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Prevalence of intestinal helminths of the Silver grunt (*Pomadasys Kaakan*) and black finned sea bream (*Spondyliosoma cantharus*) fish collected from Kuwait

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ABSTRACT

Climate change has caused extensive changes to marine ecosystems in recent decades; this might lead to an increase in marine disease. Parasite infestation in silver grunt and black finned sea bream is still few. This study aimed to determine the prevalence and intensity of intestinal helminthes of 60 sample of Silver grunt and 60 samples of black finned sea bream species collected from Kuwait fish markets. The result revealed that the total infection rate in each species was 58.3% and 33.3% respectively. The endoparasite worms found in the intestine of Silver grunt were *Procamallanus sinespinis* which recorded the highest infestation rate 26.6% with intensity 2, followed by *Aephnidiogenes senegalensis* (25%) followed by *Prosorhynchus indicus* (18.3%) with intensity 4 for each, *Anisakis physeteris* and *Aniskais simplex* with infestation rate 11.6, 8.3%, respectively and intensity of 2 and 4, respectively. In addition, the endoparasite worms found in the intestine of black finned sea bream was *Hysterothylacium fabri* (8.3%). Histopathological examination of infested intestine showed necrosis, degenerative changes, and congestion of intestinal blood vessels.

Introduction

Fisheries play great role in the national economic sector (VASEP, 2018). A total of 35% of all Kuwait's fish fauna have been recorded from the coral reef ecosystem alone. The total commercial fish's production from both local fishing and aquaculture meets only about 40-50% of the market demand (Al-Sarawi and Al-Obaid, 2002). The main commercial fish species in Kuwait belongs to 14 families such as *Pomadasys Kaakan* (Silver grunt) and *Spondyliosoma cantharus*. *Pomadasys Kaakan* (Silver grunt) or Nagroor, is one of the prime commercial species in Kuwait and throughout the Arabian Gulf (Valinassab *et al*., 2010). The grunts fish species inhabits the coastal waters in rocky areas, coral reefs and in muddy substrates to 75 m depth, as in Persian Gulf and the Gulf of Oman, the Gulf of Aden, Arabian Gulf (Valinassab *et al*., 2011). The black seabream *Spondyliosoma cantharus* is distributed in the eastern Atlantic and common in the Mediterranean Sea but rare in the Black Sea (Griffiths and Heemstra, 1995). Helminth infections have a great impact on fish industry due to the pathological effects on fish productivity and the zoonotic potential of many helminths species (Chai *et al*., 2005). Parasites have been considered as sensitive bio indicators for aquatic ecosystem health due to the direct linkage and dependence of these organisms with multiple-host life cycles to the surrounding animal communities (Hechinger *et al*., 2007). Multiple hosts fish parasites with complex life cycles are used to indicate food relationships in unaffected marine habitats (Lafferty *et al*., 2008). While the occurrence of endoparasites often decreases in polluted waters, however, ectoparasites increased (Kleinertz, and Palm, 2013).

Carnivorous fish as Silver grunt is more likely to be infected by endoparasite worms than herbivores and omnivorous fish (Sarjito, 2005). *P. Kaakan* is an active predator, feeding generally on a wide variety of fish species inhabiting coral reefs, crustaceans and Polychaeta feeds. The crustaceans, entirely *P. pelagicus*, are the single major food group that the fish feed on while the minor groups include fish, mollusks and echinoderms (Valinassab *et al*., 2011).

Prosorhynchus indicus are parasites of marine fishes closely resembles *P. facilis* while Aephnidiogenidae are parasites of marine teleosts includes seven genera as *Aephnidiogenes* which isolated from *Pomadasys* spp. (Madhavi and Bray, 2018). There were three species of this genus were reported from India as *A. senegalensis* which showed specificity to haemulid fishes and has been recorded from these hosts from a wide range of localities. Anisakid are important pathogens, it can cause problems for human and animal after consumption of raw or undercooked fish so that the accurate identification of this species is essential (Arizono *et al*., 2011). These anisakid nematodes larvae were found usually encapsulated in visceral organs and the peritoneum. It was recorded from different marine fish such as European Hake *M. merluccius lessepsianus* that there were Twenty-two (36.66%) naturally infected fish with *Anisakis* larvae (Abo-Rahma *et al*., 2016), which were isolated from the intestine and abdominal cavity of *T. araneus* and *T. radiates* and collected from the Gulf of Tunisian (Azizi *et al*, 2017). *Anisakis* sp. could be distinguished from other Anisakidae parasites by presence of boring tooth at the anterior end and by the ventriculus shape (Anshary, 2011). *Anisakis physeteris* was isolated from the gastrointestinal system of cantang groupers, with a prevalence of 1% and an intensity of 1 person/fish (Agustina *et al*., 2018).

Procamallanus (*Spirocamallanus*) *sinespinis* sp. is a new species of parasitic nematodes that was found in the intestine of Silver grunt (*Pomadasys argenteus*). It was primarily distinguished by 10–12 spiral ridges in the buccal capsule, the presence of wide caudal alae, three pairs of pedunculate preanal papillae, two unequally long spicules by the tail

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tip with a knob-like structure in the male, and the broad, rounded tail with a terminal digit-like protrusion without cuticular spikes in the female (Moravec and Justine, 2017). The genus Hysterothylacium, which may be parasitized by both marine and freshwater fish species, has roughly 101 species reported so far (Bezerra *et al*., 2020). The *S. colias* gut yielded *Hysterothylacium fabri*, which had a pale body colour and a coarse transverse striated cuticle. Shortly behind the base of the subventral lips, lateral alae thin is extended to the caudal area, and 3 lips on the anterior end, roughly equal in size. The esophagus is encircled by a nerve ring (Beatriz *et al*., 2020). Histopathological alterations extensively used as biomarkers in the evaluation of health condition of fish (Roberts, 2001).

This research aimed at determination of the prevalence and intensity of intestinal helminthes in Silver grunt and black finned sea bream species collected from Kuwait fish markets.

Materials and methods

Study area Fish samples collection

A hundred and twenty fish samples; 60 Silver grunt fish (*Pomadasys* sp.), 60 finned black seabreams were collected from Arabian gulf at Kuwait bay (Fig. 1) during the period from February to November 2019. The fish samples were collected alive or freshly dead for doing parasitological examination to fish intestine. The PM examination and parasitological examination was done at the veterinary and agriculture laboratory center, public Authority for Agriculture Affairs and Fish Resources Kuwait.

Fig. 1. Map of Arabian Gulf and Kuwait Bay.

Chemicals used for parasitological examination

Lacto phenol cotton blue (LPCB) ready use (Merck, Germany) for adult trematodes and cestodes staining. Polyvinyl lacto phenol for clearing of nematodes and acanthocephalans. Physiological saline (0.9%Nacl), and 70% ethyl alcohol.

Instruments used for parasitological examination

Binuclear light microscope (EBB-4260, Globe Scientific, USA), digital camera (OMAX, A3580U3, China), slides and covers slides, Scissors, forceps, containers, and droppers.

Fish samples were dissected and examined for the presence of helminthes. The stomach and intestine of each sample were removed and placed in a separate container for examination. The mucous membrane of each organ was scraped between the blades of forceps, the contents were washed by physiological saline then examined by magnifying lens for helminthes. The worms were counted and preserved in 70% ethanol until staining and identification.

Adult trematode

The recovered digenean parasites were stained with a simple and rapid technique using lacto phenol cotton blue (LPCB) according to the method of Henedi and El-Azazy (2013), trematode was washed in saline, flattened and fixed in 70% ethanol, according to the worm size, a suitable drop from ready to use LPCB was added on a clean slide then the worm was placed on the stain for 2-3 minutes, then covered by coverslip to make permanent slide. The coverslip was fixed on the slide by using nail polish. The adult trematodes were identified according to Madhavi and Bray (2018) and Hafeezullah and Siddiqi (1970 a, b).

Nematodes

The recovered nematodes and acanthocephalans were cleared and counted in polyvinyl Lacto phenol and identified according to Moravec and Justine (2017, 2020).

The identification of intestinal helminths was done according to Castro (1996).

Results

Parasitic examination revealed presence of different helminthes.

Subfamily: *Prosorhynchinae*.
Genus: *Prosorhynchus*
Species: *Prosorhynchus indicus*
Host: Silver grunt *Pomadasys Kaakan*
Description

It had an extended body and reached up to 3 ml in length and long neck that was free of uterine coils. Rhynchus were tiny and triangular. It had a disc-like anterior portion and a conical posterior portion. The caecum extended to the ovary's level in the back. The rearmost part of the worm's body had gonads. The testes were separated by uterine coil, in the posterior quarter of body. Cirrus-sac was small, extended to the level of the posterior testis. Ovary was present pretesticular. Vitelline fields confined to posterior half of body, between ovary and about equator It characterized by elongated body, long neck and contained much smaller eggs. As shown in Fig. 2.

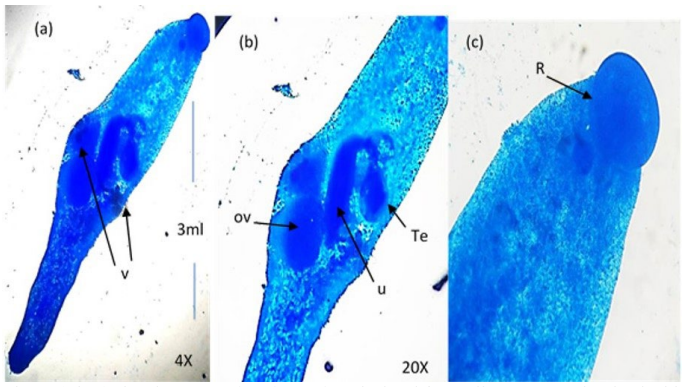

Fig. 2. (a,b,c,) showing *Prosorhynchus indicus* isolated from Silver grunt and stained with cotton blue stain; (R) Rhynchus, (v) vitellarium, (ov) ovaries, (u) uterine coils, (Te) testis & (e) eggs.

Family: Aephnidiogenidae
Genus: *Aephnidiogenes*
Species: *Aephnidiogenes senegalensis*
Host: Silver grunt *Pomadasys* sp.
Description

The worm's body was elongated, measuring 5 ml, and its tegument was narrow. The ventral sucker was smaller or comparable in size to the oral sucker, which was subglobular. The prepharynx was short and distinct. The intestine splits in the forebody, and the caeca extended almost to the posterior extremity before opening through the ani. Two testes were separated and situated in mid-hindbody. Cirrus-sac was absent. Seminal vesicle long and coiled ended with genital pore. Ovary was oval located pretesticular close to ventral sucker. Uterus located between cae-

ca mainly between anterior testis and ovary. Vitelline fields extend from posterior extremity to the level of the ovary. As shown in Fig. 3.

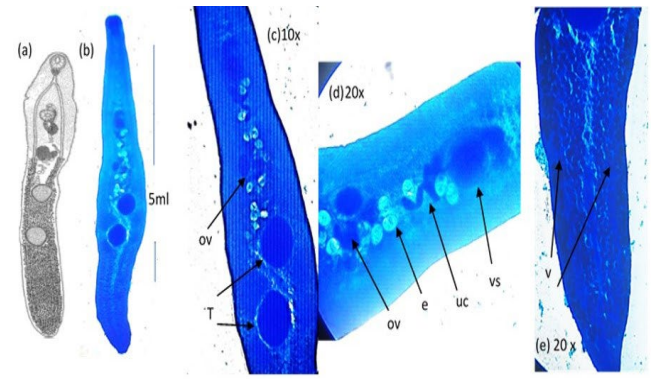

Fig. 3. (a, b, c, d) showing *Aephnidiogenes senegalensis* isolated from Silver grunt and stained with cotton blue stain; (ov) ovaries, (T) testis, (v) vitellarian field, (uc) uterine coil, (vs) ventral sucker, (e) eggs.

Family Anisakidae
Genus: *Anisakis*
Species: *Aniskais simplex*:
Host: Silver grunt *Pomadasys Kaakan*
Description

There were four small labial papillae (two dorsolateral and two ventrolateral) surrounding the mouth opening and forming longitudinal lateral grooves that were extended along the body and start next to the mouth area and end before the mucron in the typical third-larval stage of *Anisakis*, which was characterized by smooth cuticle, 1.5 cm in length, and poorly developed lips. Single or multiple papillae was found on the ventrolateral lips. The two double papillae on the dorsal lip of the larvae were distinguished by the presence of a boring tooth. the presence of an oblique esophageal-gastrointestinal junction. The ventriculus was elongated, and the intestinal caecum was missing. At the base of its ventrolateral lips, it had an excretory pore, and its short, rounded tail was mucron-filled. As shown in Fig. 4.

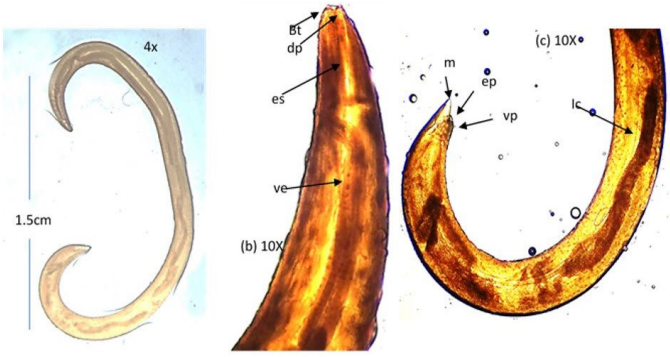

Fig. 4. a) Total length 1.5 cm, b) anterior part, c) posterior part of *Aniskais simplex* third larval stage isolated from Silver grunt *Pomadasys* intestine;(Bt) boring tooth, (dp) two dorsal lips, (es) esophagus, (ve) ventriculus, (vp) ventral lip, (ic) intestinal ceca (ep) excretory pore, (m) macron.

Species: *Anisakis physeteris*
Host: Silver grunt *Pomadasys Kaakan*
Description

This worm had an elongated cylindrical body, its total length being roughly 1.2 cm. It had a cuticle that was smooth and bears a distinctive boring tooth or larval tooth. The esophagus was 0.53 mm long, while the ventriculus was 0.11 mm long. The intestine has an oblique esophagointestinal junction and a conical tail without any mucrons. *Anisakis physeteris* worms were included in type II of *Anisakis* which had a conical tail without mucron. Presence of tail appendix. The worm founded was female larvae characterized by presence of vulva in the posterior part of the body. As shown in Fig. 5.

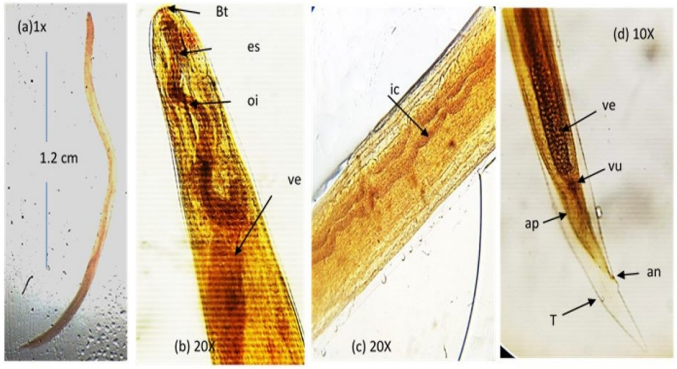

Fig. 5. a) Third Stadia Larvae of *Anisakis physeteris* isolated from Silver grunt *Pomadasys* intestine; (b) Anterior part, (c) Mid body part, (d)Posterior part, (Bt) Boring tooth, (es) Esophagus, (ve) Ventriculus, (ic) intestinal ceca,(oi) esphagointestinal junction, (vu) vulva , (an) Anal opening, (ap) Appendix, (T) Tail.

Species: *Hysterothylacium fabri*
Host: Black sea bream (*Spondyliosoma cantharus*).
Synonyms: *Ascaris fabri*, *Contracaecum fabri*
Description

H. fabri fourth-stage larvae, the body was pale, 8–10 ml in length, and had a delicately transversely striated cuticle. Shortly behind the base of the subventral lips, lateral alae thin extended to the caudal area. 3 lips on the anterior end, roughly equal in size. Esophagus was small, virtually cylindrical, and muscular. Between 1/3 and 1/4 of its length, the esophagus was encircled by a nerve ring. Excretory pore was slightly posterior to nerve ring. Ventricular appendix was longer than intestinal caecum. Ventriculus was almost spherical. Intestinal caecum was short. Tail conical was relatively short and covered by numerous spines. As shown in Fig. 6.

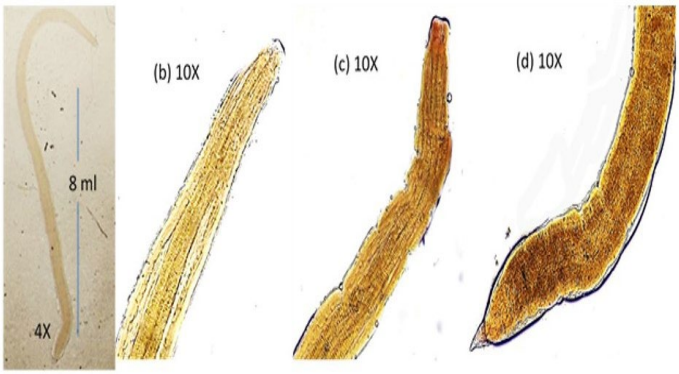

Fig. 6. *Hysterothylacium fabri* isolated from Black sea bream (*Spondyliosoma cantharus*) intestine.

Family *Camallanide*
Procamallanus (*Spirocamallanus*) *sinespinis*
Host: Silver grunt *Pomadasys Kakkan*
Description

Nematode measuring 1.4 cm in length and with a finely transversely striated cuticle. Oval mouth opening bordered by organized cephalic, Buccal capsule was orange-brown and slightly longer than wide, with simple well-developed basal ring. Had muscular esophagus which was shorter than glandular esophagus; two parts of the esophagus slightly expanded near their posterior ends. Intestine was brown color, narrow. Excretory pore approximately at level of junction of both parts of esophagus. Male had spicules similar in shape, unequal, with sharply pointed distal ends, Gubernaculum is absent. Tail was conical, with small terminal cuticular knob. As shown in Fig. 7.

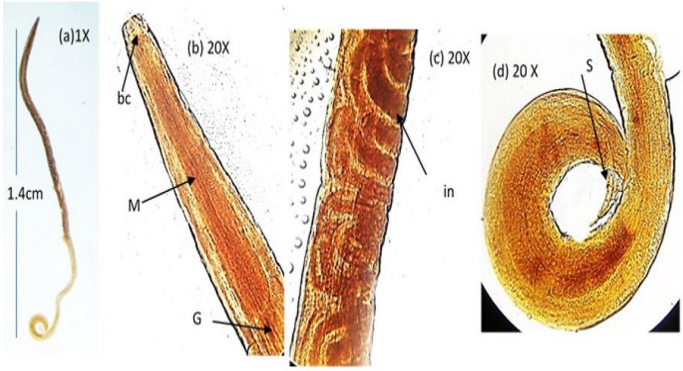

Fig. 7. *Procamallanus* (*Spirocamallanus*) *sinespinis* male isolated from Silver grunt *Pomadasys* intestine; (bc) buccal capsule, (M) muscular esophagus, (G) glandular esophagus, (in) brown intestine, (s) spicule.

Total Prevalence rate and seasonal prevalence of the recovered parasites from examined fish species

Out of the 120 fish species that were examined (60 Silver grunt *Pomadasys* and 60 Black sea bream *Spondyliosoma cantharus*), roughly 35 and 20 fish, respectively, were found to be infested with various parasitic helminth species. The overall infection rate in each species is 58.3 and 33.3% (Table 1).

It was found that summer revealed the highest levels of parasite infestation, followed by autumn and spring, while winter showed the lowest levels.

Table 1. Prevalence rate of recovered Parasites from examined fish species collected from Kuwait markets.

Fish species	Examined	Infested	%
Silver grunt *Pomadasys Kaakan*	60	35	58.30%
Black sea bream, *Spondyliosoma cantharus*	60	20	33.30%

Types of detected parasitic species in collected fish species, total prevalence and intensity of infestation

The total prevalence of each isolated parasite from Silver grunt *Pomadasys*. *Procamallanus sinespinis* recorded the highest infestation rate (26.6%) with intensity 2, followed by *Aephnidiogenes senegalensis* (25%) followed by *Prosorhynchus indicus* (18.3%) with intensity 4 for each, and *Anisakis physeteris*, *Aniskais simplex*, *Prosorhynchus epinepheli* with infestation rate 11.6, 8.3, 6.6 respectively and intensity of 2, 4, 3 respectively (Table 2).

The total prevalence of isolated *Hysterothylacium fabri* nematode from Black Sea bream was 8.3% with intensity 2 (Table 2).

Histopathological alterations

Histopathological changes if intestinal tissue of the examined fish species infested with both trematodes and nematodes showed necrosis in the villi cells, inflammation, and degeneration of intestinal layer's (mucosa, submucosa, muscularis and serosa) and sloughing of the tissue. Dilation of blood vessels and congestion. The histopathological section of silver grunt intestine revealed the presence of trematode parasite in between its intestinal villi, while sea bream intestinal villi revealed the presence of encysted metacercaria (Fig. 8).

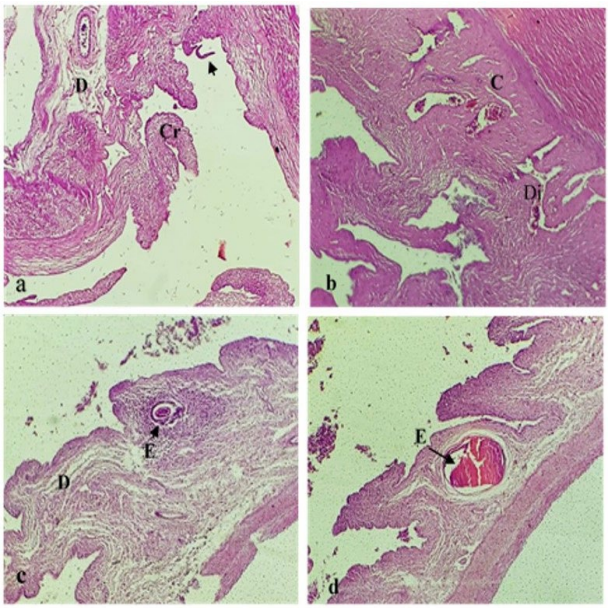

Fig. 8. a and b) 20X the intestinal tissue of silver grunt showing degeneration of intestinal tissue (D), curling of the villi (Cr), presence of trematode parasite(arrow), dilation (Di) and congestion (C) of blood vessel; c&d) 20X the intestinal tissue of black sea bream show degeneration(D) and presence of encysted metacercaria (E).

Discussion

With a wide geographic distribution, marine fish serve as hosts for ecto- and endoparasites from many families and species. The role of marine fish as hosts varies and depend on different ecological and host factors (Hassan, 2008). The main foodborne zoonoses associated with the consumption of fishery products are mainly attributable to trematodes, cestodes and nematodes. Among these parasites, the anisakid nematodes are the most important under the sanitary point of view, since they are capable to induce pathologies in humans such as anisakidosis, anisakiosis after the ingestion of raw, poor cooked/smoked or superficially salted fish meat containing infective larvae (Klimpel and Palm, 2011).

According to the description of the *Prosorhynchus indicus* species isolated from Silver grunt *Pomadasys*, it had an elongated body and a long neck. Rhynchus were tiny and triangular. Vitelline fields confined to posterior half of body, these descriptions are consistent with Madhavi and Bray (2018). The morphological description of the trematode species *Aephnidiogenes senegalensis* was found in the intestine of Silver grunt *Pomadasys* indicated that the worm body was elongated, there were two distinct testicles in the middle of the hind body. Vitelline fields were extended from the posterior extremity to level of the ovary, similar results were recorded by Madhavi and Bray (2018).

Parasitic nematodes are important pathogen associated with human and animal health. Some inhabit the marine environment, where adults are commonly found in the digestive tract of marine mammals and Larvae infect aquatic invertebrates and non-mammalian vertebrates as intermediate hosts (Klimpel and Palm, 2011). Regarding the morphological description of nematodes worms, the normal third- larval stage of *Aniskais simplex* obtained from the gut of Silver grunt *Pomadasys* was distinguished by a smooth cuticle, its lips were underdeveloped, and its body

Table 2. The detected parasitic species, their prevalence and intensity of infestation in each examined fish species.

Fish species	Type of parasite	Total prevalence (%)	Intensity of infestation per fish
Silver grunt *Pomadasys*	*Prosorhynchus indicus*	18.3	4
	Aephnidiogenes senegalensis	25	4
	Prosorhynchus epinepheli	6.6	3
	Aniskais simplex	8.3	4
	Anisakis physeteris	11.6	2
	Procamallanus sinespinis	26.6	2
Black sea bream *Spondyliosoma cantharus*	*Hysterothylacium fabri*	8.3	2

was characterized by a smooth surface. Longitudinal lateral grooves were formed by four tiny labial papillae. The dorsal lip was distinguished by the presence of a boring teeth. These morphological descriptions nearly similar to that illustrated by Borges *et al.* (2012) an in accordance with Knoff *et al*. (2017). The *Anisakis* sp. larva was isolated from the stomach wall of *Johnius coitor* and *Lutjanus johnii*, The third stage larva of *Anisakis* was characterized by the presence of a boring tooth, the excretory pore in the lip region, a large ventricle and the absence of the ventricular appendage and caecum (Rueckert *et al*., 2008).

The nematode *Anisakis physeteris* was found in the gut of Silver grunt *Pomadasys* had an elongated cylindrical body, a smooth cuticle, and characteristic boring teeth or larval teeth. In this study, the worm founded was female larvae characterized by presence of vulva in the posterior part of the body, which are similarly to findings of Knoff *et al*. (2017). In addition, the *Hysterothylacium fabri* nematode species collected from the intestine of Black Sea bream (*Spondyliosoma cantharus*) was identified by its whitish body colour and highly transversely striated cuticle. Similar description was recorded by Beatriz *et al.* (2020). The morphological description of *Procamallanus* (*Spirocamallanus*) *sinespinis* collected from Silver grunt *Pomadasys* revealed that the worm was medium-sized and had a cuticle that was finely transversely striated. Buccal capsule was orange-brown and somewhat longer than wide, male have Spicules, as described by Moravec and Justine (2017). *Procamallanus* sp. was isolated from the intestinal content of Johnius coitor, Lutjanus johnii and Scatophagus argus, belonging to the family Camallanidae which was characterized by an orange buccal capsule and a round oral opening (Rueckert *et al*., 2008).

Regarding the overall prevalence of the parasites found in the fish species under investigation, the percentage of total infestation in the fish species Silver grunt *Pomadasys* and Black sea bream *Spondyliosoma cantharus* is around 58.3% and 33.3%, respectively. This was very similar to the observation made by Azizi *et al.* (2017) among the examined trachinid fishes from the Tunisian coasts. While the results are higher than that recorded by Agustina *et al.* (2018). Silver grunt *Pomadasys* had the highest observed infestation rate followed by Black Sea bream Spondyliosoma cantharis, this result attributed to the feeding habits of each species, carnivorous fish such as grunts are more likely to be infected by endoparasite worms than herbivores and omnivorous fish such as black sea bream nearly similar to what revealed by Ruckert *et al.* (2009). As the spreading of endoparasites is may be due to the presence of invertebrates around the floating net cages that act as intermediate hosts (Sarjito, 2005).

The seasonal incidence of parasite infection in the investigated fish species' revealed that summer showed the highest levels of parasitic infestation, followed by autumn and spring, with winter had the lowest levels. This outcome almost exactly matched what was revealed by Azizi *et al*. (2017) who record that the maximum level of helminths infestation in fish was during summer followed by spring. According to Hassan (2008), this may be attributed to environmental conditions like water temperature and chemical composition, which may promote the spread of intermediate hosts like invertebrates.

Regarding the overall prevalence and severity of infection of each parasite isolated from Silver grunt *Pomadasys* fish samples, it was noted that *Procamallanus sinespinis* recorded the highest infestation rate (26.6%) with intensity 2, followed by *Aephnidiogenes senegalensis*, which was 25%The prevalence of *Prosorhynchus indicus* isolated from Silver grunt *Pomadasys* was 18.3% with intensity 4 for each, while Madhavi and Bray (2018) recorded P. indicus from Bay of Bengal. *Aniskais simplex* with prevalence rate of 8.3 % and intensity 4 per fish, while Abo-Rahma *et al*. (2016) recorded prevalence rate of 36.66% from the European Hake *M. merluccius lessepsianus*. In addition, Azizi *et al*. (2017) recorded prevalence rate (20.31%) of typical *Anisakis* larvae from *T. draco* from the Gulf of Tunisian and the intensity was varied from 1 to 10 per fish. While Agustina *et al*. (2018) record a single infestation of cantang groupers with *Anisakis physeteris* 1 % with intensity of 1 individual/fish and the recorded parasite was female compared to our study which revealed that the *Anisakis physeteris* total prevalence rate was 11.6% and the intensity of infestation was 2 per fish. Knoff *et al*. (2017) reported that Auxis thazard was parasitized with *A. simplex* and *A. physeteris*, and *Thunnus thynnus* with *A. simplex*. The prevalence was 20%, 20%, and 11.7%, respectively.

In this study *Prosorhynchus epinepheli* was isolated from Silver grunt *Pomadasys* with infestation rate 6.6 % and intensity 3 per fish.

The total prevalence of *Hysterothylacium fabri* isolated from Black Sea bream was 8.3% with intensity 2, in comparison to Azizi *et al*. (2017) who recorded *H. fabri* from *T. draco* which was collected from the Bay of Bizerte, and *T. radiatus* that was collected from the Bay of Bizerte and Mahdia.

The intestine of the examined fish species infested with adult trematode and nematodes revealed degeneration and necrosis in all intestinal layer's (mucosa, sub mucosa, muscularis and serosa), there was trematode parasite in between villi of silver grunt intestine. While there was encysted metacercaria in villi of sea bream intestine. These results were similar to the finding recorded by Eissa *et al.* (2010).

Conclusion

The findings of the present study revealed infestation of the Silver grunt and the Black Sea bream collected from Kuwait with various classes of helminthes which adversely affect such fish species.

Conflict of interest

The authors declare that they have no conflict of interest.

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