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Amyloodiniosis in Cultured Hybrid Striped Bass (*Morone saxatilis* × *M. chrysops*) in North Carolina

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Abstract.—An outbreak of amyloodiniosis in pond-cultured hybrid striped bass × white bass (female *Morone saxatilis* × male *M. chrysops*) is the northernmost documented outbreak caused by an endemic source in fish of the western Atlantic. Dinospores produced by this isolate of *Amyloodinium ocellatum* were anteroposteriorly flattened, as contrasted with the gymnodinoid dinospores of the *A. ocellatum*-type species, suggesting that more than one strain or species of *A. ocellatum* may exist.

Amyloodinium ocellatum is a euryhaline dinoflagellate that infests a wide range of marine and estuarine fishes (Sindermann 1990). It has been responsible for numerous epidemics in fish culture facilities worldwide (Paperna 1983; Barbaro and Francescon 1985), including in the USA, where it has been a serious problem in the Gulf Coast states from Texas to Florida (Lawler 1980; G. Vermeer, Florida Marine Research Institute, personal communication). We report an amyloodiniosis outbreak in hybrid striped bass × white bass (female *Morone saxatilis* × male *M. chrysops*) at a North Carolina commercial farm that began operating in 1987.

Methods

On 17 July 1987, a 2.4-hectare farm pond was stocked with juvenile hybrid striped bass (3–4 months old) that had been spawned and raised in fresh water in Arkansas. Before stocking, the pond had been filled with untreated brackish water pumped from a canal draining into the Pamlico River. On 15 June 1988, the dissolved oxygen in the pond was 1.5 mg/L; supplemental aeration was immediately initiated and the dissolved oxygen increased to more than 5 mg/L within 5 h. However, about 2 weeks later, some fish were anorexic and were flashing and congregating near the aerator. At this time, pond salinity was 9‰,

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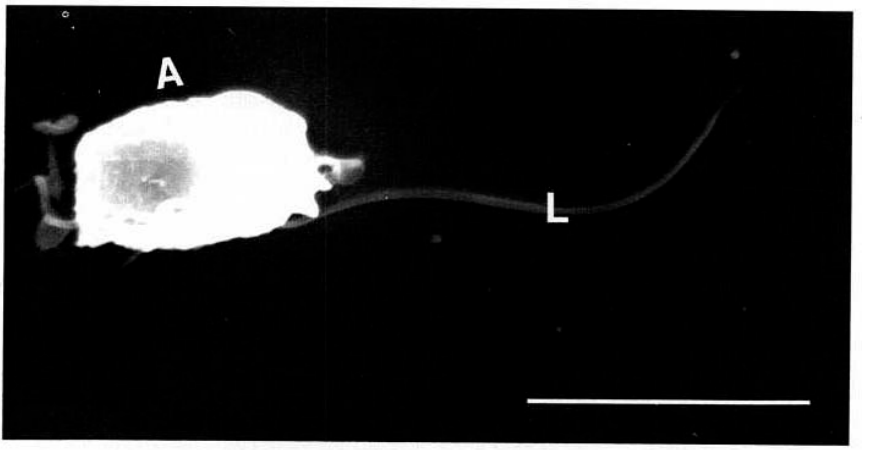

FIGURE 1.—Scanning electron micrograph of a dinosopore of the North Carolina *Amyloodinium ocellatum* isolate. A = anterior end of dinospore; L = longitudinal flagellum. Bar = 10 μm.

temperature was 25–29°C, hardness was 1,650 mg/L as $CaCO_3$, alkalinity was 50 mg/L as $CaCo_3$, and pH was 6.5–7.5.

Eleven fish (150–240 mm standard length; 80–332 g) were captured by cast net and necropsied at the Aquatic Medicine Laboratory, North Carolina State University. No other fish were examined. Kidney swabs from four fish were cultured on blood agar at room temperature. Gills were fixed in 10% neutral buffered formalin, processed routinely, and embedded in paraffin wax; 5-μm sections were made and stained with hematoxylin and eosin. Selected samples were also stained with periodic acid–Schiff, Alcian Blue (pH 6.0), Gomori methenamine–silver, or Giemsa.

A culture of the parasite was initiated using the method of Noga (1987) and propagated in a mixture of artificial seawater (IO2) and Hank's balanced salt solution (Noga 1989). Dinospores sampled from the 10th passage of this culture were prepared for scanning electron microscopy (SEM) by fixing them for 24 h at 4°C in 2% glutaraldehyde in 0.10-M sodium cacodylate trihydrate buffer. Dinospores were then slowly centrifuged at 50 × gravity for 10 min to avoid dinospore lysis. A small sample of the concentrated dinospores was added to a circular glass coverslip previously coated with poly-L-lysine; the dinospores were allowed to settle out of solution and attach to the coverslip for 1 h. Specimens were then rinsed in distilled water, dehydrated in an alcohol series, and dried to critical point with CO_2 in a Ladd Critical Point Dryer. The coverslip was mounted with two-sided tape to an SEM stud and sputter-coated in a Hummer VI Sputtering System with a 20-nm layer of gold–palladium. Specimens were viewed with a Jeol JSM-35CF Scanning Microscope.

Results

The gross necropsy showed no apparent abnormalities. Wet mounts of the gills revealed a moderate to heavy infestation of *A. ocellatum* (about 50–200 trophonts per filament). No parasites were seen in body or fin scrapings. Kidney cultures were negative after 7 d. Histological examination revealed numerous trophonts (20–57 μm along the long axis), a mild to moderate diffuse epithelial hyperplasia, and abnormally fewer goblet cells; all these features have been reported in gilthead bream *Sparus aurata* that have amyloodiniosis (Paperna 1980). However, few lesions displayed a depression at the site of parasite attachment that was considered characteristic of gill infestations in gilthead bream (Paperna 1980).

The North Carolina isolate had key characteristics of *A. ocellatum* Brown and Hovasse 1946, as described by Brown (1934), Nigrelli (1936), and Brown and Hovasse (1946), including a red stigma, rhizoids that penetrate the host cells, and a stomopode. However, the dinospores were anteroposteriorly flattened (Figure 1), and their average size was 8 μm long by 13.8 μm wide (N = 50). Except for a small percentage of deformed dinospores, virtually all parasites in our cultures had this shape.

After identifying *A. ocellatum* on the fish, a single treatment with copper sulfate was performed.

However, within the next 3 d, several thousand fish died with clinical signs of respiratory distress (i.e., aggregation near the surface of the water, rapid breathing). Three days after the copper treatment, fresh well water (0‰ salinity) was pumped into the pond. Within 2 d, the salinity was 0‰ and no more fish died.

Discussion

Both Brown (1934) and Nigrelli (1936) described *A. ocellatum* as having gymnodinoid (i.e., barrel-shaped) dinospores. The dinospores described by Nigrelli (1936) were 12 μm long by 8 μm wide, whereas those observed by Brown (1934) were either 12.5 μm long by 9 μm wide (1930 observations) or 15 μm long by 14 μm wide (1931 observations).

Neither investigator could confirm the source of infection. Brown (1934), whose fish were kept at the London Zoological Society Aquarium, speculated that parasites in her study were carried by fish from the Dutch East Indies and Bermuda. Nigrelli (1936), whose fish were kept in the New York Aquarium, described infestations from aquarium fishes that had been collected from waters of Sandy Hook Bay, New Jersey. However, species from the East Indies were also present in the New York Aquarium, although these fish did not show clinical signs of amyloodiniosis. Lawler (1980) also found that *A. ocellatum* isolated from fishes of the Mississippi Sound, Gulf of Mexico, had anteroposteriorly flattened dinospores. The taxonomic importance of this characteristic is uncertain, but it suggests that different strains of *A. ocellatum* and possibly other species of *Amyloodinium* may exist. Confirmation of this hypothesis will require a more comprehensive examination of the morphology, pathology, and other characteristics of isolates collected from other geographic regions and hosts.

Amyloodiniosis may have been initiated by the hypoxic stress that preceded the North Carolina outbreak by about 2 weeks. No problems were experienced during the first summer of growth or in two other 2.4-hectare ponds having similar densities of hybrid striped bass that did not experience hypoxia. Stress factors, such as overcrowding (Lawler 1980), are believed to precipitate outbreaks of amyloodiniosis. Furthermore, overcrowding alone may facilitate its spread.

Although *A. ocellatum* is certainly capable of causing massive and acute mortalities, copper toxicity may have exacerbated losses in this case. High levels of copper were initially added to the pond (at the beginning, free copper was 0.3 mg/L), which may have aggravated the respiratory distress caused by amyloodiniosis.

Because these fish were spawned and raised to juveniles in fresh water and because no other fish had been introduced into the farm pond from other areas, the source of the parasite was most likely the Pamlico River. To our knowledge, this is the northernmost record of an *A. ocellatum* outbreak in any culture facilities along the northern Atlantic coast. However, there is evidence that the geographic range of *A. ocellatum* can extend well into the temperate zone during warmer parts of the year. For example, it is a major problem in the culturing of striped bass and red drum *Sciaenops ocellatus* in South Carolina (W. Jenkins, South Carolina Wildlife Resources Commission, personal communication). Nigrelli (1936) reported the parasite as a latent infestation from wild fish in Sandy Hook Bay, New Jersey, although outbreaks were never observed in the wild. Interestingly, Brown (1934) reported that *A. ocellatum* could infest even coldwater species native to the British Isles, such as cod (*Gadus* sp.), when aquarium water temperatures were optimal and warmwater marine fish were present as carriers. There is also evidence that *A. ocellatum* can cause epidemics in coolwater environments, such as the northern Mediterranean Sea (Paperna 1980). If these outbreaks are caused by different strains of the parasite, it is important to prevent the introduction of such isolates into other areas, especially as marine aquaculture becomes more widespread.

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