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PATHOBIOLOGICAL AND ULTRASTRUCTURAL STUDIES OF INFESTED SPOTTED SEABASS, *DICENTRARCHUS PUNCTATUS* IN DAMIETTA REGION, EGYPT

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Abstract

The spotted seabass, *Dicentrarchus punctatus* (Bloch, 1792) is ecologically and economically important marine fish species in Damietta region. However, their infection with crustacean parasites is an important topic in the identification of these parasites and this histopathological study investigates the aspect of parasitism and diseases that may affect their well-being. Herein, we illustrate copepode parasite, *Lernanthropus Kroyeri* and isopod (*Nerocila spp*.) infecting the gill filaments of a commercial marine spotted sea bass, *D. punctatus* and detail histopathological changes to infected tissues under light and scanning electron microscopes. A total of 75 *D. punctatus* fishes were collected from Boghaz Damietta area seasonally 2022. Using a light microscope, *L. kroyeri* was mostly discovered clinging to gill filaments ventral side, close to interbranchial septum. The parasite attached itself by grabbing hold of the gill filaments with its antennae. Lamellary edoema, fusion of the secondary lamellae as a result of considerable epithelial growth, erosive changes to the branchial lamellar epithelium, and necrosis in the terminals of the primary lamellae where the parasites penetrated were also present. According to Isopoda (*Nerocila spp.*), the epidermal cells of the *D.punctatus* with isopoda infestation displayed vacuolar and ballooning degeneration with isolated superficial sloughing to deep ulcerations. Significant hyaline degeneration and Zenker's necrosis were present in the underlying muscles, along with an infiltration of localized mononuclear cells and melanophage cells. These results explained specific infection patterns of the identified parasites in relation to their hosts under LM and SEM microscopes.

Key words: Parasitic copepods, Fishes, Pathobiology, *Lernanthropus Kroyeri, isopod*

Introduction

The Mediterranean Sea fishes especially in coastal waters of Egypt are considered as highly valued fish sources (El Zokm *et al*, 2022). In addition, Egypt with its dense and fast growing population, concentrated along the River and Delta region, believes that the importance of fish and fisheries products to the nation's domestic economy and food security (Abdelhak *et al*, 2020). Egypt's aquaculture sector is expanding quickly and is currently one of the top 10 producers in the world (Thorpe, 2005).

Dicentrarchus punctatus (spotted sea bass) is the most significant marine fish species for Egyptian commerce, and frequently employed in aquaculture. *D. punctatus* is an euryhaline fish (Moreira *et al*, 1992). Hence, it is crucial fish for brackish water and marine farming. Also, the sea bass is a valuable fish species raised as main source of protein in the Mediterranean Sea areas (Tokşen, 1999). From Southern Morocco to the Norwegian coast, the spotted sea bass is another important species distributed across the Mediterranean Sea and the Eastern North Atlantic (Fritsch *et al*, 2007).

The most characteristic pathogenic effect of *L. kroyeri* are erosion and tissue disruption (Zaid *et al*, 2022). A lesion associated with female *L. kroyeri* was compatible with depression, destruction and massive filamentous tissues loss (Zayeda *et al*, 2023).

The current study aimed to re-examine morphology and evaluate infection of copepod, *L. kroyeri* on gills of *D. punctatus*, and the harmful effects of parasitic copepod, *L. kroyeri* and the isopod (*Nerocila* sp.) by using light and scanning electron microscopy.

Materials and Methods

Fishes: a total of 75 live *D. punctatus* fish were randomly selected, with body weights varied between 2021 to the end of 2022 from the Mediterranean Sea Coast, Boghaz of Damietta Governorate, Egypt. The fish were trapped alive from the catch site and put in tanks with 1/3 of their volume filled with water and the rest with air.

Clinical picture: The inspected fish were measured for length and body weight before receiving a clinical examination, either living or recently dead ones. Gross examinations were performed on the fish specimens under inquiry to identify any external parasites and any clinical abnormalities. All fish were given a post mortem (PM) examination (Amlacher, 1970).

The collected fish were examined by dissecting out the opercula with scissors to reveal the gills, which were then separated and examined by magnified hand-lens for any abnormalities. 1- A microscopic examination was done for the gill filaments after being put on a clean slide with a drop of diluted sea water (normal saline) and a cover slip was added. 3- For SEM specimens were prepared, washed repeatedly with seawater to free them from mucus, fixed in cold (4°C) 2.5% glutaraldehyde followed by dehydration, critically dried, and sputter-coated with gold/ palladium. Then, they were mounted on metal stubs and coated with a gold film by sputtering, before being examined under JEOL JSM-6510LV SEM in the Electronic Microscopy Unit, Mansoura University.

Permanent preparation for copepod identification: The detected Copepods were gathered with a dissecting needle and a fine brush, preserved in a tiny vial, and cleaned with distilled water. They were preserved in an equal mixture of 70% ethanol and 5% glycerine, fixed in 10% formalin, and passed descending in ethanol (70%, 50%, & 30%), then cleaned in glycerine, mounted in DPX, and examined (Lucky and Lucký, 1977).

For histopathological examinationof gills, they were fixed in 10% neutral formaldehyde solution. After treatment, tissue samples were processed for paraffin-sections at4-6µ, stained in haematoxylin-eosin (H x E) and examined by the light microscope (Roberts, 2001).

Results

The clinical picture of the naturally infected fish with copepods revealed moderate to severe pathogenic changes. Clinical manifestation included haemorrhagic regions on the fish's body surface, particularly around the mouth, on gill cover, at fins' bottom, and in mouth opening. When surface breathing (sucking air), opacities grew larger. *D. punctatus* gills had patches of redness and pallor (a marbling or mosaic look). Others showed petechial haemorrhages and stacked gill tips in other places. Also, gills were coated in an excessive mucous discharge. In severe cases, there were enhanced mucus secretion, haemorrhagic lines, and pale gill filaments. *L. kroyeri* was seen with hand lens or even by naked eye showed black lines in the filame nts of gill.

Meanwhile, infested *D. punctatus* showed *L. kroyeri* between the gill filaments as black lines with petechial of haemorrhage, mosaic appearance, excessive mucus secretion and pale gill filaments**.**

In the isopoda, opercula bulging were visible and resulted in entire sloughing of gill filaments in one or two gill arches. The elimination of isopoda from the infected fish's gill chamber reveals an opercula skin lesion that developed as a consequence of the former's adhesion to the latter**.**

Taxonomic characters: 1- *L. kroyeri* Van Beneden, 1851: A crustacean copepod was picked up from *D. punctatus'* gills. Both females and males copepods have their bodies longer than usual one. Female' cephalothorax rounded poster lateral corners, a dorsal shield anteriorly narrower and posteriorly boundary; considerably concave and anterior lobes expand ventrally as conspicuous. Between cephalothorax and pre-genital trunk, there was a significant constriction. The two egg sacs were plainly visible macroscopically in gills of fish, easily identified females. 2- Isopods (*Nerocila* sp.) in *D. punctatus* bronchial chambers were noted. Bronchial chambers' anterior-ventral region was covered in parasites. Gill chambers contained just one parasite, which was adhered to exterior body surface. Large, well-developed sessile eyes, body somewhat vaulted and twisted to opposite side. Cephalon is wider than long but narrower to a round top. The cephalon barely submerged in personae's centre1. A narrow antenna reaches pereonite's posterior border and approximately to midline. Antenna was longer than antennule and with far apart bases.

Pathological impacts of infections by light microscopy: a- *L. kroyeri:* The interbronchial septum and ventral surface of the gill filaments were the main locations where *L. kroyeri* was discovered. Antennae were used to grab hold of the gill filaments during attachments, and pointed forward towards gill arch to attach**.** This made it possible for the parasite's body and any extant egg strings to lie parallel to the filaments**.** The antennae were pointed forward towards the gills arch, indicating attachment**.** Thus, body and any extant egg strings were parallel to filaments**.**

Epithelial hyperplasia was frequently recorded consequence of *L. kroyeri* infection. In the microscopic examination, individuals of *L. kroyeri* were observed in gill filaments of fish hosts. Primary lamellae were destructed where *L. kroyeri* penetrated to gills. There were also lamellar edema, fusion of the secondary lamellae due to significant epithelial proliferation, mucus cell proliferation, erosion of the branchial lamellar epithelium and necrosis in tips of the primary lamellae where the parasites penetrate**.**

Histologically, regressive events predominated at the site of parasite attachment, including total surface tissue degradation exposing principal lamellae cartilage, blood vessels being exposed, and hyperplasia on by second antennae and maxillipeds grabbing activity. Infections by parasites caused primary lamellae erosions, necrosis, epithelial erosion, compressions, and hemorrhages**.** Most pathogenic changes that occurred during infections were confined, keeping almost all of the gills filaments relatively normal. Numerous of the clinical alterations linked to *L. kroyeri* were brought on by attachment, including the positioning of the antennae into the gills and the pressures the parasite's body applied.

The dimension of the host, the distance between the hemi-bronchi, and the parasite morphology all had an impact on how severe the pressure variations were. The area nearest to an inter-bronchial septum was typically the one with the least amount of space. The thickest area of the parasite's body, the anterior section of the cephalothorax, was where variations in pressure were largest**.**

b- Isopoda (*Nerocila* sp.): The epidermal cells of the *D.punctatus* with isopoda infestation displayed vacuolar and ballooning degeneration with isolated superficial sloughing to deep ulcerations. Edema and localized mononuclear cells infiltration were visible in the dermis. Considerable hyaline degeneration and Zenker's necrosis were present in the surrounding muscles, along with an infiltration of localized mononuclear cells and melanophage cells**.** With mononuclear cells infiltrating, both the primary and secondary lamellae, the gills of the isopod-infested *D. punctatus* displayed localized desquamation in the secondary lamellae**.**

b- Scanning electron microscopy showed that *L. kroyeri* by piercing activity of econdary antennae, parasites particularly attached themselves to major lamellae**.** Second antennae, maxillipeds, third, and fourth thoracic legs strengthen the integrity of attachment**.**

The most histological impact was that the tissues disruption and erosion caused by the earlier appendages clasping lamella**.** Closer to attachment site, fusion of secondary lamellae was noticed. Regressive processes predominated at the parasite attaching location, including superficial tissues degradation and the exposing of the primary lamellae cartilage as a consequence of the second antennae, second maxillae, and maxillipeds' grabbing motion.

In close proximity to the attachment point, hyperplasia of the interlamellar epithelium and partial fusion of the secondary lamellae were also detected. A lesion associated with female *L. kroyeri* was compatible with depr-ression, destruction and massive loss of filamentous tissues. Mucous secretion covered epithelial surface of primary gill lamellae**.**

Isopoda (*Nerocila* sp.): In the infected *D. punctatus*, the epidermal cells showed vacuolar and ballooning degeneration along with localized shallow to extensive ulceration.

The edema and a localized infiltration of mononuclear cells were in the dermis. The significant hyaline degeneration and Zenker's necrosis were present in the underlying muscles, along with an infiltration of localized mononuclear cells and the melanophage cells.

Details were given in tables (1, 2, 3, 4 & 5) and figures (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 & 12).

Table 1: Number of examined fish species

Fish species	Common name	Number of examined	Arabic name
D. punctatus	Spotted sea bass	75	Nokt

Table 2: Displaying of fish checked in various seasons (n=75):

Season	Autumn	Winter	Spring	Summer
D. punctatus	20	25	11	19

Table 3: Mean intensity of *L. kroyeri* and *Nerocila* sp. infestations in examined fish species (n= 75)

Fish Species	No. of infested Fish	Mean intensity
L. kroyeri	64	15.4
Nerocila sp.	11	14.6

Table 4: Seasonal prevalence of *L. kroyeri* and *Nerocila* sp. infestation among the examined fishes (n=75)

Season	Infested Fish *L. kroyeri*	Infested Fish *Nerocila* sp.	Total infested	Prevalence %
Spring	22%	11%	8	21%
Summer	39%	29%	17	29%
Autumn	30%	33%\	18	30.5%
Winter	0.090%	31%	23	7.9%

Table 5: Prevalence of *L. kroyeri* and Isopod infestations in relation to length among *D. punctatus*

Fish length (cm)	No. of examined Fish *L. kroyeri*	No. of examined Fish isopod	Prevalence (%)
10-15	15	10	16%
15-20	27	8	11%
20-25	9	6	17%
Total	51	24	27%

Discussion

Along with the *Lernaeopodidae* and *Caligidae*, the *Lernanthropidae* is the third-largest family of parasitic fish Siphonostomatoida, with more than 150 species, the vast majority of which are found in tropical waters. (Boxshall, 2011). The most widely distributed genera of both *lernanthropids*, and *Lernanthropus*, contained 111 species (Eissa, 2002). The parasitic copepod *L. kroyeri* Beneden, 1851, which infests gills of the sea bass fish *D. punctatus* (Samak, 2004).

The existence of isopoda additionally resulted in opercula bulging, which could end up resulting in the entire sloughing of the gill filaments in one or two gill arches along with the skin lesions, which followed the removal of isopoda from the opercular chambers, may have been plainly observed in the skin surface around the operculum. This agreed with Eissa *et al.* (2017), they reported the two crustacean parasites are different forms by parasitological analysis. Isopods and copepods *L. kroyeri*, the first parasitic copepod ever discovered, on the gills of *D. punctatus*. This finding agreed with (Eissa *et al.* 2012) who isolated a certain genus from a specific host and location. However, it differed from that of El-Deen *et al.* (2013), who identified *Caligus minimus Otto*, 1821 (copepod) parasitizing many fish *Moolgarda seheli* and *Mugil cephalus*.

In the present study, the parasite was detected in *D. punctatus* mouth cavities. This agreed with Ragias *et al.* (2004), who caught the same parasite in the same fish. But, it disagreed with Toksen (2007), who detected the parasite in gilthead sea bream *Sparus aurata* cultured fish gills. Also, Eissa *et al.* (2016) detected this parasite from

Dicentrarchus labrax's gills and the inner surface of the operculum.

In the present study, *Nerocila* spp. up to genus level was separated from both the *D. labrax* and *D. punctatus'* bronchial cavities. In the bronchial chambers' anterior-ventral area it was detected in the gills chambers on a single side only, not both sides, and the head basally, it was linked to the skin. This agreed with Alas *et al.* (2008), who found *Nerocila bivittata* on the skin of the Rusty Blenny *Parablennius sanguinolentus'* caudal peduncle. Also, this agreed with Akmirza (2014), who reported *N. bivittata* from the brown scanty sciana umbra skin.

In this study, the descriptive morphology of *L. kroyeri* agreed with Özel *et al.* (2004), Also, the present study, found that the cephalothoracic appendages of the parasite copepod *L. kroyeri*, comprising the second antennae, second maxillae, and maxilliped, were used for attaching both genders to host tissues. This agreed with Öktener *et al.* (2010), who found that the first and second antennae, second maxillae, maxilliped, and the second gave the cephalothorax appenda was 26%, high in autumn 33%, followed by 11% in spring. This data was than those reported by Eissa *et al.* (2017) who found that some marine fishes have 10.7% seasonal incidence of parasitic isopods. Also, Alas *et al.* (2008) reported that *Nerocila bivittata* overall seasonal prevalence was 7.4% on the caudal peduncle of the Rusty Blenny *Parablennius sanguinolentus.*

In the present study, Isopoda had the greatest prevalence in autumn followed by winter, then summer, and finally spring. This agreed with Boghdady *et al.* (2015), who found that *Nerocila* spp. seasonal occurrence in *D. punctatus* was great in autumn, followed by summer, and least was in both winter and spring. This variation might be due to geographical and climatic conditions. The present *L. kroyeri* infection was detected in 22.7% of fish. This was less than the 35% given by Manera and Dezfuli (2003) or even 47% reported by Eissa *et al*. (2012). ges. Moreover, Ho *et al.* (2011) found that *lernanthropids* attached to gill filaments using their prehensile antennae, maxillipeds, and third leg. The second antennae of *Lernanthropus* species are designed morphologically to pierce the host gill. This action is aided by the motion of the maxillipeds and the modified third pair of legs (Kabata 1979). The antenna structure was also visible under a scanning electron microscope (SEM), showing the typical prehensile and uncinate structures that the parasite uses for attaching to or feed on the host tissues (Kabata 1985).

In the present study, crustacean's infestation in *D. punctatus* at specific seasons, seasonal *Lernanthropus* infestation was 22.7%, during the other three seasons (fall, winter, and summer), with a maximum of 39% in the summer. This agreed with Boghdady *et al.* (2015), who found an increase in *Lernanthropus* infesting *D. punctatus*, with decrease in spring and marked increase in summer, winter, and fall before maximum peak.

The total seasonal prevalence of isopoda winter 31%, but it was 29% in summer and Undoubtedly, the seasonal variations more or less depend on the climatic and geographical areas as well as fauna and flora.

Conclusion

The parasites used the second antennae to pierce primary lamellae and anchor there. Regressive phenomena predominated at the parasite attaching site, including superficial tissues degradation and the exposing of the primary lamellae cartilage as a consequence of the second antennae, second maxillae, and the maxillipeds' grabbing motion.

A lesion was found in female *Lernanthropus kroyeri* and *Nerocila* sp. with depression, destruction and massive loss of filament tissues.

No doubt, applying biosafety chemicals to control copepods in water sources is a must.

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Explanation of figures

Fig.1: Adult female & male *L. kroyeri*.

Fig.2: *L. kroyeri* attached with gills

Fig.3: *Nerocila* sp.

Fig. 4: Diagram of the female copepodian parasite *Lernanthropus kroyeri* (a) ventral view; (b) antennule; (c) antenna; (d) 2nd thoracic leg; (e) maxillule. CS=cephalic shield; es=egg sac; gf=gill filament; L3= 3rd thoracic leg and L= 4th thoracic leg.

Fig. 5: Gill filaments of *Dicectrarchus punctatus* infested with *Lernanthropus kroyeri* (arrow), (a), female causing erosion and disruption of the tissue (*) and hypertrophy (arrow heads); (b), male causing severe haemorrhage and hyperplasia of the gill lamellae (*) and necrosis of gill epithelial cells (arrow heads) (H&E stain).

Fig.6: Light micrographs illustrating the gills of *Dicectrarchus punctatus* infested with parasitic copepod, *Lernanthropus kroyeri* (P) demonstrating total sloughing of the secondary lamellae, as well as gills arch edoema and mononuclear cellular infiltrating (*), necrosis (n) and erosion of gill filaments by 2nd antennae (arrows) . (H & E stain)

Fig. 7: *Nerocila* sp.

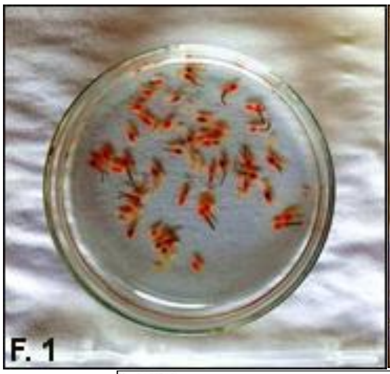
Fig. 8: Electron micrographs of *Lernanthropus kroyeri* attached to gill filament of spotted sea bass, (a) unique 3rd leg attached closed to filament as assistant organ. (b) Magnified SEM micrograph illustrating localized erosion and disruption (*) and pressure exerted by attachment caused fusion of secondary lamellae and loss of normal gill structure (arrows). cs, cephalothorax shield; M2, 2nd maxilla; m, maxilliped; A2, 2nd antenna; L1, 1st thoracic leg and L2, 2nd thoracic leg; L3, 3rd thoracic leg; L4, 4th thoracic leg & gf, gill filament.

Fig. 9: SEM of female *Lernanthropus kroyeri* in ventral view attached with gill filament of *Dicectrarchus punctatus* ; A2, 2nd antenna; CS, cephalic shield; es, egg sac; gf, gill filament; L3, 3rd thoracic leg; L4, 4th thoracic leg and m, maxilliped.

Fig. 10: Seasonal changes of mean intensity of *L. kroyeri* and *Nerocila* sp. close to Boghaz area near Damietta City.

Fig. 11: Prevalence of *L. kroyeri* among examined fishes according to season

Fig. 12: Prevalence of Isopod among examined fishes according to season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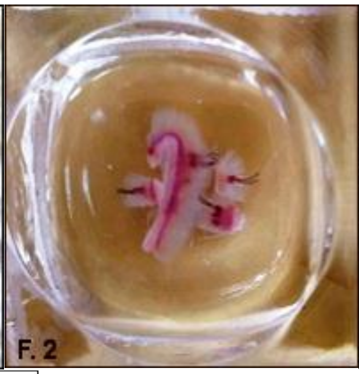
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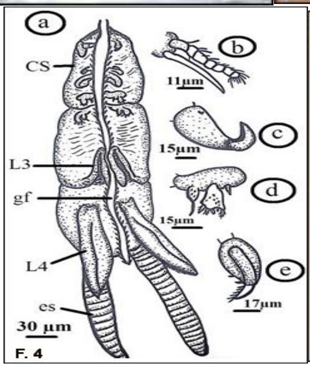
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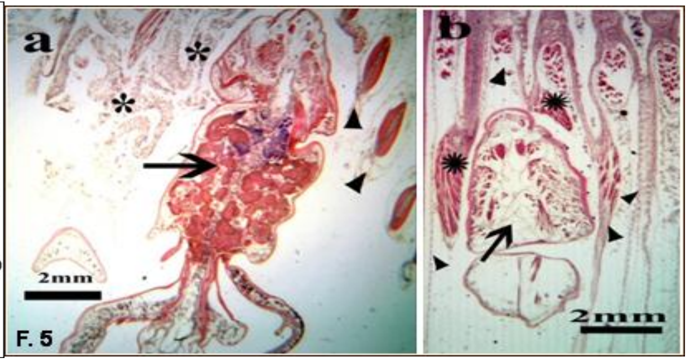
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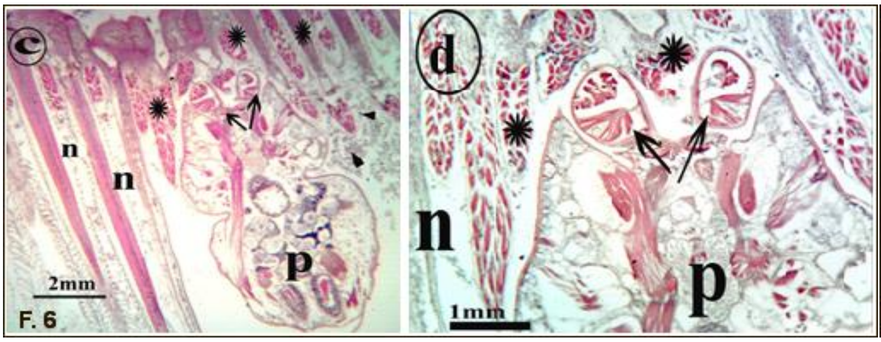
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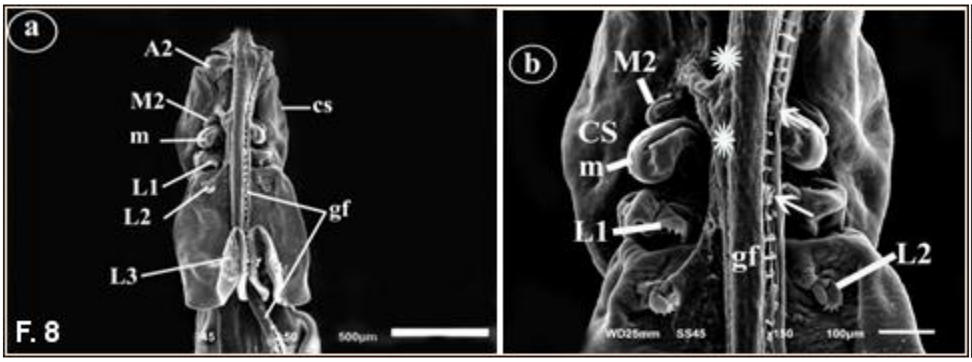
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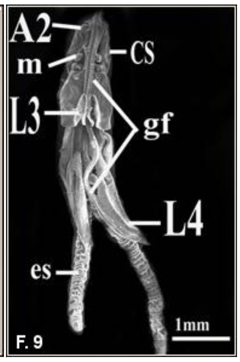
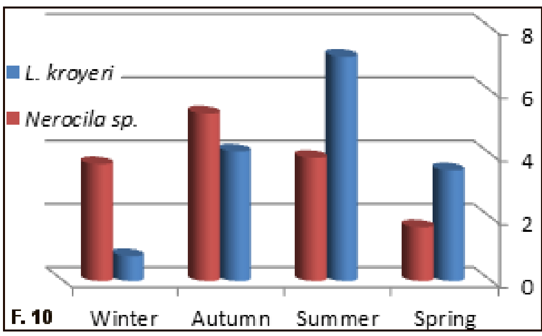
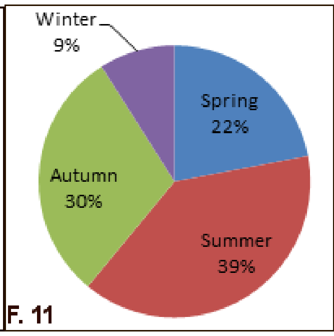
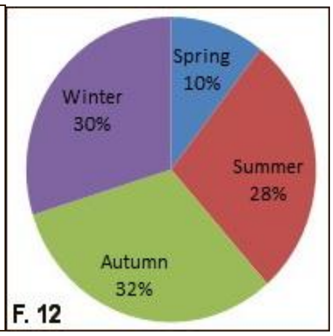
L2
100µm


A2
m
CS
L3
gf
L4
es
1mm
F. 9


L. kroyeri
Nerocila sp.
8
6
4
2
0
Winter
Autumn
Summer
Spring
F. 10


Winter 9%
Spring 22%
Autumn 30%
Summer 39%
F. 11


Spring 10%
Winter 30%
Summer 28%
Autumn 32%
F. 12