestation in the digestive system's organs, which matched that seen in the literature, can be explained [54]. Long and high-weight fish were more infected than short and low-weight fish (Figures 4 and 5); this agreed with previous study [55], who indicated that the longer and heavier fish were the more susceptible to parasitic infestation. The gradual build-up of pollutants and toxins in fish tissues can increase their vulnerability to parasite infections. Insecticide concentrations are commonly found in various wastewater sources, and research has shown their harmful effects on aquatic organisms, particularly fish, even lower levels of discharge can lead to the accumulation of pollutants in these organisms [56]. Additionally, although host age is known to influence the likelihood of parasite infection, there is very little experimental data to support the hypothesis that parasites favor potential hosts who are of a certain age [57]. Furthermore, older fish have stronger immune systems, which reduces their susceptibility to the illness brought on by these parasites [58].

In the locations where it has been found, *L. sceleratus* is regarded as a problem for fisheries and poses a serious danger to local biodiversity [13,17]. The invasion success of this exotic species may be the result of the absence of predators in the introduced geographical areas [33] or/and because then new areas are free of diseases that infects this fish.

CONCLUSION

This was the first reported study on prevalence of parasitic infestation of *L. sceleratus* in Libya and for the first time, we report the infestation of *L. sceleratus* by *Haplosplanchnida sp.* and *Cryptobia iubilans.* The results of the present study concluded that *L. sceleratus* is a new host for some native parasites, offering an additional niche for successful and increasing local populations of these parasites.

Conflict of Interest

There are no financial, personal, or professional conflicts of interest to declare.

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انتشار بعض الإصابات الطفيلية في *Lagocephalus sceleratus* في شرق ليبيا

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المستخلص

الخلفية والأهداف: تم إدراج *Lagocephalus sceleratus* (*L. sceleratus*) كأحد "أسوأ 100 نوع من الأسماك الغازية" في البحر الأبيض المتوسط. أجريت هذه الدراسة لمعرفة مدى انتشار العدوى الطفيلية في *L. sceleratus*. **طُرق الدراسة**: خلال هذه الدراسة، تم صيد مائة وعشرة سمكة في مواقع مختلفة في شرق ليبيا في الفترة من مارس إلى مايو 2022. قمنا بفحص جميع الأسماك بعد تشريحها وفحصها عيانيا وكذلك الفحص المباشر باستخدام المجهر الضوئي. قمنا بتحديد مدى انتشار الطفيليات حسب المنطقة والجنس والعمر والأعضاء المصابة. **النتائج**. أظهرت النتائج أن معدل انتشار الطفيليات كان بالنسب التالية: 10.91% فصيلة كربتوبيا ايوبيلانس (*Cryptobia iubilans*)، 2.73% فصيلة هابلوسبلانكنيدا (*Haplosplanchnida* sp.)، 5.45% فصيلة أنيساكيس (طور اليرقة الثالث) (*Anisakis* sp. (L3)). وفيما يتعلق بالجنسين، أظهرت الدراسة أن نسبة الانتشار بين الذكور والإناث بلغت 10.71% و11.11% على التوالي. كما لوحظ أن أعلى نسبة إصابة بالطفيليات كانت في منطقة سوسة 50% يليها طلميثة 38.9%، ثم منطقة خليج – البمبة 18.8%. ولم يتم تسجيل أي إصابة في عين الغزالة. تم في هذه الدراسة العثور على عدد من الطفيليات في الأمعاء، اثنتان منها وهي فصيلة كربتوبيا ايوبيلانس (*Cryptobia iubilans*)، وفصيلة هابلوسبلانكنيدا (*Haplosplanchnida* sp.)، وهذه حسب علمنا هي أول دراسة منشورة عن الإصابة بهذه الطفيليات في سمكة الارنب *L. sceleratus* في ليبيا. من غير المعروف ما إذا كانت هذه الطفيليات يمكن أن تسبب مرض لسمكة الارنب *L.sceleratus* أم لا، وهذا يحتاج إلى مزيد من البحث والدراسة. **الخاتمة**: يعتبر *L. sceleratus* مضيفًا جديدًا لبعض الطفيليات المحلية، مما يوفر مكانًا إضافيًا لنجاح وزيادة أعداد هذه الطفيليات المحلية.

الكلمات الدالة: *Haplosplanchnida* sp.، *Cryptobia iubilans*، *Lagocephalus sceleratus*، سمكة الارنب، الأمراض الطفيلية، ليبيا.