Global Veterinaria 14 (2): 219-227, 2015
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2015.14.02.9329

Infestation of Cage-Cultured Marine Fish with *Benedenia acanthopagri* (Monogenea; Capsalidae) in Eastern Province of Saudi Arabia

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Abstract: For development of Saudi's marine fish aquaculture industry in Saudi Gulf coast, Fisheries Research Center in the Eastern Province of Saudi Arabia carry out surveillance and monitoring program for aquatic diseases in mariculture systems. Present study aimed to investigate the prevalence, seasonal variations, density of parasitism, clinical and postmortem lesions with determination of organic matter in cage cultured marine fishes, Acanthopagrus *bifasciatus; Diplodus noct* and *Sparidentex hasta* with *Benedenia acanthopagri* in a private marine fish farm located at Arabian Gulf, Eastern Province, Saudi Arabia. The farm considers the oldest cage-cultured marine fish farm at the Eastern province. It had 30 Cages measured 5 x5 x 4 m. Study was performed on 640 total numbers of marine fishes, 165 fish was infested with *Benedenia acanthopagri* with total percentage 25.78. Infested fish appeared suffered from respiratory distress enhancement of opercular movements and surfacing, gulping the atmospheric air, opening mouth, hemorrhages at base of fins, caudal peduncle, caudal fins and abdominal region, scales sloughing and shallow ulcers at different parts of the body, congestion of gills with marbling appearance. The isolated parasite was identified as *Benedenia acanthopagri*. Seasonal variations of the investigated monogenea was recorded, the highest season of infestation was summer 39.16 %, followed by autumn 27.22 %, spring 21.11 % and the lowest infestation rate was recorded at winter, 12.50 %. The concentration of organic matter was closely related and correlated to the prevalence of infestation of *Benedenia acanthopagri.*

Key words: Cage culture · *Acanthopagrus bifasciatus* · *Diplodus noct* · *Sparidentex hasta* · *Benedenia acanthopagri* · Organic matter

INTRODUCTION

The Monogenea is a class of Platyhelminthes, common parasites of the skin and gills of both marine and freshwater fish [1-3]. There are many different species, most of which have a narrow host range in nature restricted to one species, genus, or family. However, this host specificity is often lost in aquaculture [4].

Heavy monogenean infestations are usually indicators of poor sanitation and deteriorating water quality (overcrowding, high ammonia or nitrite, organic pollution or low oxygen) [5, 6]. Monogeneans can rapidly reproduce under such conditions. The doubling time for viviparous monogeneans can be as little as 24 hours. Reproductive rate is also controlled by temperature, which, although not variable in a tropical aquarium (which should have a narrow range of temperature), is important in less controlled environments [7].

Monopisthocotyleans feed mainly on the superficial layers of the skin and gills. This feeding activity is irritating and thus often causes skin cloudiness or focal reddening resulting from excess mucus production, epithelial hyperplasia, or hemorrhage [8]. Even small

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numbers of parasites can elicit excess mucus production or pruritus. Some species can cause deep skin wounds and ulcers [5, 7].

General identification can be made from the number and shape of grasping hooks and the form of muscular adhesive structures for identifying different species. Most of them are oviparous, producing free-swimming hooked and ciliated larvae that hatch from eggs (oviparous). In others, larvae are produced from adult parasites (ovoviviparous) that often remain on the same fish with their parents [1, 9].

Present study aimed to achieve the following objectives: First, to investigate the prevalence, seasonal variations, density of parasitism with *Benedenia acanthopagri* in cage cultured marine fishes, *Acanthopagrus bifasciatus, Diplodus noct* and *Sparidentex hasta* in Eastern Province of Saudi Arabia. Secondly, to detect clinical and postmortem lesions, as well as, to evaluate the magnitude of injury that induced by this parasite in infected tissues and organs of the fish. Thirdly, to test the efficacy of formalin in controlling the parasite.

MATERIALS AND METHODS

Study Area: Study was carried out on a private farm located at Eastern province of Saudi Arabia on the Arabian Gulf. Twobar Seabream (*Acanthopagrus bifasciatus*), Sobity Seabream (*Sparidentex hasta*) and Red Sea Seabream (*Diplodus noct*) are the main culture fish species in this farm. It had 30 Cages, measured 5 X 5 X 4 m/cage. The stocking density of the caged fish ranged 10-12 kg/m^3 and fish were fed commercial feeds. Study was performed on 640 fish, fish species, numbers of fishes, average body weights and lengths are shown in (Table 1). Survey on cultured marine fish was performed periodically from June 2012 to May 2013.

Clinical and Postmortem Examination: The behavior, swimming, respiration and external body surface and gills were observed for the collected investigated fish then transported alive in large plastic bags to the laboratory of Fisheries Research Center in the Eastern Province [7].

Parasitological Examination: Scrapings from external body surface; skin, fins and gills of live fish were taken with sea water and examined using light compound microscope searching for external parasites. Gills arches were cut separated from the fish, gill filaments were also cut and placed in Petri dish full of seawater for accurate examination for monogenea. Isolated monogenea preserved in 70% ethyl alcohol for further examination and staining with alum carmine [10].

Total and Seasonal Prevalence: Along the study year, June 2012 to May 2013 infested marine fishes (*Acanthopagrus bifasciatus; Sparidentex hasta* and *Diplodus noct*) with *Benedenia acanthopagri* was recorded as a total prevalence, also recorded in each season with determination the intensity of infestation; Light infestation (+) (1 parasite/field), Moderate infestation (++) (2-5 parasites/ field) and Heavy infestation (+++) (more than 5 parasites/ field), in each season.

Determination of Ammonia, Nitrite and Nitrate in the Cage Cultured Water: Ammonia, nitrite and nitrate were measured in the water samples collected in each season using (Hach model DR/2000) according the operator manual. In addition, water temperature, dissolved oxygen, salinity and pH were measured using (JENWAY 9200).

Histopathological Studies of Infested Fishes with *Benedenia Acanthopagri*: Sections were taken from the affected gills of diseased fish were fixed in 10% formal saline for twenty-four hours. Sections were washed in tap water and passed in serial dilutions of alcohol (ethyl and absolute ethyl) for dehydration. Specimens were cleared in xylene and embedded in paraffin. Paraffin wax tissue blocks were prepared for sectioning at 5-7 microns thickness by sledge microtome. The obtained tissue sections were collected on glass slides, deparafinized, stained by hematoxylin and eosin then, examined using light electric microscope [11].

Table 1: Showing prevalence of *Benedenia acanthopagri* in investigated marine fish species

Fish Species	Average Length (cm)	Average body weight (gm)	No. Exam.	No. Infect.	%
Sparidentex hasta	27- 36	347 - 745	240	80	33.33
Acanthopagrus bifasciatus	18 - 29	111- 521	240	55	22.91
Diplodus noct	16 -22	73 - 190	160	30	18.75
Total	--------	---------	640	165	25.78

RESULTS

Clinical Signs and Postmortem Lesions: The presence of large numbers of *Benedenia acanthopagri* causes considerable irritation to infested fishes and results in the fish 'rubbing' themselves along the bottoms and sides of cages. The fish with severe infections had small focal haemorrhages on the body, sloughing of scales and excessive mucus secretion. Infected gills was found pale colour or congested covered with mucous layer, showing marbling appearance, occasional haemorrhagic spots were also noticed on gills.

Infested *Sparidentex hasta* fish appeared with dark color suffered from respiratory distress, enhancement of opercular movements, surfacing, gulping the atmospheric air by opening mouth (Fig. 1, A), abnormal swimming and flashing. Hemorrhages at the base of fins and the abdominal region (Fig. 1, B &C), at the caudal peduncle and caudal fins (Fig. 1, D). Skin sloughing and shallow ulcers at dorsal region (Fig. 1, E) as well as on the caudal peduncle (Fig. 2), severe congestion of gills with necrotic foci (Fig. 1, F). *Diplodus noct* fish showing congested gills with mosaic (marbling) appearance (Fig. 3).

Heavy infestation with *Benedenia acanthopagri* in summer months caused not only haemorrhagic and abrasive lesions, but also mortalities in cultured marine fish due to severe necrosis of the gill tissues resulting in suffocation and death.

Identification of the Isolated Parasites: Isolated parasites was identified mainly basing upon the characteristic morphology, it is dorsoventrally flat (no body cavity), compressed worms Platyhelminthes, hermaphrodite have male and female organs, It is oviparous laying fertilized eggs, the anterior part called prohaptor composed from large 2 adhesive organs help the parasite in movement and feeding (Fig 4). While the posterior attachment organ, or opisthaptor, appears as a single distinct unit composed of several large centrally, located sclerotized anchors and small marginal hooklets (for piercing host tissue) (Fig. 4). The isolated parasite is subclass: Monogenea, order: Monopisthocotylea, Family: Capsalidae, Genus: Benedenia species *acanthopagri* (*Benedenia acanthopagri*) [12] (Figs. 4 &5, A, B and C).

Prevalence of *Benedenia Acanthopagri* Infestation: In the period of survey from June 2012 to May 2013 out of 640 examined marine fishes 165 fish infested with *Benedenia acanthopagri* (Monogenea: Capsalidae) in all seasons of the year with total percentage 25.78 Table 1. Present study indicated that 3 species of marine fishes, *Sparidentex hasta, Acanthopagrus bifasciatus* and *Diplodus noct* was infested with *Benedenia Acanthopagri.* The highest infested species was *Sparidentex hasta* 33.33% and the lowest infested one was *Diplodus noct* 18.75 %, while *Acanthopagrus bifasciatus* infested with 22.91%.

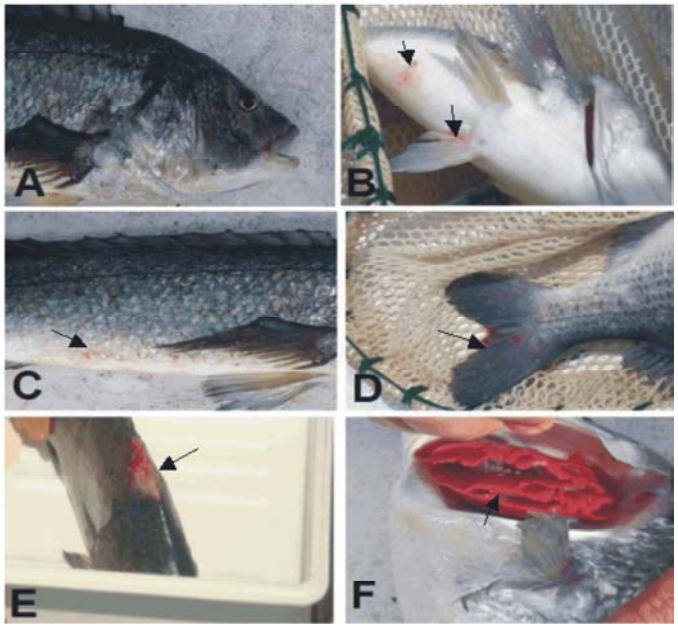

Fig 1: A. Dark color *Sparidentex hasta* fish with opening mouth, (B &C) hemorrhages at the base of pectoral fins and the abdominal region (arrows), (D) hemorrhages at the caudal peduncle and caudal fins, (E) scales sloughing and shallow ulcer at the dorsal region, (F) congestion of gills with necrotic foci.

Fig 2: *Sparidentex hasta* fish suffered from parasitic ulcer on the caudal peduncle (arrow)

Fig 3: *Diplodus noct* showing congested gills with mosaic (marbling) appearance (arrow).

Fig 4: Showing wet mount of *Benedenia acanthopagri*

Seasonal Variations of *Benedenia Acanthopagri* Infestation: The present study revealed that the highest season of infestation was summer 39.16 %, followed by autumn 27.22 % and spring 21.11 % and the lowest infestation rate was recorded at winter 12.50 % Table 2.

Degrees of Infestation During Different Seasons: There is wide range of severity in infestation during different seasons, concerning the light infestation (1 parasite /field); the highest percentage was recorded at spring 31.89% followed by summer 24.64% and winter

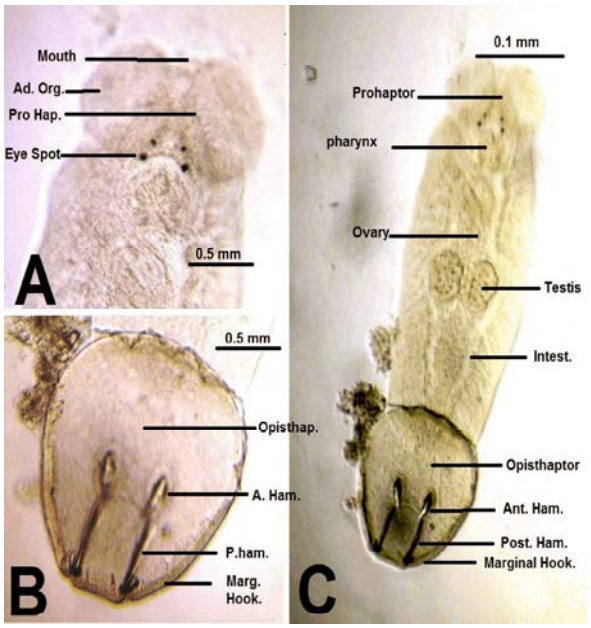

Fig 5: Wet mount of *Benedenia acanthopagri* (A) Anterior end of *Benedenia acanthopagri* adh.org.= adhesive organ, pro. Hap. = prohaptor, mouth= mouth and Eye Spot = Eye spot (B) Posterior part of *Benedenia acanthopagri*: Opisthap. = Opisthaptor, P. ham. = Posterior hamulus, Marg. Hook. = marginal hooklets, A. Ham. = anterior hamulus (C) Whole parasite: Prohaptor = prohaptor, Pharynx = pharynx, Ovary= ovary, Testis. = testis, Intest.= intestine, adh. org.= adhesive organ and Opisthaptor = opisthaptor.

23.19% while the lowest percentage was recorded at autumn 20.29%. Regarding moderate infestation (2-5 parasite /field) the highest percentage was recorded at summer 32% followed by spring 26 % and autumn 22 % while the lowest percentage was recorded at winter 20 %. On the otherhand, in heavy infestation (more than 5 parasites / field),the highest percentage was recorded at summer 43.48 % followed by autumn 32.61 % and spring 23.91 % while the lowest percentage was recorded at winter 0 % Table 3.

Organic Matter Concentration During Different Seasons: The concentrations of organic matter were closely related and correlated to the prevalence of infestation of *Benedenia acanthopagri.* The highest percentage concentration of ammonia (NH_3 mg/l) was recorded at summer 0.09 mg/l and the lowest concentration was recorded at winter 0.01 mg/l. The highest percentage concentration of Nitrate

Table 2: Showing seasonal prevalence of *Benedenia acanthopagri* in investigated marine fish species:

	winter			spring			summer			autumn		
Fish species	No. Exam	No. infect	%	No. Exam	No. infect	%	No. Exam	No. infect	%	No. Exam	No.infect	%
Sparidentex hasta	60	12	20.00	60	18	30.00	60	28	46.66	60	22	36.66
Acanthopagrus *bifasciatus*	60	6	10.00	60	11	18.33	60	23	38.33	60	15	25.00
Diplodus noct	40	3	7.5	40	6	15.00	40	13	32.50	40	8	20.00
Total	160	21	12.50	160	35	21.11	160	64	39.16	160	45	27.22

Table 3: Degrees of infestation with *Benedenia acanthopagri* during different seasons

	Light infestation (+) (1 parasite/field)		Moderate infestation (++) (2-5 parasites/ field)		Heavy infestation (+++) (more than 5 parasites/ field)	
Season	No. of infest.	%	No. of infest.	%	No. of infest.	%
Summer	17	24.64	16	32	20	43.48
Autumn	14	20.29	11	22	15	32.61
Winter	16	23.19	10	20	0	0
Spring	22	31.89	13	26	11	23.91
Total	69	41.82	50	30.30	46	27.88

Table 4: Averages of organic matter concentration during different seasons

Season	Free ammonia (NH_3) mg/l	Nitrate (NO_3) mg/l	Nitrite (NO_2) mg/l
Summer	0.09	13.66	0.50
Autumn	0.05	9.20	0.28
Winter	0.01	5.40	0.15
Spring	0.07	10.80	0.36

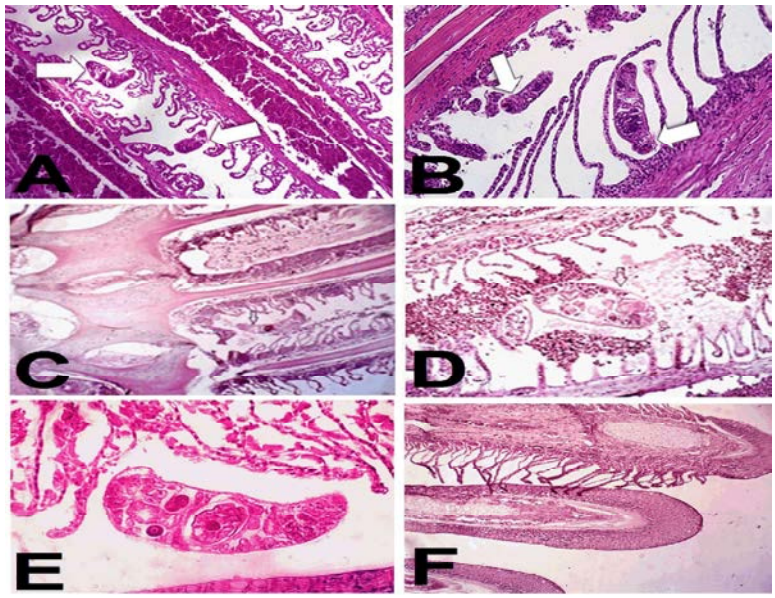

Fig 6: Showing (A&B&C and D) Presence of different sizes of parasitic *Benedenia* particles in-between the secondary gill filaments and between the primary gill lamellae with odema and congestion of primary gill core H&E stain X400 (white arrows) (E) magnified *Benedenia acanthopagri* embedded in-between the gill filaments H&E stain X400 (F) Advanced apical hyperplasia and deformity of secondary gill lamellae with hyperplastic mucous cells of lining epithelial cells of secondary gill filaments H&E stain X200

(NO_3 mg/l) was recorded at summer 13.66 mg/l and the lowest concentration was recorded at winter 5.40 mg/l. The highest percentage concentration of Nitrite (NO_2 mg/l) was recorded at summer 0.50 mg/l and the lowest concentration was recorded at winter 0.15 mg/l Table 4.

Histopathological Studies: Histopathological changes of the present study revealed, hyperplasia of the apical gill lamellae accompanied with necrosis and deformity. Gill filaments also showed congestion of branchial blood vessels of the lamellae, sloughing of the secondary gill lamellae with the presence of large numbers of gill flukes (Fig. 6 A, B, C, D, E & F).

Therapeutic Control: After 24 hour from the treatment with formalin 250 ppm as a bath. Parasitological examination revealed that the *Benedenia acanthopagri* were completely eradicated and most fish regained their normal feeding response, mortality ceased and fish began to appear healthy.

DISCUSSION

The fish exposed for many diseases like any of the organisms. In recent times, these diseases began to draw the attention of many researchers, especially after the intensification of fish farming in private fish farms. Ectoparasites are considered one of the pathogens that represent a big problem in the process of aquaculture because of the large economic losses either through direct or indirect [7].

Monogenean parasites causes mortality of fish because their life cycle is simple and direct do not need intermediate host, so they multiply rapidly and numbers are plentiful in short time, especially if there environmental factors appropriate to do so. The impact of single - parent worms affect the work of the different organs of fish such as bad breath or osmotic change, or may lead to the entry of many other pathogens, or it may lead to mortality or decrease in growth rates of fish [1, 5]. Present study aimed to investigate the prevalence, seasonal variation, intensity of parasitism, clinical and post mortem lesions and histopathological alterations with determination of organic matter in cage cultured of some marine fishes, *Acanthopagrus bifasciatus, Sparidentex hasta* and *Diplodus noct* with *Benedenia acanthopagri* in Eastern Province of Saudi Arabia on the Arabian Gulf.

Benedenia acanthopagri (Monogenea; Capsalidae) reported for the first time in Middle East from skin of *Acanthopagrus latus* (Sparidae) from fish in culture tanks at Al-Raas, Kuwait [12]. In the present study, *Benedenia acanthopagri* reported for the first time from a new three hosts, *Acanthopagrus bifasciatus, Sparidentex hasta* and *Diplodus noct* in Eastern Province of Saudi Arabia on the Arabian Gulf.

Regarding the clinical signs and post mortem lesions present study revealed that Infested *Sparidentex hasta* fish appeared with dark colour suffered from respiratory distress, enhancement of opercular movements, surfacing, gulping the atmospheric air by opening mouth, abnormal swimming and flashing. Hemorrhages at the base of fins, caudal fins, caudal peduncle and in the abdominal region. Scales sloughing, shallow ulcers at different regions of the body and sever congestion of gills with necrotic foci were also seen. In some cases, *Sparidentex hasta* fish showing parasitic ulcer on the caudal peduncle, while *Diplodus noct* fish showing congested gills with mosaic (marbling) appearance. The results nearly agree with [1, 7] and [5] who reported that Captive broodstock of grouper, Taradi collected from Red Sea coast, Jeddah demonstrated clinical infection with a monogenean parasite *Benedenia epinepheli*.It was found that the parasite has tissue tropism to the gills, fins and skin of Taradi fish. However, fishes in advanced state of infestation showed flashing, erratic swimming behavior, restlessness and off food, with development of small focal hemorrhages on the body surface. In some cases, sloughing of the skin and excessive mucus secretion were seen. The clinical sings attributed to Parasite mobility spreads wounds over a broad area reducing local damage. However, infected fish in aquaria or aquaculture cages can succumb to the direct monogenean life cycle resulting in exponential population increases with severe consequences. Monopisthocotylea infections on captive fish can be associated with reduced appetite, stunting of growth and emaciation. Captive fish can suffer stress, particularly if host densities are high or if fish are handled regularly (routine husbandry practices in aquaculture), exacerbating Monopisthocotylea infections [13]. Attachment and feeding by large numbers of Monopisthocotylea on fish 'skin' and gills can injure host epithelial cells faster than tissue regenerates. Mechanical irritation by significant 'skin' Monopisthocotylea populations probably occurs because infected fish may rub against hard substrates (tank or cage structures; bottom sediment). Progressive symptoms and damage to 'skin' by heavy infections may include dark 'skin' patches from parasite feeding activities; excess mucus secretion; compression, erosion or removal of epithelium; loose scales; dermis injuries such as haemorrhages, open sores and ulcers exposing connective and muscle tissue; osmotic problems. On gills, significant Monopisthocotylea burdens can lead to increased mucus production and epithelial inflammation [1]. Large Monopisthocotylea populations on 'skin' or

gills have been associated with mortalities of captive fish, but damage they inflict is open to secondary infections and perhaps viruses, bacteria and other microorganisms mostly kill infected hosts [5].

Concerning the identification of the isolated parasites present study displayed that the isolated parasite was identified mainly basing upon the characteristic morphology, it is dorsoventrally flat (no body cavity), compressed worms Platyhelminthes, hermaphrodite have male and female organs, It is oviparous laying fertilized eggs, The anterior part called prohaptor composed from large 2 adhesive organs help the parasite in movement and feeding. While the posterior attachment organ, or opisthaptor, appears as a single distinct unit composed of several large centrally, located sclerotized anchors and small marginal hooklets (for piercing host tissue). The isolated parasite is subclass: Monogenea, order: Monopisthocotylea, Family: Capsalidae, Genus: Benedenia species. The results confirmed by [5, 9 & 12].

Concerning the prevalence of *Benedenia acanthopagri,* the present study revealed that in the period of survey from June 2012 to May 2013 out of 640 examined marine fishes 165 fish infested with *Benedenia acanthopagri* (Monogenea: Capsalidae) in all seasons of the year with total percentage 25.78. Present study indicated that 3 species of marine fishes, *Sparidentex hasta*, Acanthopagrus *bifasciatus* and *Diplodus noct* were infested with *Benedenia Acanthopagri.* The highest infested species were *Sparidentex hasta* 33.33% and the lowest infested one was *Diplodus noct* 18.75 %*,* while *Acanthopagrus bifasciatus* infested with 22.91%. The results nearly agree with [9, 14-18] and [5] who recorded different prevalence which may be due to different fish species, locality, geographical distribution.

The present study recorded that there was wide seasonal variations of *Benedenia acanthopagri infestation* among examined fish. The highest season of infestation was summer 39.16 %, followed by autumn 27.22 %, spring 21.11 % and the lowest infestation rate was recorded at winter 12.5 %. The results nearly agree with that obtained by [19]. This may be due to the high water temperature that cause enhancement of the life cycle of *Benedenia acanthopagri* and increasing prevalence and intensities of parasitism with Benedenia [7].

Regarding the degrees of infestation during different seasons present study revealed that there is wide range of severity in infestation during different seasons, concerning light infestation (1 parasite /field), the highest percentage was recorded at spring 31.89% followed by summer 24.64% and winter 23.19% while the lowest percentage was recorded at autumn 20.29%. Regarding the moderate infestation (2-5 parasite /field) the highest percentage was recorded at summer 32% followed by spring 26 % and autumn 22 % while the lowest percentage was recorded at winter 20 %. On the otherhand, in heavy infestation (more than 5 parasites / field), the highest percentage was recorded at summer 43.48 % followed by autumn 32.61 % and spring 23.91 % while the lowest percentage was recorded at winter 0 %. Intensity of infestation firmly closed with ecological reasons and stress factors causing infestation, from present study it was noticed that high infestation is always correlated with water temperature which is usually occur in summer and spring where enhancing life cycle increased population and densities of monogenea [1, 19-21].

Concerning the organic matter concentration during different seasons, present study displayed that the concentrations of organic matter were closely related and correlated to the prevalence of infestation of *Benedenia acanthopagri*. The highest percentage concentration of ammonia (NH_3 mg/l) was recorded at summer 0.09 mg/l and the lowest concentration was recorded at winter 0.01 mg/l. The highest percentage concentration of Nitrate (NO_3 mg/l) was recorded at summer 13.66 mg/l and the lowest concentration was recorded at winter 5.40 mg/l. The highest percentage concentration of Nitrite (NO_2 mg/l) was recorded at summer 0.50 mg/l and the lowest concentration was recorded at Winter 0.15 mg/l, the results agree with [19, 13] and [7] who reported that heavy monogenean infestations are usually indicators of poor sanitation and deteriorating water quality (e.g., overcrowding, high ammonia or nitrite, organic pollution, or low oxygen). They can rapidly reproduce under such conditions. The doubling time for viviparous monogeneans can be as little as 24 hours. Reproductive rate is also controlled by temperature, which, although not variable in a tropical aquarium (which should have a narrow range of temperature).

Regarding the histopathological alteration due to infestation of *Benedenia acanthopagri* to the gills of investigated marine fish, the present study revealed, hyperplasia of the apical gill lamellae accompanied with necrosis and deformity. Gill filaments also showed congestion of branchial blood vessels of the lamellae, sloughing of the secondary gill lamellae with the presence of large numbers of gill flukes, present results nearly agree with the results obtained by [3 ,5, 7 and 11].

With regard to parasitic control in the present study, inspection of the parasitised fish (exposed to 250 ppm of commercial formalin, for one hour and examined after 24 hours indicated that fish became free of the parasite.

Mortality ceased and formalin treated fish appeared healthy. Formalin is known to control *Benedenia acanthopagri* in marine fish [5].

From the present study, it was concluded that *Benedenia acanthopagri* (Monogenea; Capsalidae) parasite recorded for the first time from the cage cultured *Sparidentex hasta, Acanthopagrus bifasciatus* and *Diplodus noct* fish in Eastern Province of Saudi Arabia on the Arabian Gulf. Infested fish was observed with respiratory distress; surfacing, gulping the atmospheric air with opening mouth showing abnormal swimming and flashing. Hemorrhages present at the base of fins, abdominal region and caudal fins. The highest prevalence and densities of infestation were recorded at summer season. The concentrations of organic matter (ammonia, Nitrite and Nitrate) were closely related and correlated to the prevalence of infestation of *Benedenia acanthopagri*. In case of outbreak with *Benedenia acanthopagri* in summer season, formalin is an effective therapeutic agent.

ACKNOWLEDGEMENT

Deepest gratitude and appreciation to *Mr. Jaber Al-Shehri*, Deputy Minister for Fisheries Affairs, Ministry of Agriculture, Kingdom of Saudi Arabia, for supporting this study. Sincerely thank for Mr. Ahmed Bin Saleh Al-Aiadh, General Director of Aquaculture Department, Ministry of Agriculture for his encouragement and guidance which emboldened to carry out and fulfillment of this work. Sincere thanks to Mr. Sami Al-Shabeb, Supervisor of Qatif Lab., for facilities he offered while conducting this work. Special thanks are to Mr. Abdulrahman Al-Zahrani, Supervisor of Jeddah Lab. for his cooperation.

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