

## FOOD AND FEEDING HABITS OF MARINE EDIBLE FISH *POMADASYs MACULATUM* (BLOCH, 1797) AND DESCRIPTION OF A NEW NEMATODE *DUJARDINASCARIS MACULATUM* N.SP.

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### ABSTRACT

*Pomadasys maculatum* (Bloch) is a marine fish of economic importance, were collected ( February 2006 to July 2007) from fresh landing of Karachi coast, Pakistan. Seventy five specimens of *P. maculatum* were subjected for analysis of food and feeding habits showed no significant seasonal variation in food contents. During the analysis of food items, nematodes were also recovered from the stomach which when compared with literature indicates the presence of a new nematode species that cause infection in the intestine. The infection in intestine may be due to the alkaline medium of the intestine. Identification of nematodes was made using conventional techniques and Scanning Electron Microscopy. Nematodes corresponded to a new species of the genus *Dujardinascaris* (Baylis, 1947). The new species along with some already known nematodes were detected in intestine of 5 fish specimens. Tissue damage by this nematode in intestine was also observed.

**Key words:** Food and feeding habits; marine fish *Pomadasys maculatum*; *Dujardinascaris maculatum*; Intestine; Nematode; new species; Tissue damage.

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### INTRODUCTION

Food and feeding habits are indispensable part of biological and taxonomic studies because it is essential for an organism's growth, development and reproduction. All this is dependent on energy that enters an organism in the form of food (Wootton, 1992). The analysis of stomach contents of fish could provide information about the niche of a particular fish in its ecosystem and this has become a standard practice in fish ecology works (Hyslope, 1980). Fishes are the predator-prey pyramid within fresh water as well as in marine water and therefore tend to be infected by a considerable range of parasites, which occur in large number. Parasite and disease reduce fish production by affecting the normal physiology of fish (Kabata, 1985).

In aquaculture situations, fish become infected with nematodes if they are fed live foods containing infective life stages or if they are raised in culture setting that promote the growth of other animals that carry the infective stages of the nematode (vector or paratenic host) or allow nematodes to complete their life cycle (intermediate hosts). Some nematodes can be transmitted directly from fish to fish. Adult nematodes are typically found in fish digestive tracts. However, depending upon the species of nematode and the species of infected fish adult and other life stages of nematodes can be found in almost any part of the fish (Roy & Young, 2006).

Literature survey reveals a lot of work done on food and feeding habits of *Pomadasys maculatum* in Pakistan and other part of the world. Histopathology of intestine of some fish species of Karachi coast has been carried out by (Bilqees and Fatima, 1995; Bilqees *et al.*, 1998; Khatoun & Bilqees, 1999; Rizwana *et al.*, 1999). But no relevant work in relation with food and feeding habits of *Pomadasys maculatum* from Karachi coast with recovery of a new nematode species and infestation in fish intestine has been reported yet.

### MATERIALS AND METHODS

#### Study Area:

Karachi is one of the major fish harbor of Pakistan, handles about 90% of fish and sea food catch in Pakistan (Pakissan.com. 2001-2007).

#### Collection of Fish specimens for dietary habits:

A total of 75 fish specimens of *Pomadasys maculatum* (Bloch) were collected during February 2006 to July 2007 from commercial landing at Karachi fish harbor, West Wharf, early in the morning after landing of fish trawlers and preserved whole in 10% formalin for later examination in Laboratory of Jinnah University for women. The fishes were identified according to Qureshi, 1955; Hoda, 1985 and Majid *et al.*, 1992. The collected fish

specimens were measured for total length which ranged between 16-50cms . After dissection of the fishes, the stomachs were removed, weighed and preserved in 8 % formalin solution. The number of the empty stomachs was recorded. The stomachs containing food items, either ½ full, ¾ or gorged, were examined under a dissecting microscope. An attempt was made to identify the food items to species level. But the level of discrimination depended on the completeness of the food items. Polychaetes, small crustaceans and some fishes were particularly difficult to identify as they were rapidly digested and were rarely in good conditions. For planktons, the microscopic preparations per filtrate were observed under the microscope for taxonomic identification.

The food items were analyzed by percentage of frequency of occurrence method (F%), the number of stomachs containing each food items is expressed as percentage of all non-empty and empty stomachs as described by Dunn, 1954.

#### **Examination of specimens for nematode parasites:**

A total of 62 fish specimens out of 75 collected specimens were subjected to parasitological examination. The body cavity of fish was opened, and the gut and liver were removed by cuts in the region of the anus and the division between esophagus and anterior stomach. The gut was then placed in a large Petri dish filled with distilled water. The section of the gut was opened with a longitudinal cut, and the whole inner surface lightly scraped to remove the parasites with mucus, if any. The nematode recovered were washed in physiological saline and preserved in 70% ethanol. For light microscopy the nematodes were cleared in glycerin. Diagrams were prepared with a camera Lucida E 200 Nikon Drawing Tube. Measurements are given length by width in millimeters. Specimens for scanning electron microscopy were fixed in cold 4% glutaraldehyde in buffer (pH=7.2) and kept in it for 24 hours, then dehydrated through a graded series of alcohols, infiltrated with amyl acetate, after critical drying mounted on stubs, coated with gold and photographs were taken with the help of SEM. Joel Japan JSM 6380A at an accelerating voltage of 15KV at Karachi University, central laboratory. The SEM measurements are in micrometers.

#### **Procedure for histopathology**

During the present study, histopathology of infected intestine of *Pomadasys maculatum* (bloch.) was carried out. For histological studies of intestine, small pieces of infected intestine were fixed in 10% formalin for 24 hours, washed several times with water, dehydrated in graded series of alcohols. Cleared in Cedar wood oil and xylene. Wax blocks were made in cavity blocks by usual method. Thick sections (6-8 microns) were cut with a rotatory microtome. After removing the wax by xylene, hydration was carried out. Sections were stained with haematoxylin and eosin, dehydrated, cleared in clove oil and xylene and mounted permanently in Canada balsam. Photographs were taken with a photomicroscope Nikon (Optiphot-2) using Fuji color film.

### **RESULTS**

#### **Dietary habits of *Pomadasys maculatum* (Bloch, 1797)**

The number of stomachs in which food items occurred was expressed as a percentage of total number of stomachs. The composition of food items and frequency percentage occurrence (F %) are categorized in Table (1-2). The food category mainly comprises of crustaceans, molluscs, teleosts, detritus, Planktons and polychaetes. Nematodes among the helminths category were also observed.

#### **NEMATODE PARASITE**

A new nematode species was identified and compared with the available literature (Yamaguti, 1961; Sood, 1989; Akhtar & Bilqees, 2006; Akhtar & Bilqees, 2008 and Akhtar & Bilqees, 2009) and through Research service of British Library, provided by Nadja Noel and Rupert Lee .

Altogether, 8 new nematode specimens including 3 males and 5 females were identified from 62 fish specimen. These nematodes were collected from the intestine. Nematodes occurred generally in those months in which the fish stomachs were gorged, full or 1/2 full. This suggests the correlation between the dietary habits and occurrence of nematode parasites.

#### **Taxonomic summary:**

Order: Ascarididea

Family: Heterocheilidae

Subfamily: Filocapsularinae

Genus: *Dujardinascaris* Baylis, 1947

(Syn. Dujardinie Gedoelst, 1916)

Type host: *Pomadasys maculatum* (Bloch, 1797)  
 Site of infection: Intestine  
 Type locality: Fish harbor, Karachi coast Pakistan  
 Prevalence: 8.6% (5 fishes infected/ 62 examined)  
 No. of specimens: 3 male and 5 female nematode  
 Intensity: 2.5  
 Cat. No.: N.11 JUW. Holotype, male  
 N.12 JUW. Allotype, female

***Dujardinascaris maculatum***  
 (Figs. 1,A-D)

**Diagnosis:** Stout, long cylindrical, dark brown worms, tapering towards both extremities with pointed posterior end in male and conical posterior end with round tip in female. Mouth is bounded by three lips, with out dentigerous ridges. Cuticle is finely striated, these striations are prominent at the anterior end in male and at posterior end in female. Esophagus is entirely muscular, intestinal caecum well developed. Anal opening is not prominent in female.

**Table 1. Food contents of *Pomadasys maculatum* (Bloch, 1797).**

S.No	Food Contents	<i>Pomadasys maculatum</i> (Dhotar) n=75
1	Polychaetes	Neries
2	Crustaceans	Amphipods, Shrimps, ( <i>Penied</i> sp) Copepodes, Crabs ( <i>Acetes</i> sp)
3	Molluscs	<i>Loligo</i> sp.
4	Platyhelminthes	-
5	Nematyhelminthes	Nematodes ( <i>Dujadinascaris</i> sp.)
6	Teleosts	<i>Leiognathus</i> sp.
7	Planktons	-
8	Detritus	-
9	Miscellaneous	Compound eyes, legs, chelae and mantis of shrimps

**Male:** (3 specimens including the holotype) Stout, elongated, dark brown, body length 9.05—10.0 and maximum width 6.07—7.02. The lips are 0.16—0.18 x 0.35—0.45 in size. Esophagus is 0.5—0.8 in length and 0.15—0.25 in width. Intestinal caecum is 0.40-0-0.49. Caudal papillae including 13 pairs precloacal, 2 pairs postcloacal and 1 adanal are present. Spicules are two, unequal 0.91—0.95 and 0.5—0.6 in length. Gubernaculum absent. excretory pore is 0.35 from anterior end. Tail is pointed, 0.06—0.07 in length, excretory pore is 0.43 from the anterior end.

**Female:** (5 female including allotype). Body is 13—19 in length and 0.42—0.50 in its breadth. Cuticular striations are very prominent in posterior half of the body, just above the tail. Lips are 0.09—0.15 x 0.18—0.30 in size. Oesophagus measures 0.55—0.64 in length and 0.20—0.22 in width. Nerve ring is at a distance of 0.32-0.36 and excretory pore is 0.45-0.52 away from the anterior end. Intestinal caecum is 0.12-0.15 in length. Vulva is pre equatorial at a distance of 0.05-0.06 from posterior extremity, opening into a muscular atrium. Vagina is muscular and slender. Excretory pore at a distance of 0.41-0.52 from anterior end. Tail is bluntly conical.

**DISCUSSION**

Present analysis of food categories and food items indicate that *P. maculatum* is a carnivorous fish. According to the present analysis of *Pomadasys maculatum* (Bloch) the crustacean (71.42%) and miscellaneous items (71.42%) ranked on the top among the other food categories on the basis of percentage of frequency of occurrence (2-3).

Principal food items (F%) in crustacean category were shrimps (71.4%), *Acetes* sp.,(71.4%) and copepods sp.(45.71). *Sepia* sp.,(35.71%) was dominating in molluscs category occupying the 2<sup>nd</sup> rank. Among the teleost group *Johnius* sp.,(35.71%), *Sardine* sp.,(14.28%) and *Leiognathus* sp.,(7.14%) were observed. Nematodes (7.14%) were dominating among helminthes (Table 2), but these were not the part of food contents.

**Table 2. Frequency of Occurrence in relation to total number of Stomachs analyzed (F, F%) of *Pomadasys maculatum* (Dhotar) N=75(Empty= 6.66%).**

Organisms identified	F	F%
<b>Polychaetes</b>		
Neries	5	7.14
<b>Crustaceans</b>		
Shrimps	50	71.4
Copepods	32	45.71
Amphipods	40	57.14
Crabs( <i>Acetes</i> sp)	50	71.4
<b>Molluscs</b>		
<i>Solen</i> sp.	6	8.57
<i>Sepia</i> sp.	25	35.71
<i>Pholas</i> sp.	12	17.14
<i>Loligo</i> sp.	20	28.57
<b>Helminthes</b>		
<b>Platyhelminthes</b>	-	-
<b>Nematyhelminthes</b>	25	35.71
Nematode ( <i>Dujrdinascaris</i> sp.)	5	7.14
<b>Teleosts</b>		
<i>Johnius</i> sp	25	35.71
<i>Sardine</i> sp.	10	14.28
<i>Leiognathus</i> sp.	5	7.14
<b>Planktons</b>	-	-
<b>Detritus</b>	-	-
<b>Miscellaneous</b> 2		
Compound eye	24	34.28
Legs	30	42.85
Chelae	50	71.42
Mantis of shrimps	26	37.14
Crustacean	28	40

An attempt was made to observe seasonal variation in dominancy of food items indicating gorged stomach of *P. maculatum* during the months of February, March and April. Molluscs and crustaceans were dominant in April, but were frequently seen throughout the year, Polychaetes were absent in August, September, October and January, but were present in March, April and May. *Loligo* sp. were seen in month of February and March. The results of the present study were compared with food and feeding habits of other Pakistani fishes. The observations agreed with the work of Khan & Huda, 1993; Ajazuddin, 2000;2001; Safi & Khan, 2005 and Imtiaz & Khan, 2005.

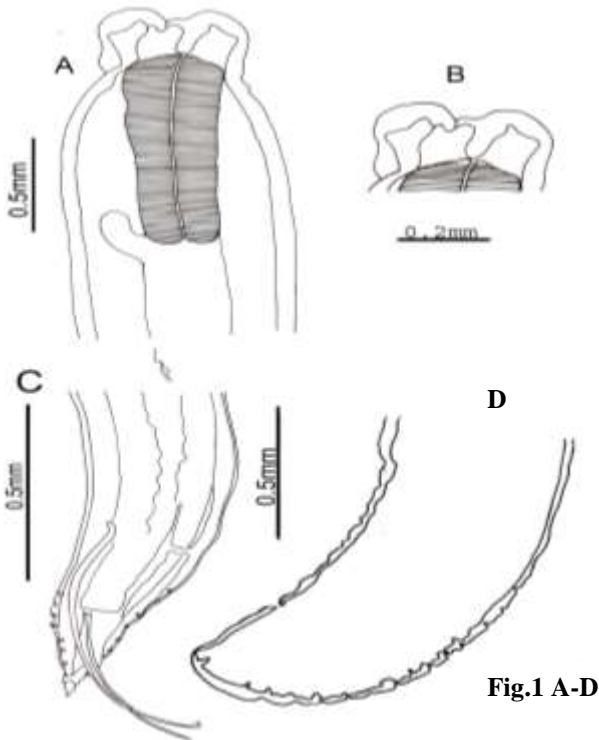


Fig.1 A-D

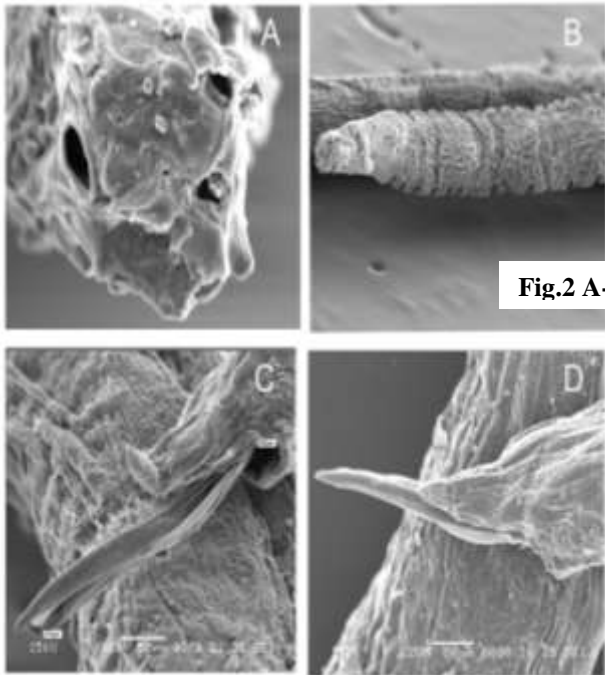


Fig.2 A-D

Fig. 1 (A-D) *Dujardinascaris maculatum* sp.n.  
A: Anterior end of female (alotype); B: Cephalic end (enlarged);  
C: Posterior end of male (holotype); D: Posterior end of female (allotype).

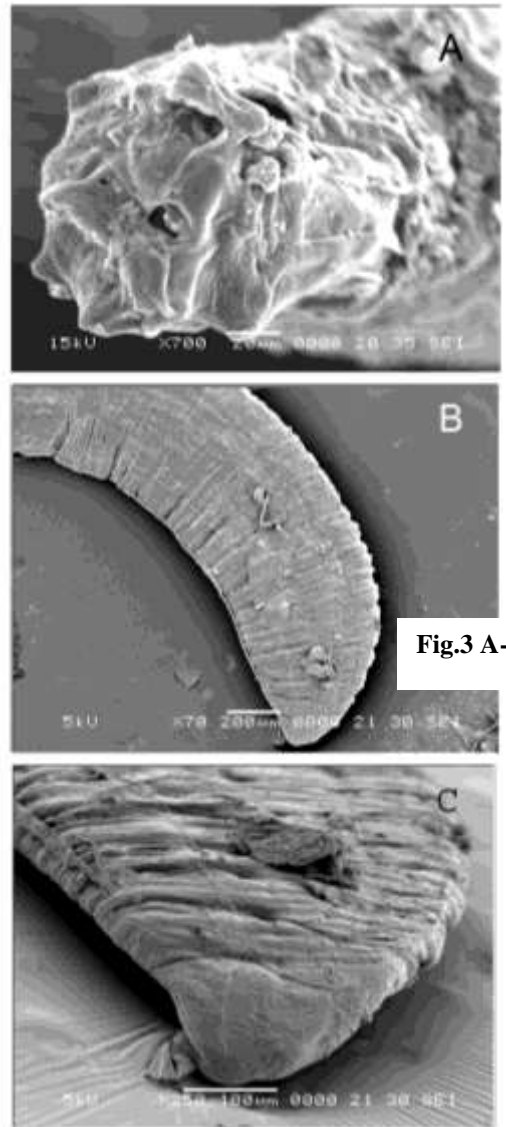


Fig.3 A-C

Fig. 3 (A-C) *Dujardinascaris maculatum* sp.n.,  
SEM micrographs of female.  
A: Cephalic end (enface view); B: Anterior end  
(lateral view); C-D: Caudal end (dorsal view).

Fig. 2 (A-D) *Dujardinascaris maculatum* sp.n., SEM  
micrographs of male.  
A: Cephalic end (enface view); B: Anterior end (lateral  
view); C-D: Caudal end (dorsal view).

The genus *Dujardinascaris* was created by Baylis in 1947. This genus normally occurs in crocodiles, lizards and alligators. Sprent (1990); Goldberg, *et al.*, (1991); Machida *et al.*, (1992); Scot *et al.* (1999); Sprent, *et al.*, (1998); Moravec (2001); Sood (1989); Bursy, *et al.*, (2005); Junker, *et al.*, (2006) and Amanda, *et al.*, (2009) Yamaguti (1961) listed two species of nematodes from fish under the genus, *Dujardinascaris*, *D. . cenotae* (Pearse, 1936) from *Rhandia guatemaensis* in Yucatan; *D. melapteruri* (Baylis, 1923) in *Melapterus electricus*. Sood (1989) presented a key to the species of *Dujardinascaris* Baylis 1947 reported from fishes in South Asia. There are seven species in this genus namely; *D. magna* Khan & Begum, (1971), in *Sciaena* sp. from Karachi coast; *D. ritai* Zaidi & Khan (1975) in *Rita rita* of fresh water fish from Lahore, *D. quadrii* Zubari and Farooq, (1976) from *Sciaena* sp., from Karachi coast; *D. Sciaena* Bilqeess *et al.*; (1977) in *Sciaena diacanthus* from Karachi coast, *D. cybii* Arya and Johnson, (1978) from fish *Cybium guttatum* from India and *D. karachiensis* Bilqeess *et al.*; (2004) from *Pomadasys olivaceum*, Akhtar & Bilqeess (2008) reported *D.jello* from *Sphyraena jello* and *D. mujibi* (2009) was described from *Sphyraena forsteri*. The present species *Dujardinascaris maculatum* is compared with described species of genus *Dujardinascaris* Baylis and is regarded a new species name *D.maculatum* refers to the fish host.

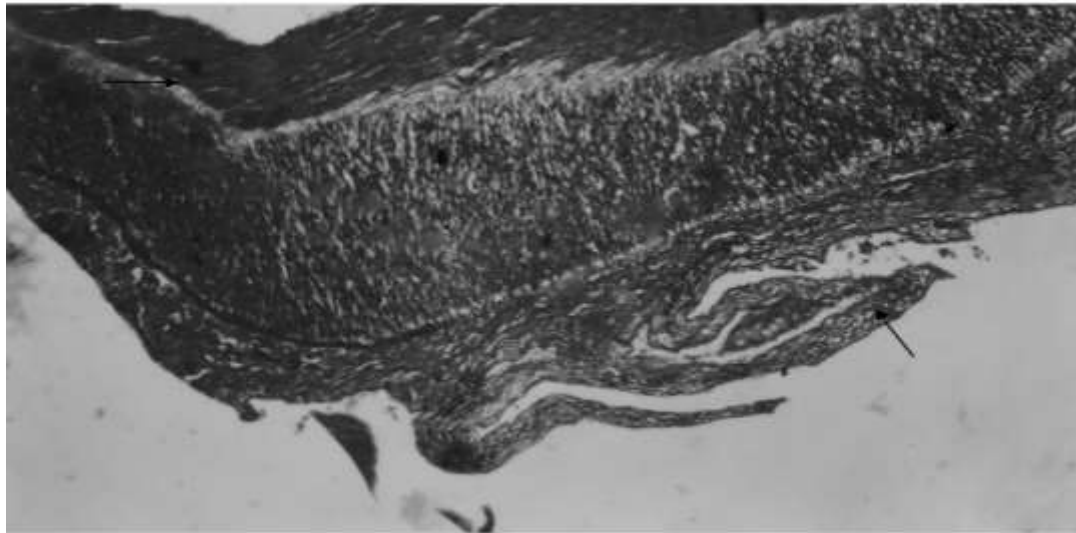


Fig.4. Transverse section of intestine of *Pomadasys maculatum* showing destruction of villi, submucosa and epithelial layers (arrow) (X20).

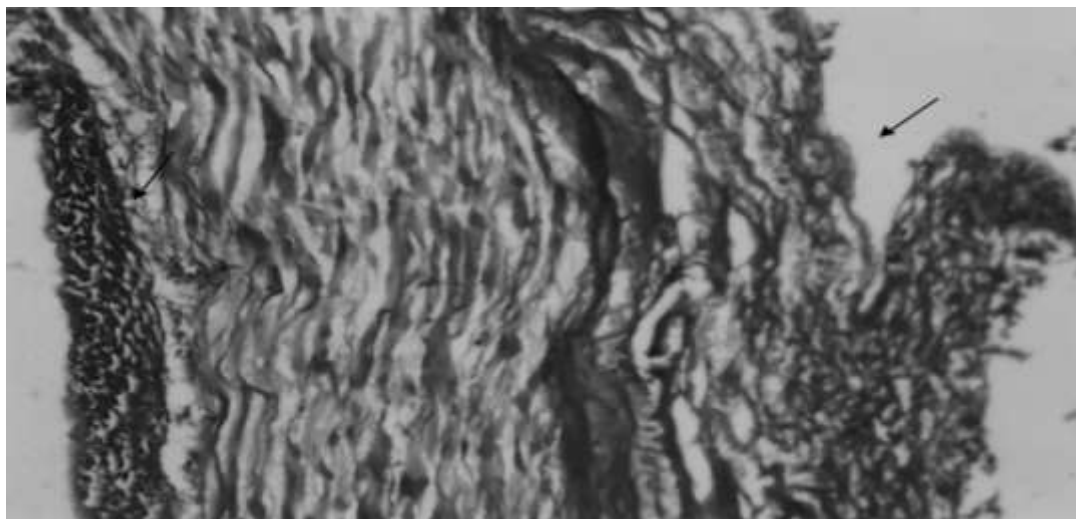


Fig.5. Transverse section of intestine of *Pomadasys maculatum* showing fibrosis, atrophy and vacuolated empty spaces (arrow) (X50).

#### Histopathology of intestine of *Pomadasys maculatum* (Figs. 4-5)

Histopathology of both stomach and intestine were carried out. It was found that nematode effect on intestine rather than stomach which may be related with the acidity of the stomach while infestation in the intestine may be

due to alkalinity. Intestine of infected fish (*Pomadasys maculatum*) was found infected by nematode larvae, causing mechanical destruction in the whole thickness of the intestine. Observations revealed destruction of villi (Fig 4) and submucosa. The epithelial cells at the tip of villi were atrophied leaving empty spaces and also showed other alterations (Fig.5).

**Table 4. Morphological variations in the new species of the genus *Dujardinascaris* Baylis, 1947 (All measurements are in mm).**

Species	<i>D. mujibi</i> (male)	<i>D. mujibi</i> (female)	<i>D. jello</i> (male)	<i>D. jello</i> (female)	<i>D. maculatum</i> (male)	<i>D. maculatum</i> (female)
Host	<i>Sphyraena forsteri</i>	<i>Sphyraena forsteri</i>	<i>Sphyraena jello</i>	<i>Sphyraena jello</i>	<i>Pomadasys maculatum</i>	<i>Pomadasys maculatum</i>
Body(L)	30-31	50.05--51.85	26.0--27.5	31.20--33.25	9.05-10.0	13-19
Body(W)	1.22-1.34	1.05-1.07	0.43-0.48	1.30-1.34	6.07—7.02	0.42-0.50
Head diameter	0.05-0.14	0.14-0.16x 0.22-0.24	0.07—0.09x 0.10-0.13	0.15—18x 0.25-0.27	0.16—0.18x 0.35-0.45	0.09—0.15x 0.18-0.30
Muscular esophagus(L)	3.60--4.19	3.60—4.70	1.84-1.87	1.50--1.60	0.50--0.80	0.55-0.64
Muscular esophagus(W)	0.17-0.22	0.17-0.20	0.15-0.18	0.14-0.18	0.15-0.25	0.20-0.22
Nerve ring from anterior region	0.16-0.23	0.24-0.36	0.17-0.28	0.18-0.30		0.32-0.36
Intestinal cecum (L)	3.00--3.50	2.30--2.80	1.60-1.72	1.68-1.79	.40-0.49-	0.12—0.15
Intestinal cecum (W)	0.20	0.13--0.17	-	-	-	-
Spicule 1 (L)	2.20-2.22	-	1.69-1.70	-	0.91-0.95	-
Spicule 2 (L)	2.20-2.22	-	1.80-2.00	-	0.50--0.60	-
No of caudal papillae	12-14	-	17	-	13--16	-
Pre-anal	9-10	-	7	-	13	-
Post cloacal	2	-	2	-	2	
Adanal Pedunculate	2	-	2 6	-	1	-
Valva from posterior extremity	-	0.95—0.98	-	0.54--0.60	-	0.05—0.06
Tail(L)	0.10-0.12	-	0.01	-	0.060—0.07	0.05

The food items were analyzed by frequency of occurrence method (F%), the number of stomachs containing each food items is expressed as percentage of all non-empty empty stomach (Dunn, 1954).

**CONCLUSION**

No significant variations in food contents throughout the year were recorded except that full stomach showed high parasitic infection with various parasites in which nematodes were dominant leading to new species. The prevalence of infection was 8.6% and intensity was recorded 2.5. The infestation in the intestine rather than stomach may be related with acidity of the stomach. This may also be related nematode larvae that are injurious to host tissue in terms of morphological alteration, growth and reproduction.

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