Journal of
Oceanography and Marine Research

Mahmoud et al., Oceanography 2014, 2:4
DOI: 10.4172/2332-2632.1000129

Research Article Open Access

Parasitological and Comparative Pathological Studies on Monogenean Infestation of Cultured Sea Bream (*Sparus aurata*, Spariidae) in Egypt

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Abstract

This investigation was realized between December 2012 to November 2013. A total of 400 Sea bream *(Sparus aurata)* were sampled with history of loss of appetite and sluggish movement with increased breathing frequency, were investigated in terms of monogenean parasites .The prevalence, seasonal dynamics, mean intensity as well as the histopathological influence of the detected monogeneans were estimated. The standard parasitological methods were used in the survey and yielded four monogenean species on the Sea bream gills: *Furnestinia Echeneis* (58.59% of invaded fish), *Encotyllabe spari* 32.81%, *Sparicotyle chrysophrii* 25.78% *and Choriocotyle chrysophrii* 17.19%. The total prevalence was 32% and the highest rate was recorded during summer. With the exception of *Encotyllabe spari*, the detected parasites are new records for the monogenean fauna of Egypt (new geographical record) and also Sea bream (*Sparus aurata)* is a new host record for the monogenean *Choriocotyle chrysophrii*.

Histopathological examination revealed different pathological changes in the affected branchial tissue. The lesions were depended on the type of the detected monogenea. Necrosis of the gill lamellar epithelium, hyperplasia of mucous secreting cells and focal areas of denuded epithelium were the most common lesions noticed at the sites of parasites attachment.

Keywords: Sea bream, Cultured, Monogenea, Histopathology, Egypt.

Introduction

The intensification of aquaculture and globalization of the seafood trade have led to remarkable development in the aquaculture industry which play a role in the development of many national economics and in meeting demands for aquatic animal production [1]. The industry has been plagued with disease problems caused by bacterial, viral, fungal and parasitic pathogens which are associated with mortality causing substantial economic losses. Although it is well understood that parasites have a major impact on marine aquaculture, yet relatively few published reports about marine fish parasites in Egypt and are mostly restricted to wild fish [2-4]. Sea bream (*Sparus aurata*) is marine Fish with economic value and wide spread all over the world especially in the Mediterranean Sea. In Egypt, farming of Sea bream is at an early stage of development, it has started in late 1980 `s and is still restricted to few farms and float cages in the Mediterranean Sea [5].

Among the most species rich classes of fish parasites, are monogenean trematodes which commonly inhabet fish gills and skin. There are two types of monogeneans based upon the opisthohaptor morphology: Monopisthocotylean monogeneans such as *Furnestinia echeneis* and *Encotyllabe spari* which are globally frequent in wild populations and can be pathogenic to cultured fish, [6,7] and Polyopisthocotyleans ones which are pathogenic to economically important fish around the world, [8,9] Amongst them, *Choriocotyle chrysophrii* and also *Sparicotyle chrysophrii* which was recorded as the most threatening ectoparasites for Sea bream (*Sparus aurata*) culture [10,11]. High infestation with monogeneans through their attachment and feeding, may lead to significant fish mortality as a consequence of respiratory distress, tissue damage and secondary bacterial and fungal infections. In addition, many traits such as monoxeny, rapid reproduction and hermaphroditism enable them to cause serious damage in both wild species and farmed stocks [12-14].

From pathological point of view, the induced lesions depend on the type of monogenea and Some of them induce closely similar tissue changes [15,16]. Monogeneans 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 [17] Several blood-feeding polyopisthocotylean monogeneans have been shown to induce gill damage and mortality [18,19].

The present investigation was done on cultured Sea bream, *Sparus aurata* from a private marine fish farm in Domietta province, Egypt depending on a history of respiratory distress manifested by sluggish movement with increased breathing frequency. The aim was to identify the monogenean fauna parasitic on cultured Sea bream (*Sparus aurata*), their prevalence, seasonal dynamics as well as the histopathological changes induced by each of the detected species.

Material and Methods

Fish

During the period from December 2012 to January 2013, 400 fish specimens of cultured Sea bream, *Sparus aurata* (Sparidae) were obtained seasonally from a private fish farm in Ezbet Elborg area (in corresponding to Mediterranean sea), Domietta province. Fish body

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Received October 28, 2014; **Accepted** November 26, 2014; **Published** December 03, 2014

Citation: Mahmoud NE, Mahmoud AM, Fahmy MM (2014) Parasitological and Comparative Pathological Studies on Monogenean Infestation of Cultured Sea Bream (*Sparus aurata*, Spariidae) in Egypt. Oceanography 2: 129. doi: 10.4172/2332-2632.1000129

weights and lengths were ranged from 50 up to 200 gm and 15 to 25 cm respectively. Samples were transported to the laboratory of parasitology department; Faculty of veterinary medicine, Cairo University in aerated tanks partially filled with its natural water and was kept alive until investigation.

Parasitological examination

1 Macroscopic examination

Skin surface, fins and gills were examined by naked eyes and with the help of dissecting microscope for any attached parasites, lesions or external changes.

2 Microscopic examinations

Four to sex mucous smears were immediately prepared from the skin and fins with the aid of microscopic slides and subsequently examined with the aid of a dissecting microscope. Fish were killed by severing the spinal cord behind the head. Once euthanized, gills were carefully removed and placed in separate petri dishes containing normal saline to remove any excess gill mucus, and they were examined for monogenean parasites under a stereomicroscope. Live parasites were slightly compressed between a slide and a cover slip prior to being examined under a light microscope. The collected monogeneans were fixed in 4% formalin for permanent whole mount preparation [20].

Measurements were taken on fixed specimens using ocular micrometer calibrated against a stage micrometer. Prevalence, mean intensity and measurements followed the guidelines of [21]. Microphotographs were taken. Identification of the parasites was made according to the works of [22,23] and available literatures.

Histopathological examination

Tissue specimens from gills were fixed in 10% neutral buffered formalin for routine histopathological examinations. The fixed samples were washed in tap water overnight and exposed to ascending concentrations of ethanol (70, 80, 90 and 100%), cleared in xylene and embedded in paraffin. Tissue slides of 5 µm thick sections were prepared and stained by hematoxylin and eosin (H&E). The histopathological preparation was performed according to [24,25].

Results

Results of parasitological examination

1-Macroscopic examination:

The affected gills appeared pale with excessive mucous secretions forming claudy film of slime.

2-Microscopic examination:

Four species of monogenea belonged to four families were detected in gills of cultured Sea bream (*Sparus aurata*).

Morphological description of the detected monogenea species

Encotyllabe spari [23] **(Monopisthocotylea: Capsalidae) Description** (Figure 1): Body is cylindrical, measuring 1.06 -2.02 mm in length (excluding the peduncle of the opisthaptor) and 0.23 – 0.30mm as maximum width. Anteriorly, two oval anterior suckers are surrounded by membranous lobe with ventral infolded margens. Pharynx is 0.05 -0.07mm in diameter and at its level, two pairs of eye spots are present. The caeca run posteriorly with many ramifying branches. Opisthaptor is the form of muscular disk connected to the body by muscular peduncle provided with a thin marginal membrane and armed with one pair of large stout anchors (0.08- 0.13 mm long), one pair of simple anchors (0.01-0.02 mm long) and 14 marginal hooks (0.01-0.02 mm long). Two oval testes are situated pre-equatorial measuring 0.02-0.11 mm by 0.04-0.15 mm. Cerrus pouch is elliptical. Ovary is ovalm pre- testicular measuring 0.03-0.05 by 0.02-0.03mm. Vetelline follicles are extensive, filling lateral and post testicular region of the body.

Furnestinia Echeneis **(Monopisthocotylea: Diplectanidae)**

Description (Figure 2) **:** The body is 0.5- 0.8 mm in length and 0.1- 0.2 mm in width in ovary level. One pair of tri-lobed head organs and two pairs of eye spots are present anteriorly. Pharynx is rounded (0.03 -0.05mm in diameter). Haptor is 0.1- 0.2mm in diameter with lamellar shaped dorsal and ventral squamodiscs having numerous concentric rows of paired lamellae and two pairs of anchors supported

Figure 1: Encotyllabi spari *(Left: Whole worm Right: Opisthaptor).*

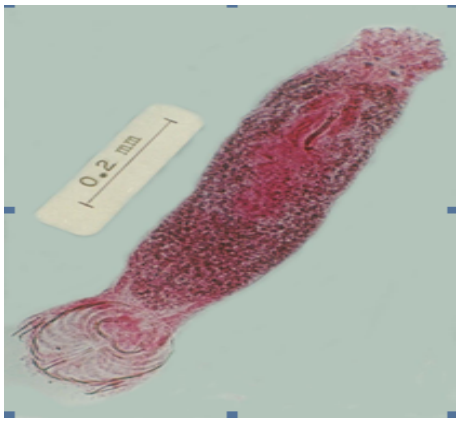

Figure 2: Furnestinia echeneis.

Figure 3: *Choriocotyle chrysophrii.*

with one middle and two lateral bars. Testis is large, lying in the middle of the body, measuring 0.06 -0.08 by 0.07 -0.09 mm. Single prostatic reservoir and a sclerotized copulatory organ are clearly appeared. Ovary is pre-testicular, looping around the intestinal limb. The vitellaria are follicular extending from the pharynx level to the haptor.

Family Diclidophoridae

***Choriocotyle chrysophrii*, Description** (Figure 3 by Mahmoud, 1994): Body is fusiform with attenuated anterior end. It measures 2.43 – 4.01 mm long and 0.52 – 0.73 wide. The prohaptor has one pair of suchers . Pharynx is large (0. 05 – 0.08 x 0.7- 0.9 mm). Genital pore is median, lying on the level of oesophogial biforcation and armed with hooks of 0.1- 0.2mm long. The opisthaptor is terminal with four pairs of pedunculated clamps with a terminal lappet between the last one. The clamp has chitinious pieces and curved lateral bars. Testes are

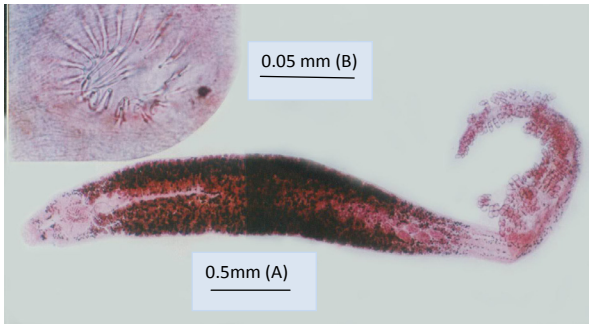

Figure 4: ***Spariocotyle chrysophrii*** *(A: Whole worm B: Genital atrium).*

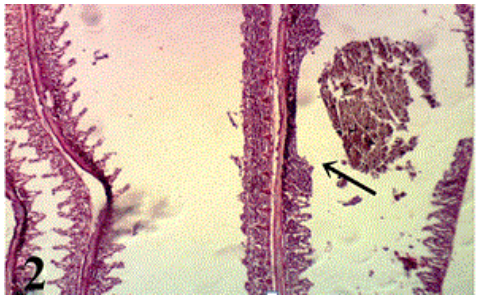

Figure 5: Gills of sea bream (*Sparus aurata*) showing necrosis and desquamated epithelium with remnants of the parasite(arrow). H& E stain x 100.

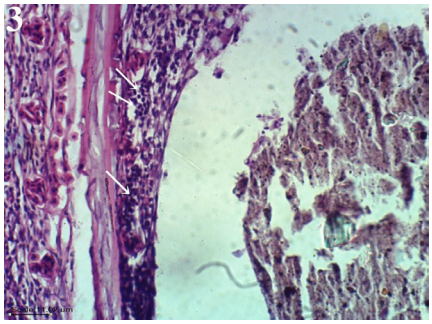

Figure 6: Higher magnification of fig 5+. showing mononuclear cells infiltration in the necrotic tissue of secondary gill lamellae (arrow). H& E stain x 400.

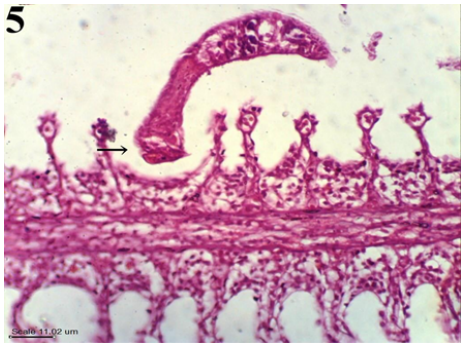

Figure 7: Gills of sea bream (*Sparus aurata*) showing showing *Furnestinia echeneis* parasite attached to secondary gill lamellae (arrow). H& E stain x 400.

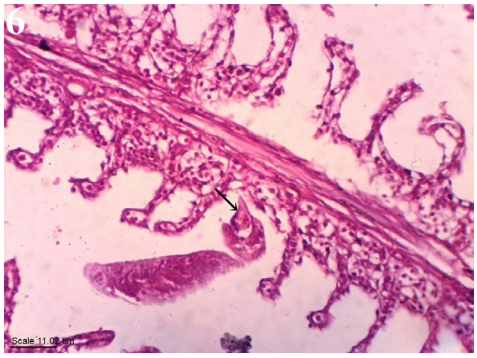

Figure 8: Gills of sea bream *(Sparus aurata)* showing sercum- scibed area of detached epithelium (arrow). H& E stain x 400.

Figure 9: Gills of sea bream *(Sparus aurata)* showing larvae of the parasite in the gill raker near the carilagenous part of the gill arch (arrow). H& E stain x 400.

numerous (18 -25 in number) and post ovarian. Vitelline follicle fills most of the body. Ovary is median and folded.Vagina is absent.

***Sparicotyle chrysophrii* (Polyopisthocotylea: Microcotylidae)**

Description (Figure 4 by Mahmoud and Shaheed. (1998): Body is 4.3 -5.5 mm in length, 0.5-0.7 mm in width in ovary level. Anterior there are two buccal suckes with septum and numbers of minute spines. Pharynx is 0.03 -0.04 in diameter. Oesophagus has two lateral diverticula. Intestinal caeca extends posteriorly with inner and outer diverticula. Haptor is triangular in profile and bordered with numerous (60 -70) pair of small clambs of uniform structure. Testes are ovoid in

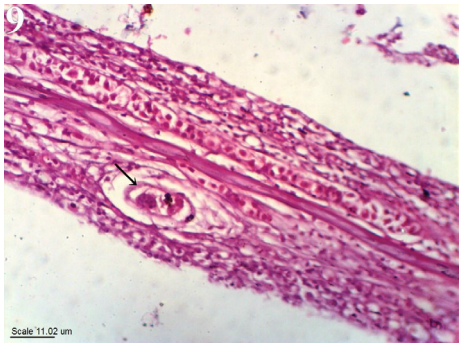

Figure 10: Gills of sea bream (*Sparus aurata*) showing larvae of the parasite in the secondary gill lamellae (arrow). Note: Lamellar fusion and hyperplasia. H& E stain x 400.

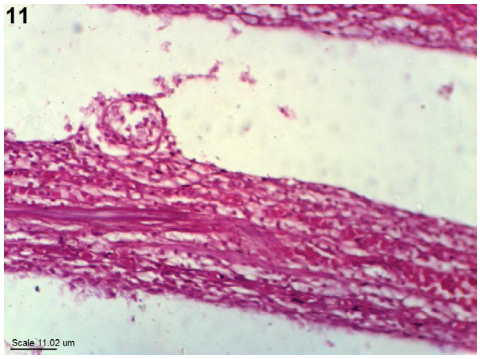

Figure 11: Gills of sea bream (*Sparus aurata*) showing necrosis and desquamation of the epithelium together with lamellar fusion and hyperplasia (arrow). H& E stain x 400.

shape, vary in number (15-20) and each measuring 0.06 – 0.08 mm. Genital atrium is armed with 34- 40 spines with curved tips. Ovary is inverted U shaped and lying pre-testicular. Vitellaria are follicular and coextensive.

Prevalence and seasonal dynamics of monogenean species infestation: (Table 1).

In the present study, out of the examined 400 *Sparus auratus* fish, 128 (32.0%) were found infested with monogenea. The highest rate of infestation was recorded during summer (49%) while the lowest was during winter (19%).

Monopisthocotylean species (*Furnestinia echeneis* and *Encotyllabi spari)* were the most abundant infesting 18.75% and 10.50% of the examined fish respectively. Mixed infestation was noticed between all detected species.

Results of histological examination

The histopathological examination revealed severe pathological lesions in some of the examined cases while in others, the changes were mild. The lesions ranged from mucous cells hyperplasia to complete necrosis of the branchial epithelium or even desquamation. Haemorrhage and oedema were also noticed.

The examined lamellar tissue infested with *Encotyllabi spari* showed marked branchitis characterized by mononuclear cells infiltrating the branchial epithelium. The affected parts appeared with necrosis and desquamated epithelium with remnants of the parasite (Figure 5). In such cases mononuclear cells infiltration was not uncommon (Figure 6).

In case of *Furnestinia echeneis* infestation, the lesions were relatively milder and focal. The parasites were easily detected in the affected gills (Figure 7). The sites of parasite attachment showed sircum-scribed area of detached epithelium (Figure 8), it seems to be resulted from feeding of the parasite on the lamellar tissue. However the inflammatory reactions and fusion of the secondary lamellae were scanty.

The infestation with *Choriocotyle chrysophrii* was characteristic. The eggs of the parasites were embedded in the bronchial tissue. In such cases there was no evidence of lamellar fusion or hyperplasia while lamellar edema was a common finding. Some of the examined cases revealed bronchitis and lamellar fusion, such lesions were accompanied with detection of larvae in the bronchial tissue either in secondary gill lamellae and/ or gill rakers (Figures 9, 10).

The infestation with *Spariocotyle chrysophrii* induced severe pathological lesions in the bronchial epithelium. The lamellar tissue showed necrosis and desquamation of the epithelium together with lamellar fusion and hyperplasia (Figure 11,12). In such cases hemorrhage and edema were not uncommon.

Discussion

In the present study, examination of 400 *Sparus aurata* samples for monogenean parasites was done depending on a history of respiratory manifestation symptoms. .Macroscopic examination revealed marked paleness of the infested fish gills with excessive mucous secretions forming claudy film of slime, the result which agree with that noticed by Paperna,I.(1991) for monogenean gill affection

Season	No of fish		No. of infested fish with			
	Examined	Infested (%)	*Encotyllabe spari*	*Furnestinia echeneis*	*Sparicotyle chrysophrii*	*Choriocotyle chrysophrii*
Winter	100	19	4	10	5	2
Spring	100	33	9	16	8	4
Summer	100	48	20	34	13	11
Autumn	100	28	9	15	7	5
Total (%)	400	128 (32)	42 (10.50)	75 (18.75)	33 (8.25)	22.0 (5.5)

Table 1: Prevalence and seasonal dynamics of the detected monogenean species.

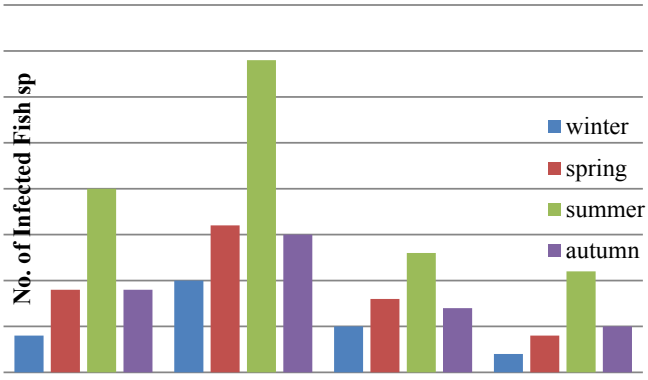

Chart 1: Prevalence and Seasonal dynamics of the detected monogenean sp.

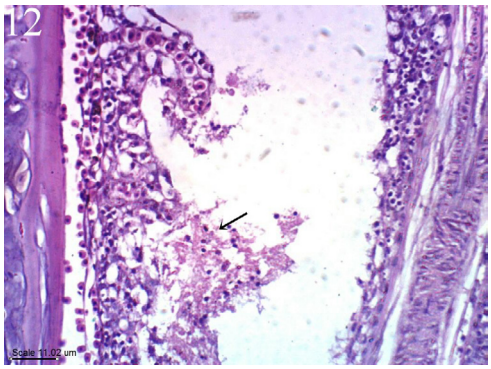

Figure 12: Gills of sea bream (*Sparus aurata*) showing advanced lamellar necrosis (arrow). H& E stain x 400.

Four types of monogenean parasites were isolated from the gills of sea bream and identified as *Encotyllabe spari*, *Furnestinia Echeneis, Choriocotyle chrysophrii* and *Sparicotyle chrisophrii* depending on the morphological characteristics studied by light microscopy. The percentage of infected sea breams with monogenean parasites during the present survey is 32%. Previous study done by [34] reported nearly the same rate (32,21%) while that reported by [29,30] were markedly different and were 4.44% and 49,80% respectively. The highest rate of infestation was recorded during summer (49%) while the lowest was during winter (19%), this is might be attributed to the suitability of the water temperature to the reproduction and abundance of the detected monogenean parasites.

Encotyllabe spari was detected in 32.81% of the infested fish; this rate is much higher than that reported by Pellicer. In the Mediterranean Sea (2) and by Kardousha [26], (8%) in Arabian Gulf**.** The detected *Encotyllabe spari was* morphologically similar to those described by Yamaguti et al [24,27]. This species is geographically widespread with a wide range of hosts; it was reported in the Atlantic Ocean by Khon [28], in the Persian Gulf [27]. This is the second record of this monogenean species in Egypt as the first one was by Mahmoud and Shaheed from *Serranus fasciatus* in Gulf of Suez.

Furnestinia echeneis in the present survey, showed the highest rate among the infested fish (58.59%) with the maximum rate during summer. In contrast**,** Revarsat [6] denoted that, rate of infestation with this parasite increase in spring, this is might be attributed to the difference in environmental condition and locality. Higher prevalence of *F. echeneis* (73.2%) in the wild sea bream population was recorded by Mladineo, among cage-reared sea bream in Mediterranean Sea. No cases of mortality were recorded among the infested fish in spite of the high intensity of F. echeneis their gills, the data which came in agreement with Quaglio [29]. On the other hand, *F. echeneis* caused high mortality due to sever necrosis on the gill and mass mucous secretion in *Siganus auratus,* [30] also in natural sea bream in Red Sea and Acabe Bay, [31].

Sparicotyle chrisophrii was recorded in relatively lower rate (8.25%) among the examined fish. This species was previously recorded by [2] with infection rate of 4,4% of sea bream gills and noticed the significant increase in number of infected individuals with the temperature increase.Tthe recorded rate in this study is different from that presented by Mladineo [28] which stated 32.21%, [26] (53.2%) **.** [8] and [38] also detected this parasite on the sea bream gills.

The recovered *Choriocotyle chrysophrii* recorded the prevalence of 5.5% and sea bream was recorded as new host of this monogenea species as the species were previously recorded in other wide range of hosts as mentioned by [34].

Record of mixed infection between all detected monogenea species in the present investigation supports the finding of [35,36].

In present study, the pathological changes in the gills were different depend up on the type of the parasite which detected in the gills. The lesions were noticed in secondary gill lamellae and gill rakers in the area of gill arch, in this concern**,** Paperna [16] studied the pathogenesis and effect of some monogea infestation on the mortality of farmed fish and paid attention to the relation between the type of monogenea, site of attachment on the gills and mortality rate. He indicated that the sites of proliferation are dependent on the preferred sites of the monogenean species. Some prefers the tips of the gill filaments and causes mass mortality.

In this study, the detected lesions in case of *Encotyllabi spari* were severe and characterized by bronchitis, necrosis and desquamated epithelium. However, such lesions were nearly similar to those of *Spariocotyle chrysophrii* infestation. Molnar recorded similar pathological lesions caused by *D. vastator* and *D. lamellatus,* where severe hyperplasia of the epithelium of gill filaments was a common finding. Molnar added that, feeding on epithelial cells and anchorage (attachment) by the monogeneans cause severe destruction of the gills resulting in hemorrhage and metaplasia of the gill tissue.

The infestation with *Choriocotyle chrysophrii* showed characteristic eggs and/ or larvae embedded in the bronchial tissue, this result was in parallel with the finding of Whittington [37], who recorded that a few capsalids attach egg bundles to gill filaments.

No pathological signs are referred to *F. echeneis* infections, also with 50 specimens/gill arch infection intensity [29]. But in heavily infestation shows hyperplasia of gill epithelium with thickening of lamellae up to fusion. The gills show diffused degeneration and necrosis in the filament epithelial tissue [6].

S. chrysophrii shows a high pathogenicity at low infection intensity (8 parasites/gill arch) with gross lesions such as gill and systemic anemia already noticeable at necropsy. In this case histology shows severe hyperplasia of gill epithelium with thickening of lamellae up to fusion, and heavy sloughing off of the epithelial cells. Moreover the gills show diffused degeneration and necrosis in the residual epithelial tissue. The hematophagous attitude of *S. chrysophrii* is evident for the presence of several erythrocytes in the parasite gut [6,29].

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