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Mixed parasitic infestation in D*icentrarchus labrax, Dicentrarchus punctatus* and *Sparus aurata* in Suez Canal

Eissa IAM[1], Mona M Ismail[2], Mona M Abd El-Wahab[3], Amina A Dessouki[4], Heba I Abdel-Mawla[5], Rehab A Qorany[6]
[1, 2] Faculty of Veterinary Medicine, Department of Fish Diseases and Management, Suez Canal University, Ismailia, Egypt
[3] Department of Fish Health, Animal Health Research Institute, Ismailia Provincial Lab, Ismailia, Egypt
[4] Faculty of Veterinary Medicine, Department of pathology, Suez, Canal University, Ismailia, Egypt
[5, 6] Department of Fish Diseases, Animal Health Research Institute, Ismailia Provincial Lab, Ismailia, Egypt

Abstract
A total of 286 marine fishes of “*Dicentrarchus labrax*, *Dicentrarchus punctatus* and *Sparus aurata*” were collected from Suez Canal in Ismailia Province, Egypt. Abundant mixed parasitic infestation implicating Cymothoid isopods, Lernanthropus, Caligus, Praniza larvae of Gnathiids, Monogeneans and Digeneans was frequently seen. There were double and tripartite parasitism and a quaternary parasitism especially by crustaceans. The total prevalence of mixed parasitic infestation was 23.42% among examined fishes, and *D. labrax* is a prospect host for mixed parasitic infestations (56.52%). The histopathological picture was recorded as a completed detachment or degeneration of primary gill lamellae, severe congestion and hyperplasia. Results of this study provided the parasites involved in mixed infestation and their effect on the fish health.

Keywords: mixed parasitic infestation, fish parasites, *dicentrarchus labrax*, *dicentrarchus punctatus*, *Sparus aurata*, Egypt

Introduction

Egypt is one of the coastal countries that must take advantage from fish proteins where it is the main component of daily food of many people in Egypt (Jannat *et al*., 2010) [25]. GAFRD (2014) [21]. stated that, *D. labrax and S. aurata* are the main components of Egyptian mariculture. *Sparus aurata*, is an important demersal species, which is the major marine aquaculture fishes in the Mediterranean area, jointly with *Dicentrarchus labrax* (Crosetti *et al.*, 2014) [14]. Seabasses (*D. labrax* and *D. punctatus*) are common in the Mediterranean Sea and found in marine to slightly brackish water. (Morretti *et al.*, 1999) [34]. They have similar morphologies in their early periods, *D. labrax* grows to a better commercial length than *D. punctatus* (Bonhomme, 2002). Parasitic infestation is of major economic significance and could adversely affect the health of fishes which consumed as a food by human (Aneesh *et al.*, 2013) [4]. Marine parasites are of extensive ecological and economic importance in both wild and marine aquaculture fish (Zaid *et al.*, 2018) [61]. Fish parasites make up a remarkable part of marine variety of life, three to four parasite species can be observed in a single fish (Klimpel *et al.*, 2009) [29]. The mixed infestations jointly by four parasitic crustacean species on banded needle fish, *Strongylura leiura* recorded by (Aneesh *et al.*, 2013) [4]. There was double infestation of parasites in *Acanthopagrus bifasciatus*, in the gills with monogenea and copepods or monogenetic trematodes and isopods while in *Pomacanthus maculosus* had a double infestation in the intestine with trematodes and nematodes (Amna M. Hassan, 2018) [2]. Therefore, the present study was planned out to determine the influence of mixed infestation in *D. labrax, D. punctatus and S. aurata* in Suez Canal area in Ismailia Province and its effect on the host.

Material and Methods

Samples Collection: A total of 286 marine fishes of “92*Dicentrarchus labrax*, 101*Dicentrarchus punctatus* and 93*Sparus aurata*” were collected in different seasons from Suez Canal in Ismailia Province. These fish species were collected between September (2017) to the end of August (2018). The three fish species will be obtained by the aid of fishermen (wild) then will be transported immediately to the laboratory of Animal Health Research Institute alive in thick polyethylene bags and tanks containing 1/3 of its volume the water from the site of capture where the remaining volume was filled with air.

Table (1): Showing number of examined fish species:

Fish species	Common name	No. of exam. fish	Arabic name
D. labrax	Sea bass	92	Karous
D. punctatus	Spotted sea bass	101	Nokt
S. aurata	Sea bream	93	Denees
Total		286	

A. **Identification of marine fish species:** It was adopted according to (Fischer and Bianchi, 1984) [20].
B. **Clinical picture:** Clinical examination was made externally and internally on the live or freshly dead fish for detection of any clinical abnormalities according to (Conroy and Hermann, 1981) [11].
C. **Parasitological examination:** Fish specimens were examined macroscopically and microscopically for external and internal parasites as soon as possible after they were sacrificed. The identification was performed, using a dissection microscope and a stereo microscope Leica-S6D, according to Pillai (1985) [42].

D. Preparation of permanent specimens for identification

1. **Crustacea:** The detected crustacea collected by dissecting needle and a fine brush then kept in a small vial, washed and cleaned by distilled water. They fixed in 3% formalin and preserved in equal amount of 70% alcohol – 5% glycerin in test tube and permanent amounts prepared by passage in descending grades of alcohol (70%, 50%, 30%), cleared in glycerin and mounted in glycerin- gelatin, according to (Lucky, 1977) [31].
2. **Monogenetic trematodes:** The detected worms (separated or within gill tissue) were fixed in formalin 3% then a drop of glycerin alcohol (1:4), dehydrated in ascending grades of ethyl alcohol (3, 50, 70, 80, 90, 100%), cleared with clove oil, then xylene to remove the oil (each step take 15-30 minutes) and mounted in Canada balsam then left to dry in horizontal position in hot air oven (Negm-Eldin and Saleh, 1995).
3. **Digenetic trematodes:** The collected digenetic trematodes were washed in distilled water and fixed by 5% formalin between two slides after relaxation and stained with Semichon`s acetocarmine, dehydrated in ascending grades of ethyl alcohol and mounted in Canada balsam according to the methods adapted by (Schmidt, 1992) [49].

E. Identification of the isolated parasites: Crustacean parasites were identified according to **(**Noga, 2010) [39], monogenetic trematodes were identified according to (Santos *et al.*, 2000) [48], (Jithendran *et al.*, 2005) [26]. and (Justine, 2009) and digenetic trematodes were identified according to (Yamaguti, 1934) [52]. and (Nahhas *et al*., 1998) [36].

F. Histopathological examination: Specimens for histopathological techniques were freshly taken from affected organs and tissues of naturally infected fishes. Specimens were trimmed and fixed in 10% phosphate buffered formalin. Then washing in tap water for a day, dehydrated in different concentrations of alcohol, and cleared in xylol then instill in paraffin wax, and cut into thin sections 5-micron thickness. Sections were stained by H&E stain and examined microscopically (Roberts, 2001) [46].

Results and Discussion

1. Clinical picture of naturally infested fishes

The clinical picture in the naturally infested fishes with crustaceans collected from Suez Canal area, showed moderate to severe pathognomonic clinical abnormalities as co-existence of bilateral different species of isopods (*Nerocila orbignyi* and *Nerocila bivittate*), there were opened mouth and degeneration of the gill filaments at the site of the attachment of isopods (fig. 1). This agreed with (Brusca, 1981) [9]. who reported that there were cases occur, as bilateral or multiple infestations of the gills with cymothoid species, in which the host fish is placed in physically stressful environments.

Co-existence of isopoda (*Nerocila bivittata*) and *Lernanthropus kroyeri* detected in the seabass and (*Levonica redmanii*) co-existed with *Lernanthropus kroyeri in the* spotted seabass (plate 1). Also, Existence of isopoda, *Lernanthropus kroyeri* and *Caligus sp.* at the same *D. labrax* and *D. punctatus* fish. This result agreed with (Aneesh *et al.*, 2014) [5]. who recorded the presence of cymothoid isopod (*Cymothoa frontalis), Lernanthropus tylosuri* and *Caligodes lacinatus* in the same belonid fish *Strongylura strongylura*. Cohabitation of *Lernanthropus kroyeri*, *Caligus sp.* and monogenetic trematode at the same fish (plate 2). This result in the same direction with (Engi Abd Al-Galill, 2016) [18]. who recorded mixed infestations of monogenean with crustacean in *Dicentrarchus labrax* and *Epinephelus aeneus* which collected from Mediterranean Sea in Port Said governorate. In *D. punctatus*, *Caligus minimus* detected in the buccal cavity (dorsal palate of the jaw). Whereas, in *S. aurata* were seen at the base of pectoral and anal fins and detection of petechial haemorrhage on the skin, erosions, loss of some scales. This parallel to Venmathi (2009) [55]. and Noor El-Deen *et al.* (2013) [44]. and disagreed with Akif and Kayis (2015) who reported that *C. minimus* isolated from *D. labrax* found in the mouth and mainly on the tongue but not found on the skin and fins.

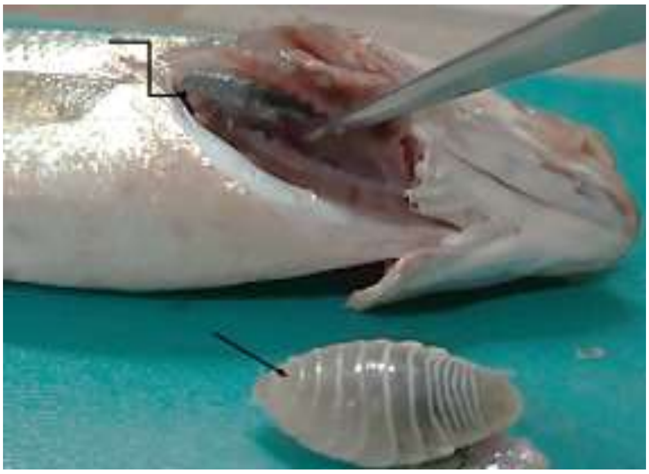

Fig 1: *D. labrax* showing *Nerocila orbignyi* (arrow) and *Nerocila bivittate* (elbow arrow).

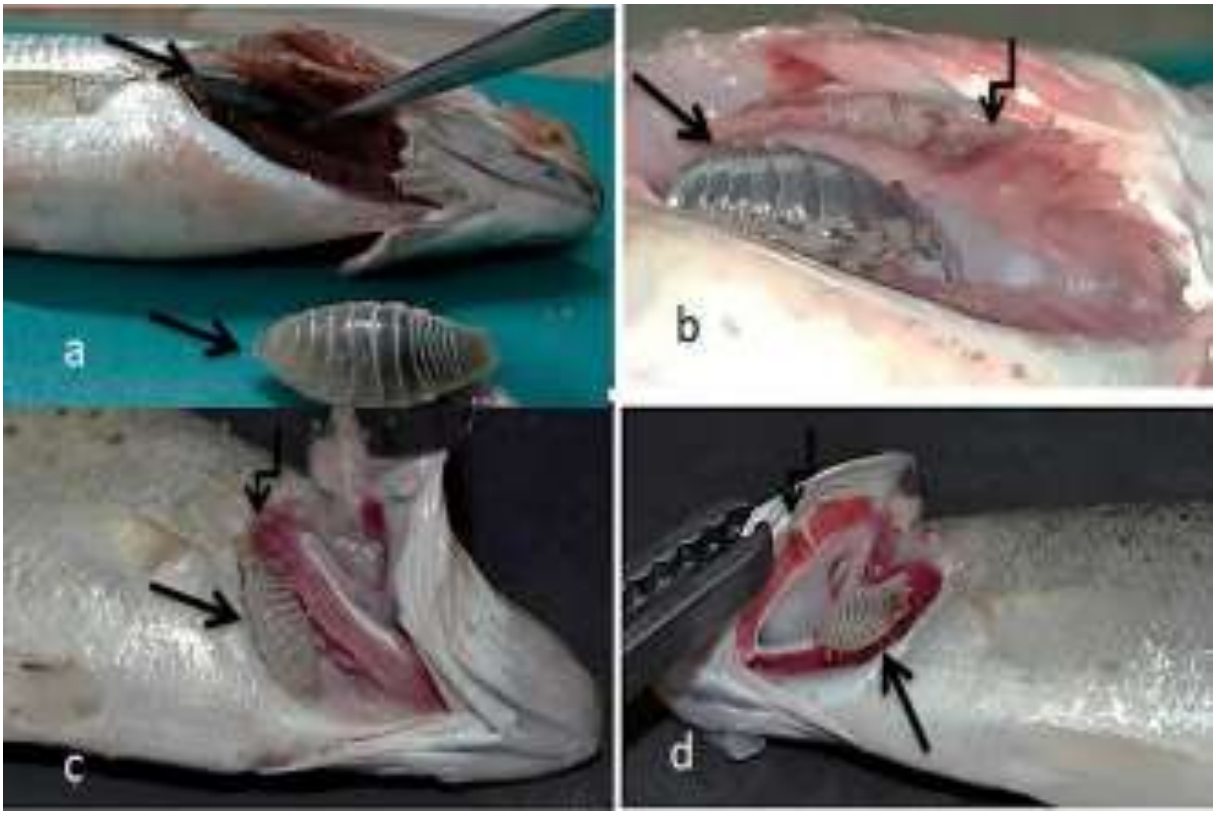

Fig 2: (a) and (b) *D. labrax* revealing isopods (arrow) and *Lernanthropus kroyeri* (elbow arrow). (c) and (d) *D. punctatus* showing isopods (arrow) and *Lernanthropus kroyeri* (elbow arrow).

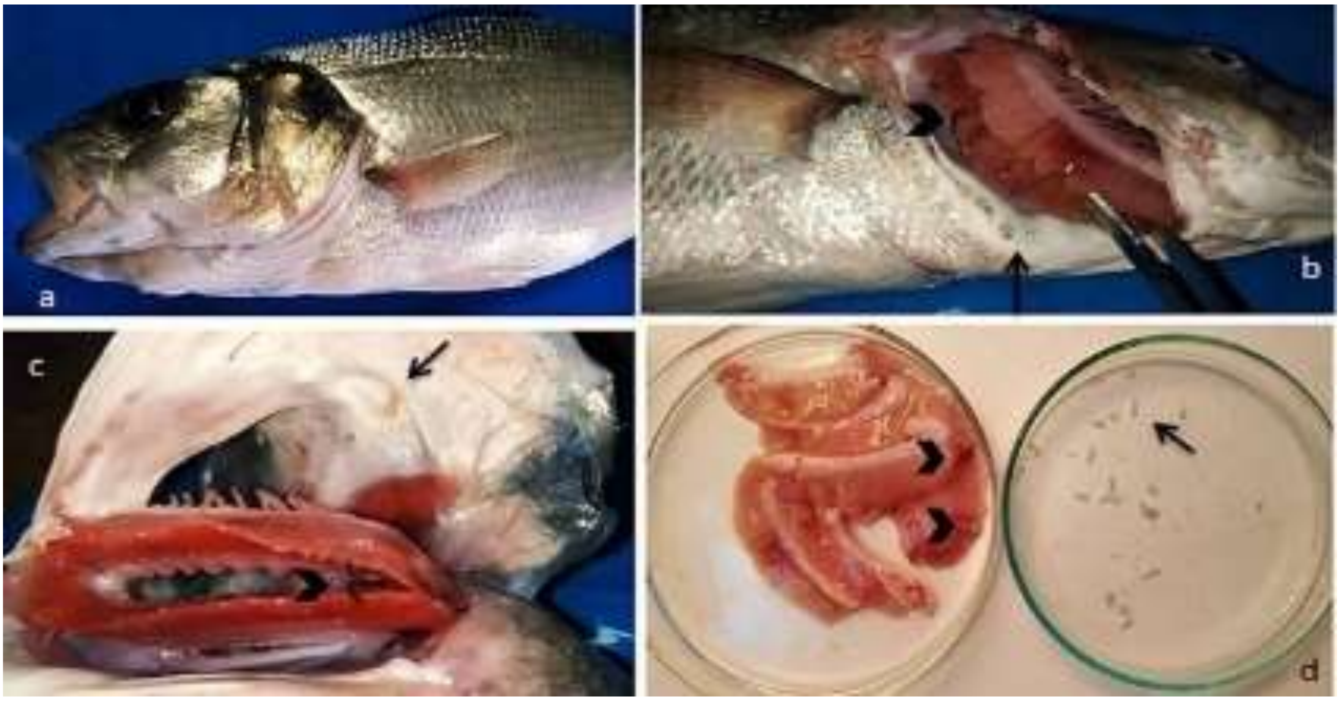

Fig 3: *D. labrax* showing a- opened mouth. b- *Lernanthropus kroyeri* (arrow head) and *Caligus sp.*(arrow). c- *Lernanthropus kroyeri* (arrow head) and *Caligus sp.*(arrow). d- Petri dish: *Lernanthropus kroyeri* (arrow head) in between gill filaments and *Caligus sp.* (arrow).

Moreover, digenean parasites detected in the branchial cavity co-habituated with *Lernanthropus kroyeri* and *Caligus sp.* at the same infested *D. labrax*. The presence of digenean trematodes in the present study; in the branchial cavity, on the inner surface of the operculum and beside the tips of gill filaments of *D.labrax* and *D.punctatus* respectively, this result supported by Rohde *et al.* (1980) [40]. who found the adult digeneans firmly attached with their ventral suckers to the spines of the gill arches or rackers, also, (Cribb, 2005) [13]**.** and **(**Klimpel *et al.*, 2019) [29]. recorded that digeneans are primarily parasites of the gut in fishes but they also occur under the scales, on the gills, in the swim bladder, body cavity, urinary bladder, gall bladder, flesh, ovary and circulatory system. Also, detection of white lesion and yellowish colouration on the gill filaments, white and dark spots on the liver and congested spleen and heart which was examined under microscope and revealed unidentified metacercarial cysts.

2. Parasitological examination

Fish samples were examined macroscopically and microscopically:

A-Crustaceans:1-Adult isopods: **a- *Nerocila bivittate:*** isolated from gills of *D. labrax* and *S. aurata* **(Plate 3).** **b- *Nerocila orbignyi*:** isolated from gills of *D. labrex*. **c- *Renocila thresherorum*:** isolated from gills of *D. labrex* and *D. punctatus*. **d- *Levonica redmanii*:** isolated from gills of *D. labrax* and *D. punctatus* **(Plate 4).** 2- **Praniza larva of Gnathiids:** isolated from *D. labrax* **(Plate 5).** **3- *Lernanthropus Kroyeri:*** isolated from *D. labrex* and *D. punctatus* **(Fig. 2).** **5- *Caligus minimus*:** isolated from *D. labrex, D. punctatus and S. aurata*. **(Plate 6).**	**4- *Caligus longipedis*:** isolated from *D. labrex* **(Plate 7).** B- **Monogeneans**: 1-***Diplectanum aequans***: isolated from *D. labrex*. **C- Digeneans:** **1-Branchial cavity Digeneans:** ***a- Tangenarchopsis chinensis***: isolated from the branchial cavity of *D. labrex* **(Fig. 3).** **2- Internal Organs Digeneans:** a- ***Tangenarchopsis chinensis*:** Whole digenean isolated from the stomach of *D. labrax* **(Fig. 3).** b-***Lecithochirium angustiovum*:** Whole digenean isolated from the intestine of *D. labrax* **(Fig. 4).**	**B.2. Metacercarial cyst:** **1- Unidentified excysted metacercarial cyst:** isolated from the skin of lower jaw of *D. labrax*. **2- Unidentified encysted metacercarial cyst:** isolated from liver and heart of *S. aurata*.

Fig 4: a- Dorsal view of *Nerocila bivittata*. b- Ventral view of *Nerocila bivittata*.

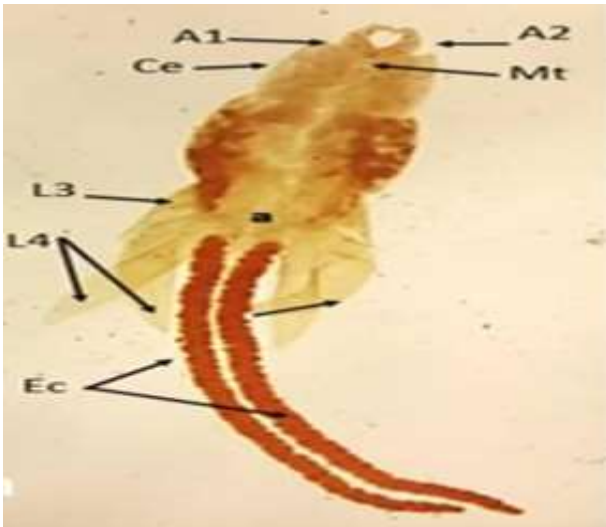

Fig 5: *Lernanthropus Kroyeri*: isolated from *D. labrax and D. punctatus*. a: whole female copepode. b: Anterior part of female copepod. A1: first antenna; A2: second antenna; Mt: mouth tube; M: maxilliped; M2: second maxilla; Ce: cephalothorax; L1; 1st thoracic leg; L2: 2nd leg; L3: 3rd leg; L4: 4th leg; a: abdomen; SS: spermatophore sac; Up: Uropods; Ec: egg sac.

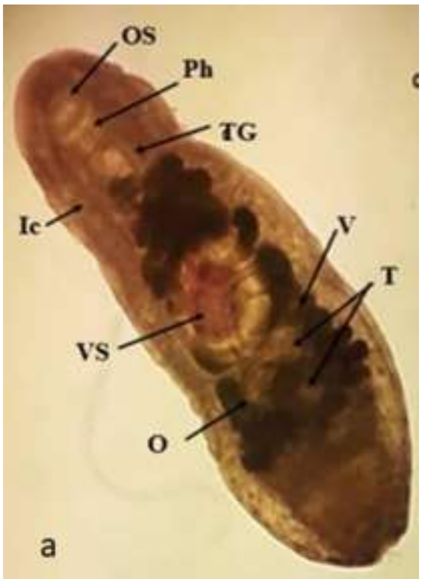

Fig 6: *Tangenarchopsis chinensis:* stained digenea with Acetocarmine. b: unstained fluke. OS: Oral sucker, Ph: Pharynx, Vs: Ventral sucker, V: Vitellaria, O: Ovary, T: testes TG: Terminal genitalia, IC: intestinal caeca.

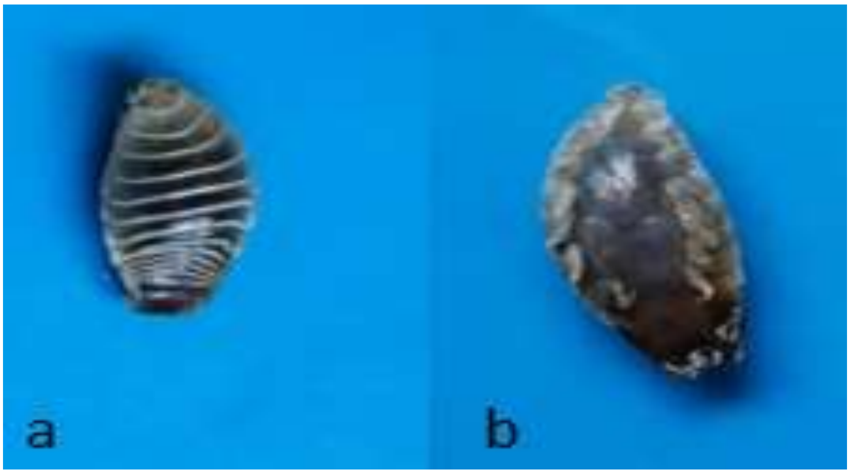

Fig 7: a- Dorsal view of *Levonica redmanii*. b- Ventral view of *Levonica redmanii*.

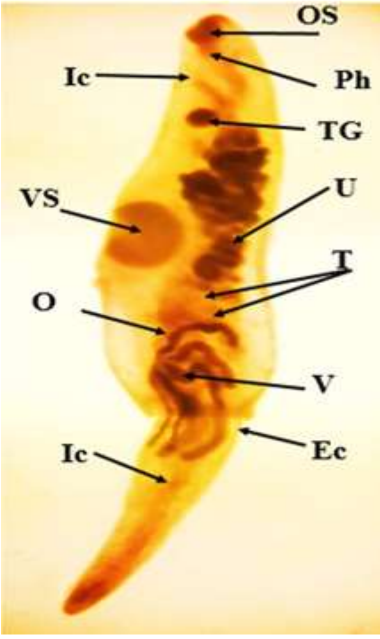

Fig 8: *Lecithochirium angustiivum:* OS: Oral sucker, Ph: Pharynx, Vs: Ventral sucker, U: uterus, V: Vitellaria, O: Ovary, T: testes TG: Terminal genitalia, IC: intestinal caeca, EC: evaginated ecsoma

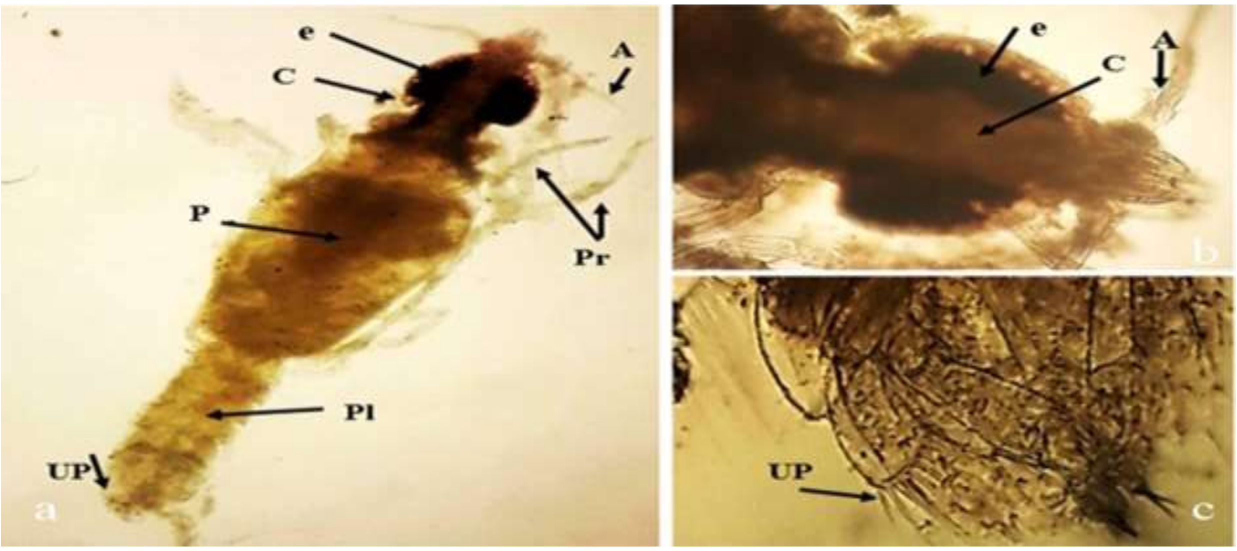

Fig 9: Paraniza larva: a: Whole Paraniza larva. b: Anterior part of Gnathid isopod showing cephalon and pereon c: posterior part showing Telson and uropods with fringing setae. A: antenna; e: eye; C: cephalon; P: Pilion; Pl: pleotelson; Pr: periopodes; Up: uropods.

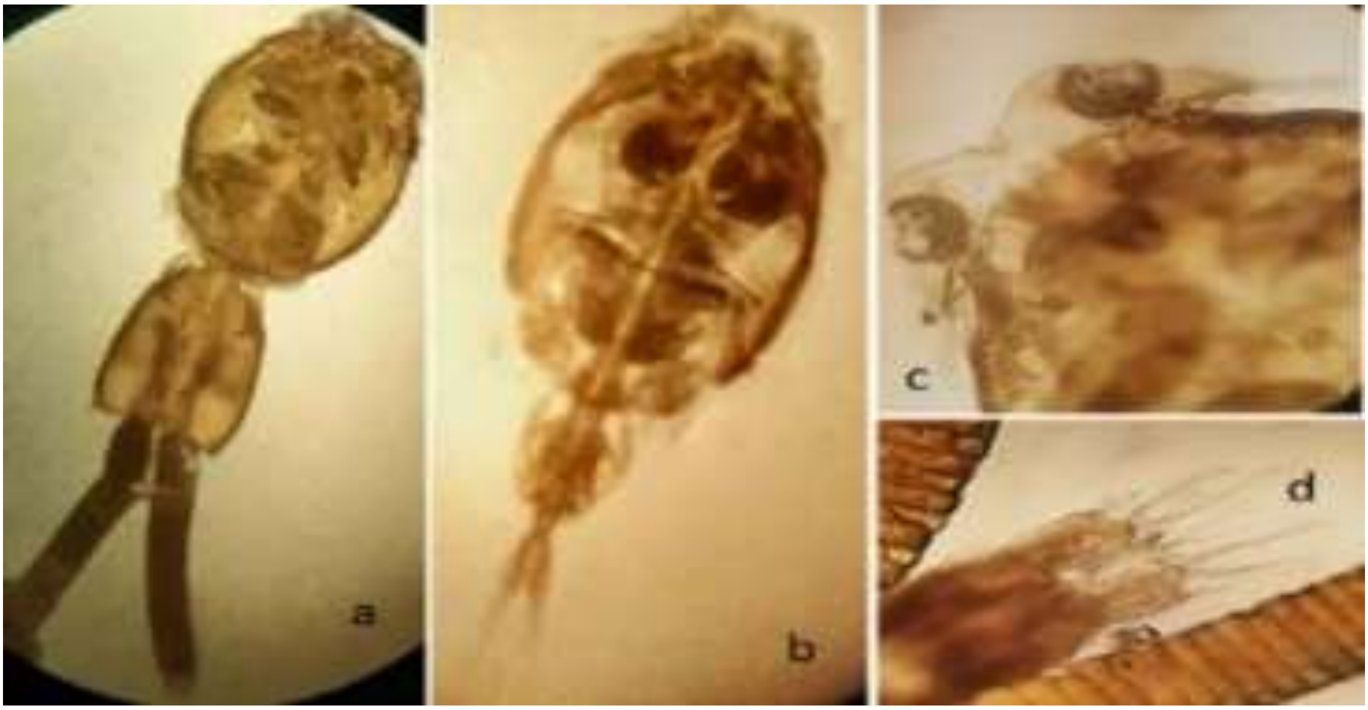

Fig 10: *Caligus minimus*: isolated from *D. labrex* and *D. punctatus.* a: whole female copepode. b: whole Male copepod. c: Cephalothorax of *Caligus minimus*. d: posterior end of male showing caudal rami.

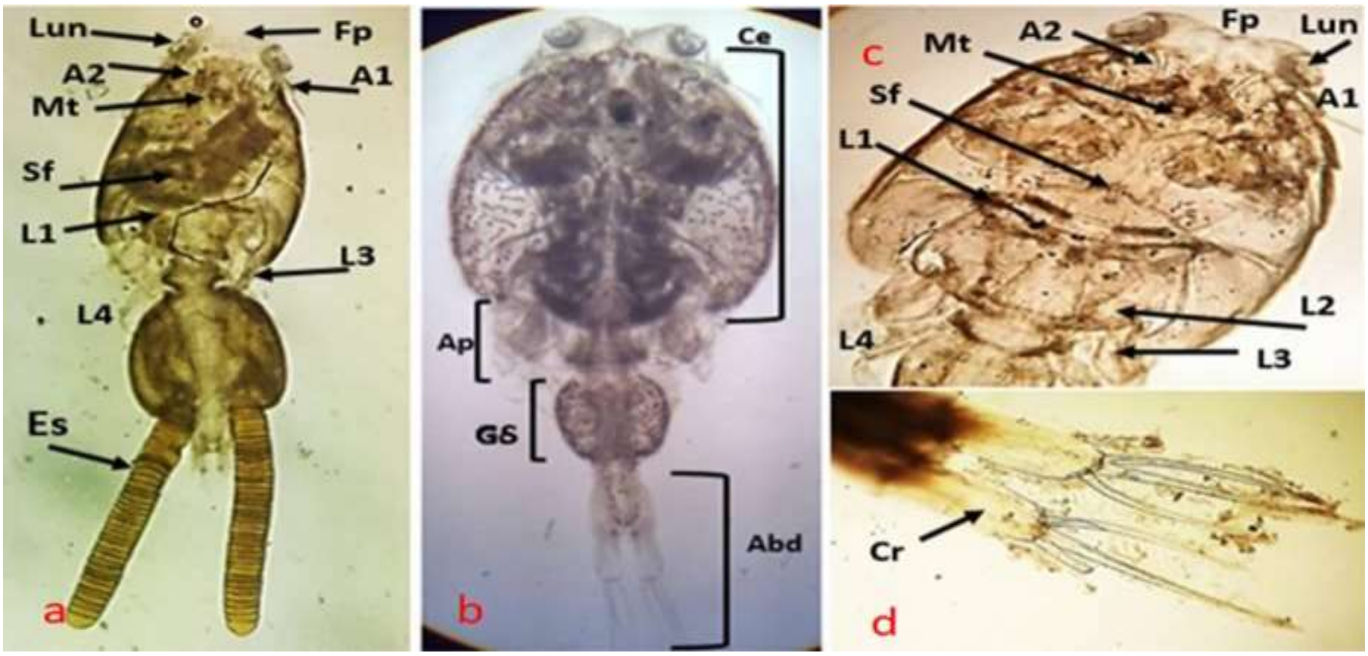

Fig 11: *Caligus longipedis* isolated from *D. labrex*. a: Whole female copepode. b: Whole male copepod. c: Cephalothorax of *Caligus longipedis*. d: posterior end of male showing caudal rami. Lun: lunule; Fp: Frontal plate; A1: first antenna; A2: second antenna; Mt: mouth tube; M: maxilliped; M2: second maxilla; Sf;: Sternal furca; L1:1st thoracic leg; L2: 2nd leg; L3: 3rd leg; L4: 4th leg; Es: egg sac; Ce: cephalothorax; Ap: Apron; GS: genital segment; abd: abdomen; Cr: Caudal rami.

3. Prevalence of mixed infestation among examined and infested fishes:

Table (2): Prevalence of mixed infestation among examined fishes:

Fish Type	Mixed Infestation										
	I.& another I. sp.	I. & L.	I. &C.	I, L. &C.	P., L., C.& D.	L. &C.	L., C.& M.	L.& D.	D.&C.	D.&C.& L.	Total
D. labrax n (92)	4 4.34%	7 7.6%	2 2.17%	1 1.08%	1 1.08%	23 25%	2 2.17%	5 5.43%	4 4.34%	3 3.26%	52 56.52%
D. punctatus n (101)	0	6 5.94%	0	0	0	2 1.98%	0	5 4.95%	0	0	13 12.87%
S. aurata n (93)	0	0	0	0	0	0	0	0	2 2.15%	0	2 2.15%
Total n (286)	4 1.39%	13 4.54%	2 0.69%	1 0.34%	1 0.34%	25 8.74%	2 0.69%	10 3.49%	6 2.09%	3 1.04%	67 23.42%

n: (number of examined fish). I.: Isopoda, L.: Lernanthropus, C.: Caligus, P.: Praniza larvae, M.: Monogenean, D.: Digenean.

Table (3): Prevalence of mixed infestation among infested fishes:

Fish Type	Mixed Infestation										
	I. & another I. sp.	I. & L.	I. &C.	I, L. &C.	P., L., C.& D.	L. &C.	L., C.& M.	L.& D.	D.&C.	D.&C.& L.	Total
D. labrax n(66)	4 6.06%	7 10.61%	2 3.03%	1 1.52%	1 1.52%	23 34.85%	2 3.03%	5 7.58%	4 6.06%	3 4.55%	52 78.79%
D. punctatus n(68)	0	6 8.82%	0	0	0	2 2.94%	0	5 7.35%	0	0	13 19.12%
S. aurata n(9)	0	0	0	0	0	0	0	0	2 22.22%	0	2 22.22%
Total n(143)	4 2.8%	13 9.09%	2 1.4%	1 0.7%	1 0.7%	25 17.48%	2 1.4%	10 6.99%	6 4.2%	3 2.1%	67 46.85%

n: (number of infested fish), I.: Isopoda, L.: Lernanthropus, C.: Caligus, P.: Praniza larvae, M.: Monogenean, D.: Digenean.

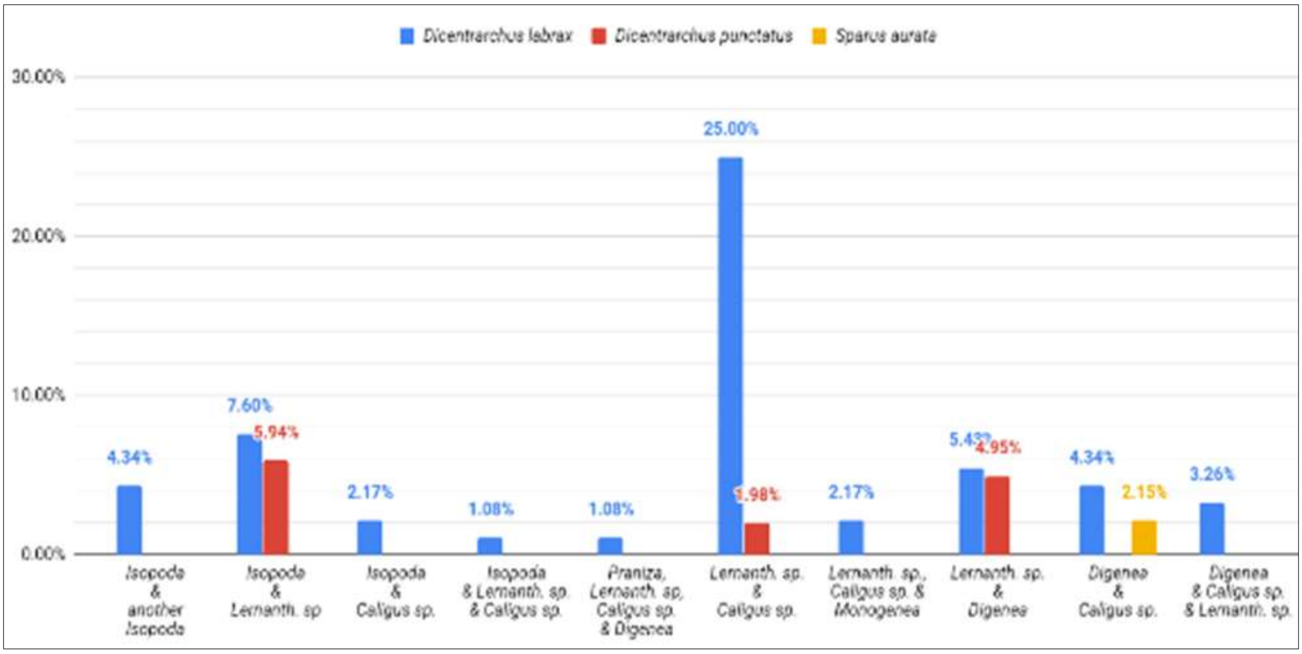

Fig 12: Prevalence of mixed infestation among examined fishes.

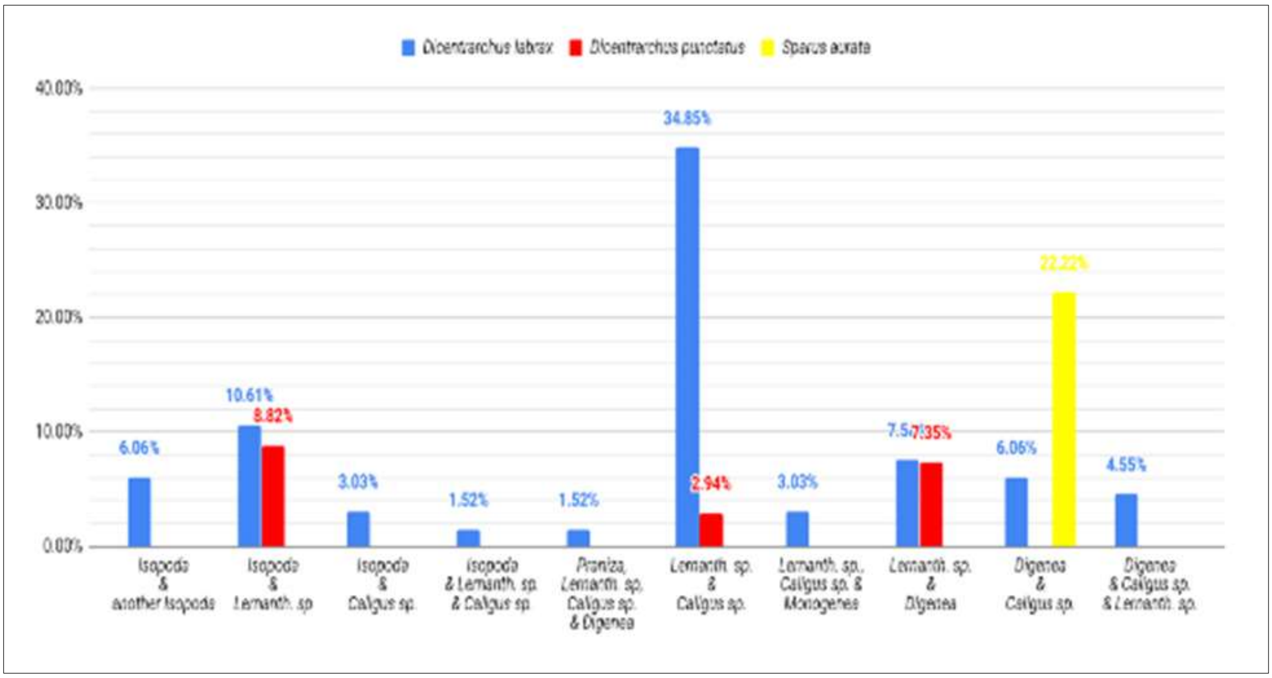

Fig 13: Prevalence of mixed infestation among infested fishes.

Convergently 0.34% of the examined (1 out of 286) fishes showed the presence of quadruple parasitism, by (Praniza larvae, Lernanthropus, Caligus and Digeneans) during autumn season (Tables 2). While, there was triple parasitism at the same examined fish by any of the three parasites in three different combinations: 1.08% (Isopoda, Lernanthropus and Caligus), 0.69% (Lernanthropus, Caligus and Monogenea) and 1.04% (Digenea, Caligus and Lernanthropus).However, three examined fishes revealed double parasitic infestation in six different combinations as 1.39% (Isopoda & another Isopoda species), 4.54% (Isopoda & Lernanthropus), 0.69% (Isopoda & Caligus), 8.74% (Lernanthropus & Caligus), 3.49% (Lernanthropus & Digenea), 2.09% (Digenea & Caligus). The single parasitic infestation was 26.57% (76 infested fishes out of 286). The present study reports the frequent occurrence of double and triple parasitism and quadruple parasitism, especially by the species of parasitic crustaceans including (isopods, copepods), monogenetic trematodes and digenetic trematodes on the host fish *D. labrax* and in some cases in *D. punctatus* and *S. aurata*; this result was supported by (Klimpel *et al.*, 2009) [29]. and (Aneesh *et al.*, 2014) [5]. The total prevalence of mixed infestations among examined fishes was (23.42%), this result was higher than that obtained by Engi Abd Al-Galill (2016) [18]. as it was (4%), while this result lower than that obtained by (Aneesh *et al.*, 2014) [5]. as it was (95%). This differences in prevalence and abundance of the infection varied with location where fishes collected, the climate temperature, crowding, habitat and the type of fishes. In spite of few reports on double parasitism, our knowledge on the occurrence of mixed parasitism especially crustaceans is very poor (Aneesh *et al.*, 2014) [5].

3. Histopathological examination of naturally infested fishes

Parasitic crustaceans have passive influences on the host fishes by the attachment of the parasites and their feeding activities which leading to emaciated and diseased fishes (Bharadhirajan *et al.*, 2013) [6]. Different pathological lesions were observed in gill filaments of *D. labrax n*aturally infested with isopoda in case of one parasite or more. Mild to moderate degeneration was observed in some cases while in other severe cases, the primary gill lamellae were completely detached or degenerated. Severe hyperplasia and adhesion of secondary lamellae was seen along with congestion and mild to moderate mononuclear cell infiltrations. Mild hemorrhages were seen in some infested cases (Fig. 5). This result similar with Nadia Ali and Aboyadak (2018) and nearly agreed with (Nisreen *et al.*, 2016) [38], Shaheen *et al.* (2017) [50], Helal and Yousef (2018) [23].

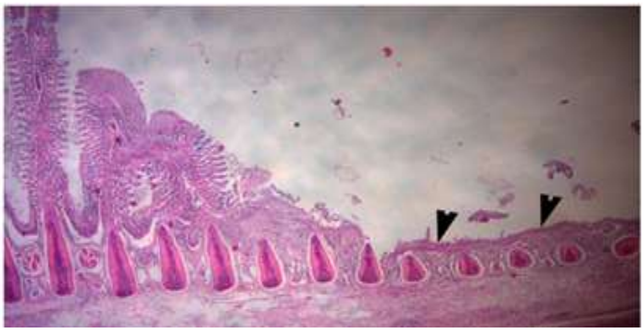

Fig 14: (a) Gills of *D. labrax* infested by Isopoda showing severe atrophy of primary and secondary gill lamellae (arrow heads), severe adhesion, hyperplasia of secondary gill lamellae (arrows). H&E. (a) X 100.

On the other hand, the gills of *D. labrax* and *D. punctatus* naturally infested with *Lernanthropus kroyeri* showed severe erosion, desquamation and necrosis of the secondary lamellae. Atrophy at the site of *Lernanthropus* attached were also seen. Adhesion due to hyperplasia of secondary lamellae with severe leukocytic infiltrations mainly with eosinophils and lymphocytes (Fig. 6). this result in accordance to Toksen (2007) [53], Jithendran *et al.* (2008) [27], Yardimci and Pekmezci (2012) [60].

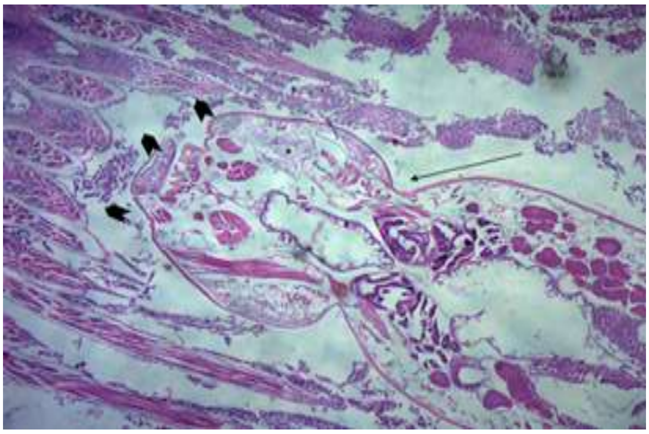

Fig 15: Gills of *D. labrax* infested with *Lernanthropus kroyeri* (arrows) showing sections of the parasite *Lernanthropus kroyeri* that causing severe atrophy of the gill lamellae (arrow heads) along with hyperplasia of gill epithelial cells admixed with inflammatory cells. (asterisk). H&E. X 100.

In particular, the gills of the *D. labrax* fish infested with mixed infestation of isopoda, *Lernanthropus kroyeri* and *Caligus sp.* revealed massive degeneration of gill lamellae, necrosis and atrophy. Moreover, there were severe congestion and severe hyperplasia. In addition, there were adhesions of some lamellae along with leukocytic infiltrations (Plate 8). This result agreed with Manera and Dezfuli (2003) [32]. who reported caligids and lernanthropids can often cause erosion, desquamation and necrosis of the gill filaments and, in cases of heavy infection, this may lead to asphyxiation, anemia, and secondary bacteria (Toksen *et al.*, 2006) [52].

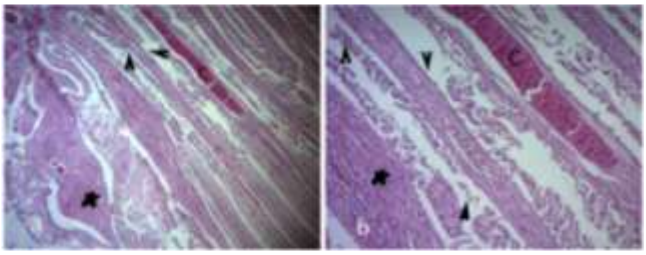

Fig 16: Gills of the *D. labrax* fish infested with mixed infestation of isopoda, *Lernanthropus kroyeri* and *Caligus sp.* showing massive degeneration of gill lamellae necrosis and atrophy (arrow heads) severe congestion (**C**) and severe hyperplasia, adhesions of some lamellae along with leukocytic infiltrations (asterisk). H&E. (a) X 100, (b) X 200.

In the intestine of infested *D. labrax* with digenetic trematode species revealed, cross section of parasitic worms in serosa and submucosa along with leukocytic infiltrations mainly eosinophils and enlargement, thickening and inflammation of its walls. This result nearly similar to the result obtained by

Heba Abdel-Mawla and Nesreen Yousef (2019) [23].

Conclusion

In conclusion, amongst the three examined fishes, *D. labrax*, is a potential host for mixed parasitic infestations. The repeated occurrence of double, triple parasitism and quadruple parasitic infestation found on the *D. labrax* indicated that it is not an incidental episode. The histopathological examination revealed that the fish health affected with the mixed infestation. Based on the findings of the present study, more investigations are highly recommended.

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