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Survey of major fish pathogens and their prevalence in the fishing area of Shalateen, Red Sea, Egypt

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Abstract
During the spring of 2019, a marine fish survey from Shalateen port was conducted to detect the prevalent parasites, bacteria, and fungi pathogens in order to fill the gap in data on fish pathogens in this area. Gross examination revealed no lesions except excessive mucus secretion in some samples which were usually associated with monogenean infestations. The overall prevalence of parasitic infection was 18.5%. Bigeye snapper had the highest percentage (71.4%), followed by Arabian monocle bream (33.3%) and Yellowstripe goatfish (39.3 %). The presence of Vibrio and Photobacterium species is indicated by the use of selective medium for bacterial isolation. In terms of fungal infection, Aspergillus was the most prevalent genus, represented by three species: *A. flavus* (40 CFU/g), *A. niger* (57 CFU/g), and *A. fumigatus* (20 CFU/g). Some other genera were recorded as *Cladosporium* sp. (25 CFU/g), *Alternaria* (13 CFU/g) and *Penicillium* sp. (6 CFU/g). All examined fish species had a high record of yeast species.

Keywords: red sea, Shalateen, fish, pathogens, survey

Introduction
The Red Sea coast is very significant on the Egyptian fisheries sector (Azab *et al.* 2015) [1]. The triangle of Halayeb and Shalateen, marked by the presence of coral reef terraces in many regions, aids in breaking waves and serves as a defense and feeding zone for a wide variety of fishes. Shalateen is the main fishing site in this area beside Abu Ramad and Halayeb (Mahmoud 2005) (Hatem *et al.* 2009) [2], fish collected from this area transferred directly to Hurghada, Cairo and other cities in Egypt (Tesfamichael and Pauly 2016) [3]. Fish parasites cause economic loss in addition to the threat of zoonotic species that have been reported from various geographical regions causing human infections (Park *et al.* 2009) [4]. Bacterial pathogens are the most significant microbial agents affecting marine fishes (Moustafa *et al.* 2010) [5] but a few studies have involved the marine species to cause disease outbreaks (Alicia *et al.* 2005) [6]. Fungal diseases caused damage on cultured fish, shellfish, and, in some cases, wild marine animals. Members of the genera Fusarium, Ochroconis, Exophiala, Scytalidium, Plectosporium, and Acremonium are among the main groups of mitosporic fungi (Hatai 2012) [7]. One of the major challenges in fish pathogen diagnosis and disease control is the lack of rapid, efficient, and dependable methods for diagnosing and identifying fish pathogens (Lievens 2011) [8]. The present study was oriented to detect primary survey of pathogens in shalateen fishing port including parasites, bacteria and fungi.

Materials and Methods
Study area
Shalateen port 520 Km south of Hurghada at Elba National Park was chosen to collect fish samples. The fishing landing site location latitude (N: 23° 09' 07.31") and longitude (E: 35° 36' 51.14") (Fig. 1).

Fish sampling and Identification
Fish samples from five different species were collected live from boat livewells in the Shalateen fishing port for the current study (Table 1).

The examined fish were identified according to Randall (1983) [9]. Bacterial and fungus isolation were carried out in the animal health research institute laboratory, Shalateen station.

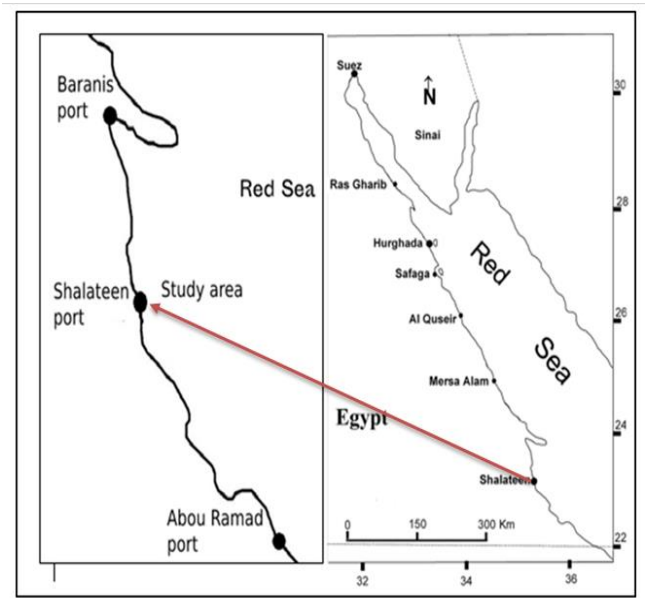

Fig 1: The map of the study area

Clinical examination

The clinical examination was performed on live or freshly dead fish; specimens under investigation were grossly examined for determination of any external disease symptoms, according to the methods described by Noga (2010) [10].

Parasitological examination:

According to Syme (1966) [11], macroscopic examination was used to detect any abnormalities in various parts of the fish body using naked eyes and a hand lens. This examination included skin, fins, operculum, eyes, gas bladder, and musculature. The specimens were thoroughly checked for parasitic infection in the body surface; fins, head, gills and oral cavities using dissecting microscope according to Lucky (1977) [12]. Wet compression preparations of the examined fish were done from internal organs Liver, spleen, kidneys, heart and gonads, especially those showed gross lesions. These wet preparations were carried out by compressing small parts of the organ between two slides and microscopically inspected.

Detection of Bacterial pathogens

For bacterial examinations samples from gills, liver, spleen, kidney and external lesions from fish were cultured on tryptic soy agar (TSA) with 1.5% (w/v) NaCl as general medium. Thiosulfate-citrate-bile salts-sucrose agar (TCBS), Aeromonas agar base medium supplemented with ampicillin, Pseudomonas agar base medium supplemented 2 % NaCl and Azide blood agar supplemented with 2 % NaCl as selective media. All inoculated media were incubated at 22 ºC for 48 hours.

Detection of fungal pathogens

Fish samples from skin, gills, liver and kidney were inoculated onto Potato Dextrose agar (PDA) (potato extract, 4.0 g; Dextrose, 20.0 g; Agar, 20.0 g; seawater 1,000 ml), 500 mg/l of chlormenphicol was introduced into the media to prevent bacterial growth. Cultures were incubated at 28°C and observed after 7-14 days for fungal development. For each sample, the developing fungi were isolated, counted and identified according to Raper and Fennell (1965) [14], Pitt (1979) [14], Moubasher (1993) [15], Pitt and Hocking (2004) [16], De Hoog *et al*. (2000) [17] and Colin *et al*. (2012) [18].

Histopathological examination:

Intestinal pieces were collected due to their high parasitic load, immediately transferred to histological cassettes and fixed in 10% neutral buffered formalin. Sections (5 µm thickness) were cut and mounted over glass microscopic slides (Ahmed *et al*, 2019) [19]. Slides stained with Haematoxylin and eosin according to Fischer *et al* (2008) [20], Titford (2009) [21], and Musumeci (2014) [22].

Results and Discussion

Environmental parameters

The Red Sea is one of the world's most important aquatic biodiversity and endemic repository, with warm water temperatures ranging from 21 to 30 ° C (Hawkins and Roberts 1994; Lieske and Myers 2004) [23, 24]. and salinity levels of 42.5 ppt (Sofianos *et al*. 2002) [25]. In the present study physical water quality parameters; temperature, pH, salinity, TDS and dissolved oxygen generally showed the same patterns which were reported by (EL-Shenawy *et al*. 2006 and Fahmy *et al*. 2003) [26, 27], salinity showed minor variation (38.9 µg/l). All nutrients had lower values than those reported by Abdelmongy and El-Moselhy[28] in the northern Red Sea (2015) (Table2).

Table 1: Total number of Red sea fish examined during the present study.

No.	English name	Scientific name	Common name	Total No.
1	Yellowstripe goatfish	*Mulloidichthys flavolineatus* (Lacepede, 1801)	عنبر بلدي	48
2	Klunziger"s wrasse	*Thalassoma rueppellii* (Klunzinger, 1871)	ملاص ابو ربيع	18
3	Arabian monoclebream	*Scolopsis ghanam* (Forsskal, 1775)	سمان او غانم	24
4	Bigeye snapper	*Lutjanus lutjanus* (Bloch, 1790)	بهار شخرم	7
5	Lethrinus latinus	*Pinkear emperor* (Lacepède, 1802)	شعور شركسى	9

Table 2: Environmental parameters recorded in the study area.

Parameters	Results
Temp. (C°)	23.1
pH (pH unit)	8.1
DO (mg/L)	8.4
Sal. (ppt)	38.9
TDS (mg/L)	37.8
NO_3 (µg/L)	0.62
NO_2 (µg/L)	0.064
NH_4(µg/L)	0.1
PO_4 (µg/L)	0.22

Clinical examination

There were no pathognomonic signs in infested fishes in this study, except for excessive mucus secretion with sometimes-small necrosis of gills in some goatfishes, which was associated with gill monogenean infestations. Other symptoms such as haemorrhages, abrasions, skin ulcers, and eye opacity were not attributed to parasitic infestation and appeared to be the result of improper handling and fishing.

Parasitic infections

A clinical examination of the examined fishes revealed no abnormalities; however, a P.M examination revealed excessive mucus secretion in the gills with sometimes-small necrosis; the spleen and kidneys revealed no abnormalities; and the stomach revealed slight congestion of its wall due to digenetic trematode infestation in Bigeye snapper. Gill monogenean was isolated from Yellowstripe goatfish, Bigeye snapper and Arabian monocle bream. Gastrointestinal digenea were isolated from the stomach of Bigeye snapper and intestine of Yellowstripe goatfish. Detected Monogenean was isolated from *Lutjanus lutjanus* and was belonging to Family: Hemiuridae Looss, 1899ents, and Genus Erilepturus, *Erilepturus sp*. It is characterized by its ovoid thick shelled embryonated eggs (Fig. 2).

The total prevalence of parasitic infection was 18.5%. The highest percentage was in Bigeye snapper (71.4%) followed by Arabian monoclebream (33.3%) then Yellowstripe goatfish (7.3%) Table (4). Intestinal histological examination of yellowstripe goatfish showed high trematodes invasion inside the intestinal lumen. Degenerative changes were recorded in the form of sloughed epithelium which found in the lumen of the intestine. Inflammatory reactions were seen in the form of inflammatory cells and red blood cells infiltration within the intestinal villi (Fig. 3).

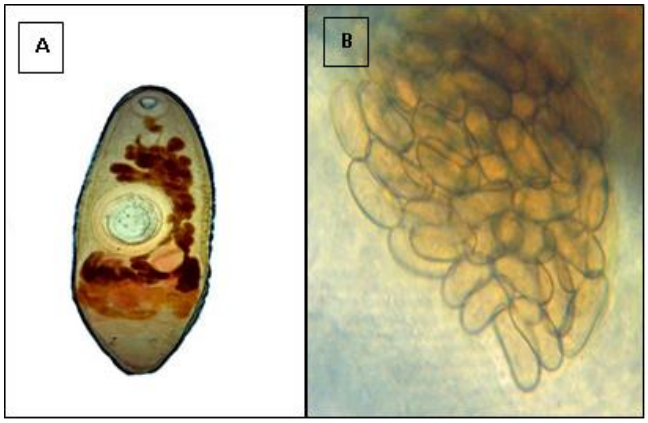

Fig 2: Showing *Erilepturus* sp isolated from *Lutjanus lutjanus*: A. Whole fluke, B. Ovoid thick-shelled emberyonated eggs.

Table 3: Prevalence of bacterial infections in the examined fish samples.

Fish species	Examined	Infected	%
Yellowstripe goatfish	112	44	39.3
Bigeye snapper	36	13	36.1
Total	148	57	38.5

Table 4: Isolates positive on selective media.

Fish	Yellowstripe goatfish				Bigeye snapper				Total isolates
Organ	Liver	Kidney	Spleen	Gills	Liver	Kidney	Spleen	Gills	
TCBS medium	8	5	4	7	2	1	1	2	30
HSU medium	6	1	4	5	3	1	-	2	22

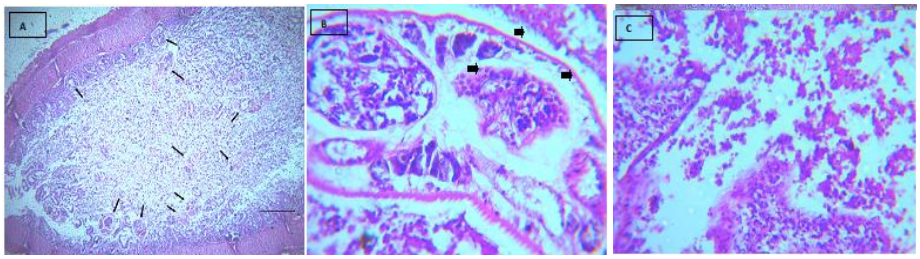

Fig 3: a) showing the distribution of Trematodes within intestinal lumen of yellowstripe goatfish, (black arrows). H&E, 4x. b) Adult trematode appears clearly in intestinal lumen H&E, 40x. c) Sloughed epithelium in the intestinal lumen of yellowstripe goat fish, thick black arrows H&E, 40x.

Microbial infections

Bacteria

Examined fish were sub clinically infected as there were no external or internal lesions, while some isolates were positive when incubated on general and selective media; BHI agar. Positive isolates on BHI agar, were inoculated on available selective media; Thiosulfate-citrate-bile salts-sucrose agar (TCBS) for *Vibrio* and *Photobacterium* species and Hsu-shotts medium for *Tenacibaculum maritimum*. Identification of both groups of isolates which are positive on BHI agar and negative on the selective media, details shown in Tables from 3 to 5. Saeed *et al* 1987 [29] used BH agar media containing 3 % salt (NaC1) for isolation of *pseudomonas* associated with disease in cultured *siganus rivulatus* in the Red sea.

El-Galil and Mohamed (2012) [30] used Trypticas soya agar (TSA), and thiosulphatecitrate- bile salt-sucrose agar (TCBS) to isolate vibrio species *V. harveyi*, V. *parahemolyticum*, *V. anguillarum* and *V. furnissii* and Flexibacter maritimus

medium (FMM) for *Tenacibaculum maritimum* bacteria from Red sea fish species. Abou-Elela *et al* 2009 [31] used TCBS media to count Vibrio sp. from Red sea water and *V. parahaemolyticus*, was dominant, *V. alginolyticus.* Several pathogenic bacteria were reported from Red Sea fish in Egypt, *Vibrio anguillarum* and *V. alginolyticus* were the dominant cause for the naturally infected in Epinephelus tuvina, Siganus rivulatus, collected from the Suez bay (Moustafa *et al.* 2010) [5]. Also, shrimps from Suez Bay showed infections with *V. alginolytcus*, *V. parahaemolyticus* and *V. fluvialis* (Abd El-baky 2012) [32], *V. alginolyticus* Bird wrasse fish in the indoor aquaria (El-Galil and Mohamed 2012) [30] *V. harveyi* from Arabian surgeon fish (Hashem and El-Barbary 2013) [33], cause outbreak in aquarium-maintained stingrays (Emam *et al* 2019) [34]. Vibriosis in Red Sea presents a serious concern to marine life and human health (Mustafa 2015 and Darwish *et al.*, 2021) [35, 36]. Bacterial indicators total coliform (TC) *E. coli* (EC) and fecal streptococci (FS) were detected for shalateen fishing port from 1998 to 2004 and the count exceeded the acceptable levels in the last five years of that study (EL-Shenawy *et al.* 2006) [26].

Table 5: Prevalence of fungus infections in the examined fish samples.

Fish species	Examined	Infected	%
Yellowstripe goatfish	20	8	40
Arabian monoclebream	18	9	50
Klunziger"s wrasse	18	16	88
Total	56	33	60

Fungi

Fungi isolated from fish skin, gills, liver and kidney of examined showed that *Aspergillus* was the most common genus presented by 3 species of which *A. flavus* (40 CFU/g); *A. niger* (57) and *A. fumigatus* (20), followed by *Cladosporium* Spp. (25), *Alternaria alternata* (13) and *penicillium* sp. (6). Yeast sp. show high record in all examined fish species. The highest total count was recorded from gills (87) and skin (82) (Table 6).

Present study results were generally similar to those reported by Abdel-Sater *et al.* (2018) [37] were *Aspergillus*, *Penicillium*, *Cladosporium* and *Exophiala* the most common genera isolated from Red sea fish. Also, *Aspergillus*, *Penicillium*, *Cladosporium* were isolated from Red sea sediment (Khallil *et al.* 1991 and Abd-Elaah 1998) [38, 39].

Table 6: Total count of fungi isolated from some Red Sea fish from samples shalateen port on PDA at 28°C on the basis of Raper & fennel (1965).

Species	Organ				Total Count
	Skin	Gills	Liver	Kidney	
Aspergillus flavus	15	6	10	9	40
A. fumigatus	12	5	3	0	20
A. niger	18	16	11	12	57
Alternaria alternata	7	0	4	2	13
Cladosporium Spp.	0	25	0	0	25
Penicillium Sp.	0	0	6	0	6
Yeast Spp.	30	35	22	18	105
Total count	82	87	56	41	

Conclusion:

Study of different fish species from Shalateen region revealed the presence of multiple parasitic, bacterial and fungal species. We believe that a wide scale study is necessary for the detection of pathogenic infection in different fish species present in Shalateen region.

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