GILL LESIONS ASSOCIATED WITH *ERPOCOTYLE TIBURONIS* (MONOGENEA: HEXABOTHRIIDAE) ON WILD AND AQUARIUM-HELD BONNETHEAD SHARKS (*SPHYRNA TIBURO*)

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ABSTRACT: Gill lesions associated with infections of *Erpocotyle tiburonis* (Brooks, 1934) (Monogenea: Hexabothriidae) on wild bonnethead sharks (*Sphyrna tiburo* (L., 1758) (Carcharhiniformes: Sphyrinidae)) were compared with those on aquarium-held ones using light and scanning electron microscopy. Uninfected gill filaments had slender, triangular, smooth-surfaced lamellae and interlamellar water channels that were approximately equal in size. Four wild sharks were each infected by 3–11 widely separated adult *E. tiburonis,* and 1 of these sharks hosted a juvenile specimen. Lamellae flanking or touching adult *E. tiburonis* were pushed aside or bent, but were otherwise identical to those of uninfected filaments. Two aquarium-held sharks were each infected by hundreds of juvenile and adult *E. tiburonis.* In these sharks, lamellae near juveniles were pushed apart or bent, but were otherwise normal, whereas a thick, ragged-surfaced layer of hyperplastic epithelium both filled interlamellar water channels and partially or completely covered lamellae near adults. Results of this study suggest that the intense infections of *E. tiburonis* were facilitated by captivity and caused severe hyperplastic lesions that ultimately led to the death of the sharks by reducing or blocking the respiratory water flow over lamellae and thus reducing the exchange of gases and ions across the lamellar epithelium. In contrast, the wild sharks were infected by fewer worms and exhibited relatively minor lesions.

Fishes in hatcheries (e.g., Pratt, 1919), ponds (e.g., Paperna et al., 1984), floating sea cages (e.g., Kaneko et al., 1988), and public aquaria (e.g., Nigrelli, 1940; Poynton et al., 1997) may develop intense monogenean infections linked to host morbidity and mortality. Members of Hexabothriidae Price, 1942 (Monogenea) infect the gills of elasmobranchs and holocephalans (Boeger and Kritsky, 1989), and some of their hosts are commonly maintained in public aquaria, e.g., hammerhead sharks (Sphyrinidae), requiem sharks (Carcharhinidae), and houndsharks (Triakidae). At least 1 probable hexabothriid species has been associated with disease in a captive shark (Cheung, 1993). However, microscopic details of lesions associated with hexabothriid infections on wild or aquarium-held elasmobranchs have not been described. Herein, gill lesions associated with the hexabothriid *Erpocotyle tiburonis* (Brooks, 1934) on wild and aquarium-held bonnethead sharks, *Sphyrna tiburo* (L., 1758) (Carcharhiniformes: Sphyrinidae), are described and compared.

MATERIALS AND METHODS

Four young-of-the-year bonnethead sharks (each about 480 mm in total length) were caught in a gill net East of Panama City in St. Joe Bay (29°39′67″N, 85°11′15″W) on 27 April 2000 and killed by severing the cervical spinal cord. Gill arches from each shark were excised, and monogeneans attached to gills were observed before fixing each gill arch in 10% neutral buffered formalin. A neonate female bonnethead shark (290 mm in total length) died at the Tennessee Aquarium on 30 September 1999, 38 days after its arrival, and was fixed whole in 10% neutral buffered formalin. This shark was born in a fenced lagoon, and the pregnant mother of this shark was captured off Marathon, Florida. Another female bonnethead shark (640 mm in total length) that originated from off Marathon died at the Mystic Aquarium on 14 November 1999, 156 days after its arrival, and its gill arches were excised and fixed in 10% neutral buffered formalin.

A dissecting microscope was used to locate infected and uninfected areas from all formalin-fixed gills and to approximate the numbers and locations of juvenile and adult monogeneans on gill filaments. Selected

gill arches were bisected such that half was used for light microscopy (LM) and half was used for scanning electron microscopy (SEM). Gill intended for LM was trimmed and placed in 3.0 cm × 2.5 cm × 0.5 cm plastic tissue cassettes, processed routinely for paraffin embedding, sectioned at 4 μm intervals, stained with hematoxylin and eosin, and mounted on glass slides. Gill intended for SEM was sliced into 0.3–0.5-cm cubes, subjected to 3 10-min washes with 0.1 M *N*-(2-hydroxyethyl) piperazine-*N*′-(2-ethanesulfonic acid) (HEPES) buffer, postfixed in 1% osmium tetroxide in 0.1 M HEPES buffer for 16 hr, subjected to 3 10-min washes with distilled ultrafiltered water, dehydrated in a graded ethanol series, stored overnight in 100% ethanol, critical point dried for 3.5 hr, mounted on metal stubs using silver paint, and sputter-coated with gold–palladium.

Representative juvenile and adult monogeneans from areas of gill that were not to be sectioned were removed and dehydrated in a graded ethanol series. Most of these worms were then cleared in clove oil, whole-mounted on glass slides in neutral Canada balsam, and studied with the aid of a compound light microscope equipped with Nomarski optics. A juvenile specimen and an adult specimen from the dehydrated lot were immersed in hexamethyldisilazane (HMDS) for 15 min, dried for 10 min using a vacuum to remove the HMDS, mounted on metal stubs using 2-sided sticky tape, sputter-coated with gold–palladium, and examined using a scanning electron microscope.

RESULTS

All of the examined juvenile and adult monogeneans (Figs. 1–5) conformed to the description of *E. tiburonis* (see Brooks, 1934). The bulb-like oral sucker is terminal and possesses a smooth surface, and a zone of finely reticulated tegument surrounds the mouth (Fig. 3). Haptoral suckers of adults are arranged in 3 tandem pairs (Fig. 1), and those of juveniles are radially arranged (Fig. 2). Minute papillae cover the inner surface of each haptoral sucker (Fig. 2), and a flap of tegument nearly encircles the rim of each haptoral sucker (Figs. 1, 2). Haptoral sclerites are embedded in the roof of each sucker (Figs. 1, 2). Muscle fibers run through the body–haptor peduncle and connect to the proximal portion of each of these sucker sclerites. Appendix hooklets are directed ventrally and not exposed. The bifurcate distal tip of the appendix possesses paired thimble-shaped protrusions, each with a reticulated tegument (Fig. 5) superficially similar to that surrounding the mouth.

Uninfected gill filaments from both wild and aquarium-held bonnethead sharks appeared normal and had evenly spaced,
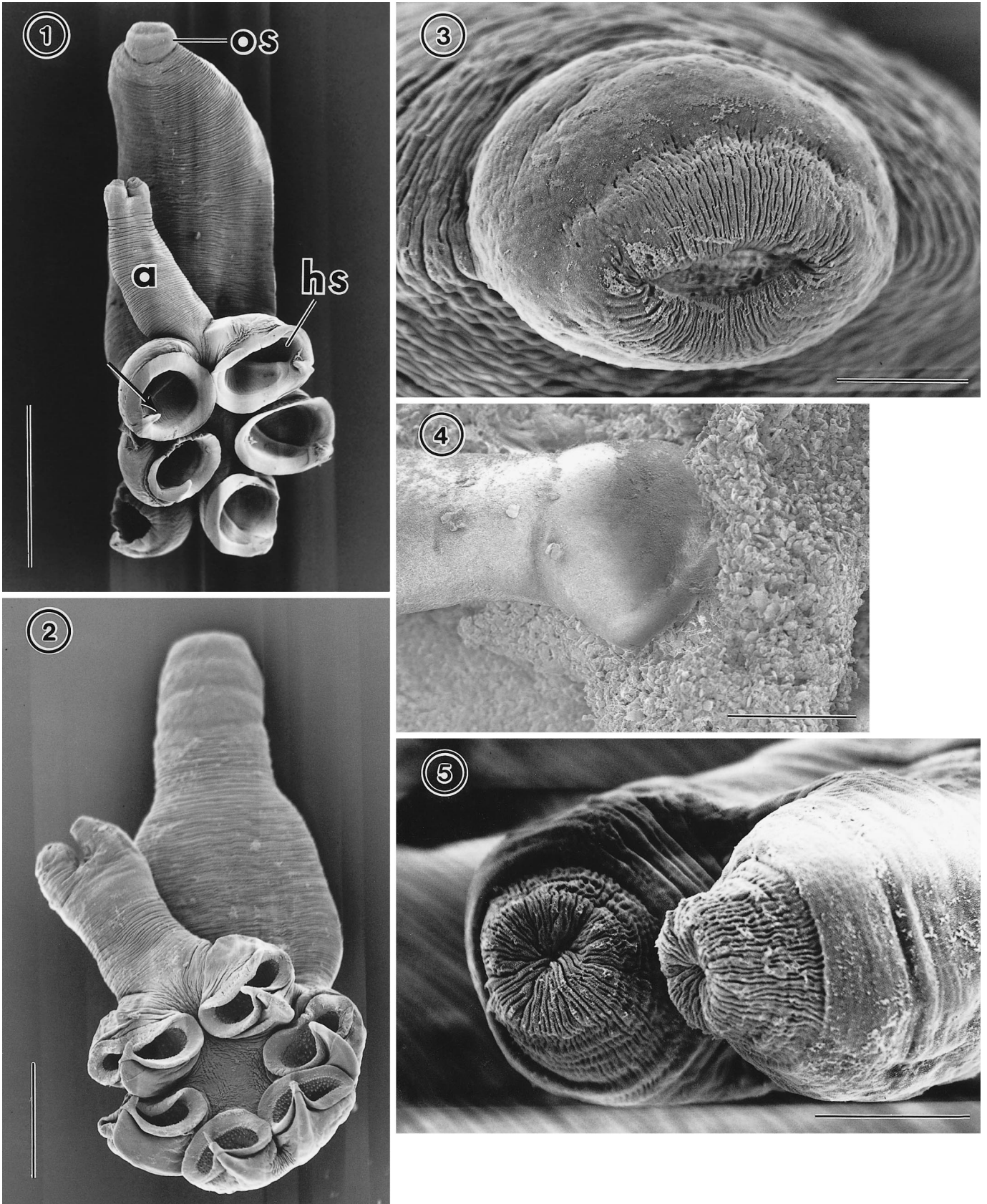
FIGURES 1–5.   Scanning electron micrograph of *Erpocotyle tiburonis* (Brooks, 1934) from gills of aquarium-held bonnethead sharks, *Sphyrna tiburo*. **1.** Adult, ventral view: oral sucker (os), haptoral sucker (hs), tip of haptoral sclerite (arrow), appendix (a). Scale bar = 600 μm. **2.** Juvenile, ventral view of radially arranged haptoral suckers and numerous papillae on inner surface of haptoral suckers. Scale bar = 100 μm. **3.** Adult oral sucker, en face view of smooth and reticulated tegument surrounding mouth. Scale bar = 60 μm. **4.** Adult, lateral view of oral sucker applied to hyperplastic gill epithelium of host. Scale bar = 100 μm. **5.** Adult, lateral view of appendix tips. Scale bar = 60 μm.
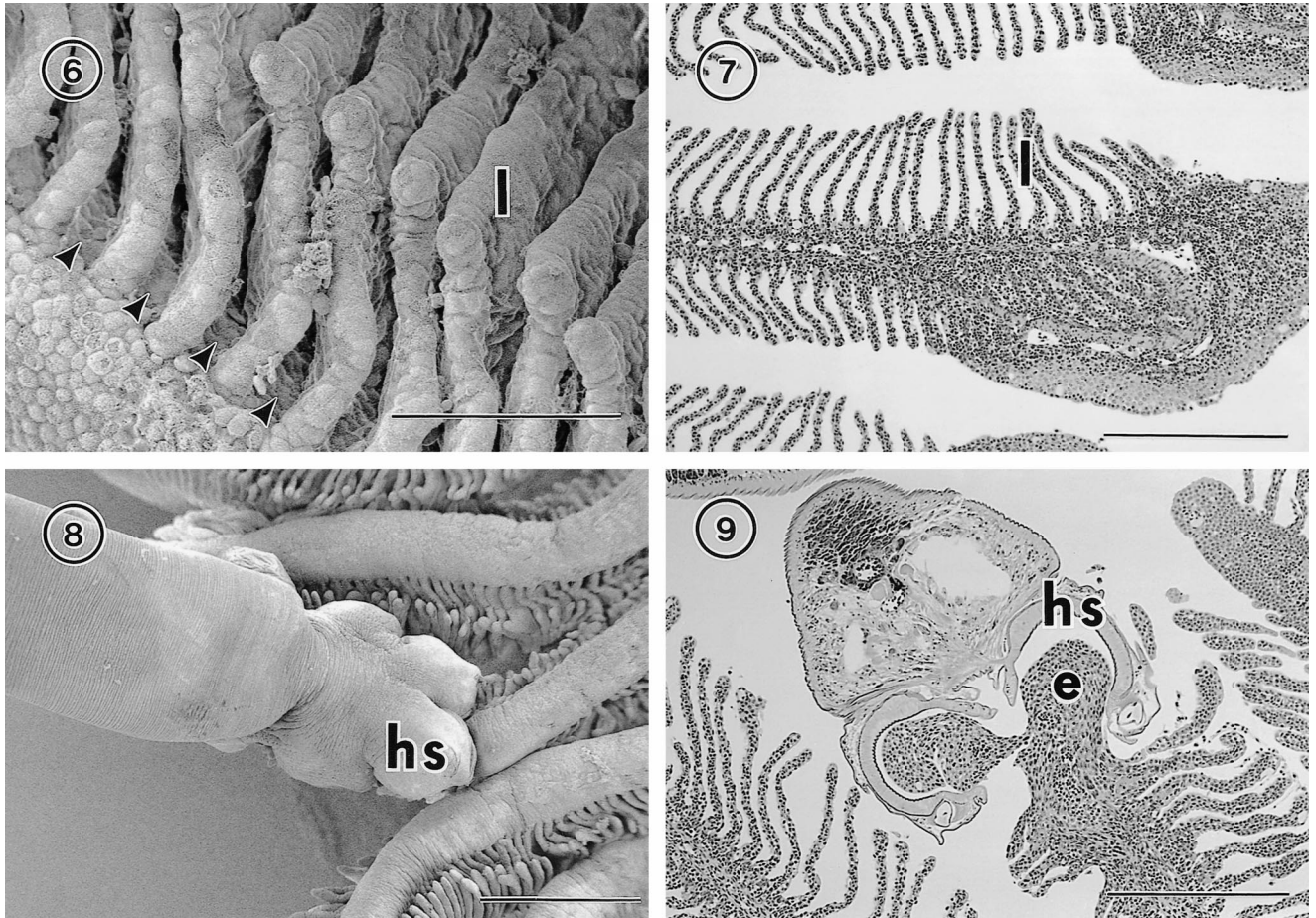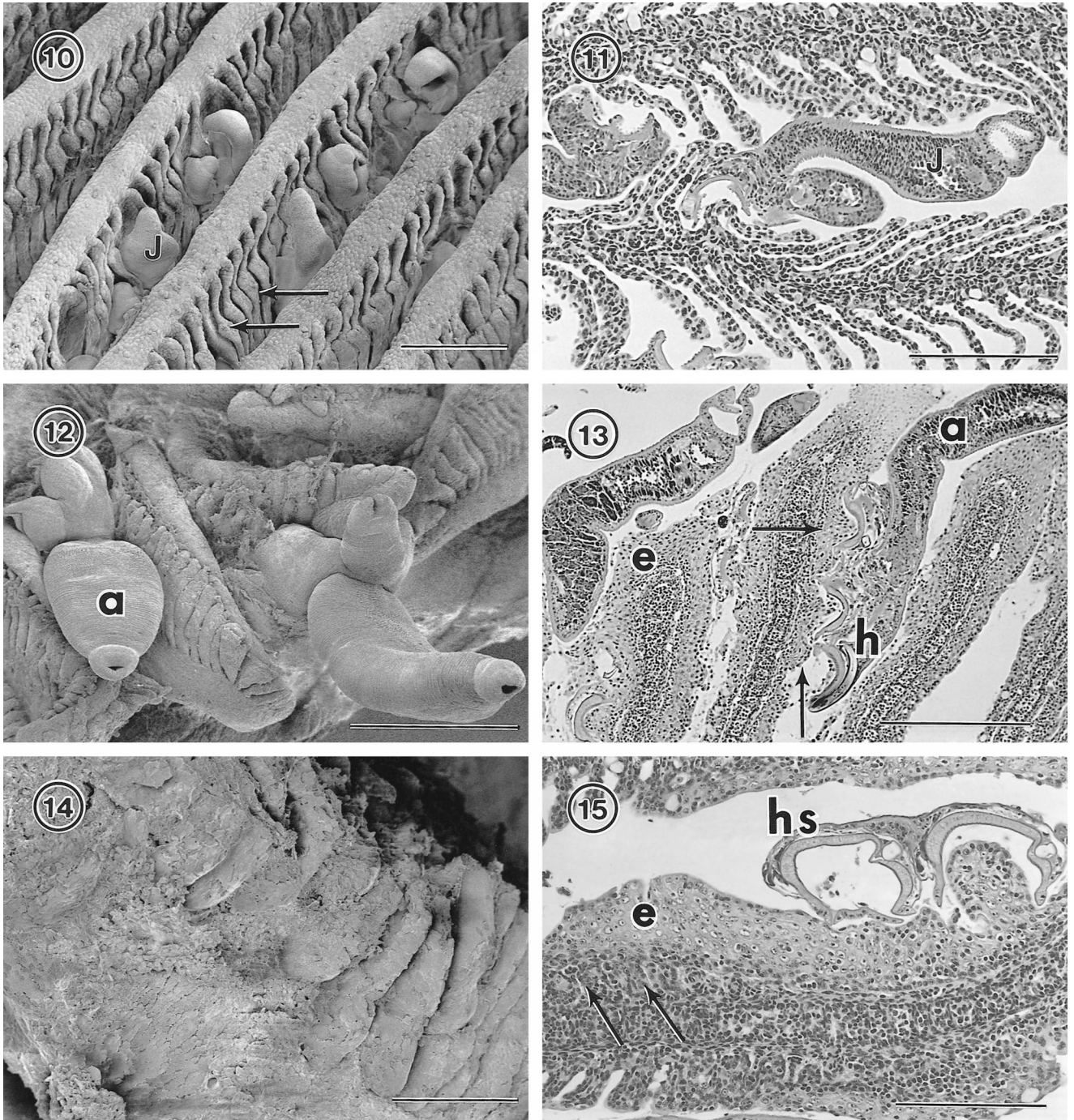
FIGURES 6–9.   Gill from wild bonnethead sharks, *Sphyrna tiburo*. **6.** Scanning electron micrograph showing uninfected interlamellar water channels (arrowheads), and lamellae (l). Scale bar = 500 μm. **7.** Section of uninfected distal portion of gill filament. Note that lamellae (l) form regularly spaced arrays and that both interlamellar and interfilament water channels are unobstructed. Scale bar = 400 μm. **8.** Scanning electron micrograph of haptor and posterior portion of an adult *Erpocotyle tiburonis* (Brooks, 1934) attached to a gill filament. Note that haptoral suckers (hs) are attached distally about the efferent portion of a filament. Scale bar = 500 μm. **9.** Tissue section showing attachment site of adult *E. tiburonis* in the distal third of a gill filament. Note that haptoral suckers (hs) grasp the filament from each side and are filled with epithelium (e). Nearby lamellae appear normal. Scale bar = 400 μm.

thin, triangular, smooth-surfaced lamellae separated by interlamellar water channels that were approximately equal in size (Figs. 6, 7). Stratified squamous epithelium covered the distal tips of these filaments (Fig. 7).

Wild bonnethead sharks appeared healthy, i.e., without external abnormalities and not emaciated. Their gills lacked gross lesions and had similarly colored filaments consisting of red lamellae that each possessed a strip of lightly colored capping tissue. The wild sharks were infected by 3, 3, 11, and 5 widely separated adult *E. tiburonis* that were located along the distal third of gill filaments. One of these sharks was infected by a juvenile *E. tiburonis* located within the proximal two-thirds of a filament; however, a tissue section from this region was not obtained. No juveniles were observed in gill sections from the wild sharks. Live adult *E. tiburonis* were conspicuous on gills and each possessed a brownish-gray, diverticulate intestine. Adults probed surrounding gill filaments by extending and contracting their bodies while twisting about the haptor. They attached with their right and left haptoral suckers clamped to opposite sides of filaments (Figs. 8, 9), and haptoral suckers of some adults were filled with the stratified squamous epithelium

of the filament tips (Fig. 9). Lamellae along the distal third of infected filaments appeared normal, i.e., similar to lamellae in uninfected areas, although some were pushed aside by adult *E. tiburonis*.

Aquarium-held bonnethead sharks were emaciated and displayed signs of irritation, i.e., erratic swimming patterns, flashing, rapid respiration, and piping, about the time of death. Approximately several hundred juvenile *E. tiburonis* and scores of adult *E. tiburonis* infected each of these sharks. Juveniles were wedged between lamellae along the proximal two-thirds of gill filaments with the long axis of the body typically running parallel to that of the lamellae (Fig. 10). Some lamellae near juveniles appeared normal; however, lamellae separating neighboring juveniles were pushed together or compressed. Other lamellae were drawn within haptoral suckers of juveniles or pressed between juveniles and the tissue of the central filament axis (Fig. 11). The anterior end of some juveniles contacted lamellae of flanking filaments, bridging the space between filaments. As a result, some interlamellar water channels in these regions were reduced in size or eliminated. A single juvenile was attached to the epithelium of the buccal cavity near the

FIGURES 10–15. Gill of aquarium-held bonnethead sharks, *Sphyrna tiburo* infected by *Erpocotyle tiburonis* (Brooks, 1934). **10.** Scanning electron micrograph along the proximal two-thirds of gill filaments showing juvenile *E. tiburonis* (J) attached between and oriented parallel to lamellae (arrows). Scale bar = 200 μm. **11.** Tissue section along the proximal two-thirds of filaments. Note that lamellae near juvenile *E. tiburonis* (J) are well-defined and slender and that some interlamellar water channels seem reduced in size because of apparent compression or bending caused by worms. Scale bar = 200 μm. **12.** Scanning electron micrograph of several adult *E. tiburonis* (a) attached along the distal third of filaments. Note that worms block spaces between filaments. Scale bar = 500 μm. **13.** Tissue section along distal third of filaments where several adult *E. tiburonis* are attached. Note that lamellae are not evident within mounds of hyperplastic epithelium (e), and that the haptor (h) of an adult *E. tiburonis* (a) is attached to hyperplastic epithelium (arrows). Scale bar = 400 μm. **14.** Scanning electron micrograph of gill near adult *E. tiburonis* (not shown). Hyperplastic epithelium covers lamellae and fills interlamellar water channels. Scale bar = 200 μm. **15.** Tissue section of attachment site of adult *E. tiburonis* showing hyperplastic epithelium (e) and haptoral suckers (hs). Note that hyperplastic epithelium fills inter-lamellar water channels (arrows) and some lamellae are absent beneath and about the suckers. Scale bar = 200 μm.

most posterior left gill arch; however, a tissue section of this region was not processed for histological examination. Adults were alone or loosely clustered and attached about the capping tissue surrounding the efferent arterioles of filaments or more ventrally along the distal third of filaments (Figs. 12, 13). The epithelium near adults was thick, i.e., hyperplastic, and possessed a ragged surface. Typically in these sites, well-defined lamellae were not evident (Fig. 13), or hyperplastic epithelium covered lamellae and partially or completely filled interlamellar water channels (Figs. 14, 15). Haptoral suckers of some adults were attached to and filled by hyperplasic epithelium (Figs. 13, 15). Hemorrhage, thrombosis, or necrosis of gill was not evident, and in no shark was a vascular core of a filament breached by an adult or juvenile *E. tiburonis.*

DISCUSSION

Intense infection by *E. tiburonis* in aquarium-held bonnethead sharks was associated with proliferative gill lesions that probably altered the flow of respiratory water over lamellae, thereby reducing the exchange of gases and ions across the lamellar epithelium. Physiological complications associated with these alterations most likely killed these sharks. Evidence of secondary infection was not evident in the aquarium-held bonnethead sharks. The gill epithelium of sharks is typically flat and 1–2 cells thick. Respiratory water flows from the buccal cavity, around the gill arches, and along the filaments, with some water flowing between the lamellae and exiting along the interfilament excurrent water channels and the gill slits (Benz, 1984). The results of the present study suggest that the many juvenile and adult *E. tiburonis* located between both filaments and lamellae may have blocked water flow over portions of the respiratory epithelium. The thick hyperplastic gill epithelium near adult *E. tiburonis* in aquarium-held sharks also may have reduced respiratory water flow by occluding interlamellar water channels and by narrowing the interfilament excurrent water channels, as well as reduced gas and ion diffusion between respiratory water and blood. Lamellae were missing along distal portions of some infected filaments, or, if present, they were covered by hyperplastic epithelium. It is doubtful that respiratory gases and ions could be exchanged normally in either of these regions. However, in contrast to the intense infections and severe gill lesions observed on the aquarium-held bonnethead sharks, wild bonnethead sharks were infected by relatively few, widely separated adult *E. tiburonis.* Wild sharks possessed unobstructed spaces between filaments and lamellae, i.e., interlamellar water channels and interfilament excurrent water channels, a normal respiratory epithelium, and a seemingly full complement of lamellae. In the present study, there was no evidence of a gill lesion in a wild shark that could have interfered with the normal flow of respiratory water or the respiratory exchange of gases and ions. These observations suggest that in nature the parasite–host relationship between *E. tiburonis* and *S. tiburo* normally may be relatively inconsequential. Although the effect of an individual worm is probably the same in both wild and aquarium-held hosts, these results indicate that the intensity of infection dictates the severity of the lesion.

Supplemental observations on the morphological features of *E. tiburonis* that are relevant to attachment are also reported herein. The tegumental flap surrounding the rim of each hap-

toral sucker may function as a seal that facilitates the maintenance of negative pressure within the sucker (Figs. 1, 2). Furthermore, when the muscles that run through the haptor peduncle and to the proximal portion of each haptoral sclerite contract, the proximal end of each sclerite probably rises from the host surface, in turn pivoting the sharp distal tip toward and thus embedding it in the gill. The function of the appendix may be to facilitate movement. The distal suckers of the appendix may grapple host tissue before the haptoral suckers release their grip, and then the appendix may contract and drag the body and haptor to a new location on the host. Brooks (1934) stated that the haptoral suckers of *E. tiburonis* were arranged in a circle; however, the results of the present study indicate that these suckers are radially arranged only in juveniles and that they appear as tandem pairs in adults. The tandem arrangement of haptoral suckers in adults may allow for more efficient clamping to opposing sides of the capping tissue that overlies the efferent arterioles of filaments (Figs. 8, 9), whereas the radial suckers of smaller worms may facilitate attachment to lamellae (Fig. 11).

The diet of a species of *Erpocotyle* has not been determined. However, using spectroscopic and histochemical methods, Llewellyn (1954) showed that 8 polyopisthocotylineans, including the hexabothriid *Hexabothrium appendiculatum* (Kuhn, 1829), fed on blood. Hargis (1959) observed probable undigested shark red blood cells in the gut of 5 of 6 specimens of the hexabothriid *Heteronchocotyle leucas* Hargis, 1955. Halton and Jennings (1965) listed the food and feeding habits of several representatives from Monopisthocotylinea and Polyopisthocotylinea and concluded that the polyopisthocotylineans that they studied fed predominantly on blood, but that the polyopisthocotylineans *Diplozoon paradoxum* Nordman, 1832 (Diplozoidae), *Diclidophora merlangi* (Nordman, 1832) (Diclidophoridae), and *Pseudodactylocotyla palmata* (Leuckart, 1830) (Dactylocotylidae) supplemented their blood diets by ingesting gill tissue and mucus. The gill sections in our study exhibited no evidence of blood feeding by *E. tiburonis.* The circulatory system of infected gill filaments in both wild and aquarium-held bonnethead sharks was not breached, and there was no sign of hemorrhage or thrombosis. Adult *E. tiburonis* attached along the distal third of filaments of aquarium-held sharks had access to hyperplastic epithelium (Figs. 13, 14), and the oral sucker of an adult worm was in apposition to a mound of epithelial cells (Fig. 4). However, that this worm had fed on these cells was not confirmed. Microscopic observations of live worms attached to freshly excised gill would probably help elucidate this parasite's feeding behavior and diet.

Differences in the distribution of and lesions caused by juvenile and adult *E. tiburonis* on wild versus aquarium-held sharks may be clues about the life history of this parasite. Juveniles were attached along the proximal two-thirds of gill filaments, and adults were usually attached along the distal third of filaments. Hyperplasia was not observed in the proximal portions of filaments near juveniles; however, severe hyperplasia occurred near adults attached along the distal portions of filaments. If severity of hyperplasia is positively correlated with duration of local infection, a lack of hyperplasia near juveniles could suggest a relatively brief attachment. Kearn (1984) showed that postlarvae of *Entobdella soleae* (van Beneden and Hesse, 1863) (Capsalidae) colonized the dorsal surface of the

common sole, *Solea solea* (L., 1758) (Soleidae), before migrating toward the head of the sole and onto the ventral surface where they matured and mated. Kearn (1998) suggested that mate-finding in monogeneans should be easier if the breeding population is concentrated at a particular site on the host. If oncomiracidia of *E. tiburonis* initially colonize the proximal portions of gill filaments, they might later migrate downstream as postlarvae toward the distal tips of the filaments to mature, copulate, and produce eggs. From the distal tips of gill filaments water exits the branchial chamber via the nearby gill slits, and thus adults located along the distal tips may have greater success in releasing their eggs or oncomiracidia into the external environment. In addition, the large size and attachment positions of adult *E. tiburonis* on the gill filaments would seem to ensure that eggs would be released into the nonrespiratory water that flows along the gill filaments above the lamellae. This may be significant because this water flow pathway is a more direct route out of the branchial chamber and into the milieu than that of the respiratory water (Benz, 1984).

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