Histopathological reactions of the blue shark, *Prionace glauca*, to postlarvae of *Hepatoxyylon trichiuri* (Cestoda: Trypanorhyncha: Hepatoxyllidae) in relationship to scolex morphology

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Abstract. Postlarvae of the cestode *Hepatoxyylon trichiuri* (Holten, 1802) were found attached to the surface of viscera of *Prionace glauca* (Linnaeus, 1758) taken from Atlantic coastal waters off the south coast of Massachusetts, USA. Gross anatomy of the attachment site shows a quadriradiate rim surrounding a deep pit with holes corresponding to the penetration site of each tentacle. Hyperplasia of the liver capsule into outgrowths at the attachment site conform to the attachment of the bothridia. The cuboidal epithelium of the liver capsule became columnar forming papillary outgrowths, with a dense fibrotic reaction beneath the attachment site and infiltration by leukocytes and pigment containing granulocytic cells. Blood sinuoids beneath the attachment site are greatly enlarged. Postlarvae attached to the surface of the epigonal organs of three blue sharks were not accompanied by reactions. SEM examination of the scolex of *H. trichiuri* postlarvae revealed fused pairs of bothridia within infolded muscular lateral rims, porese tegument devoid of microtriches and a bilateral plane of symmetry in the tentacular armature.

Linton (1897, 1906, 1909, 1912, 1923) appears to have been the first author to draw attention to the effects of cestode larvae, especially trypanorhynchs, on their marine fish hosts (Williams 1967). Rees (1967) reviewed the pathogenesis of adult cestodes noting the more injurious effects of those armed with hooks citing studies on onchocealeiid tetraphyllideans and diphyllyaleans parasitic in elasmobranches. She further noted that, in general, the pathogenesis of fish cestodes had received little attention compared to cestodes of other vertebrate classes. Numerous papers have been devoted to the diversity of cestode holdfasts (Linton 1938) and the modes of attachment of adult cestodes to the mucosa of the elasmobranch spiral intestine (Euzet 1954, 1959, Rees 1961, Rees and Williams 1965, Williams 1966, 1968a, 1968b, 1984, Williams and Campbell 1977a), but none of these included trypanorhynchs. The pathogenic effects of adult trypanorhynchs were described by Williams (1982) in comparing mucosal topography and the distribution of adult cestodes in the spiral intestine of hosts representing three families of elasmobranches (see Williams and Campbell 1977b), while McVivar (1972) suggested that histopathogenicity caused by elasmobranch cestodes was associated with their size, mobility, and mode of attachment. Borucinska and Cairns (1993) subsequently described the histopathogenicity of two adult trypanorhynchs from the muscosa of the nurse shark. Descriptions of the histopathology of trypanorhynch cestodes in extra-enteric sites are unreported. Opportunities to observe the effects of trypanorhynchs in superficial sites are uncommon because the larvae typically burrow into the muscles or mesenteries of their intermediate or paratenic hosts. In general, trypanorhynchs producing postlarvae (without blastocyst) move freely, tentacles everted, within the intestinal lumen and a variety of extraintestinal locations. One example, described herein, is the large plerocercus of the trypanorhynch *Hepatoxyylon trichiuri* (Holten, 1802), a species parasitizing large sharks such as *Prionace glauca* (Linnaeus, 1758) in both postlarval and adult stages, and as a postlarva in numerous teleosts (Dollfus 1942).

MATERIALS AND METHODS

Tissues examined were obtained from 23 blue sharks, *Prionace glauca*, ranging in total length from 3 to almost 4 metres. Sharks were taken on baited long-lines aboard the R.V. Corsair during the months of June-July of 1974-1982 near Martha's Vineyard and south of Noman's Island. Sharks were dissected at sea and postlarvae were excised with the surrounding 2 cm² of tissue at the attachment site. Control tissue samples of approximately 1 cm² were taken from unaffected organs. All fixation was in 10% formalin buffered in sea water. Tissue sections of 6 specimens, cut at 8-10 µm because of the large size of the scolex, were prepared from paraaffin embedded specimens dehydrated in a tertiary-buty alcohol/ethanol series. Stains of Harris' hematoxylin counterstained with eosin Y, and Mallory's connective tissue stain were used to stain sections cut on an American Optics rotary microtome and affixed to slides with Haupt's gelatin adhesive. The tentacles and bothridia of several specimens were hand sectioned, stained with Harris’ hematoxylin or fast green, dehydrated and mounted in Canada balsam prior to

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Figs. 1-4. Attachment of *Hepatozylon trichiuri* postlarvae to epigonal organ and liver. **Fig. 1.** Postlarva attached to epigonal organ (x 4). **Fig. 2.** Postlarva detached from surface of liver. Note quadripartite tissue outgrowths at bothridial attachment site and holes made by penetration of tentacles (x 4). **Fig. 3.** Tentacle embedded into epigonal organ (x 200). **Fig. 4.** Sagittal section through liver attachment site. A thickened surface epithelium overlies fibrous layer containing numerous enlarged vascular passages (x 100). **Fig. 5.** Detail of liver capsule at top right in Fig. 4. Note thickened epithelium modified into numerous papillary outgrowths (x 400).
examination and photography with an Olympus BHB microscope. Specimens for scanning electron microscopy (SEM) were dehydrated in an ethanol series, critical point dried in a Samdri 780A (Tousimis), mounted on stubs coated with conductive silver paint, sputter coated in a Hummer VI-A and examined in a JSM 820 (JEOL) scanning electron microscope.

RESULTS

The discernable effects of Hepatoxylon trichiuri postlarvae, up to 3 cm long, on the epigonal organ consist of a pitlike depression in the organ surface conforming to the apex of the scolex. The short tentacles, fully penetrated, cause distention of the surface (Fig. 1). Upon microscopic inspection following staining evidence of a host response is not apparent. Despite focal compression at the attachment site, perforation of the organ capsule and destruction of the tissue immediately around the tentacle and hooks, there was no evidence of hemorrhage, hyperaemia, hyperplasia, infiltration by inflammatory cells or signs of encapsulation (Figs. 3,8). Instead the penetrated region shows the aggregation of lymphocytes and granulocytes characteristic of the epigonal organ's lymphomyeloid function.

In the liver by contrast, hyperplasia was apparent on the surface, four papillomatous outgrowths, caused by the two bothridia, bracket a depression containing four holes made by the penetration of the tentacles (Fig. 2). The liver surface has been contorted by the muscular action of the bothridia and the underlying connective tissue of the capsule is thickened (Fig. 4). Chronic irritation has resulted in fibrosis extending from the liver capsule into the underlying liver parenchyma (Figs. 4,5,7). The cuboidal epithelium of the liver capsule (Fig. 6) has become hypertrophied and proliferated into numerous papillary outgrowths (Fig. 5). Increased vascularization, distortion and enlargement of hepatic sinusoids were evident adjacent to the attachment sites (Fig. 4). Numerous lymphocytes and large pigmented granulocytes were present adjacent to the fibrous layers. The thickening of the surface layers is associated with hyperplasia and metaplasia especially at the lateral margins where the capsule epithelium has transformed into columnar cells on papillae (Fig. 5).

SEM observations of the scolex of H. trichiuri show that the tentacle apertures lie within the medial boundaries of the bothridial margins and that the bothridial margins continue as ridges across the apex of the scolex and are contiguous with the bothridial margins on the opposite side. Numerous small pores, verified at higher magnifications, occupy the space medial to the apical ridges and on the adherent surface of the bothridium (Fig. 9). The tegument is smooth overall being devoid of microtriches. Four short globose tentacles, armed with 15-16 large hooks per half spiral row (Fig. 10), are completely embedded in the organ surface. The largest hooks have total lengths of 160 to 220 μm agreeing with the characterization distinguishing the species from H. megacephalum (Rudolph, 1819) as provided by Dollfus (1942 – p. 87). The armature of Hepatoxylon is homeoacanthous with hooks arranged in quinconxes (Fig. 10). A change in hook form in H. trichiuri (Fig. 10) can be seen in comparison to the uncinate hooks of the internal face (at left) and the straighter falciform shape of the hooks as the row approaches the external face (at right). The change in hook form without change of pattern represents a subtle change from rotational pattern isometry toward glide reflection isometry in which the hooks of the pattern can only be translated in two directions along the axis of the tentacle (Campbell and Bevridge 1994).

DISCUSSION

Hepatoxylon trichiuri postlarvae have been reported from the viscera and body cavity of numerous large teleosts and elasmobranchs and the adult worms from the stomach and spiral intestine of sharks (Dollfus 1942). Presumably in the life history, first-stage larvae are ingested with unknown prey and then emerge within the gastrointestinal tract of the fish, burrow through the gut wall by the action of their tentacles and bothridia, enter the coelomic cavity and attach to the surface of organs. Apparently, large sharks are invaded by the postlarvae when they ingest infected fish as prey. Unlike the small, delicate larvae of most trypanorhynch postlarvae of H. trichiuri are large and muscular attaining lengths of 2 to near 10 cm by 5 to 8 mm wide.

Observed host responses to the tentacles and bothridia on the liver and epigonal organ of Prionace glauca reflect the different cellular constitution and response in these organs (Figs. 1-8). In its coelomic location the tissue injury caused by the postlarva arises through the penetration of the tentacle hooks and the apical margins of the slit-like bothridia. The histological appearance of the epigonal organ at the attachment site of H. trichiuri is unremarkable when compared with sections from uninfected sharks. Both light and electron microscope studies of lymphomyeloid tissues in sharks have shown that the epigonal organ primarily contains developing and mature eosinophilic granulocytes with lesser numbers of blast cells, plasma cells and occasional aggregations of lymphocytes (Mattisson and Fange 1982, Fange and Pulsford 1983). It has been proposed that the presence of large numbers of degranulated granulocytes in the epigonal organ may indicate their storage over a long period of time (Fange and Pulsford 1983). Despite the fact that eosinophilic granulocytes are numerous in the peripheral blood, Morrow (1978) could not find evidence of them attacking the nematode Proleptus obtusus, a parasite of the dogfish, in the presence or absence
of antibody. The absence of any activity around *H. tri-
chiuri* supports Morrow's (1978) observation and ques-
tions cell function in the inflammatory reaction to
parasites in other organs of *P. glauca*. 

Castro (1981, 1982) noted that parasites of the gas-
trointestinal tract may induce inflammation by three
types of injurious stimuli, chemical, mechanical and antigenic. Despite its location at the organ surface where the cestode larva makes minimal physical contact, *H. trichiuri* has effectuated similar histopathological changes to those observed within the gastrointestinal tract. The effects of *H. trichiuri* on the liver involve modification of mucosal epithelial cell function and necrosis resulting in a granulomatous reaction culminating in fibrous encapsulation. Focal destruction of the liver capsule by the tentacles followed by bothridial attachment and the inflammatory response indicate that the mechanical process of invasion is the primary event. The consequence of invasion is scarring with damage to tissues around the capsule as indicated by the vascular changes and infiltration of leukocytes. Superficially, at the attachment site, the pit-like depression bordered by four bulbous outgrowths is evident that the bothridia seen on the scolex function as four fused into pairs despite the fact that each bothridium is bounded by a single muscular rim (Fig. 9).

The spatial arrangement of hooks viewed creates the illusion of rotational pattern isometry if the armature were transformed into a 2-dimensional figure. However, Campbell and Beveridge (1994) showed that among the trypanorhynch with homeoacanthous armatures a plane of bilateral symmetry can and often does exist as evidenced by the change in hook form, size, number or spatial arrangement. The subtle change in form seen in *H. trichiuri* imposes a plane of reflection (bilateral) symmetry on the pattern causing a transformation to glide reflection isometry characteristic of heteroacanthous and poeciloacanthous armatures. In studying examples such as *H. trichiuri*, made readily apparent with SEM, it is obvious that the three major types of armatures of trypanorhynch, each previously treated as unique, are graded into one another by subtle changes in pattern symmetry. Comparison of the attachment of *H. trichiuri* with the observations by Williams (1984), Borucinska and Caira (1993), H. Palm (pers. comm.) and personal observations of living trypanorhynchus causes us to hypothesize that the physical size of the tentacles and the number, form and arrangement of the tentacular hooks may be functionally related to their use. Short stout tentacles like those of species of *Nybelinia* are used in a direct boring action by alternate extension and retraction of the tentacles. *Hepatoxylon* may behave in a similar manner by eversion and inflation of the tentacles as evident in variable extension and diameter (Fig. 9; Dollfus 1942 - p. 93). Species of eutetrahrhynchids and grillotidens often have long slender tentacles, with enlarged hooks on one surface, that make superficial attachment in the mucosal crypts and may be more important in locomotion than anchoring the worm and possibly allow control of the projection of the small strobila into the gut lumen.

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REFERENCES


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