Studies on Hatschekiasis in Some Red Sea Coral Reef Fishes at Aquarium of Hurghada Area

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Abstract:

A total number of 77 marine fishes of 3 species represented as 23 Abu Sabib (*Cheilinus lunulatus)*, 24 kosher Abu Lolo (*Epinephelus fasciatus)* and 30 Sigan (*Siganus luridus)* were collected seasonally between May 2014 and the end of April 2015 from coral reef sites along the shore of the National Institute of Oceanography and Fisheries (NIOF) at Hurghada's Red sea. They were investigated for detection of parasitic copepods. It was revealed that 31.16% of the examined marine fishes were infested. The detected species were belong to Genus Hatschekia Poche1902 from *Cheilinus lunulatus* with a prevalence78.26%, and from *Siganus luridus* with prevalence 20% while not detected in *Epinephelus fasciatus*. The total and seasonal prevalence of infestation in each fish species in relation to body weights and lengths were recorded. Besides, the histopathological alterations were studied and discussed.

Key word: copepod**,** Abu Sabib, Sigan, Hatschekia sp., prevalence.

Introduction:

Internal and external parasites may result in emaciated and physically weakened hosts that become susceptible to other causative agents of disease ***(Eissa 2002 and Hossam et al.,2012).*** In the aquaculture industry throughout the world, the parasitic copepods are important as Pathogens causing heavy mortality or acting as disease inducers, by creating a portal for entry of bacterial or other pathogens ***(Johnson et al. 2004).***

They damage the gills by feeding on the delicate tissue of the gill lamellae or on the blood circulating within the lamellae, leading to a loss of respiratory surface area

(Lester & Hayward 2006 and Palacios-Fuentes et al. 2012). The attachment of copepods and the action of their mouthparts, provoke gill edema, hyperplasia and inflammation **(Andrews et al. 2010).**

The present study was aimed to throw the light on the clinical picture, total and seasonal prevalence of the crustacean parasitic disease Hatschekiasis in some Red Sea coral reef fishes at aquarium of Hurghada Area and in relationship to body weights and lengths.

Materials and Methods:

Fishes:

A total of 77 marine fishes of 3 species represented as 23 *Cheilinus lunulatus* ,24 *Epinephelus fasciatus* and 30 *Siganus luridus* of different body weights collected seasonally from coral reef sites along the shore of the National Institute of Oceanography and Fisheries (NIOF) at Hurghada's Red sea. Fishes were obtained by aid of fishermen and fishing gears and transported alive to the Aquarium.

Aquaria:

Fully prepared glass aquaria (100 x 60 X 70 cm.) were used for holding fishes. They were supplied with sea water from sea shore of Hurghada's institute (NIOF). Continuous aeration maintained in each aquarium using an air pump. Thermostatic heaters (Type CMI, Germany) were used along the course of the study in winter, early spring and late autumn to maintain the temperature 23±1°C, while in late spring, summer and early autumn the temperature were the room temperature.

Clinical picture:

First, body weight and body length of the examined fishes were recorded and the clinical examination was carried out on the live fishes or freshly dead ones. Fish specimens under investigation were grossly examined for determination of any clinical abnormalities and any external parasites and for demonstration of the internal abnormalities were performed on all fish according to ***Conroy and Hermann (1981).***

Parasitological examination:

1. Macroscopic examination:

Macroscopic examination was done for detection of any abnormalities in different parts of fish body by naked eyes and hand lens. Skin, fins, gills, mouth, eyes and opercula were dissected and examined for presence of parasitic crustaceans.

2. Microscopic examinations:

Freshly sacrificed fish was scraped with a scalpel blade from just behind the operculum to the tip of the tail fin. Scales and mucus were transferred to slides with a drop of marine water and cover slip to prevent drying and examined microscopically for detection of any ectoparasitic crustacean. The branchial region was dissected and examined by the naked eyes then under dissecting microscope. Gill arches were removed to a slide and

proceed to cut away the cartilaginous arch to separate gill lamellae, few drops of marine water were added to ensure a uniform distribution of the filaments under the entire cover slip ***(Lucky 1977).***

3. Permanent slides and smears preparations:

The attached copepods in gills were detached by a dissecting needle and a fine brush, kept in small vial and washed with distilled water, fixed in 3% formalin and preserved in equal amount of 70% alcohol-5% glycerin in test tube. Permanent mounts were prepared by passage in descending grades of alcohol (70, 50 and 30%), cleared in glycerin and mounted in glycerin-gelatin then examined microscopically. The isolated crustacean parasites were identified according to the methods adopted by ***Lucky (1977)*** and ***Woo (2006)***.

Results

Clinical picture:

The clinical signs in the naturally infested fishes revealed no pathognomonic clinical abnormalities. *Siganus luridus* gills had a marbling (mosaic) appearance (congested and pale areas) with excessive mucus secretion, presence of crustacean on gills forming black lines and black spots between gill filaments and gill tips were sticking with greyish coloration. *Cheilinus lunulatus* showed sticking of the gills (Plate 1).

Results of parasitological examination:

A crustacean species was collected from the gills of *Siganus luridus and C.lunulatus* only as Female of Hatschekia sp. But the females isolated from *S. luridus* were adult with body elongate comprised distinct cephalothoracic head, short neck region consisting of first and second pedigerous somites and long cylindrical trunk. Trunk cylindrical, about 4 times longer than wide; internal tissues separated from cuticle and appearing sheathed with transparent cuticular membrane. Egg-sacs were arranged in one row, as the seeds of a pea, containing up to (8- 15 eggs). Antennules were 4 - segmented; first segment longest, Antenna (Claw-like) 3-segmented shorter than antennule: basal segment unarmed; middle segment elongate, with surface of cuticle pitted; distal segment short, tapering distally forming curved claw. Legs 1 and 2 were biramous. While females isolated from *C.lunulatus* were similar of females isolated from *S. luridus* except difference on size and segmentation of antennules and antenna and number of eggs per egg sac, mean body length of female including egg sacs were shorter than female's body length crustacean isolated from *S. luridus*. Egg-sacs were pair of one row, containing up to (3-4 eggs). Antennules were 3 segmented. Antenna (Claw-like) was 2-segmented (Plate 2). Based on the morphological and parasitological examination, the isolated parasites were belonged to:-

Order: Siphonostomatoida
Family: Hatschekiidae Kabata, 1979
Genus: Hatschekia Poche, 1902
Hatschekia spp

Prevalence of Hatschekia spp. among the examined fishes:

Table (1) shows that the total prevalence of Hatschekia spp. among examined fishes was 31.16% as it was 78.26% in the examined *C.lunulatus* and 20% in *Siganus luridus* and 0% in *E. fasciatus*. The seasonal prevalence of was (20%) in autumn, (35%) in winter, (25%) spring and (40.9%) in summer (Table 2). The prevalence in relation to body weights and lengths is illustrated in Tables (3 & 4)

Histopathological findings:

Gills of *S. luridus* and *C. lunulatus* infested with crustacean Hatschekia spp. were observed detached from gill and was not observed in gill lesion. The lesions were desquamation of epithelial lining, necrosis of secondary lamellae and in some cases this parasite elicited an unusual host response, in the form of proliferation of gill filament cartilage support (Plate 3).

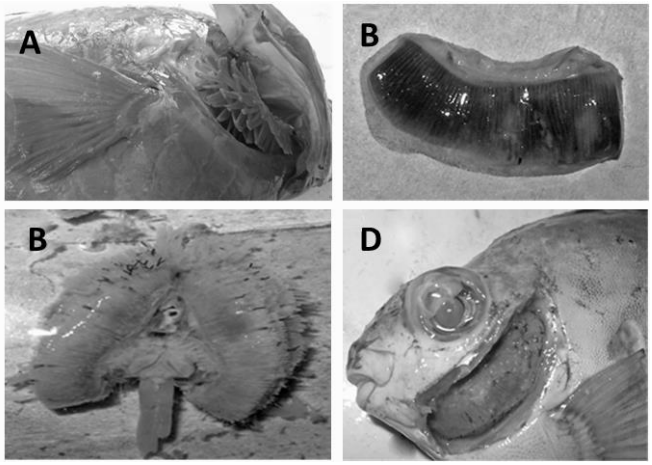

Plate (1): Sticky gills were seen in *Cheilinus lunulatus* **(A)** with massive areas of white necrotic tissues marbled by congested gill filaments (mosaic appearance) (arrows) **(B)**. Gills of *Siganus luridus* fish revealed presence of crustacean egg sacs in form of black lines and spots between gill filaments associated with excessive mucus secretion. **Note** black spots of crustacean eggs between gill filaments (arrows) **(C&D).**

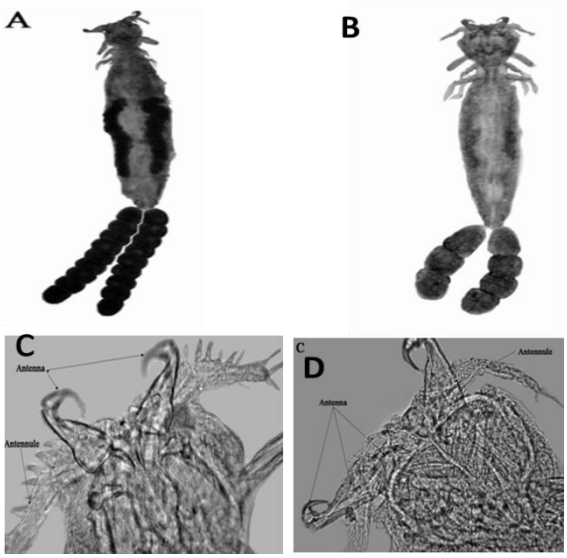

Plate (2). Female of Hatschekia sp. Whole female of *Hatschekia sp.* isolated from *S. luridus* (**A**) and from *C. lunulatus* **(B)** (x4). Anterior part of cephalothoracic head of Hatschekia sp. was varied in shape and segmentation of antenna (arrows) and antennule (arrows) isolated from *C. lunulatus* (**C)** and those isolated from *Siganus luridus* **(D)** (x40)

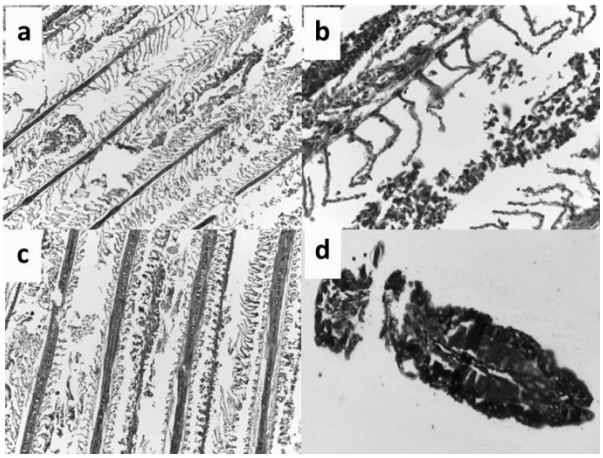

Plate (3). Gills of *S. luridus* infested with Hatschekia sp. revealed **(a, b)** desquamation of epithelia lining and necrosis of secondary lamellae (arrows), (H& E stain, 4X, b (10X)), **(c)** proliferations of chondrocytes in gill filament support of primary lamellae (arrow), (H&E stain, 10X) and (**d)** Cross section of the Hatschekia sp was observed detached from gill (H&E stains, 40 X).

Table (1): *Showing Prevalence of Hatschekia sp among examined fishes:*

Fish species	No. of examined fish	No. of infested fish	%
C. lunulatus	23	18	78.26
S luridus	30	6	20
E. fasciatus	24	0	0
Total	77	24	31.16

Table (2): *Showing Seasonal prevalence of Hatschekia sp infestation among the examined fishes*

Season	Autumn	Winter	Spring	Summer	Total (n = 53)
S *luridus*	0 (0%) ***(n = 0)***	0 (0%) ***(n = 4)***	1 (8.3%) ***(n = 12)***	5 (35.7%) ***(n = 14)***	6(20%) ***(n = 30)***
C.lunulatus	3 (60%) ***(n = 5)***	7 (70%) ***(n = 10)***	4 (100%) ***(n = 4)***	4 (100%) ***(n = 4)***	18(78.26%) ***(n = 23)***
E.fasciatus	0(0%) ***(n = 10)***	0(0%) ***(n = 6)***	0 (0%) ***(n = 4)***	0 (0%) ***(n = 4)***	0 (0%) ***(n = 24)***
Total	3(20%)	7(35%)	5(25%)	9(40.9%)	24 (31.16%)

Table (3) *Prevalence of Hatschekia sp in relation to total length and body weight among Siganus spp*

Total body length (cm)	No. examined	Crustacean infestation	
		No. infected	%
10- 15	8	0	0
15- 20	17	6	35.29
20-25	5	0	0
Total	30	6	20
Fish body weight (g)	**No. examined**	**Crustacean infestation**	
		No. infected	%
Less than 100	20	2	10
100-200	10	4	40
Total	30	6	20

Table (4) *Prevalence of Hatschekia spp. in relation to total length and body weight in C.lunulatus*

Total body length (cm)	No. examined	Crustacean infestation	
		No. infected	%
15- 20	2	2	100
20- 25	10	6	60
25- 35	11	10	90.9
Total	23	18	78.26
Fish body weight (g)	**No. examined**	**Crustacean infestation**	
		No. infected	**%**
100-200	4	3	75
200-300	10	6	60
300-500	9	9	100
Total	23	18	78.26

Discussion:

The main clinical signs observed in the infested fishes by gill crustacean were manifested as excessive mucous production. Any slight structural damage of gills can render a fish very vulnerable to osmoregulation as well as respiratory difficulties as fish gills which are responsible for regulating the exchange of salt and water and play a major role in excretion of nitrogenous waste products. These results nearly agreed with those reported by ***Mahy Ghobashy (2000) and Eissa (2002).*** Regarding the Postmortem examination of infested fishes by gill crustacean infestation, it was revealed congestion and paler areas (Marbling) of gills with excessive mucous secretions, sticking of the gill tips and grayish discoloration appeared and presence of crustacean on gills forming black lines and black spots between gill filaments. This result agreed with ***Omaima (1993), Montero et al. (2004), Lester and Hayward (2006) and Purivirojkul and Areechon (2008).*** These lesions may be attributed to the hard fixation of crustacean parasites with their claws and their activity and feeding. Excessive mucous secretion may be to dilute the irritation and act as a defense mechanism against the infestation. Marbling appearance may be caused due to destruction of the efferent vessels by copepod claws fixation where the blood pressure is low and extensive hemorrhages are caused the very hard clotting of blood brings about rapid occlusion of the vessel, thrombus is formed resulting in ischemia which in turn leads to necrosis in some areas occurred due to the inflammation and congestion of some areas with progressive degeneration of the other parts of the gill filaments giving the appearance of such phenomenon ***(Eissa, 2002).***

The parasitic crustacean Hatschekia spp. were isolated from gills of *Siganus luridus* and *Cheilinus lunulatus* and identified according to the morphological similarities with descriptions of ***Akmirza (2012)*** who isolate the same genus but from conger eel and ***El-Rashidy and Boxshall (2011)*** who obtained the same genus from gills of same fish species (*S. luridus*) and disagree with ***Purivirojkul and Areechon (2008),*** who obtained the same genus (Hatchekia sp and *Hatschekia caudate*) from " *Scolopsis dubiosus*" and " *Lutianus vitta*", respectively. The site of infestation and locality was in accordance with that mentioned by ***Tadros et al. (2014).***but the host (*Nemipterus japonicus*) different. The parasite measurements and morphological characters are nearly similar to *Hatschekia gracilis* that obtained by the same authors**.**

Regarding gill Hatschekiasis, the total prevalence was 31.16%. This result is higher than that obtained by ***Badawy (2001), Abd El-Aal (2003), Maather El-Lamie (2007)*** as the prevalences were 2.25, 10.43,

15.67 and 17.5% respectively. This difference may be attributed to the locality from which fish samples obtained and fish species. The present findings nearly agreed with ***Manera and Dezfuli (2003), Purivirojkul and Areechon (2008) Abdel-Mawla and El-Ekiab (2012)*** *and* ***Eissa et al. (2012)*** as the prevalence of copepod parasites infestation were 35, 23.02, 42 and 47%. for gill copepods only. These few differences from previous authors may be attributed to difference of the locality and fish species. The total prevalence of copepod infestation in some previous studies may involve the buccal cavity, skin and not only the gill copepodiasis. Also, may be attributed to the isolated species of gill copepods as in this study differed from species of gill copepods isolated by previous authors. Regarding the seasonal prevalence of crustacean infestation, the peak was highest in summer 40.9% followed by winter 35% then spring 25% and autumn 20%. It nearly agreed with results obtained by ***Badawy (1994) and Eissa et al. (2012)*** in which they recorded the summer season as the season of the highest infestation rate. But disagreed with ***Maather El- Lamie (2007)*** who recorded the highest infection of seabass with copepod in autumn (76%) followed by winter (68%) and ***Boualleg et al. (2010)*** who recorded the highest infestation of *Mullus barbatus* with copepods in autumn and winter in the same rate (31.6%) and ***Abdel-Mawla and El-Ekiab (2012)*** who recorded seasonally, the prevalence of gill crustaceans as 52, 48, 40 and 28% in spring, autumn, summer, and winter respectively. *M. labrax* only was infested with a peak at autumn 76%, followed by winter 68% then spring 28% and summer 16%. This variation in prevalence may be attributed to the unequal samples and different sites from which fish were collected and the differences in types of copepods.

C. lunulatus showed no clear relationship between prevalence of Hatschekia and the increase in fish weights as prevalence of gill copepods increased up to fish of 100-200g, and it decreased at 200-300 g then increased again with host size up to fish of 300-500g. It was probably because changes in the size of gill filaments affect their attachment capability, enhancing the possibility of being detached by respiratory currents ***(Timi and Lanfranchi, 2006*** *and* ***Maather El-Lamie (2007)***.

Concerning the pathological changes of gills of *S. luridus* due to affection with the crustacean copepod Hatchekia spp., it revealed desquamation of epithelia lining and necrosis of secondary lamellae and proliferation of gill filament cartilage as well as mononuclear cell infiltration. These results are nearly agreed with that obtained by ***Mahy Ghobashy (2000), Manera and Dezfuli (2003), Abu Samak (2005), Vinobaba (2007),)*** *and*

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دراسات عن مرض الهـاتشكيا في بعض أسماك الشعاب المرجانية بالأكواريوم في البحر الأحمر

تم تجميع 77 سمكة بحرية متمثلة في 23 ملاص أبو سبيب و 24 كشر ابو لولو و30سيجان شبيحى من مناطق الشعاب المرجانية على طول الشاطئ من المعهد القومي لعلوم البحار والمصايد فرع الغردقة في البحر الأحمر موسميا بين مايو 2014 وحتى نهاية أبريل 2015. تم فحصها لوجود مجدافيات الخياشيم الطفيلية(كوبيبودا). و قد تبين أن 31.16٪ من الأسماك البحرية مصابة بكوبيبودا من جنس هـاتستشيكا بنسبة إصابة في اسماك ملاص ابو سبيب 78.26٪، ومن السيجان نسبة إصابة 20٪ بينما في كشر ابو لولو لم تسجل أي اصابات. تم رصد نسب الاصابة الكلية والموسمية وعلاقتها بالاوزان والأطوال لكل نوع على حدة بالاضافة الى تسجيل التغيرات النسجومرضية ومناقشتها.