First record of *Benedenia sciaenae* (Monogenea: Capsalidae) infecting the brown-spotted grouper fish *Epinephelus chlorostigma* (Family: Serranidae) from the Red Sea in Egypt

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Abstract: *Benedenia* (Capsalidae) is a genus of important oral and cutaneous fluke parasite of aquarium, cultured and marine fish. In the present study, the morphological and morphometric characterization of *Benedenia sciaenae,* a monogenean parasite infecting the gills of the brown-spotted grouper fish *Epinephelus chlorostigma* were described by means of light microscopy as a first description from *Epinephelus chlorostigma*. 215 out of 290 (74.1%) fish samples were found to be infected with this ectoparasitic capsalid causing pathogenic and epizootic events. The adult worm is flattened, elongated with an anterior adhesive organ enclosing two anterolateral adhesive structures, each one possesses three lobes which aids for adhesive secretions while the enlarged posterior end enclosing haptor. The adult worm measured about 0.52 -0.67 (mean 0.59 ±0.03) mm in total length and 0.33 – 0.49 (mean 0.38 ±0.02) mm in width. Haptor width measured 0.25-0.29 (mean 0.26 ± 0.02) mm; its hard parts consist of two pairs of hamuli and the accessory sclerites. The anterior hamulus measured 0.027-0.034 (mean 0.31±0.002) mm long while the posterior one measured 0.030-0.040 (mean 0.036±0.002) mm and each of the accessory pieces measured 0.032-0.044 (mean 0.040±0.002) mm long. Results showed that the general morphology of the present *Benedenia* sp. resembles that of *B. sciaenae* described previously in Turkeys from *Argyrosomus regius* fish host with the dimensions of body more or less similar. Also, there were significant correlations ($P \leq 0.05$) between fish length, weight and parasite abundance per fish. Number of monogeneans was increased with host size and age to fish of intermediate length and weight, and then it decreased probably because changes in size of gill filaments affect their attachment capability, enhancing the possibility of being detached by respiratory currents.
[Kareem Morsy; Sayed Abdel-Monem; FathyAbdel-Ghaffar; Abdel-Rahman Bashtar; Ali Al-Ghamdi and Rania Abdel-Gaber **First record of *Benedenia sciaenae* (Monogenea: Capsalidae) infecting the brown-spotted grouper fish *Epinephelus chlorostigma* (Family: Serranidae) from the Red Sea in Egypt]** Life Science Journal,. 2011; 8(4):245-252] (ISSN: 1097-8135).

Key words: *Benedenia sciaenae* – Monogenea – Capsalidae - *Epinephelus chlorostigma*- Red Sea – Light microscopy.

1 Introduction

Monogenea is a class of platyhelminthes parasitic mostly on external surfaces and gills of freshwater and marine fishes (Whittington *et al*., 2004). Boeger and Kritsky (2001) recognized 53 families based on morphological characters. Most of monogeneans are highly host specific (Hargis, 1955 and Lawler, 1981), which aids in the specific identification of worms from a particular host. Benedeniide monogeneans belonging to the family Capsalidae are pathogenic to fish under culture (Thoney and Hargis, 1991; Chisholm *et al.,* 2004). Some of these large capsalids are known to be concealed on their hosts, a phenomenon first reported by Van Beneden (1856**)** for *Benedenia sciaenae* from *Sciaena quila* (Sciaenidae) of the Belgian coast. Several taxonomic and morphological studies have provided some knowledge about geographical distribution, host range of Benedeniinae (Whittington and Horton, 1996; Egorova, 1997; Whittington *et al*., 2001a). Tokşen *et al.* (2007) studied the Infestation of *Benedenia sciaenae* van Beneden, 1856 of Cultured Meagre (*Argyrosomus regius* as a new host record) in Turkeys, they described this parasite belonging to Benedeniinae due to the presence of one pair of accessory sclerites and two pairs of hamuli. *Benedenia seriolae*, has been a longstanding pathogen of species in intensive culture in Japan (Whittington *et al*., 2001b). Approximately 20% of the total production costs for farmed Carangidae species in Japan are spent to control *B. seriolae* (see Ernst *et al*., 2002). Ogawa *et al.*(1995) study the development, infection rate and pathogenicity of *Benedenia hoshinai* which infects the Japanese striped knifejaw *Oplegnathus fasciatus* cultured in a net pen in Nagasaki, he found that the intensity of infection was estimated to be 80-220 worms per fish causing ulcers at the infection sites which may decrease the growth and productivity of the infected fish leading to death. Also he found that the number of worms on the host sharply decreased as they grew bigger than 1.5 mm long, suggesting that most small

worms detached themselves from the host before reaching that size.

The present investigation aims to study the prevalence of natural infection with monogenean parasites in addition to their morphological and morphometric characterization by means of light microscopy. Also, the relationship between length, weight of infected fish and the extent to which they are parasitized by this monogenean was studied to determine if the frequency and intensity of parasitism changed over time.

2 Material and Methods

A total of 290 specimens of *Epinephelus chlorostigma* (Forsskal, 1775) fish (family: Lutjanidae Valenciennes 1828) (size range: 10 – 25 cm, mean 18.5 ± 7.15cm; body weight 100 – 250 g, mean 205 ± 20 g) were caught from the coasts along Gulf of Suez and Hurghada city, Red Sea, Egypt. Samples were obtained at irregular intervals in 2010-2011. Fish were identified according to Randall (1983) and their modern names follow Froese and Pauly (2011). They were immediately transported in water tanks to the parasitology laboratory, Zoology Department, Faculty of Science, Cairo University. To prevent the loss of mobile and temporary ectoparasites, the captured fish were kept alive in aquaria filled with the same water source and examined within few hours. Firstly, the characteristics of the host such as standard length and weight were noted. Skin surface, fins and gills were then examined by naked eyes and with the help of dissecting microscope for any attached parasites, lesions or external changes. After removing opercula and exposing gill arches, each gill was removed carefully from the fish, immersed in normal saline to remove any excess gill mucus. Monogenean parasites were recovered with a Pasteur pipette using a dissecting binocular microscope. The monogeneans were fixed in 4% formalin and the worms were washed with distilled water to remove excess fixative. Worm identification was confirmed by mounting specimens on slides in drops of ammonium picrate glycerine under cover slips, and examining hard parts using light microscopy. For permanent whole mount preparation, some of the fixed and flattened specimens were stained with acid carmine followed by washing in a ascending alcohol series and then cleared in clove oil, xylene and then mounted with Canada balsam (Ergens and Dulmaa, 1969). For each manogenean parasite, the sclerotized parts of the haptor were drawn using Camera Lucida and measured using an ocular micrometer calibrated against a stage micrometer slide according to Gussev, 1985 (Bychovskaya –Pavlovskaya *et al*., 1962). Ten specimens were measured for the range and the mean ± standard deviation (SD). Prevalence, mean abundance and measurements followed the guidelines of Bush *et al*. (1997). In order to determine if number of parasites present was related to fish length and weight, correlation test was performed to determine if there is any association between these parameters and parasite load. The total and lengths of the fish were measured in centimeter (cm) using a measuring board. Fish samples were weighed to the nearest gram (g) using weighing balance. To satisfy the assumption of the statistical analyses used, all infection data number of parasites per length and weight of host were analyzed by t test at 95% confidence level to achieve homoscedasticity or linearity. Values of $p<0.05$ were considered as statistically significant.

3 Results

Two hundred and fifteen (215) Out of 290 *E.* chlorostigma fish with a prevalence of 74.1% were infected with the ectoparasitic *Benedenia* sp. The parasite have flattened, elongated symmetrical body tapering anteriorly and enlarging to haptor level posteriorly (Figs. 1,2). The total body length was 0.52 -0.67 (mean 0.59 ±0.03) mm while its width at mid level of testes measured 0.33 – 0.49 (mean 0.38 ±0.02) mm. Two pairs of eye spots were present anterior to pharynx (Fig. 3), the anterior pair being smaller than the posterior one. Pharynx measured about 0.08 – 0.21 (mean 0.10 ±0.02) mm in diameter. Two ellipsoidal equatorial testes were observed at the mid part of the body, each measured 0.12 – 0.18 (mean 0.16±0.02) mm in diameter. Ovary pretesticular and oval in shape. Vetellaria follicular occupying almost the entire available space of the body proper. Two anteroventral attachment organs were observed anteriorly and measured 0.046 – 0.068 (mean 0.06 ± 0.003) mm for each (Fig. 3). Posteriorly, the worm is armed with a disc-like haptor supplied with hooks (Figs.1,2). These haptor was oval, aseptate, the basic arrangement comprises a saucer-shaped attachment organ measured 0.25-0.29 (mean 0.26 ±0.02) mm and armed with three pairs of median sclerites that are usually large and 14 small hooklets at the periphery of the haptor proper and a thin membranous marginal valve around the edge (Figs. 3,4). Median sclerites comprise a central pair of accessory sclerites located anteriorly and two pairs of ventrally directed hamuli as anterior and a posterior pairs (Figs.4,5). Accessory sclerites (1 pair) stout, scoop-shaped with pointed tips directed anteriorly measuring 0.032-0.044 (mean 0.040±0.002) mm long (Fig. 6). Anterior hamuli long, slender, curved posteriorly, but straight at anterior end and measure 0.027-0.034 (mean

0.31±0.002) mm long. Posterior hamuli slender, with smoothly recurved posterior terminus measuring 0.030-0.040 (mean 0.036±0.002) mm . Marginal hooklets arranged radially in haptor region and measured 0.002-0.005 (mean 0.003±0.001) mm (Fig. 7). Line diagram of the worm body, haptor, sclerites, hamuli and hooklets was shown in (Fig. 8).

The highest prevalence of infection was obtained in the examined fish species within the standard length (15 – 20cm) and weight (70 – 95g) ranges as shown in (Figs. 9, 10). The parasites number was lowest in the smallest and lightweight fishes (13.0 – 17.0 cm, 20 – 60g). An increase was recorded in fishes having 18.00 – 23.00 cm body length and 62 – 85g weight, followed by a decline in larger and heavyier fishes (more than 25 cm, 100 g). Overall, the results of this experiment supported the hypothesis that increased fish length and weight are correlated with an increased number of parasites per fish.

Taxonomic summary

Type-host: *Epinephelus* chlorostigma (Family: Serranidae).

Site of infection: infecting the gills of fish host.

Type-locality: Gulf of Suez and Hurghada city, Red Sea, Egypt

Prevalence: 290 fish samples were examined for monogenean parasites, 215 (74.1%) fish were infected.

Materials deposited: Slides were deposited at Zoology Department museum, Zoology Department, Faculty of Science, Cairo University, Egypt.

Etymology: The specific genus name relates to the phenomenon first reported by Diesing (1858) for *Benedenia* sp.

4. Discussions

Gill filaments and gill lamellae of the host fish act as an important and predominant source of food and provide a relatively safe shelter for gill monogeneans as well as other ectoparasites dwelling the surface of the host gills. The gill apparatus possesses numerous attachment sites that accommodate the haptoral elements of the invading monogenean worms and provide these parasites with components of the gill tissues (blood, epithelial cells or mucous). The Benedeniinae are the largest of nine capsalid subfamilies, includes genera with an aseptate, apapillate haptor and a pair of discrete testes. Thirty three nominal benedeniine genera were previously reported worldwide and no records of Benedeniinae worm in Egypt, these are *Benedenia* (Diesing, 1858); *Allobenedenia* (Yamaguti, 1963); *Allometabenedeniella* (Velasquez**,** 1982); *Dioncopseudobenedenia* (Yamaguti 1965); *Lagenivagino pseudobenedenia* (Yamaguti, 1966); *Oligoncobenedenia* (Yamaguti, 1965); *Pseudallobenedenia* (Yamaguti, 1966) and *Tareenia*, (Hussey, 1986) *Allobenedenia* and *Allometabenedeniella* were transferred to the *Trochopodinae* by Price, 1936; *Menziesia* Gibson, 1976; *Benedeniella* Johnston, 1929, *Calicobenedenia* Kritsky and Fennessy, 1999 and *Trimusculotrema* Whittington and Barton, 1990 are considered to belong in *Entobdellinae* Bychowsky, 1957. Morphological characteristics of the described worm here resembled that of the monogenean, *Benedenia* sp described by Tripathi (1957) who reported that heavily infested fish showed excessive mucus secretions on the body surface. The morphology of the anterior ends of the majority of benedeniine genera is similar because they bear characteristic anterior attachment organs (Whittington and Barton, 1990; Whittington and Horton, 1996).

Whittington *et al.* (1994) have reported that the anterolateral region of each attachment organ is divided into three adjacent zones. The morphology of the disc – shaped, sucker-like anterior attachment organs suggests that these structures are capable of generating suction. the arrangement of the three adhesive zones on each side of the head among monogeneans appears to be a relatively common morphological feature (Kearn, 1994).

Among the capsalids, the number of the anterior attachment zones on each side of the head could be of taxonomic importance. The basic, cup-like suctorial haptor of adult capsalids help in the secure attachment to rough surfaces, this haptor is composed of one pair of median sclerites and two pairs of ventrally directed hamuli, an anterior and a posterior pair. There is evidence from some species that median sclerites and hooklets do effect mechanical attachment of the parasite to host tissue, but in other species, the capacity of the capsalid haptor to generate suction has contributed appreciably to their expansion across a wide range of host surfaces (Kearn, 1994). The arrangement of musculature external to the haptor and the way these elements interact play a major role in how the haptor generates suction. Kearn (1994) concluded that the presence of a marginal valve is critical to maintaining suction. There is unsubstantiated evidence that *Trimusculotrema* species, which lack a marginal valve and also lack large median sclerites in the haptor, may use an adhesive to attach to host ray skin. Heavy infection of *B. epinepheli* caused not only haemorrhagic and abrasive lesions, but also mortalities in cultured marine fish due to severe necrosis of the gill tissues possibly resulting in suffocation (Leong, 2001).The present described species resembles *Benedenia sciaenae* Van Beneden,

1856 reported from cultured meagre (*Argyrosomus regius*) in Turkey in its general morphology but with nearly large dimensions in the present study.

From the present study, the absence of parasites on small sized fish may be due to the small size of scales in fishes, where the parasites cannot maintain proper hold onto the body of the host. Moreover, a decrease in the parasites burden seen in the very large fishes is observed. One reason for this might be the random selection of specimens that is; more of the juveniles and sub-adults were examined as compared to the adult fishes. Another reason for it may be due to the development of acquired immunity as reported by (Etchegoin and Sardella, 1990; Tasawar and Khurshid, 1999; Tasawar and Naseem, 1999; Tasawar and Shazad, 2001; Akinsanya *et al*., 2007). However, as reported by Roberts *et al.,* (2000), bigger fishes provides larger surface area for infection than smaller ones. It is therefore a plausible explanation that the big fishes provide a good ground for the parasites to multiply over time. An increase in size is a reflection of increase in length which is considered a measure of age (Boxshall, 1974; Torres *et al*., 1977; Kabata and Whitaker, 1981; Etchegoin and Sardella, 1990). The higher parasitism observed in sub-adults over juveniles might be as a result of change in diet of the fish from weeds, seeds, phyto and zooplanktons as juveniles to insect larvae, snails, crustaceans, worms and fish as adulthood is attained (Anderson & Gordon, 1982). Another possible reason for the higher parasitism in the sub –adults over juveniles may be that of activity. The sub-adults as expected would be more active than the juveniles and probably even adults. As such, are able to compete better for food than the other age groups which mean that more contact with food and hence a higher tendency of getting infected with parasites.

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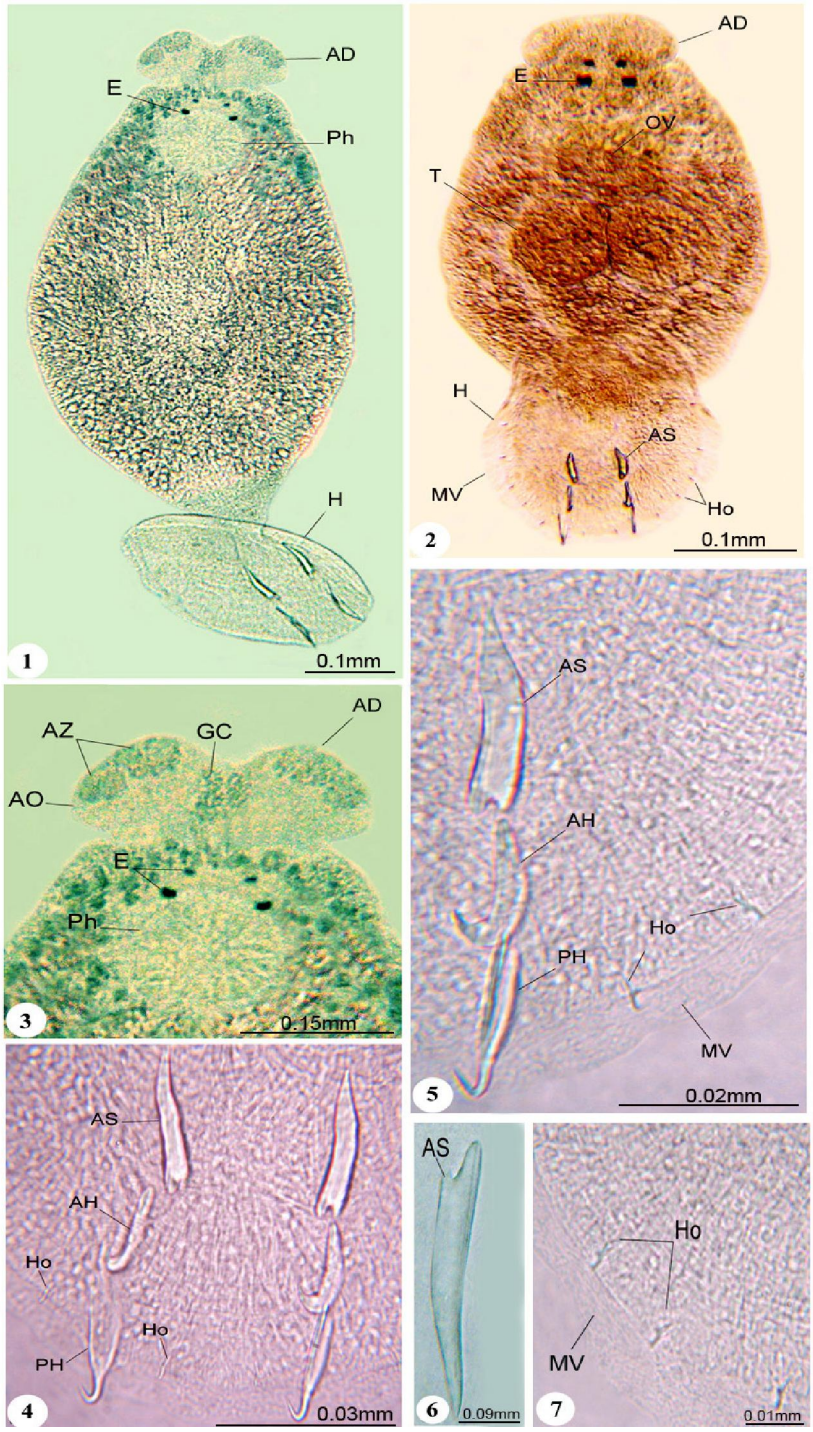

Figs.1-7 Photomicrographs of the adult *Benedenia sciaenae* showing: ***1***, ***2*** the adult worm with a flattened body consist of the anterior adhesive organs (AD), two pairs of eyes (E), large pharynx (Ph), the haptor region (H), two large equatorial testes (T) and a pretesticular ovary (O). The haptor (H) is bordered by a marginal valve (MV) and contains one pair of accessory sclerites (AS) and two pairs of hamuli. The periphery of haptor is supported by a large number of hookelts (Ho). ***3*** A high magnification of the anterior body region of the worm showing the anterior attachment zones (AZ), each consists of anterior attachment organs (AO). A zone of gland cells (GC) also seen between these organs. *4, 5* High magnifications of the haptor region showing haptoral elements as one pair of accessory sclerites (AS) located anteriorly, two pairs of hamuli, an anterior pair (AH) and a posterior one (PH).

Haptor is bordered by a marginal valve (MV) supported with hooklets (Ho). ***6*** The accessory sclerite (AS). *7* The marginal valve (MV) with tl

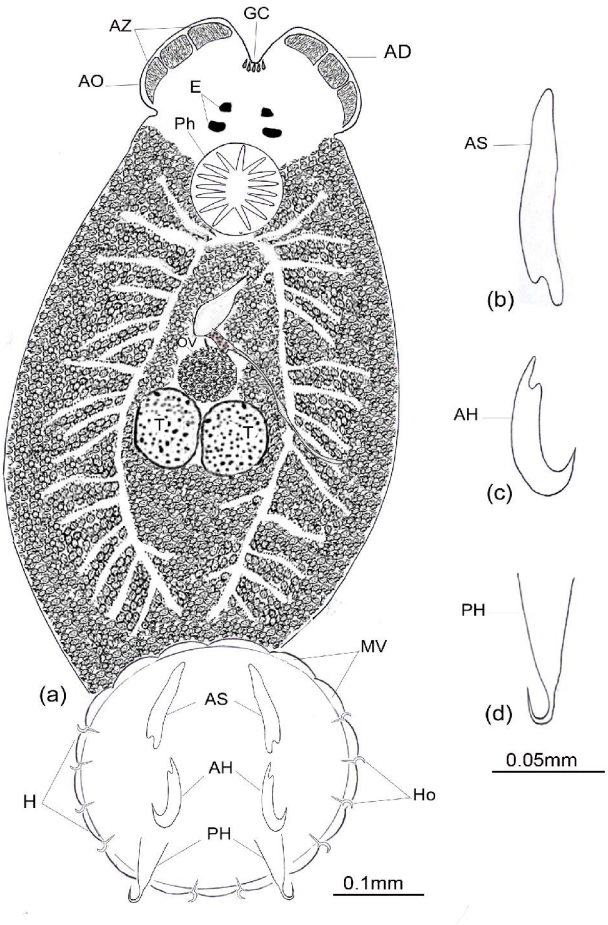

Fig.8. Schematic drawings of the adult Benedenia bohari showing the structure of its body. *a* The adult worm with anterior adhesive organ (AD) containing two pairs of eyes (E), the anterior attachement zones (AZ) and organs (AO) with its gland cells (GC) and pharynx (Ph). The haptor (H) is composed of one pair of accessory sclerites (AS), two pairs of hamuli anterior (AH) and posterior ones (PH), b-c High magnifications of the *b* Accessory sclerite (AS). *C* Anterior Hamulus (AH). *d* Posterior Hamulus (PH).

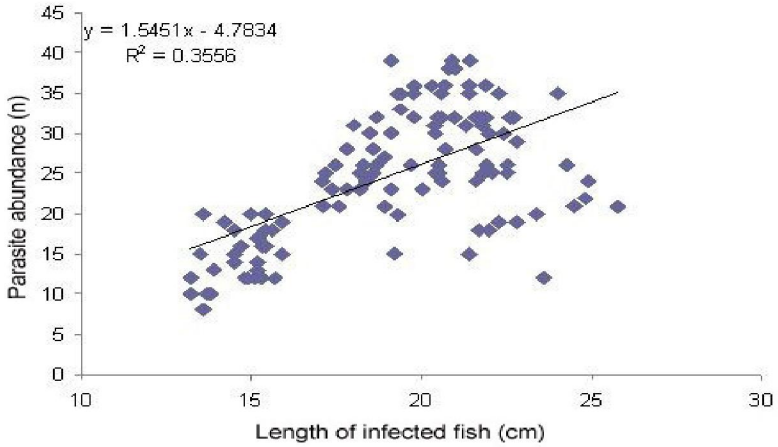

Fig. 9. Relationship between the number of parasites per fish as a function of fish length. Positive correlation was observed, the number of parasites increased by increasing length of the infected fish till a limit where at high fish length, the parasite abundance decreased.

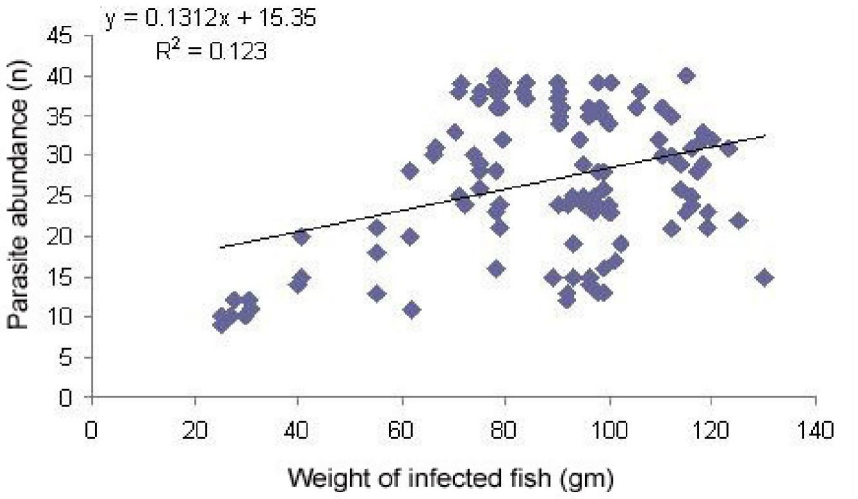

Fig. 10. Relationship between the number of parasites per fish as a function of fish weight. Positive correlation was observed between parasite abundance and weight of the infected fish till a limit where at high fish weight the number of parasites decreased.