GERF Bulletin of Biosciences 2016
ISSN: 2229-6433 June, 7(1):1-5

Research Article

A preliminary study on gill parasites of gilthead sea bream *Sparus aurata* (Linnaeus 1758) (Pisces: Teleostei) from the eastern Tunisian sea-cage aquaculture

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Open Access

Received: 4 March 2016
Accepted: 21 May 2016
Published Online: 30 June 2016

Citation:

Rabet C M, Ensibi C, Dhaouadi R and Yahia O K. A preliminary study on gill parasites of gilthead sea bream *Sparus aurata* (Linnaeus 1758) (Pisces: Teleostei) from the eastern Tunisian sea-cage aquaculture. GERF Bull Biosc. June. 2016; 7(1):1-5.

Conflict of Interests:

The authors declare no conflict of interests.

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Abstract

Parasites have recently been highlight at serious pathogenic problems in cultured fish. Many of them have long been recognized to have the potential to affect the growth, fecundity and survival of hosts. With the development of marine aquaculture, the importance of parasitic study has become more evident. This study evaluated the occurrence of gill parasites in gilthead sea bream (*Sparus aurata*), farmed in the eastern coast of Tunisia. A total of 147 fish were caught in fish farm for parasitological analysis. Out of the total of the fish examined, 11 were parasitized by at least one parasite species. Histological analysis revealed that the gills of *Sparus aurata* were affected by three different parasites: *Monogenea*, *Ciliophora* and *Epitheliocystis*. This is the first report of farmed sea bream gill's parasites in Tunisia.

Key words: *Monogenea*, *Ciliophora*, *Epitheliocystis*, parasites, *Sparus aurata*, aquaculture

Introduction

Aquaculture has been expending and intensifying in almost all regions of the world. However, rapid development of fish culture in marine cages has been associated with an emergence of parasitological infestations (1). Parasites played an important role as a factor limiting the productivity of aquaculture (2). They are often responsible for major economic losses for aquaculture activities and have gained increasing importance as pathogens in marine fish (3).

Fish parasites debilitate their hosts, depress their sexual display and reproductive abilities, disrupt predator-avoidance behavior and may cause severe fish mortalities leading to reduce fish-marketability (4,5,6). Recently, fish parasites have become useful for fish-stock identification and as bio-indicators of fish feeding and migratory patterns (7,8).

Numerous studies on the parasites of marine fish were carried out, specifically on species with a great economical interest such as *Dicentrarchus labrax* (9,10), *Mugil cephalus* (11,12), *Liza aurata* (13) and *Anguilla anguilla* (14). Ectoparasites such as gill parasites figure among parameters which are involved in fish diseases. From a pathological point of view, gill parasites cause severe gill lesions like destruction and necrosis of a large part of the tissue, leading to hemorrhage and anemia (15,16).

Gilthead sea bream, *Sparus aurata* are one of the most important and successfully farmed Mediterranean fish species. The total global production volume of reared gilthead sea bream has increased with two folds over the past decade, from 82,152 tons in 2001 to 154,821 tons in 2011 (17). In Tunisia, the fish breeding has widely progressed, from 619 tonnes in 2000 to 7,125 tonnes in 2012 (18). Despite, the historical and commercial activities related to *Sparus aurata* in Tunisia, few studies have been reported on their parasites.

The objective of this study is to determine gill ectoparasites in economically important fish *Sparus aurata*, from the eastern Tunisian sea-cage aquaculture. This will help to solve some of the problems of fish diseases that can perturb their health and productivity, both in the wild and fish culture.

Materials and Methods

Collection and analysis of water samples

During fish collection, the physical-chemical parameters of the water were measured using a multi-meter water checker. The pH ranged from 7.6 to 8.3, temperature from 13.4 to 15.4, Salinity from 36.8 to 37.0 PSU.

Animals sampling and external examinations

Gilthead sea bream (*Sparus aurata* Linnaeus 1758) were spawned and reared at a commercial sea-cage aquaculture facility in East Coast of Tunisia. The samples were collected as a part of routine farm health monitoring. A total of 147 adult specimens (Fig. 1) were sampled during March 2014. The fish were randomly collected directly from cages during usual fishing operation and immediately transported to the laboratory for analysis. The weight of the fish were taken using digital weighing balance and recorded. The standard length (SL), the total length (TL) and the fork length (FL) were measured. After the dissection, the fish were examined by a careful observation of the external surface with the naked eye. Gill fragments were collected for fresh examination to search for cysts or adult parasites.

Fig 1: *Sparus aurata* (M'Rabet, 2014)

Histopathological examinations

Arches of gills were carefully removed from each fish (Fig. 2). Pieces of gill tissue were fixed 48 hours on formaldehyde solution. Then, series of dehydration and impregnation with paraffin were made. Each sample was placed in a mold and covered with paraffin in fusion (60°C). The whole was dropped to be cooled in order to make the solidification and tear off easier. In the out came of cooling, we start the preparation of section (thick of 2 to 5 µm). The coloration was undertaken with haematoxylin-eosin and we finished with drying under room temperature (14). The stained sections were observed under light microscope and micro photographs were taken for gill pathological observations.

Fig 2: Sampling of gills

Analysis of parasitic infestation

The analysis of parasitic infestation for finding the prevalence and index of infestation were carried out according to Aladetohun et al. (12).

$$prevalence = \frac{\text{number of fish infected}}{\text{total number of hosts examined}}$$

$$index\ of\ infestation = \frac{\text{number of fish infected} * \text{number of parasite collected}}{\text{total number of hosts examined}}$$

Results and discussion

The standard length of fish was 248 cm ± 2.32 and the weight means was 243.7 ± 7.45 g externally examination showed that all fish used for the study appeared to be in good health with no signs on skin. But, macroscopic observation of the gills showed that the gills of infected fish appeared reddish-white in colour with excessive mucous secretion.

The histological examination of S. aurata gills revealed that eleven fish had been attested with the presence of gill parasites: two fish with *Monogenea* , five fish with *Trichodina* and four fish with *Epitheliocystis*. The different parasites observed were designed as follows: *Furnestinia echeneis*: monogenean of the subclass monopisthocotylea; *Trichodina lepsii* Lom, 1962 and *Epitheliocystis*.

The analysis for finding the prevalence and index of infestation were carried out (Table 1). *Trichodina sp*. had the highest prevalence rate (3.40%) while, *Epitheliocystis*. and *Furnestinia echeneis*. had incidence rate of 2.72 and 1. 36 %, respectively. Furthermore, the index of parasite groups in this study were also calculated and highest index was of *Trichodina sp*. (0.374) while, *Furnestinia echeneis* had the lowest value of 0.149.

Table 1: Frequency distribution of gill-parasites found in fish

Parasites	Frequency	Prevalence	No. of fish examined	Index of infestation
Monogenea	2	1.36	147	0.149
Trichodina sp	5	3.40	147	0.374
Epitheliocystis	4	2.72	147	0.299

Monogenean of the subclass *Monopisthocotylea* was found in gill's specimen. Two fishes examined have been damaged with *Monogenea* (Fig. 3). The general morphology of this parasite was in agreement with the description of Euzet and Audouin (19). The body is elongated, with a posterior haptor having sclerotized elements (hooks and clamp) (Fig. 4). The parasite is attached to the apices of primary gill filaments tissue by its opisthohaptor inducing degeneration of the epithelia at the attachment site.

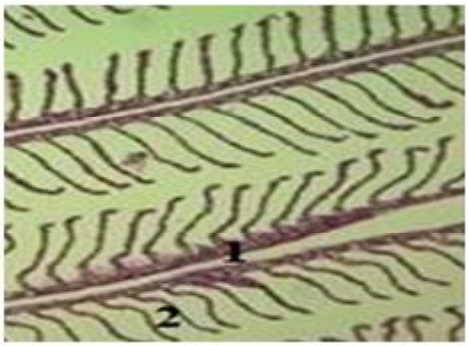

Fig 3: Photomicrographs of *Sparus aurata* gills none infected. (1) Primary gill filaments, (2) secondary gill filament. Hematoxylin and eosin stain (100×).

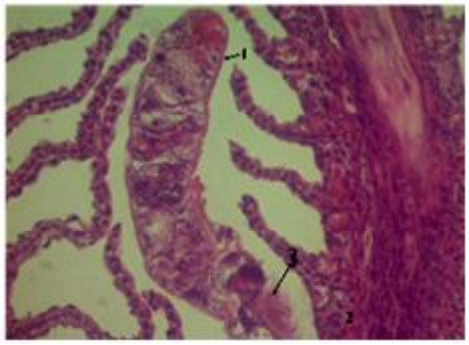

Fig 4: Photomicrographs of *Sparus aurata* gills infected with the *Monogenean Furnestinia echeneis*. Monogenean(1) can be seen attached with their opisthaptors(3) to the apices of primary gill filaments(2). (400×). Hematoxylin and eosin stain.

Monogenean gill parasites are an important class of trematoda of Phylum Platyhelminths. Majority of them are ectoparasites and have direct lifecycle. In most of cases, monogenean parasites are host-specific. *Monogeneans* mostly infect gills of fish and are found on gill filaments, gill rakers or the lateral surfaces of gill arches (20).

This report coincides with that of Antonelli et al., (21) in their work on parasite of cultured gilthead sea bream *Sparus aurata* from Corsica. Also, Bahri (22) reported that wild sea bream collected from North Lake of Tunis are infected with *Monogenea*: *Furnestinia echeneis.*

***Trichodina sp.*:** The gills of five *Saprus aurata* were infested by *Trichodina sp*. (Fig. 5). In one sample, we observed a parasite infestation with three *Trichodina*. This species was characterised by its smaller size, 32-37.6 ìm in diameter; the adhesive disc, concave, 20.8-28 ìm in diameter, consisted of 20 to 22 denticles (11). *Trichodina* observed

during this study showed significant resemblance in size and shape with those identified from the gills of *Mugil cephalus* and *Liza aurata* from Ghar El Melh lagoon as well as from the gills of *Liza saliens* from Ichkeul Lake (11). Bahri (22) have reported that this ciliate was the most prevalent species in wild *Sparus aurata* from North Lake of Tunis and note that infested gills revealed significant damages such as oedema, necrosis and sloughing off of epithelial cells.

Fig 5: Photomicrographs of *Sparus aurata* gills infected with *Trichodina lepsii* (arrow) (400×). Hematoxylin and eosin stain.

Epitheliocystis: We observed infestation of *Epitheliocystis* in four gill-samples. Round cysts from 10 to 40 ìm diameter were observed between the second gill lamellar (Fig. 6). Epitheliocystis has been reported in numerous fish species such as Atlantic salmon (23), red sea bream *Pagrus major* (24), sea bream (25). This is the first report of *Epitheliocystis* in farmed sea bream (*Sparus aurata*) in Tunisia. Cysts usually were present in the middle, base, and tip of the gill filament but mostly concentrated in the middle of the gill filament (26).

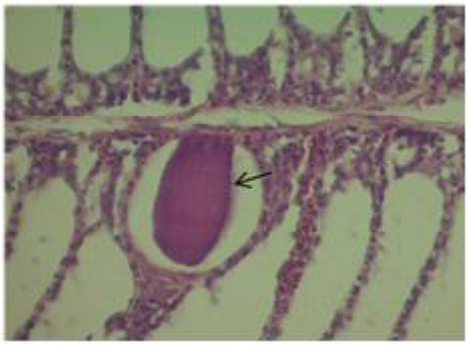

Fig 6: Photomicrographs of *Epitheliocystis* inclusions (arrow) in the between of second gill lamella of *Sparus aurata*. (400×). Hematoxylin and eosin stain.

Conclusion

This is the first report of farmed sea bream parasites in Tunisia. In the present study we have reported three parasite species which have been previously described by other authors worldwide: *Furnestinia echeneis*, *Trichodina lepsii* and *Epitheliocystis*. The taxonomic identity of these parasites based on molecular analysis is needed. Sea bream is the most species in fish farming. In Tunisia, on 2013, 26 farmed fish are working, producing more than 7,125 tons/year. Further parasitical investigation should be undertaken for a better understanding and monitoring of the threats towards the developing intensive aquaculture activity in Tunisia.

Author contributions

Dr. Charaf M'rabet designed the study, performed the autopsies, performed the histology experiments, analysed the data, and wrote the manuscript. Dr. Cherif Ensibi analysed the data and wrote the manuscript. Dr. Raouf Dhaouadi performed the histology experiments. Pr. Ons Daly Yahia Kéfi, planned the study and revised the manuscript. All authors read and approved the final manuscript.

Acknowledgements

The authors gratefully acknowledge the constructive comments on the paper, offered by the anonymous referee. We are grateful to the ENPI–CBCMED project MEDJELLYRISK for the financial support (ref: I-A/1.3/098).

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