IMPACTS OF *CLEIDODISCUS ACULEATUS,* (MONOGENEA ANCYROCEPHALINAE) ON *CYPRINUS CARPIO*

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Abstract

Cleidodiscus aculeatus infection was associated with mass mortalities of *Cyprinus carpio* reared in tanks during the period 2006–2007. All dead fish had high parasite abundance (mean abundance ± S.D. = 148.3±22.5) and entangled in gills. The parasitological analysis of 30 live fish collected from the infested tanks showed that 73.2% of fish harboured parasite with intensities ranging between 5 and 12 parasites per fish. A relationship was observed between the abundance of the parasite and the condition factor (r_S=0.175, n=30, p>0.5). However, the hematocrit values were significantly lower in the infested fish. The relationship between parasite abundance and hematocrit values was negative and statistically significant (r_S=−0.625, n=30, p<0.01). The affected gills revealed that the parasites attached to the host grasping one or two lamellae with each clamp of the haptor, which led to lamellar synechiae, lamellar clubbing and disruption of epithelial and vascular structures.

Cortisol level in infected *Cyprinus carpio* (150±4.5 g body weight) with (mean intensities 7 and 9 parasites per fish) was studied at two temperature levels, 20±1.6°C and 25±1.2°C, respectively. Infected fish at 25±1.2°C, showed an elevated level of cortisol compared to uninfected fish. At 25±1.2°C, the cortisol level in infected fish compared to uninfected was insignificantly increased. A mild to moderate Trichodina infection was also detected in gills. Combined effects of gill damage and parasite blood feeding could be related to the recorded anemia and, finally, the death of the fish.

INTRODUCTION

Ectoparasites can easily multiply and disperse in confined areas, in a very high intensity (Rohde, 1984 and Thoney and Hargis, 1991).Monogeneans are generally found as parasites on or in cold-blooded vertebrates, mainly the elasmobranches, bony fish and in some amphibians and reptiles. In fish, the majority of monogeneans are parasitic on the gills or skin. These parasites are site- and host-specific, generally occurring in relatively low numbers (El-Naggar and Serag (1985). However, the establishment of a heavy monogenean infection, particularly under unfavorable culture conditions, may give rise to mass epizootics with severe economic loss (Ramadan, 2000).

Many species of monogenea have been recorded to provoke massive losses in fish cultures. Usually, blood-feeding monopisthocotyleans have been considered harmful to their hosts, which feed on mucus and epithelium (Thoney and Hargis, 1991), but outbreaks of mortality have also been reported in association with microcotylids (Faisal and Imam, 1990). In addition, monogeneans such as gyrodactylids are frequently encountered ectoparasites of *Oncorhynchus mykiss* and are known to cause epidermal injuries in that host Buchmann and sciani, (1997) and Mahfouz, (1997).

Numerous investigations have demonstrated that fish respond to harmful stimuli by releasing cortisol from their interrenal cells (Nielsen and Buchmann, 1997 and Pottinger *et al.*, 1999). In addition, several studies have indicated that increased cortisol levels in plasma of stressed fish elevate the susceptibility of the affected hosts to various infectious organisms (Lindenstrùm and Buchmann, 1998). Thus, *C.carpio* is rendered vulnerable to the pathogenic fungus *Saprolegnia parasitica* when administered cortisol (Pottinger and Day, 1999). Brown trout, normally refractive to *Gyrodactylus salaris*, becomes susceptible following injection with hydrocortisone (Harris *et al.*, 2000). Also, synthetic corticosteroids such as triamcinolone acetonide and dexamethasone increase the susceptibility of the host to infections with *Ichthyophthirius multifiliis* (Houghton and Matthews, 1990) and

G.derjavini (Lindenstrùm and Buchmann, 1998), respectively. However, pathogens themselves could act as stressors and initiate corticosteroid release in fish. It is noteworthy that biotic factors like irritating algae have been found to induce cortisol production in exposed rainbow trout (Yang & Albright, 1995) and it is possible that a number of parasites could have a similar effect on their host. Parasitic crustaceans on host skin were indeed found to induce cortisol release in Atlantic salmon (Mustafa *et al.*, 2000).

The present study was conducted to investigate the impact of monogenea (*Cleidodiscus aculeatus*) on hematocrite and Cortisol levels as well as pathological alterations in infested *C. carpio* together with providing detailed description to the parasite.

MATERIAL AND METHODS

Experiments were conducted in aerated glass aquaria (total volume 16 L with a content of 8 L water) that continuously provided with well aerated water (pH 7.4 - 7.5)with nitrate content less than 10 mg /L, no nitrite and ammonia, and a total hardness of 24 degree .

The temperature in some aquaria was adjusted to 20±1.8°C and in other were 25±.12°C using temperature controlled thermostats. The aquaria were covered with dark cloth and placed in dark, temperature controlled rooms to reduce irrelevant and stressful stimuli.

Cyprinus carpio reared in tanks at CLAR, were controlled daily and alterations of their morphology and behavior were recorded. Dead fish were gathered, recorded and examined macroscopically. During 2006 and 2007, the tanks were subjected to anthelmintic treatments which are usually used to control monogeneans in aquaculture; using solutions of formalin300 ppm for 30 min and mebendazole 0.4 ppm for (24 h) (Thoney and Hargis, 1991). Parasites from a sample of 22 *C. carpio* were collected, identified, and counted. Samples of the substratum of each affected tank were also collected and examined for the

presence of eggs. A second sample of 15 carp from the affected tanks was studied in order to observe possible physiological or anatomical effects in *C. carpio* related to the presence of *C. aculeatus*. Fish were killed with a blow on the head, sized and weighed. The Fulton's condition factor was calculated (weight/length^3) ×100= (K = W/L^{3}T x100 (Lagler, 1978). Blood samples were taken and the hematocrit value of each individual fish was determined. Each fish (skin, gills, viscera and eyes) was examined under a stereomicroscope. Samples of liver, spleen and the four left gill arches were collected and fixed in buffered 10% saline formalin. Intensity of *C. aculeatus* in individual fish was calculated by multiplying by 2 the number of parasites recorded on the four gill arches of the right side. The possible relation between the abundance of *C. aculeatus* and both the haematocrit value and the condition factor of each fish was studied using Spearman's correlation. Samples of gills, spleen and liver were processed for paraffin wax sections, stained with haematoxylin and eosin and studied under the light microscope (LM).

Pathogen free *C. carpio* fish were acclimated in the laboratory aquaria for30 days before use. The body lengths of the fish varied from 13.5 cm to 22.0 cm and body weights from 130 to160g. All fish in the experiment (infected and uninfected) were measured and exposed to exactly equivalent conditions apart from the experimental parameter varied.

Infection procedure

Before the start of the experiment, a total of 40 fish (eight replicate samples, five fish in each) were recovered for recordings. Then, duplicate samples of fish (five fish in each sample from each aquarium) were taken on day 3. Thus, a total of 100 fish were sampled. All fish were collected, immediately anaesthetized with MS-222 (85±100 mg l/1) and examined under the dissection microscope. The number of parasites (if present) on the fish was then counted. Subsequently, length and weight measurements were taken and Cortisol content in fish blood was correlated with the plasma content, Caudal vein puncture was used to recover plasma from the fish (Lindenstrùm and Buchmann, 2000) and immediately after a

piece of the dorsal caudal muscle was excised and processed as described above. Subsequently the cortisol was measured in the plasma and supernatant.

C. carpio was infected by cohabitation. Donor fish with a known number of parasites were tagged by fin cuts and placed (2-3 per aquarium) with uninfected fish. Thus, uninfected fish were in this way exposed to 10-12 parasites per fish. Artificial stress induced (stressful conditions) for selected groups, were fish once a day (days 0, 1, 2 and 3) caught and kept out of water for 5 min with intervals of 30 s in the water for recovery Ramadan,(2000).

Plasma cortisol concentrations of fish were measured with a commercial competitive ELISA kit (Lindenstrùm and Buchmann, 2000). Only one sample from each fish was tested .Experimental design two experiments, one at 20±1.6°C and one at 25±1.2°C, were conducted. At each of these temperature levels, duplicate groups of fish were uninfected, infected with *C. aculeatus*, or exposed to stressful conditions.

Statistics Means and standard deviations were calculated for parameters (weight, length, infection intensity, cortisol concentration) in every group. Differences between groups were evaluated with a Student's t-test. The Spearman rank correlation coefficient was used to express the association between tissue and serum cortisol. Differences were considered significant when $P<0.05$ Bush, *et al.*, (1997).

RESULTS

Description

Cleidodiscus aculeatus (Van Cleave and Mueller, 1932) Figure (1) &Table (1)

The worm is somewhat flattened dorsoventerally, with narrowly trunk elliptically in outline. Eyes spots are 4 in number which located in two levels where the posterior pair larger. Gut is bifurcated, without diverticulae rami confluent posteriorly. As most Ancrocephalids have a morphological character in common: The transverse haptoral bars articulate to form a single supported for the hamuli

and penis type recognition associated with high degree of specificity for major host texa Male copulatory complex comprises sclerotized, tubular penis with more or less curved shaft and more or less flattened base; accessory pieces well sclerotized and curved distally toward distal tip of penis and base bifid. Gonads located near the middle of the body. Cirrus usually a simple cuticularized tube Accessory piece is always present, generally articulated basally with cirrus. Vagina is present and open on left margin near middle of trunk. vitellaria of numerous, small discrete follicles which arranged in pair of lateral bands extending from pharyngeal region to or into the peduncle. The bands are confluent posteriorly and sometimes anteriorly. Haptor generally distinct, discoidal or subhexagonal; armed with 2 pair of bars and 7 pairs of hooklets. Anchors with superficial roots of each pair connected by transverse bar. Bars is none articulated with each other

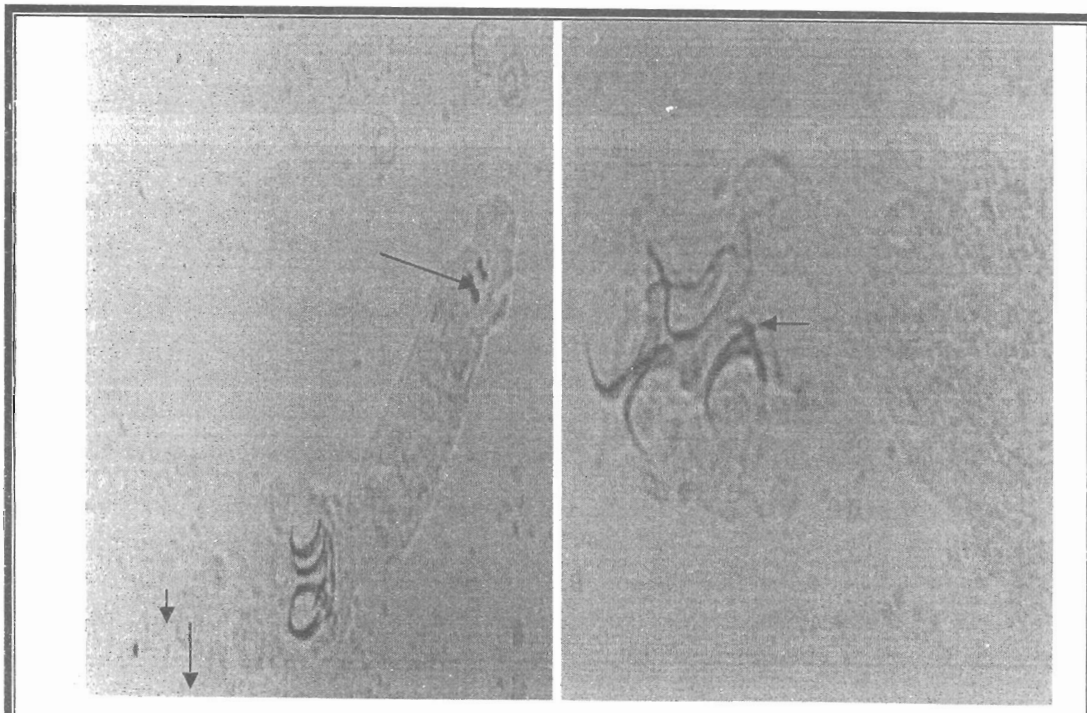

Figure 1.Wet mounts of *Cleidodiscus aculeatus and Trichodina* sp.) x100

Table 1. Measurements of *Cleidodiscus aculeatus*.

Body	length	581 um
	Width	93 um
Prohaptor	length	045um
	Width	075um
Opisthaptor	length	45um
	Width	75um
Dorsal hamulus		32um
Ventral hamulus		31 um
Dorsal trans. bar length		25 um
Ventral trans. bar length		39 um
Marginal hooklets length		7 pairs (35.1 um)
Penis length		38 um
Accessory piece		28(20-29)um

A mortality rate of 42.1% was observed for a total of 514 *C. carpio* in the infested tanks, during spring and summer of 2006 and 2007. The mortality in non-treated fish was 55.2% while in the treated fish it was 31.3% (Table 2). Most of the age classes were affected (3 to 8 months).

The external aspect of the fish was apparently healthy, with respect to both the coloration and weight. No anomalies in the behavior of affected individuals were observed, except for some slow swimming. However, hypersecretion of mucus on gills, accompanied by a large quantity of parasites (Fig. 1) and egg strings of *C. aculeatus* entangled in gill filaments, was observed in all dead fish. Egg strings were also very abundant in the substratum and the water of the tanks. The mean intensity of *C. aculeatus* (Fig.1) in the sample of 23 dead carps was very high. The appearance of the viscera was normal, with the exception of a slightly paler tonality of the spleen and, especially, of the liver of some fish, suggesting a possible anemia condition. Whitish plaques were observed in the zones of the gills where the parasites were attached (Fig. 2).

Table 2. Percentage mortality per tank of *Cyprinus carpio* associated with the presence of *C.aculeatus*.

Number of fish per tank (N)	Fish age(3-8 months)	Date	Treatment	Mortality (%)
Non treated tanks				
15	5+ and 6+	March	-	100
119	3+ and 5+	may\June	-	45
110	6+ and 8+	July	-	20
244		Total	-	55.2
Treated tanks				
130	8+	March	formalin	35
122	6+	may\June	mebendazole	32
18	6+	July	mebendazole	29
270		Total		31.3
514		Total		42.1

Out of the 15 specimens of carp fish collected from the infested tanks showed that 42.1% of fish burden with *C. aculeatus* with intensities ranging between 5 and 18 parasites per fish (Table 3). To some extent a relation was observed between the abundance of the parasite and the condition factor (r_S=0.175, n=15, p>0.5) Table (3). However, the haematocrit values were significantly lower in the fish infested with monogeneans (p=0.0156, see also Table (3). In fact, the relationship between parasite abundance and haematocrit values was negative and statistically significant (r_S=−0.625, n=15, p<0.01).

Table 3. parasite abundance and host parameters assessed for killed *Cyprinus carpio* associated with the presence of *C. aculeatus* However, these values were clearly correlated (R: 0.536, P <0:05).

Fish	Parasite abundance	Fish length(cm)	Fish weight(g)	Condition factor	haematocrit values%
infested fish	15±0.01	13±0.44	83±2.41	3.8±1.91	11
	9±0.31	13±0.26	72±1.33	3.2±1.61	19
	18±0.26	15±0.20	85±3.32	2.5±2.01	12
	5±0.37	21±0.38	96±4.41	1.03±1.01	20
	12±0.51	10±0. 12	69±2.91	6.9±1.01	12
	9±0.20	12±0.31	64±1.88	3.7±2.11	19
Non infested fish	0	15±0.33	93±5.75	2.8±1.51	20
	0	12±0.29	88±2.43	5.1±1.21	25
	0	11±0.24	65±2.36	4.9±1.11	22
	0	22±0.30	115±3.72	1.1±1.44	27
	0	24±0.21	125±2.92	7.1±1.03	28
	0	20±0.54	101±1.14	1.3±1.34	28
	0	13±0.02	85±2.017	3.8±1.61	29
	0	15±0.80	86±4.01	2.54±0.89	34
	0	14±0.04	90±9.07	3.2±1.34	38

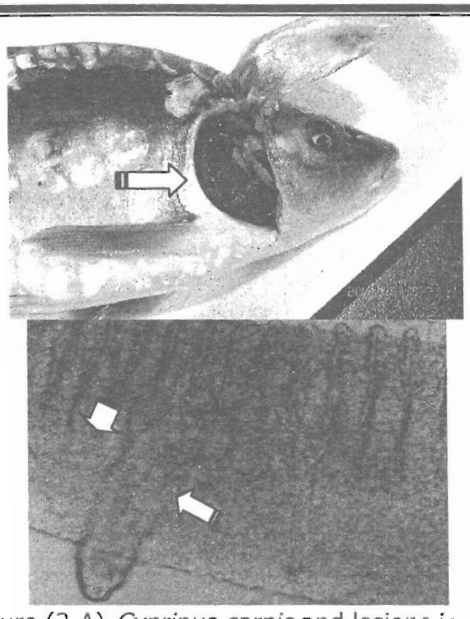

Figure (2-A) *Cyprinus carpio* and lesions in gills
Figure (2-B) *C. aculeatus* attaching to gill lamellae.
Haptor clamps grasped on lamellae, (arrowhead).

The external aspect of the fish was apparently healthy, with respect to both the coloration and weight. No anomalies in the behaviors of affected individuals were observed, except for some slow swimming and slightly secretion of mucus on gills, accompanied by a large quantity of parasites Figure (2-A) and the parasite attached to the gill lamellae where the hapator grasp on one or two lamellae Figure (2-B). However, In the late stage (Exhaustion period) and before death fish showed slow movements and sunken eye, fin and tail rot, sloughing of skin and of mucus, fish displayed a rough dirty appearance and Anemia and egg strings of *C. aculeatus* entangled in gill filaments, was observed in all dead fish (Figure 3). Egg strings were also very abundant in the substratum and the water of the tanks. The mean intensity of *C. aculeatus* in the sample of 23 dead carps was very high. The appearance of the viscera was normal, with the exception of a slightly paler tonality of the spleen and, especially, of the liver of some fish, suggesting a possible anemia condition. Whitish plaques were observed in the zones of the gills

where the parasites were attached Figure (4).

Figure 3. Late stage of infested carp showing hypersecretion of mucus , rough dirty appearance and, anemia

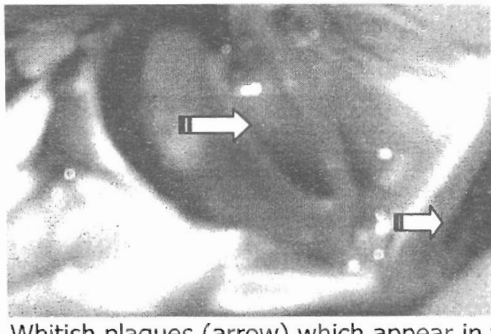

Figure 4. Whitish plaques (arrow) which appear in the zones of the filaments where *C. aculeatus* were attached

Figure (5) shows the gills heavily parasitized by *C. aculeatus.* Mild to moderate proliferate hyperplasia was also evident. Parasites were attached to the gill by means of their haptor clamps, with each clamp grasping one or two lamellae. Lamellar synechia of adjacent lamellae within a single clamp was often observed. Distortion of lamellar structure, synechiae and subsequent lamellar fusion commonly occurred in the vicinity of the attachment zone, although no severe proliferative cell response to the parasite was observed. The haptor of *C. aculeatus* exhibiting two rows of clamps and when the parasite attached to the host, the clamps grasped the lamellar tips, with one or two lamellae being inserted into them like the teeth of a zip. Cysts corresponding to the proliferative cell response of Trichodina sp. were also observed at the vicinity of the trailing edge of the gill filament Figure (6). The compression of the lamellar tips by the clamp

sclerites induced lamellar clubbing and occasionally led to the rupture of distal epithelial and vascular structures, particularly those of the marginal vessel (Figure 6 and 7).

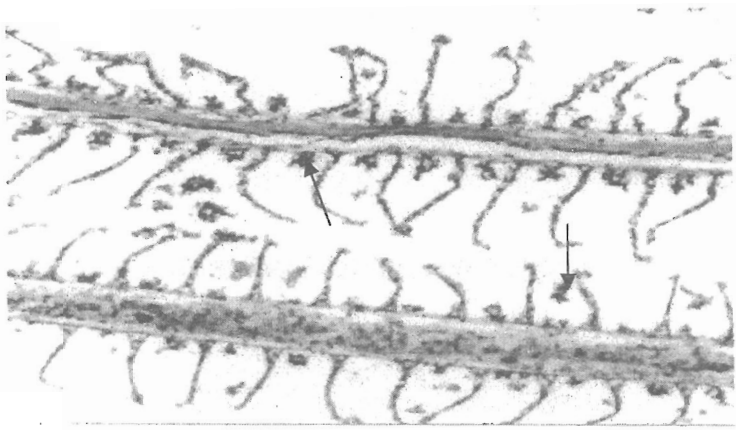

Figure 5. *Cleidodiscus aculeatus* attaching to the gill lamellae of severely parasitized fish. The clamps of the haptor grasped one or two lamellae, which led to lamellar synechia (arrowhead). (×150).

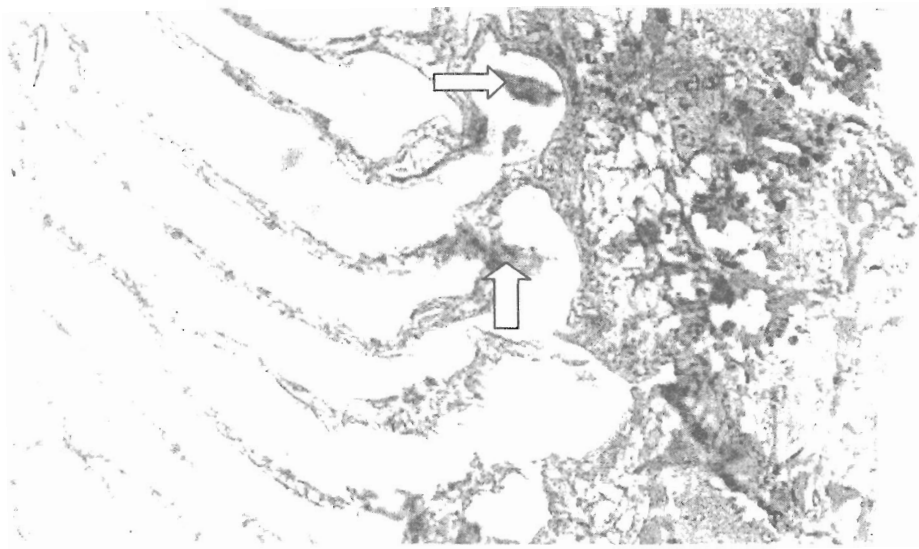

Figure 6. Mild to moderate hyperplasia in infested fish gills (arrows). (×200).

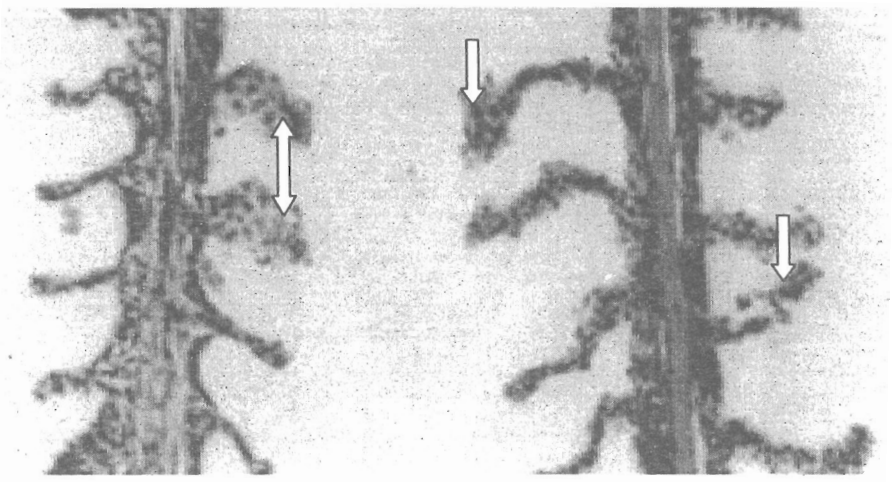

Fig. 7. Gill lamellae of *Cyprinus carpio infested* with *Cleidodiscus aculeatus*. Showing disruption of the lamellar structure, lamellar fusion, lamellar clubbing (arrowheads) and proliferative cell response. (arrows). ×500

Fish infected by cohabitation at the two temperature levels 20°C and 25°C obtained mean infections of parasites per fish, respectively. All exposed fish were infected. Duplicate groups did not differ with respect to cortisol level and were combined. Thus, all groups mentioned below each consist of ten fish. On day 0 cortisol concentrations in plasma from the four baseline groups were expressed as means of 21.5, 28.4, 24.7 and 25.1ng ml/1. Cortisol levels were significantly elevated after 3 days in groups exposed to handling. Thus, at 20°C the concentration reached a mean of 37.5ng ml/1, whereas parasite infected fish showed a significantly increased level (28.1ng ml/1) compared with controls (20.1ng ml/1). Also at 25°C the stressful stimuli (handling) caused significantly increased Cortisol concentrations (47.5ng ml/1). This was also seen in infected fish (35.2ng ml/1) compared with controls (23.1ng ml/1). However, at this higher temperature the concentrations in all groups were generally higher than at 20 °C Table (4).

Table 4. Cortisol concentrations in ng ml/L in plasma of *C. carpio* (means of ten fish and two duplicate groups combined) following 3 days with parasites, stress or with no exposure.

Temperature(°C)	20±1.6(°C)	25±1.2(°C)
Parasitized	28.1	35.2
Stressed	37.5	47.2
Control	20.1	23.1

DISCUSSION

Relevant effects on fish hosts related to the presence of *Cleidodiscus aculeatus* have not been reported in cultures of *Cyprinus carpio*. However, important losses of *Cyprinus carpio*, possibly associated with the presence of *C. aculeatus*, have also been recently observed in cultures at two different localities in the Port-Said province *C. carpio* (Ramadan, 2000) and now in Abbassa fish farm. In the case of *C. carpio* despite the healthy external appearance of the fish, their gills showed general signs of severe monogenean infestation, such as hypersecretion of mucus or lesions of the lamellar gill epithelium and vascular structures. Hyperplasia of the gill epithelium with fusion of adjacent gill lamellae has also been observed in association with the presence of parasite. Lesions caused to *C. carpio* gill tissue by *C. aculeatus* described in the present work, as well as mechanical obstruction of water fluxes between filaments due to the presence of the parasites might undoubtedly induce gill dysfunction. Nevertheless, the role played in respiratory and osmoregulatory impairment by hyperplasia (Mahfouz, (1997). The fact that hematocrit values were negatively correlated with the abundance of the parasites can be related to the blood-feeding activity of *Z. seriolae*. monoopistocothyleans are essentially hematophagous and, when they are very numerous, can diminish considerably the volume of blood of the host (Llewelyn, 1954). Moreover, the rupture of the marginal vessels of gill lamellae caused by clamps, and the subsequent microhaemorrhages may have also contributed to the lower hematocrit values shown in *C. aculeatus* infected *C.*

carpio. The synergic detrimental effect of the anemic condition and the gill damage caused by the parasite might have seriously compromised both gas exchange and oxygen transport to tissues of diseased *C. carpio*.

Concerning the mechanisms of transmission and infection establishment, a large numbers of parasites and eggs revealed in both the tanks and gills indicate a high rate of reproduction and easy transmission of *C. aculeatus* under aquaculture conditions. the case may be resample the specific characteristics of the eggs of *Allencotyla mcintoshi* as described by (Montero, 2001). The long filaments (strings) on both poles of eggs can easily be attached to gill filaments and join together forming light masses that get hooked up to the handling nets used in aquaculture, thus spreading the infestation among tanks. Also Ogawa and Yokoyama (1998) observed similar patterns in strings of eggs of *H. heterocerca*. These traits, combined with the long survival time of *C. aculeatus* and the relatively high resistance of its eggs to desiccation, might facilitate the transmission of this parasite. So it is therefore strongly suggested that, the disinfection of the nets, tank surfaces and pipes in the aquaculture articles.

A variety of species of dactylogyroids found in a lot of fish families have been assigned to *Ancyrocephalus*, but in fact are representatives of diverse (still undefined) generic entities. *Cichlidogyrus, Onchobdella* (with one species adapted to infect the skin Paperna, 1968) and *Enterogyrus* (endoparasitic in the stomach) are host specific to diverse cichlid fish species. The genera *Annulotrema* and *Characidotrema* are host specific to characid fish. *Afrocleidodiscus* occurs in both Characidae and Citharinidae and fish of the latter family are also hosts for *Nanotrema*. Several genera occur in siluriforms, some are congeneric (*Quadriacanthus*) and others have a definite affiliation to dactylogyroids infecting Southeast Asian siluroids. The detailed morphological characters coincided with the description provided by Muller (1934) and (Ha Ky, 1968; Paperna, 1979) with some differences in head organs and cephalic glands according to species.

It is noteworthy that cortisol levels in stressed fish at 20±1.6C were generally lower compared with similar fish at 25±1.2C. This could be explained by

a higher metabolism at 25±1.2C, whereby produced cortisol could be broken down or lost by diffusion at a higher rate than seen at 20±1.6C Alternatively, a low temperature could by itself act as a stressor as suggested by Wagner et al. (1997). Concerning the sampling technique, it should be mentioned that MS-222 treatment will block further cortisol production in fish during anaesthesia (Thomas &Robertson, 1991). Thus, the sampling method, including MS-222 treatment immediately after capture, used in the present study is unlikely to affect measured cortisol concentrations. It is relevant to test the effect of Dactylogyridae infections on fish because these hosts are far more vulnerable to infection than larger fish. However, it was shown that handling stress induced a marked increase of body fluid cortisol at both temperature levels. Similar handling stress was previously found to increase the cortisol levels in the plasma of larger fish (Thomas and Robertson, 1991; Nielsen and Buchmann, 1997; Mahfouz, (1997); Pottinger *et al.,* 1999). The parasite infection did also affect the host at 25±1.2°C but to a lesser degree than was seen with handling. Thus, cortisol concentration increased about 12ng ml/1 at 25±1.2°C and 10ng ml/1 at 25±1.2°C after infection. The infection level (9 parasites per fish) used in this experiment was relatively low and it cannot be excluded that higher levels might prove more stressful. Thus, the parasite *C. aculeatus* attaches to the host epidermis by the insertion of 16 marginal hooklets into the epithelial cells and feeds by browsing the epithelium whereby marked injuries appear in the skin (Buchmann and Bresciani, 1997). It would be expected that cortisol release is positively related to the intensity of infection. Extensive damage is caused by skin-parasitizing parasitic copepods such as sea lice and these do elicit a stress response. Recently, Mustafa *et al.* (2000) detected a marked increase in plasma cortisol of Atlantic salmon (*Salmo salar*) post-smolts following heavy infections with sea lice, *Lepeophtheirus salmonis*, which will add to the notion that ectoparasites can be considered as stressors. The present investigation indicates that all infections (even at low intensities) with gyrodactylids are at leas occasionally, pathogenic and immunosuppressive. It was suggested by Cusack and Cone (1986) that gyrodactylids can act as vectors for various viral and

bacterial pathogens. These could easily penetrate the host through worm induced portals in the host skin and infect the fish. Increased cortisol levels due to the irritating effect of the parasite could then be responsible for suppression of the host response towards the pathogen. Kurogi and Iida (1999) showed that stressed tilapia fish with increased plasma cortisol exhibit inferior activity of neutrophils, important effect or cells in the inflammatory response. In addition, several other humoral and cellular immune parameters are likewise depressed by cortisol (Pickering, 1993) and this increases the susceptibility of hosts to pathogens such as *Saprolegnia parasitica* (Pottinger and Day, 1999). Therefore, it cannot be excluded that parasites can have a double role in pathogenesis. This would include both production of entries for pathogens and subsequently suppression of the host response against secondary infections. The reason that the carp *C. carpio* despite cortisol production, end up rejecting *C.aculeatus* (Lindenstrùm and chmann, 2000)

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التأثيرات المحتملة لكيليودسكس اكيولاتس على اسماك المبروك العادي

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١. *قسم صحة الأسماك- المعمل المركزي لبحوث الثروة السمكية - مركز البحوث الزراعية*
٢. قسم الامراض الباطنه والمعديه والاسماك كلية الطب البيطرى – جامعة المنصورة.

تمت الدراسةلاهمية العدوى المرتبطه باعداد الوفيات فى المبروك العادى المربى فى الاحواض الدائريه فى المعمل المركزى لبحوث الثروة السمكيه بالعباسه خلال الفترة من ٢٠٠٦- ٢٠٠٧ ، وقد وجد انه عدد الطفيليات مرتبط ارتباطا وثيقا بطفيل الكيليودسكس اكيولاتس *Cleidodiscus aculeatus* .

كذا فقد وجد ان هناك وفره فى الحمل الطفيلى فى كل الاسماك الميته وكانت تلك الطفيليات منغمسه فى الخياشيم.

وبالتحليل الطفيلى لعدد ٣٠ سمكه تمك تجميعها من الاحواض الدائريه المصابه تبين بان نسبه ٧٣ز٢% من الاسماك تحمل ثقل طفيلى يتراوح من ٥-١٣ طفيل للسمكه الواحدة.

ايضا فقد تبين وجود علاقه بين الوفرة الطفيليه وعامل الشرط (روبية = ٠,١٧٥ ، ن = ١٧ ، ف> ٠,٥) ومع ذلك فان القيمه الدمويه hematocrit (PVC) كانت اقل بكثير فى الاسماك المصابه احادية العائل.

وفى ضوء المسح النسيجى للخياشيم المصابه بطفيل السيليودسكس اكيولاتس تبين ان الديدان تلتصق بخياشيم العائل وتشتبك بواحد او اثنين من افراد الخيشوم gill lamellae بكل مشبك من مشابك الدودة haptor ،الامر الذى ادى الى الامتزاج الخلوى لرقائق الخياشيم synechiaes والتجمع الخلوى للخياشيم واضطراب الهيكل الطلائى والاوعيه الدمويه عن القيا بوظائفها الحيويهز كذلك لوحظ وجود عدوى تتراوح من خفيفه الى معتدلة من الاصابه بطفيل التريكودينا(الهدبى الاولى) وعليه فان الاثار المجتمعه لهدم الخياشيم كذا والتغذيه التطفليه على دم الاسماك يمكن ان يعزى اليه فقر الدم وموت الاسماك المحقق فى النهايه.

اما بخصوص الانتاج الملاحظ للهيدروكورتيزون لاسماك المبروك العادى (١٥٠ ± ١,٦ ملجرام من وزن الجسم) المصاب بطفيل الكيليودسكس اكيولاتس *Cleidodiscus aculeatus* فقد تمت دراسة تحت مستويين من درجات الحراره مستويات اثنين ، ٢٠ ± ١,٦ درجة مئويه و ٢٥ درجة مئويه ± ١,٨ على التوالي.

وتبين ان الاسماك المصابه اظهرت ارتفاع مستوى الهيدروكورتيزون مقارنة بالاسماك غير المصابه.ومع ذلك فإن تركيز الهيدروكورتيزون كان اقل مما كان عليه في الاسماك المعرضه للاجهاد اليدوى، فعند درجة حرارة ٢٥ درجة مئويه كان مستوى تركيز الهيدروكورتيزون مرتفع معنويا فى الاسماك المصابه عنه فى الاسماك غير المصابه.

ومن وجه نظر الباحثين يحتاج البحث إلى مزيد من الإمكانيات التقنية التى قلصت من نتائج البحث وعليه يتم استكماله بدراسات أخري .