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Source: Journal of Wildlife Diseases, 41(3) : 647-653

Published By: Wildlife Disease Association

URL: <https://doi.org/10.7589/0090-3558-41.3.647>

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## Epidemic Trichodinosis Associated with Severe Epidermal Hyperplasia in Largemouth Bass, *Micropterus salmoides*, from North Carolina, USA

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**ABSTRACT:** An epidemic of trichodinosis associated with severe epidermal hyperplasia occurred in adult largemouth bass (*Micropterus salmoides*) from the Chowan River drainage, North Carolina (USA) in late winter to early spring 2002. Initial reports by anglers of fish with a “jelly-like slime coat” on the skin prompted an electrofishing survey in which about 10% of sampled largemouth bass had a very thick, bluish-white “mucoïd layer” on the body and fins. Moderate to heavy infestations of the ciliate *Trichodina* were detected in wet mounts of skin from five of five fish having the mucoïd layer; these fish also had significant gill infestations. An additional two fish with only mild reddening and four asymptomatic fish (no skin lesions) had mild skin infestations but no gill infestations. Two asymptomatic fish had no skin parasites. Four fish with the mucoïd layer were necropsied and had extremely severe epidermal hyperplasia on the body and fins. The hyperplastic epidermis had relatively few mucus cells and typically was about 5–10 times thicker than healthy epidermis. The upper four fifths of the epidermis consisted of finely vacuolated, highly flattened, somewhat disorganized epithelial cells. No other significant clinical or histopathologic abnormalities were detected. No systemic infection by pathogenic bacteria was noted. The environmental cause of the epidemic is uncertain but the lesions suggest that some chronic stressor was involved.

**Key words:** Epidermal hyperplasia, largemouth bass, mucoïd layer, trichodinids.

The Chowan River is formed from the confluence of the Blackwater and Nottoway rivers in southeastern Virginia, USA, and then flows 75 km southeastward before emptying into Albemarle Sound near Edenton, North Carolina. Approximately 2000 km<sup>2</sup> of the drainage basin is located in northeastern North Carolina with >87% of the land cover consisting of for-

est or agriculture (North Carolina Division of Water Quality [NCDWQ], 2002). The Chowan River supports one of coastal North Carolina’s most popular largemouth bass (LMB, *Micropterus salmoides*) sport fisheries. A recreational angling creel survey conducted on the Chowan River between 1 July 2001 and 30 June 2002 estimated that 82,000 angler hours were spent in pursuit of LMB. Total estimated catch of LMB over the 12-mo period was 46,955 (SE = 6,265) fish with 12,604 (SE = 3,209) harvested by anglers (Dockendorf et al., 2004).

In early 2002, a protozoan ectoparasite (trichodinid) epidemic affected about 10% of sampled LMB in two tributaries of the lower Chowan River. The first indication of the epidemic was from an angler who reported catching several LMB with what was described as a “jelly-like slime coat” in Pembroke Creek, a tributary of the Chowan River, North Carolina, in February 2002. In late March, anglers reported seeing affected LMB in Rockyhock Creek, another tributary of the Chowan River, which is approximately 4 km upstream of Pembroke Creek. On 24 March 2002, four of 40 LMB (10%) caught in a sport fishing tournament conducted in the lower Chowan River exhibited similar gross lesions, with anglers reporting that the affected fish came from areas in or near Pembroke (36°03’N, 76°37’W) and Rockyhock (36°06’N, 76°41’W) creeks. Unconfirmed reports by fishermen also sighted LMB with lesions in the contiguous Scuppernon, Alligator, and Pasquotank rivers. By

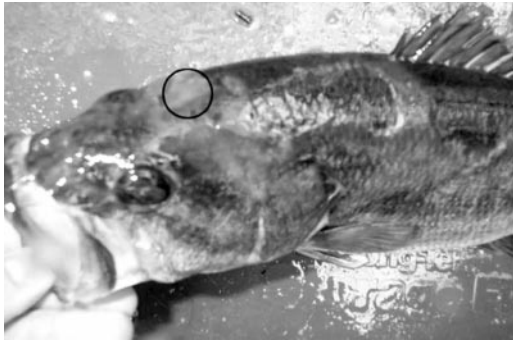


FIGURE 1. Adult largemouth bass with the mucoïd layer (severe epidermal hyperplasia) on the skin. Note the severe opacity of the skin (circled area) that covers a large area of the body. Live specimen.

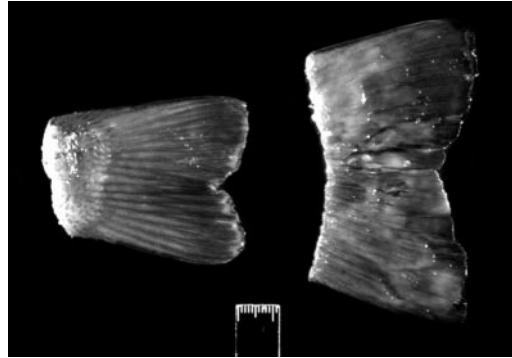


FIGURE 2. Normal caudal fin (left) and caudal fin having severe epidermal hyperplasia (right). Formalin-fixed specimens. Ruler = 1 cm.

late April, the epidemic subsided. No gross lesions were observed by North Carolina Wildlife Resources Commission (NCWRC) personnel in electrofishing surveys of 289 adult LMB from the Chowan, Roanoke, Pasquotank, Perquimans, and Scuppernong rivers in late April 2002, or in a sampling of 14 LMB from Rockyhock Creek on 1 May 2002.

Near the end of the epidemic, when we became aware of the epidemic, we performed water quality sampling in the affected area. All water quality measurements were conducted at one meter using an YSI Model 85 Handheld System (Yellow Springs Instruments, Yellow Springs, Ohio, USA). No parameters measured in Pembroke or Rockyhock creeks during the epidemic revealed any obvious anomalies. Water temperatures during 26 March 2002 to 1 May 2002 were 16.9–22.9 C, with dissolved oxygen (DO) >4.0 mg/l. Conductivities were higher than expected during early spring (range 1434–1990 microsiemens/cm) due to persistent drought conditions, although salinities measured at the time were near 1.0 practical salinity units (psu).

Twenty-one LMB were collected from Pembroke Creek on 1 April 2002 and 20 LMB from Rockyhock Creek on 8 April 2002 (30–45 cm total length) by electrofishing. Immediately after each collection, all fish with gross lesions, as well as some

apparently healthy fish, were transported alive without sedation to the North Carolina State University College of Veterinary Medicine (NCSU–CVM). All appeared to behave normally. Three of four fish brought to the NCSU–CVM on 1 April 2002 and two of nine fish submitted on 8 April 2002 (i.e., five of the 41 collected) had prominent, mucoïd, semitransparent to opaque patches on the body and fins (which we termed a “mucoïd layer”) (Figs. 1, 2). The mucoïd layer was of variable size but could be very extensive, in some cases covering from ~15 to 75% of the body surface (Fig. 1). Unlike normal mucus, the mucoïd layer was solid in consistency. However, wet mounts of the mucoïd layer did not reveal the presence of any epithelial cells, only amorphous, acellular material, leading us to believe initially that the mucoïd layer was composed of mucus. We further investigated this possibility (see below). Of the five fish with a mucoïd layer, four had a moderate to severe trichodinid (Phylum Ciliophora: Subclass Peritrichia: Order Mobilina: Family Trichodinidae: *Trichodina*) infestation on the skin and/or gills; the other fish had a mild skin and gill infestation (Table 1). The remaining eight fish examined either had no gross skin lesions or mild areas of reddening, especially on the flanks, but also on the fins; most had a few trichodinids on the skin but usually none on the gills (Table 1). A dactyl-

TABLE 1. Skin lesions and trichodinid infestation intensity on largemouth bass collected from the Chowan River drainage.

Collection Date	Location <sup>a</sup>	Fish no.	Predominant gross skin lesion	Trichodinid intensity <sup>c</sup>	
				Skin	Gill
1 April 02	P	02-0001	Mucoid layer <sup>b</sup>	+++	+++ <sup>d</sup>
	P	02-0002	Mucoid layer <sup>b</sup>	++	+ <sup>d</sup>
	P	02-0003	Mucoid layer <sup>b</sup>	++	+ <sup>d</sup>
	P	02-0004	No lesions	0	+
8 April 02	R	02-0005	Mucoid layer	+++	++
	R	02-0006	Mucoid layer <sup>b</sup>	+	+
	R	02-0007	Reddening	+	0
	R	02-0010	Reddening	+	0
	R	02-0008	No lesions	+	NE
	R	02-0009	No lesions	+	0 <sup>d</sup>
	R	02-0011	No lesions	+	0
	R	02-0012	No lesions	0	0
	R	02-0013	No lesions	+	0

<sup>a</sup> P = Pembroke Creek, R = Rockyhock Creek.

<sup>b</sup> Also had some reddening on other areas of the body.

<sup>c</sup> Trichodinid intensity is based upon number of parasites per 100× microscopic field (mean of 3-10 fields). + = mild (1-9 parasites per 100× field), ++ = moderate (10-59 parasites per 100× field), +++ = severe (60-200 parasites per 100× field), NE: not examined.

<sup>d</sup> Also had 1-3 dactylogyrids per slide in gill biopsy.

ogyrid monogenean infestation was seen on three fish. Skin of six fish with typical lesions (including lesion and apparently normal skin on each fish) and one clinically healthy fish were sampled for bacteria using a sterile culture swab that was immediately streaked onto a blood agar plate. Blood cultures were taken aseptically by bleeding the fish from the caudal vessels,

TABLE 2. Number of bacteria cultured from peripheral blood, apparently normal skin, and skin lesions of LMB.

Fish no.	Lesions <sup>a</sup>	Blood <sup>b</sup>	Number of colonies <sup>b</sup>	
			Normal skin	Lesion skin
02-0001	M	NG	NG	ND
02-0002	M	NG	35	NG
02-0003	M	NG	1	NG
02-0005	M	NG	NG	>100 <sup>c</sup>
02-0006	M	NG	11	8 <sup>d</sup>
02-0007	R	NG	ND	2
02-0004	NL	NG	2	—

<sup>a</sup> M: Mucoid layer; NL : No lesion; R = Reddening.

<sup>b</sup> NG: No growth; ND: Not done. All cultures with multiple colonies had at least two different colony types; hemolytic and/or non-hemolytic colonies were present.

<sup>c</sup> Ulcer.

<sup>d</sup> Reddened area.

immediately placing a drop of blood on a blood agar plate, and streaking the blood on the plate with a sterile swab. All cultures were incubated at room temperature. Few bacteria colonies were present in cultures from either skin lesions or areas of clinically normal skin, except for a single skin lesion that yielded >100 colonies (Table 2). Colony types were very diverse and there was no single colony type that predominated; thus, bacterial identification was not attempted. No fish had any evidence of bacteremia.

A complete necropsy was performed on four individuals with a mucoid layer, three from the 1 April 2002 collection and one from the 8 April 2002 collection. After euthanization with buffered tricaine, skin, gill, liver, head kidney, spleen, heart, and gastrointestinal tract were fixed in 10% neutral buffered formalin and processed as described previously (Noga et al., 1990). Skin was also refixed in Trump's fluid and processed as described previously for scanning electron microscopy (SEM) (Udomkusonsri et al., 2004). We also examined body and fin skin from five clinically nor-



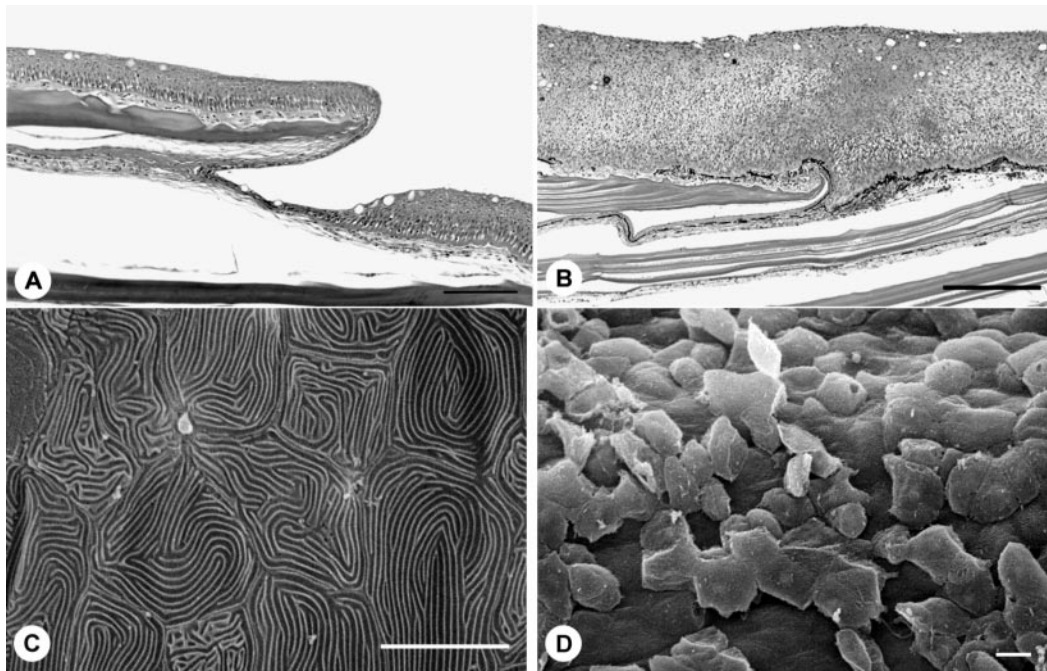


FIGURE 3. Histological sections of (A) normal skin and (B) skin having a mucoid layer (severe epidermal hyperplasia). Note the severe spongiosis, as well as the irregular, eroded surface of diseased skin. Thickness of the normal skin is  $\sim 45 \mu\text{m}$  whereas that of the diseased skin is  $\sim 400 \mu\text{m}$ . Bars: A =  $100 \mu\text{m}$ , B =  $250 \mu\text{m}$ . (C) Scanning electron micrograph of normal skin and (D) SEM of diseased skin. Note the highly eroded epithelial cells and loss of microridges on the diseased skin. Bars =  $10 \mu\text{m}$ .

mal individuals collected from the Roanoke River near Williamston ( $35^{\circ}51'N$ ,  $77^{\circ}01'W$ ) on 6 August 2002 to compare with skin lesions from affected fish; this area had not had any reports of a similar disease.

Microscopically, in all four fish having the mucoid layer, there was severe epithelial hyperplasia (Fig. 3a, b). One half to two thirds of the mucoid layer was spongiotic. There were very few mucous cells in the outermost layer of the epithelium. Immature mucous cells, eosinophilic granular cells, and lymphocytes were scattered throughout the hyperplastic epithelium. Several mitotic figures were seen. Examination of the mucoid layer of two fish under SEM showed that the surface of the skin was uneven and eroded, without obvious microridges on its surface (Figs. 3c, d). Histopathology of the viscera was unremarkable.

The effect of this epidemic on the large-

mouth bass population in the Chowan is uncertain. Because affected fish were still actively striking lures, it appears that they were not anorectic. No kills were observed during this event and we found no evidence of significant secondary bacterial infections, but mortalities might have gone undetected, especially if chronic. However, the epidemic eventually subsided spontaneously. Although the reason for the spontaneous recovery is unknown, it is most likely related to increased immunocompetence, possibly due to rising water temperatures, increased food availability, or other factors that favor a healthier population.

Except for the colonial ciliate *Heteropolaria* (= *Epistylis*), which has often caused epidemics in a number of feral fish (Esch et al., 1976), epidemics of protozoan ectoparasites in wild fish populations are rare. However, in prior years, the Chowan River fishery has experienced ectoparasite

epidemics. In the early 1980s the copepod *Lernaea cruciata* was highly prevalent in red skin erosions and ulcers on LMB and other centrarchids (Noga, 1986). Other parasites were less prevalent in these lesions, including monogeneans and *Heteropolaria*. However, mild to moderate, sub-clinical trichodinid infestations have been observed in other native fish species in the Albemarle-Pamlico estuary (Haeseker et al., 1996), as well as other areas (Khan, 2004).

Although very common in cultured fish (Khan, 1991), we know of no published reports of trichodinosis epidemics in fishery populations except for one reported from an unidentified freshwater lake in Minnesota, USA in late spring 1999, in which an estimated 5,000–14,000 white bass (*Morone chrysops*) died with heavy gill infestations (ProMED-mail, 1999). We were unable to determine the trichodinid species causing the Chowan epidemic, but trichodinids typically have a similar clinical presentation (Kahn, 1991). They are classical opportunists and thus some predisposing environmental factor was likely present (Lom, 1995). Trichodinids also commonly cause disease in late winter to early spring (Lom, 1995; Khan, 2004). The Chowan River basin has had frequent (but localized) episodes of hypoxia (defined as  $<0.5$  mg DO/l), with the latest detected event being in July 2001, resulting in a fish kill involving over 2,000 fish (NCWRC, unpubl. data). Although we did not observe such highly stressful DO levels during the epidemic, acute hypoxia might have been missed with our limited sampling. However, problems with hypoxia and anoxia in the Chowan drainage and nearby Pasquotank and Roanoke drainages typically occur during the warmer months and are not usually observed during spring (NCWRC, unpubl. data).

The epidemic also appeared to be resolving by the time we sampled and thus we might have missed an acute initiating event. However, the chronic host response in this trichodinosis epidemic suggests that

an acute stress was probably not involved. One possible chronic stressor is elevated salinity, which had persisted in the lower Chowan River drainage through the prior winter; salinities in mainstem areas of the lower Chowan River were 5.5 psu as late as 3 April 2002. Although salinities commonly meet or exceed this value during periods of low rainfall, such levels usually do not persist in the drainage throughout the winter. Pembroke and Rockyhock creeks are the two lowest tributaries in the system, and it is probable that winter salinities might have been higher than the 1.0 psu measurements that we observed near the end of the epidemic. We have observed epidemics of another ectoparasite, the leech *Myzobdella lugubris*, on LMB exposed to abnormally high salinity in Currituck Sound, North Carolina (Noga et al., 1990). Elevated salt levels can also depress certain local immune defenses (Smith et al., 1996). However, annual electrofishing surveys conducted in brackish water systems across northeastern North Carolina commonly collect adult LMB at salinities ranging up to 5 psu and no other gross evidence of trichodinid infestations have been observed (NCWRC, unpubl. data). Similarly, research from Alabama and Louisiana riverine ecosystems suggests that LMB are well adapted to brackish environments (salinities 1–3 psu) as evidenced by high condition factors, specifically body weight (Hallerman et al., 1986; Meador and Kelso, 1990).

Another possible stressor is organic pollution. The Chowan River basin has been designated a nutrient-sensitive ecosystem due to excessive nutrient input from anthropogenic sources (NCDWQ, 1997), and excessive organic loading is commonly associated with opportunistic infestations such as trichodinids (Coyle et al., 1997). Salinity stress and nutrient loading might be coupled, because poor flushing due to drought conditions tend to affect the smaller tributaries such as Rockyhock and Pembroke creeks more than the Chowan River mainstem. This situation could have

been exacerbated if LMB from mainstem areas of the lower Chowan River were crowding into these two creeks to escape salinity intrusion. When subjected to varying salinity levels, adult LMB exhibit a distinct preference for salinities near 3 psu, and have been observed moving substantial distances in search of lower salinity water when salinities exceed 5 psu (Meador and Kelso, 1989). The influence of high salinities on movement of LMB in the Chowan River was unknown before and during the epidemic; however, salinities exceeding 5 psu were measured in mainstem areas of the Chowan River during the winter months and might have initiated migration of fish into adjacent tributaries. Crowding of LMB has been documented as fish from higher salinities move into areas of lower salinity already occupied by LMB and other fish (Swingle and Bland, 1974; Hallerman et al., 1986). Densities of adult LMB in Pembroke and Rockyhock creeks during the epidemic exceeded 25 fish per electrofishing hour (NCWRC, unpubl. data), catch rates that indicate high relative abundance in the Chowan drainage, and might have contributed further to the probability of a disease outbreak.

A common characteristic of ectoparasite infestations is thickening of the cuticle (i.e., the epidermis and overlying mucus layer). This response is believed to be due to damage from feeding activity of the parasite (Lom and Dykova, 1992). When severe, it presents grossly as a whitish opacity of the cuticle/skin. To our knowledge, the response that we observed in this trichodinosis epidemic in LMB was one of the most severe thickenings of the cuticle that has ever been documented and also suggests that these lesions were chronic. The only other parasite that has been documented to produce such severe lesions is an unclassified dinoflagellate parasite infesting stickleback (*Gasterosteus aculeatus*), which induces an extremely severe epidermal hyperplasia on the skin, resulting in a grossly visible, white "gelatinous coating" that can cover a large area of the

body (Reimchen and Buckland-Nicks, 1989); this chronic, proliferative host response eventually encloses the parasites.

The gross appearance of the cuticular thickening in trichodinosis and other common ectoparasites has been considered to be caused largely by excess mucus production (Lom and Dykova, 1992), hence the terms "blue slime" or "bluish cast" (Lom, 1995). For example, Khan (1991) reported that captive Atlantic salmon (*Salmo salar*) experiencing a severe trichodinosis epidemic had gross evidence of both "whitish pustules" and "an opaque film of heavy mucus." Formaldehyde-fixed histological skin sections had both epidermal hyperplasia and excess mucus production, but the relative importance of each response was not given. Because the mucus layer is easily lost when using aqueous fixatives such as aldehydes (Speare and Mirsalimi, 1992), we cannot accurately assess the involvement of mucus in either the Atlantic salmon or LMB responses. However, epidermal hyperplasia clearly played an important role in causing this gross lesion in LMB, and the highly similar appearance of lesions before (Fig. 1) and after (Fig. 2) fixation, as well as the relatively few mucous cells in the hyperplastic epidermis, suggests that involvement of excess mucus production in these lesions was insignificant.

To our knowledge, the gross appearance of lesions as well as the specific involvement (and relative importance) of mucus versus epidermal cells in creating this highly common response has never been investigated. We were unable to identify epidermal cells on wet mounts of skin scrapings of LMB in this trichodinosis epidemic. We speculate that the spongiotic epidermis in these fish was very fragile and lysed when squashed under a wet mount, similar to the lysis that occurs when fish-parasitic turbellarians are squashed under a wet mount (Noga et al., 1999). This raises the question whether epidermal hyperplasia might be misdiagnosed as excess mucus production in clinical cases if his-

topathology is not performed. This question is more than of academic interest because it has important implications for how fish protect themselves against many important skin diseases and what role mucus truly plays in that response.

We thank L. Henry (North Carolina Division of Marine Fisheries) and two anonymous reviewers for helpful suggestions. This work was supported in part by Grant #NA46RG0087 from the National Sea Grant College Program, National Oceanic and Atmospheric Administration, to the North Carolina Sea Grant College Program to E.J.N. and in part by the Pukyong National University Research Abroad Fund (Republic of Korea) to MDH. P. Udomkunsri was a Fellow of the Royal Thai Government.

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Received for publication 30 August 2004.