ACTA ICHTHYOLOGICA ET PISCATORIA (2004) 34 (2): 115–127

OPERCULAR LESION IN WILD BLACK DRUM, *POGONIAS CROMIS* (LINNAEUS, 1766), ASSOCIATED WITH ATTACHMENT OF THE SEA LOUSE *SCIAENOPHILUS TENUIS* (COPEPODA: SIPHONOSTOMATOIDA: CALIGIDAE)

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Frasca Jr., S., Kirsipuu, V.L., Russell, S., Bullard, S.A., Benz, G.W. 2004. Opercular lesion in wild black drum, *Pogonias cromis* (Linnaeus, 1766), associated with attachment of the sea louse *Sciaenophilus tenuis* (Copepoda: Siphonostomatoida: Caligidae). Acta Ichthyol. Piscat. 34 (2): 115–127.

Background. Sea lice (Copepoda: Caligidae) are important pathogens in aquaculture, and because more fish species are being intensively cultured, more species of sea lice are recognized as pathogens. The aim of the present study was to gather baseline data regarding the effects of sea lice on a valuable sciaenid (Sciaenidae), the black drum, *Pogonias cromis*, by describing lesions associated with naturally occurring infections of *Sciaenophilus tenuis* van Beneden, 1855.

Materials and methods. Gross and histological examinations of copepods and lesions were made using light and scanning electron microscopy from samples collected from eight infected black drum captured in Mississippi Sound, northern Gulf of Mexico.

Results. Adult females of *S. tenuis* were associated with a mucosal lesion on interopercula. Female copepods attached to folds of mucosa on the posterior half of interopercula with cephalothoraces directed anteriorly in parallel with the longitudinal axis of fish. All attached male copepods grasped the abdomen or genital complex of females and were not in contact with the host. Maxillipeds of female copepods were embedded in epithelium or subepithelial connective tissue and functioned as the primary attachment appendages. Epithelial hyperplasia, fibrosis of subepithelial connective tissue, and chronic inflammatory infiltrates including presumed eosinophilic granular cells surrounded maxillipeds, indicative of long-term, focal, parasite–host interaction.

Conclusion. Aquaculture managers should regard *S. tenuis* as a potentially serious pathogen if fish develop intense *S. tenuis* infections associated with extensive gross lesions.

Key words: lesion, sea lice, parasite, *Sciaenophilus*, Caligidae, black drum, *Pogonias*, Sciaenidae, aquaculture

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INTRODUCTION

Black drum, *Pogonias cromis* (Linnaeus, 1766), is a common estuarine and nearshore species ranging from Massachusetts to Argentina (Pattillo et al. 1997). This largest of sciaenids (Sciaenidae) can grow to 120-cm total length (Matlock 1990) and live for 55 years (Murphy and Taylor 1989). Black drum were considered undesirable table fish until Chef Paul Prudhomme popularized “blackened redfish” in the late 1970s. Subsequently, high market values for “redfish” or red drum, *Sciaenops ocellatus* (Linnaeus, 1766), and harvest restrictions shifted some fishery attention to black drum as a market substitute (Geaghan and Garson 1993, Leard et al. 1993). Historically and today, many black drum are caught in the Gulf of Mexico, where the species is recreationally and commercially important (Shipp 1988, Pattillo et al. 1997). Little aquaculture effort focuses on black drum; however, red drum is cultured within and beyond its native range (Lutz 1999).

Caligidae (Siphonostomatoida) contains at least 445 species of copepods commonly referred to as sea lice (Ho 2000). Sea lice may harm wild fishes near infested aquaculture facilities and cause staggering production losses to aquaculture operations (Boxshall and Defaye 1993, Birkeland and Jakobsen 1997, MacKinnon 1997, Ho 2000). More species of sea lice will likely cause disease as more fish species are cultured (Ho 2000). Sea louse infection of wild fish ranging near an aquaculture facility can serve as a parasite reservoir that negatively impacts fish production, and intense sea louse infections of farmed fish may increase exposure of wild fishes to these parasites (Boxshall and Defaye 1993, Birkeland and Jakobsen 1997, MacKinnon 1997). Studying sea louse infections from a locality isolated from aquaculture provides useful baseline information on the presumed “natural” parasite–host relationship, which can lend comparative insight into the disease process in farmed fish as well as supply data regarding changes in parasite abundance and pathogenicity within fish populations.

The sea louse, *Sciaenophilus tenuis* van Beneden, 1852 (Caligidae) infects sciaenids in the northern and southern Atlantic Ocean (including the Mediterranean Sea) and the northern Indian Ocean (Kabata 1979, Dojiri 1983, Pillai 1985). In the Gulf of Mexico, *S. tenuis* has been reported by Bere (1936), Heegaard (1966), and Overstreet (1983) from banded drum, *Larimus fasciatus* Holbrook, 1855, black drum, and red drum, respectively. Lesions and pathologic responses to sea louse infection were described in several other teleosts (e.g. see Boxshall 1977, Johnson and Albright 1992a, 1992b), but not in a sciaenid. This report describes a lesion in black drum associated with naturally occurring infections of *S. tenuis* in Mississippi Sound, a region lacking commercial sciaenid aquaculture.

MATERIALS AND METHODS

Eight black drum, 22–109 cm total length, were caught on hook and line in Mississippi Sound (northern Gulf of Mexico) off Point Cadet Plaza, Biloxi, Mississippi (30º23.677' N, 88º58.697' W) between 24 October and 11 November 1999. Fish were maintained alive before being euthanized with tricaine-methanesulfonate (MS-222) immediately before necropsy. Twelve infected interopercula were each fixed in 10% neutral buffered formalin and examined using a stereomicroscope. For identification purposes, several copepods were cleared in lactic acid, into which a pinch of lignin pink had been dissolved, and examined using bright-field and phase-contrast microscopy and the wooden slide technique of Humes and Gooding (1964). Representative fixed samples of infected host tissue (copepods in situ), uninfected host tissue, and copepods detached from host tissue intended for histology were decalcified overnight in a 5% trichloroacetic acid solution, routinely embedded in paraffin, serially sectioned at 4-µm intervals, mounted on glass slides, and stained with hematoxylin and eosin. Slides were examined with a compound microscope and the digestive tracts of 20 sectioned copepods (19 females, 1 male) were examined for gut contents. Tissue samples and individual copepods intended for scanning electron microscopy were washed 3 times (10-min each) with 0.1 M *N*-(2-hydroxyethyl) piperazine-*N'*-(2-ethanesulfonic acid) (HEPES) buffer, post-fixed in 1% osmium tetroxide in 0.1 M HEPES buffer for 16 h, washed 3 times (10-min each) with ultrafiltered distilled water, dehydrated in a graded ethanol series, stored overnight in 100% ethanol, critical point dried for 3.5 h, mounted on metal stubs using silver paint, and sputter-coated with gold-palladium. Anatomical terms for copepods conform mainly to Kabata (1979).

RESULTS

One hundred and twenty adult female (115 ovigerous or senescent appearing with empty eggs sacs; 5 non-ovigerous) and 12 adult male *S. tenuis* were collected. There were 2 to 31 ($\bar{x} \pm s = 10.0 \pm 8.79$; 100% of samples infected) female copepods attached to the mucosa of the inner surface of each interoperculum. Female copepods were located in a single column or tight cluster on the posterior half of interopercula with cephalothoraces facing anteriorly and in parallel with the longitudinal axis of the fish (Fig. 1). The mucosa beneath female copepod attachment sites was thrown into multiple, prominent, closely apposed folds (Fig. 2) except in 2 samples (intensity = 3 in both cases) where smooth mucosa and no gross lesions were associated with infection. Regions of the cephalothoracic shield of some female copepods were covered by fields of closely packed, short, rod-shaped bacteria (Fig. 3). Few male copepods were collected, with 0 to 10 males ($\bar{x} \pm s = 1.0 \pm 2.74$; 25% of samples infected) associated with an interoperculum. Two unattached male copepods were in a sample container; however, all others were attached to the abdomen or genital complex of females (one per female), and none were in contact with the host.

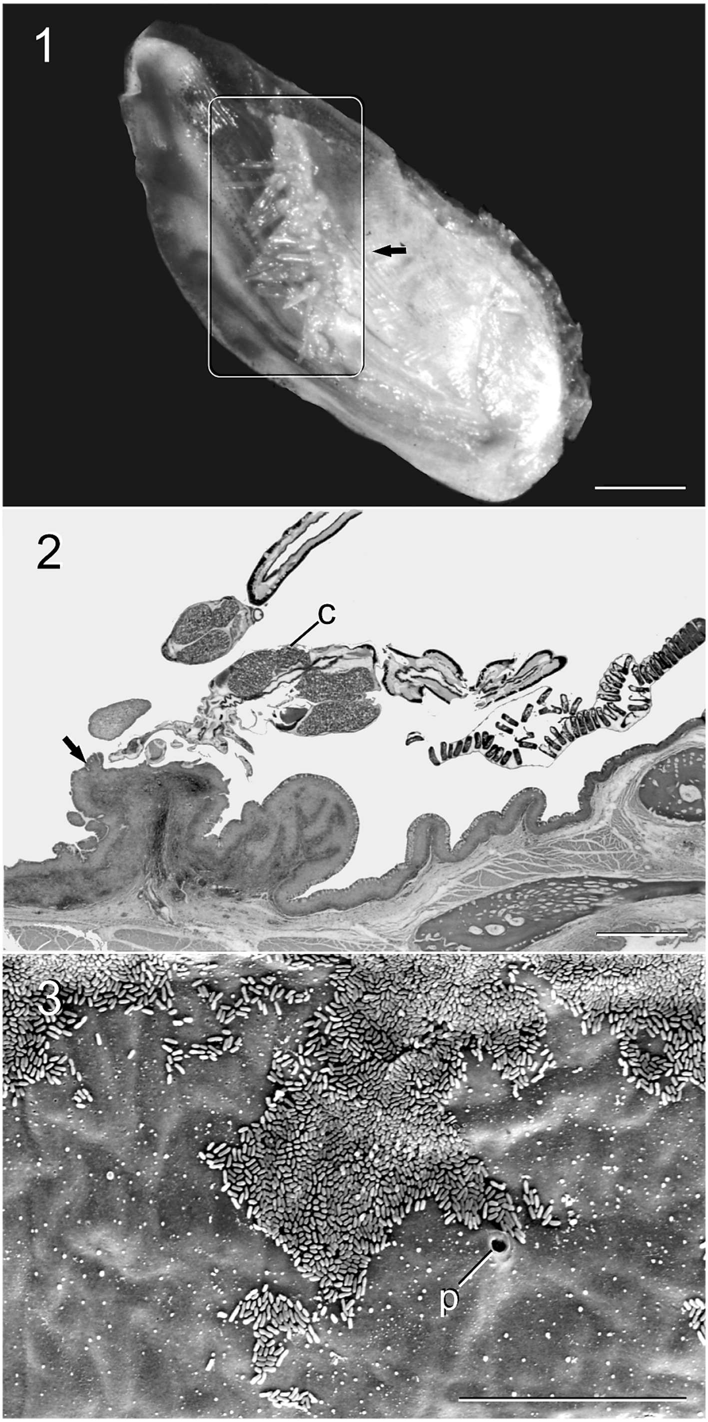
Females of *S. tenuis* caused deep impressions in the epithelial surface, while the nearby mucosa was rugose (Fig. 4). Epithelium sometimes covered the edges of copepod cephalothoraces (Figs. 4–6). There was fibrosis of subepithelial connective tissue beneath epithelial impressions (Fig. 5). Well-defined impressions of several copepod appendages and the mouth tube were evident in epithelium (Fig. 7); however, maxillipeds were the only copepod structures that significantly pierced the mucosa.

Maxilliped claws were located either in epithelium, reaching to the stratum basale and basement membrane (Figs. 5, 8), or in subepithelial connective tissue, accompanied by fibrosis and inflammatory infiltrates (Fig. 6). Subchela of maxillipeds occupied a conical, smooth-surfaced pit in the mucosa (Figs. 8, 9). Neither bacteria nor fungi were observed in these pits.

Epithelium beneath and surrounding attachment sites was hyperplastic, consisting of increased layers of spinous epithelial cells and broad-based epithelial pegs; increased numbers of mucus-secreting cells were present along the epithelial surface adjacent to, but not beneath, copepods (Fig. 10). In folds of mucosa beneath attachment sites, there was fibrosis of subepithelial connective tissue accompanied by perivascular and interstitial infiltrates of chronic inflammatory cells including lymphocytes, macrophages, and numerous granulocytes with eosinophilic cytoplasmic granules, which resembled the eosinophilic granular cells (EGCs) of other teleosts (Figs. 11, 12).

Gut contents were observed in 16 (84.2%) of 19 examined female copepods. Within the gut of these 16 copepods, red blood cells (1–3 per copepod) were in 3 (18.8%) copepods, granulocytes with eosinophilic cytoplasmic granules (1–15 per copepod) were in 4 (25%) copepods, and scant to moderate amounts of wispy or granular, eosinophilic material (possibly hemolymph) were in 14 (87.5%) copepods. No gut contents were observed in the single male copepod examined.

Figs. 1–3. *Sciaenophilus tenuis* and black drum, *Pogonias cromis*. **Fig. 1.** A column of adult female *S. tenuis* (box) attached to raised mucosa face anteriorly, i.e. into respiratory water flow (arrow), on the inner surface of an interoperculum; scale bar = 1 cm. **Fig. 2.** Sagittal section of an ovigerous *S. tenuis* (c) attached to the surface of a mucosal fold (arrow); interopercular mucosa is thrown into folds beneath the copepod, which correspond to the raised tissue seen grossly about copepods in Fig. 1 (note: a section of a second copepod is also in view above the labeled copepod); scale bar = 1 mm. **Fig. 3.** Scanning electron photomicrograph of the dorsal surface of the cephalothoracic shield of an adult female *S. tenuis* covered with closely packed, short, rod-shaped bacteria; the small perforation (p) in the cephalothorax is a cuticular pore emitting a sensory setule; scale bar = 20 µm



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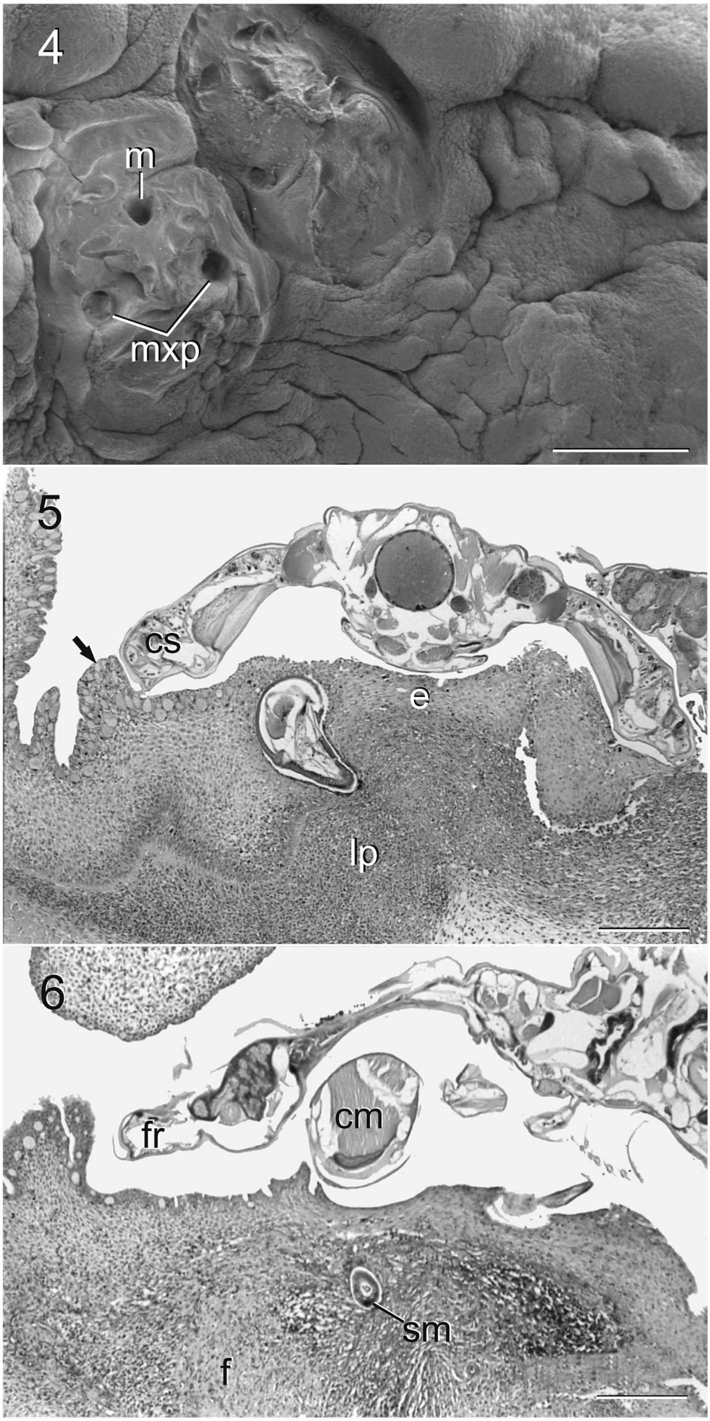

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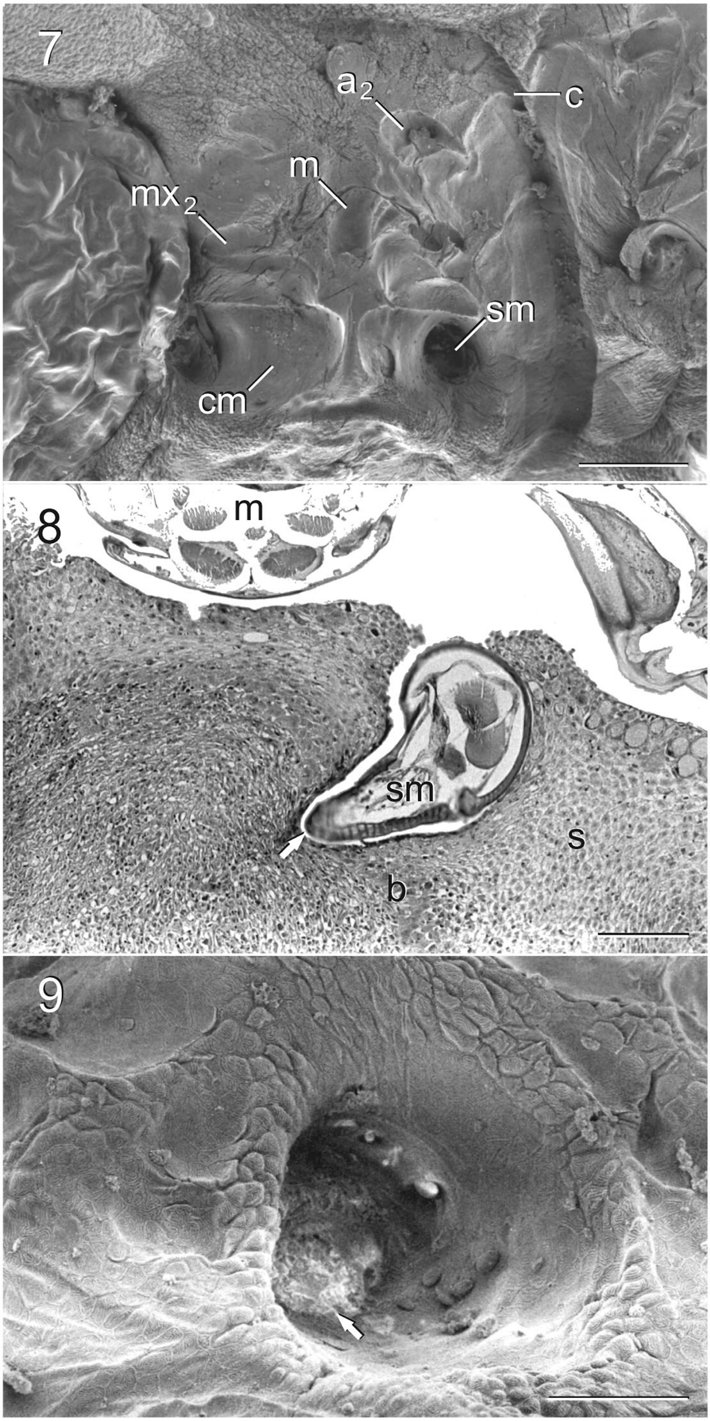
DISCUSSION

Mucosal lesions caused by attachment of *S. tenuis* resulted in localized mucosal rugosity, epithelial hyperplasia, fibrosis, and inflammatory infiltrates consisting of lymphocytes, macrophages, and granulocytes with eosinophilic cytoplasmic granules resembling EGCs, which together are evidence of lesion chronicity (Collins 1999). The absence of bacteria or fungi in lesions suggests that persistent irritation by the maxillipeds of female *S. tenuis* caused the fibrosis and inflammatory cell infiltrates associated with copepod attachment. Recruitment of presumed EGCs to sites of attachment may further characterize the inflammatory response in this host–parasite relationship. Eosinophilic granular cells are functionally similar to mammalian mucosal mast cells (Reite 1998) and are associated with chronic inflammatory responses in other teleosts (Roberts et al. 1972; Ferguson 1989). These cells were identified in abundance within lesions during episodes of chronic disease (Ferguson 1989) and parasitic infection (Blackstock and Pickering 1980, Sharp et al. 1989), and Boxshall (1977) observed them in skin lesions associated with infection by *Lepeophtheirus pectoralis* (Caligidae) on the flounder *Platichthys flesus* (Linnaeus, 1758). The presumed EGCs in black drum infected with *S. tenuis* could be contributory to an inflammatory, perhaps even allergic, reaction to yet undetermined mediators from the copepod.

Figs. 4–6. *Sciaenophilus tenuis* and black drum, *Pogonias cromis*. **Fig. 4.** Attachment impressions on the epithelial surface of the interoperculum beneath the cephalothoraces of two adult female *S. tenuis*; the 3 deepest pits in each impression correspond to where a parasite's mouth tube (m) and maxillipeds (mxp) resided (one of two impressions labeled); mucosa adjacent to copepod attachment sites is thrown into multiple, closely apposed folds; scale bar = 500 µm. **Fig. 5.** Transverse section through the cephalothorax of an attached adult female *S. tenuis*; host epithelium (e) is hyperplastic in the region of the attachment and appears compressed beneath the copepod; epithelium extends along the lateral edges (arrow) of the cephalothoracic shield (cs); lamina propria (lp) beneath the epithelium appears expanded by dense fibrous connective tissue; scale bar = 200 µm. **Fig. 6.** Sagittal section through the cephalothorax of an adult female *S. tenuis* attached to the interoperculum; epithelium beneath the frontal region of the cephalothorax (fr) and the corpus maxillipedis (cm) appear compressed; subchela of the maxilliped (sm) is located beneath compressed epithelium in the lamina propria, which is expanded by dense fibrous connective tissue (f) containing inflammatory infiltrates; scale bar = 200 µm

The inflammatory response to *S. tenuis* attachment suggests an association between stationary copepods and black drum. Other stationary copepods (or those presumed to be so) were associated with similar lesions on other fishes (Joy and Jones 1973, Boxshall 1977, Borucinska and Benz 1999). In low-intensity infections, feeding and attachment of sea lice are associated with lesions that have not been shown to be pathogenic. However, in high-intensity infections when sea lice attach and feed over a wide portion of the host, disease probably results from the cumulative effects of many lesions that promote osmotic imbalance and infection by bacteria, fungi, or both (MacKinnon 1997). According to the literature, infections of adult *S. tenuis* are restricted to the opercular regions of hosts (Scott and Scott 1913, Bere 1936, Dojiri 1983), but there is no published information regarding the attachment site of the parasitic larvae of *S. tenuis*. The herein described, naturally occurring lesion caused by the infection of *S. tenuis* in black drum is focal and limited to the mucosa. However, aquaculture may facilitate high intensity infections of larval and adult *S. tenuis* on cultured drum, resulting in locally extensive to diffuse, perhaps more deeply penetrating, lesions that may force aquaculture managers to treat *S. tenuis* as a potentially serious pathogen.

Figs. 7–9. *Sciaenophilus tenuis* and black drum, *Pogonias cromis*. **Fig. 7.** Impression in epithelium of the interoperculum beneath an adult female *S. tenuis*; individual impressions and pits corresponding to various copepod appendages and cuticular features are evident: right lateral margin of the cephalothorax (c), second antenna (a_2), mouth tube (m), second maxilla (mx_2), corpus maxillipedis (cm), and subchela of the maxilliped (sm); scale bar = 200 µm. **Fig. 8.** Transverse section through primary attachment region of an adult female *S. tenuis* on the interoperculum; subchela of the maxilliped (sm) pierces the stratum spinosum (s) to the level of the stratum basale (b), and epithelial cells and underlying connective tissue about the subchela appear compressed (arrow); copepod mouth tube (m) lies above host epithelium; scale bar = 100 µm. **Fig. 9.** Deep conical pit in the mucosa of interoperculum that contained a maxilliped of *S. tenuis*; fibrous connective tissue of the lamina propria appears at bottom of lesion (arrow); scale bar = 50 µm



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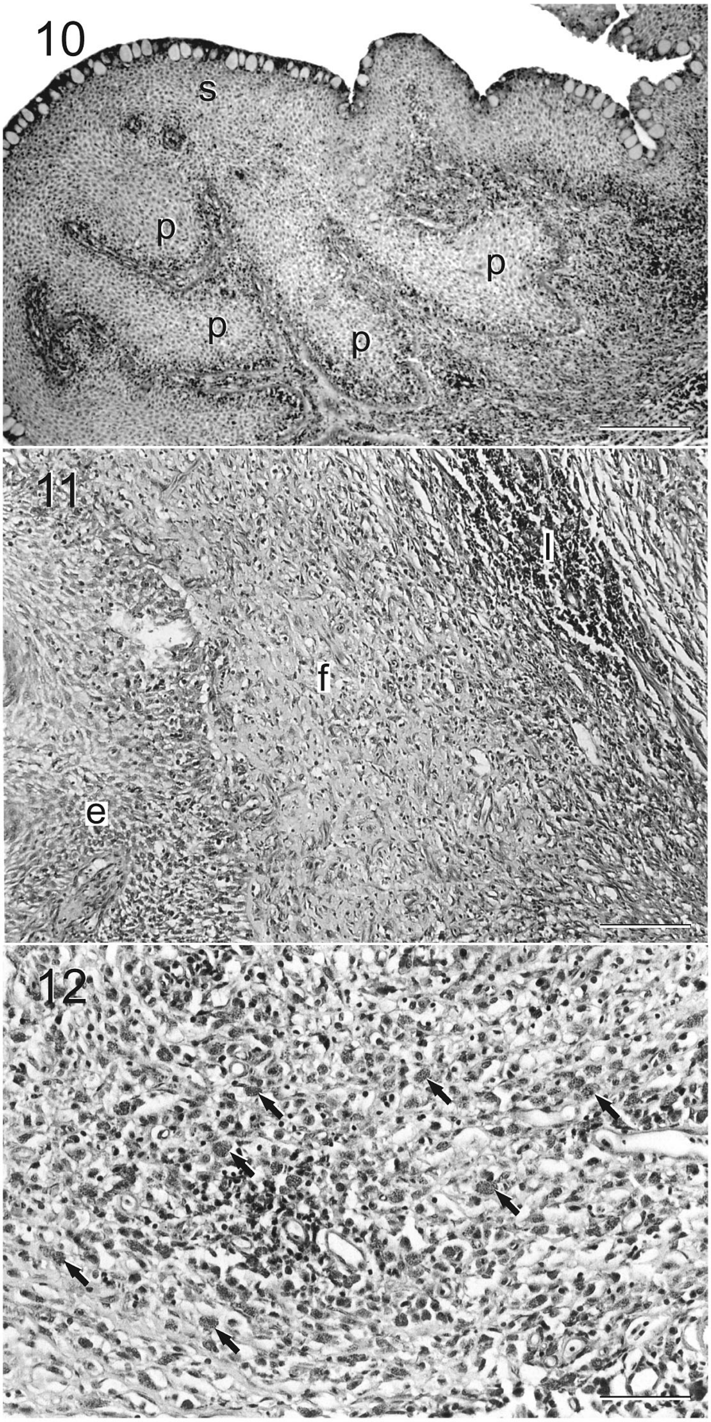

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ACKNOWLEDGMENTS

We thank Jim Romanow and others at the Electron Microscopy Laboratory (University of Connecticut) for technical assistance, Ione Jackman and Sallyann Gemme (Histology Laboratory, University of Connecticut) for preparation of histological sections, Alex Bothell (University Center for Instructional Media and Technology, University of Connecticut) for assistance with figure preparation, and Middle Tennessee State University, the Tennessee Aquarium Research Institute, and the Gulf Coast Research Laboratory (GCRL, Department of Coastal Sciences, The University of Southern Mississippi) for general support. S.A.B. additionally thanks Robin. M. Overstreet (GCRL) for support (via National Oceanic and Atmospheric Association, National Marine Fisheries Service Award NA06FL0501) during the work period.

Figs. 10–12. *Sciaenophilus tenuis* and black drum, *Pogonias cromis*. **Fig. 10.** Transverse section of a mucosal fold of an interoperculum adjacent to copepod attachment site; the stratum spinosum (s) is widened by hyperplasia of spinous epithelial cells and there are multiple epithelial pegs (p) extending into the underlying connective tissue; scale bar = 200 µm. **Fig. 11.** Interopercular mucosa at copepod attachment site; beneath hyperplastic epithelium (e), lamina propria is expanded by fibrous connective tissue (f) and infiltrates of lymphocytes (l); scale bar = 100 µm. **Fig. 12.** Infected interoperculum; presumed eosinophilic granular cells (e.g. arrows) accompany macrophages and lymphocytes in the chronic inflammatory infiltrate of subepithelial connective tissue; scale bar = 50 µm

REFERENCES

Bere R., 1936. Parasitic copepods from Gulf of Mexico fish. American Midland Naturalist **17**: 577–625.

Birkeland K., Jakobsen P.J., 1997. Salmon lice, *Lepeophtheirus salmonis*, infestation as a causal agent of premature return to rivers and estuaries by sea trout, *Salmo trutta*, juveniles. Environmental Biology of Fishes **49**: 129–137.

Blackstock N., Pickering A.D., 1980. Acidophilic granular cells in the epidermis of the brown trout, *Salmo trutta* L. Cell and Tissue Research **210**: 359–369.

Borucinska J.D., Benz, G.W., 1999. Lesions associated with attachment of the parasitic copepod *Phyllothyreus cornutus* (Pandaridae: Siphonostomatoida) to interbranchial septa of blue sharks. Journal of Aquatic Animal Health **11**: 290–295.

Boxshall G.A., 1977. The histopathology of infection by *Lepeophtheirus pectoralis* (Müller) (Copepoda: Caligidae). Journal of Fish Biology **10**: 411–415.

Boxshall G.A., Defaye D. (eds.) 1993. Pathogens of wild and farmed fish: sea lice. Ellis Horwood, New York.

Collins T., 1999. Acute and chronic inflammation. pp. 50–88. *In:* Cotran R.S., Kumar V., Collins T., (eds.) Robbins pathologic basis of disease, sixth edition. W.B. Saunders Company, Philadelphia.

Dojiri M., 1983. Revision of the genera of the Caligidae (Siphonostomatoida), copepods predominantly parasitic on marine fishes. Ph D Dissertation. Boston University, Boston.

Ferguson H.W., 1989. Gills and pseudobranchs. pp. 11–40. *In:* Ferguson H.W., (ed.) Systemic pathology of fish: a text and atlas of comparative tissue responses in diseases of teleosts. Iowa State University Press, Ames.

Geaghan J., Garson G., 1993. Assessment of the status of black drum stock on the Gulf Coast. pp. 1–38. *In:* Leard R., Matheson R.E., Meador K., Keithly W.R., Luquet C., Van Hoose M.S., Dyer C., Gordon S., Robertson J.E., Horn D., Scheffler R.R., (eds.) The black drum fishery of the Gulf of Mexico, United States: a regional management plan. Publication 28, Gulf States Marine Fisheries Commission, Ocean Springs.

Heegaard P., 1966. Parasitic copepods from Texas. Videnskabelige Meddelelser fra Dansk Naturhistorisk Forening i Kjøbenhavn **129**: 187–197.

Ho J.-s., 2000. The major problem of cage aquaculture in Asia relating to sea lice. *In:* Liao I.C., Lin C.K., (eds.) Cage aquaculture in Asia. Proceedings of the First International Symposium on Cage Aquaculture in Asia, Taiwan Fisheries Research Institute, Tungkang, Pingtung.

Humes A.G., Gooding R.U., 1964. A method for studying the external anatomy of copepods. Crustaceana **6**: 238–240.

Johnson S.C., Albright, L.J., 1992a. Comparative susceptibility and histopathology of the response of naïve Atlantic, Chinook and coho salmon to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms **14**: 179–193.

Johnson S.C., Albright, L.J., 1992b. Effects of cortisol implants on the susceptibility and the histopathology of the responses of naïve coho salmon *Oncorhynchus kisutch* to experimental infection with *Lepeophtheirus salmonis* (Copepoda: Caligidae). Diseases of Aquatic Organisms **14**: 195–205.

Joy J.E., Jones L.P., 1973. Observations on the inflammatory response within the dermis of a white bass, *Morone chrysops* (Rafinesque), infected with *Lernaea cruciata* (Copepoda: Caligidae). Journal of Fish Biology **5**: 21–24.

Kabata Z., 1979. Parasitic Copepoda of British fishes. The Ray Society, London.

Leard, R., Matheson R.E., Meador K., Keithly W.R., Luquet C., Van Hoose M.S., Dyer C., Gordon S., Robertson J.E., Horn D., Scheffler R.R., (eds.) 1993. The black drum fishery of the Gulf of Mexico, United States: a regional management plan. Publication 28, Gulf States Marine Fisheries Commission, Ocean Springs.

Lutz C.G., 1999. Red drum: a re-emerging aquaculture species. Aquaculture Magazine **25** (4): 38–45.

MacKinnon B.M., 1997. Sea lice: a review. World Aquaculture 1997 (September): 5–10.

Matlock G.C., 1990. Maximum total length and age of black drum, *Pogonias cromis* (Osteichthyes: Sciaenidae), off Texas. Northeast Gulf Science **11**: 171–174.

Murphy M.D., Taylor R.G., 1989. Reproduction and growth of black drum, *Pogonias cromis*, in north-east Florida. Northeast Gulf Science **10**: 127–137.

Overstreet R.M., 1983. Aspects of the biology of the red drum, *Sciaenops ocellatus*, in Mississippi. Gulf Research Reports, Supplement 1: 45–68.

Pattillo M. E., Czapla T.E., Nelson D.M., Monaco M.E., 1997. Distribution and abundance of fishes and invertebrates in Gulf of Mexico estuaries, volume II: species life history summaries. Estuarine Living Marine Resources Report 11, National Oceanic and Atmospheric Administration and National Ocean Service Strategic Environmental Assessments Division, Silver Spring.

Pillai N.K., 1985. The fauna of India: copepod parasites of marine fishes. Zoological Survey of India, Calcutta.

Reite O.B., 1998. Mast cells/eosinophilic granule cells of teleostean fish: a review focusing on staining properties and functional responses. Fish and Shellfish Immunology **8**: 489–513.

Roberts R.J., Young H., Milne J.A., 1972. Studies on the skin of plaice (*Pleuronectes platessa* L.). 1. The structure and ultrastructure of normal plaice skin. Journal of Fish Biology **4**: 87–98.

Scott T., Scott A., 1913. The British parasitic Copepoda. Volume I. The Ray Society, London.

Sharp G.J.E., Pike A.W., Secombes C.J., 1989. The immune response of wild rainbow trout, *Salmo gairdneri* Richardson, to naturally acquired plerocercoid infections of *Diphyllobothrium dendriticum* (Nitzsch, 1824) and *D. ditremun* (Creplin, 1825). Journal of Fish Biology **35**: 781–793.

Shipp R.L., 1988. Guide to fishes of the Gulf of Mexico. Dauphin Island Sea Laboratory, Dauphin Island.

Received: 30 November 2004
Accepted: 22 December 2004