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Surface Area of "Blotchy Bass" Spots Change Over Time in Smallmouth Bass (*Micropterus dolomieu* Lacepède, 1802)

Kelsey Young[1], Steven Seiler[1], Coja Yamashita[2], and Steve Davis[3]

[1]Department of Biological Sciences, Lock Haven University of Pennsylvania
[2]Pennsylvania Fish and Boat Commission, Harrisburg, Pennsylvania;
[3]USFWS Northeast Fishery Center, Lamar, PA

ABSTRACT

Blotchy bass syndrome is the occurrence of black ink-like spots on the skin of *Micropterus* species. The cause is unknown but some reports from anglers suggest spot occurrence increases with colder water temperature. Despite several decades of being aware of the spots, no studies have formally monitored changes in spot size or prevalence on individual fish. The objective of this study is to observe and to quantify blotchy bass spots in smallmouth bass to determine if the spots change over time. Thirty-one smallmouth bass (*Micropterus dolomieu*) were collected by electrofishing from the West Branch Susquehanna River near Selinsgrove, PA, and transported to the Northeast Fishery Center at Lamar, PA. Twenty fish exhibiting spots, the melanistic group, and eleven fish showing no sign of spots, the normal group, were mixed into five tanks fed by spring water. Because temperature is speculated to contribute to changes in blotchy bass appearances, we artificially increased water temperatures in some tanks using inline heaters. To monitor changes in blotchy bass spots, we photographed each fish at biweekly or monthly intervals. Photographs from the beginning and end dates were observed to determine quantitative changes in blotchy bass spots. We documented gains and reductions in blotchy bass spot area in both normal and melanistic fish. Bass in the heated tanks did not show a clear change in the area of spots relative to fish in unheated tanks. Our study documents that the area of spot on melanistic bass does change over time, with some fish gaining and some fish losing spot area. Further studies are needed to fully understand the ultimate cause of these changes.

Keywords: temperature, melanistic bass

Blotchy bass syndrome, or melanistic bass, is the occurrence of black ink-like spots on the skin of *Micropterus* species). Spots occur predominantly on bass larger than 29.9 cm and more frequently on bass over 38.1cm (Carlson 1986). In 1986, a group of biologists documented that the occurrence of this condition was along the east coast of the United States and a few additional states such as Missouri, Iowa, Tennessee, and Ohio that contained major inland rivers (Carlson 1986). The condition has been reported to be more prevalent in cool months compared to summer months (Carlson 1986); however, it is unclear whether fish experience an increase in spot prevalence or if the condition is simply reported more commonly by fisherman catching fish with spots during spring and fall. The definitive cause for melanistic spots on fish has not been identified however; several studies have proposed possible causes. Okihiro et al. (1993) described various xenobiotics that may contribute to black pigmented lesions in pacific rockfish (*Sebastes* spp.). Virological, bacteriological, and histopathological assessments between non-spotted and spotted North Sea dabs (*Limanda limanda*) revealed a higher frequency of lymphocyte infiltration in the areas of skin with spots, a sign of an active

immune response (Noguera et al. 2013). Another study suggests that spots may be related to stress responses in the environment because spotted fish consistently had lower levels of cortisol (Kittilsen et al. 2009). These studies do not suggest that primary causes of melanistic spots are contagious.

In 2012, samples of smallmouth bass (*Micropterus dolomieu*) with blotchy bass syndrome were submitted to the U.S Geological Survey National Fish Health Research Laboratory in West Virginia. A histological evaluation of the spots revealed that melanin-containing cells had migrated and proliferated in the epidermis layer of the skin; whereas in normal bass skin, the melanin-containing cells are located in the dermis (Blazer 2012). The cells were pleomorphic, meaning these cells had many different shapes and were loosely arranged compared to normal melanin-containing cells that are elongated and densely packed (Blazer 2012). Given this most recent histological analysis, blotchy bass spots do not appear to be melanomas (Blazer 2012).

Blotchy bass syndrome is an anomaly to fish biologists and anglers in the eastern United States because the cause and behavior of these spots is unknown. Despite several decades of reporting the occurrence of blotchy bass from riverways, no studies have formally monitored changes in spot prevalence on individual fish. The main objectives of this study are to observe and to quantify changes in blotchy bass spots in smallmouth bass in captive fish as a step toward better understanding how these spots develop. Our secondary objective is to determine if any changes in spots are associated with water temperature.

METHODS

Thirty-one sexually mature smallmouth bass (*Micropterus dolomieu*) were collected by electrofishing at the Pennsylvania Fish and Boat Commission (PFBC) Shady Nook access of the Susquehanna in Selinsgrove, PA on 3 April 2015 and transported to the Northeast Fishery Center (NEFC) facility at Lamar, PA. Twenty fish exhibited spots and eleven fish showed no sign of spots. On 9 April 2015, passive integrated transponder (PIT) tags were injected near the dorsal fin after sedating each fish with tricane methanesulfanate (MS-222). While still under anesthesia, the left and right sides of each fish were photographed. Each photograph included a ruler and an ID tag with the fish's individual PIT number (Figure 1A). The fish were randomly separated into five tanks labelled A, B, C, D, and E. Two normal fish (no spots apparent) and four melanistic fish (show spots) were placed in tanks A, B, C, and E. Three normal fish and four melanistic fish were placed in tank D. Fish were fed small rainbow trout (*Oncorhynchus mykiss*) or nightcrawlers

Figure 1. Photographing and analyzing left and right sides of a melanistic smallmouth bass after being anesthetized with tricane methanesulfanate (MS-222). A. Photographing the right side of a smallmouth bass with a ruler and (PIT) tag number specific to the individual fish. B. Identifying the 10cm reference length in tpsDig2. C. Using the pen feature in tpsDig2 to outline a zoomed in view of a blotchy bass spot on the operculum of a smallmouth bass.

(*Lumbricus terrestris*) on a regular schedule throughout the study period by staff at the US Fish and Wildlife Service based on the approximate weight of the fish in each tank.

The tanks were constantly supplied with water from a spring on the Northeast Fishery Center property. In April and May, the water temperature in the tanks averaged at 11.3°C and 13.9°C, respectively. Because temperature has been suggested as a factor related to changes in the prevalence of spots, we artificially raised the temperature of some tanks using an inline heater for several weeks beginning in June. Tanks A, B, and C were heated at an average of 20.3°C from 23 June 2015 to 15 August 2015. Tanks D and E remained at ambient temperature of the spring (average14.9°C) during this time period.

To monitor changes in blotchy bass spots, we photographed each fish at biweekly or monthly intervals. Each fish was sedated with MS-222 long enough to photograph the left and right sides. Fish were then placed back in their respective tanks for recovery. Photographs were observed each time to determine if any substantial qualitative changes spots occurred since the previous photograph was taken. To quantify changes in spot area, photographs from 9 April 2015 and 1 October 2015 were compared using TPSDig2 (Rohlf 2000). To measure surface area covered by spots, each spot was outlined by using the pen tool in TPSDig2 (Rohlf 2000) by setting the reference scale to 10.0 cm, zooming in on the spot as close as possible, then circling each spot with the pen tool (Figure 1). The TPSDig2 program calculated the area (cm^2) of the spot. The area of the spots on the left and right side of each fish were summed to get a total area of blotchy bass spots for each fish. We calculated the difference in surface area covered in spots of the left and the right side from the first and last photograph. Although care was taken while digitizing the margin of each spot, some small error in the measurement of the surface area for each spot was expected. Therefore we considered a fish as gaining or losing spot area if the change was greater than or equal to $1cm^2$ on a side. When fish exhibited a gain of surface area on one side and loss on the other side, we categorized it as mixed. Some fish died from unknown reasons before 1 October 2015; in these instances, the last photograph taken before they died was used to calculate spot coverage.

RESULTS

Over the course of the study, seven smallmouth bass died. Of the seven bass that died, six were melanistic and one was normal, however five of these bass exhibited a loss of melanistic spot area and two were classified as mixed based on their spot changes in the photograph taken before they died (Figure 3). All seven bass that died were from tanks where the water was heated, however two of the deaths occurred before the inline heaters were installed. The remaining five fish that died were from tank B and included one normal bass and four melanistic bass.

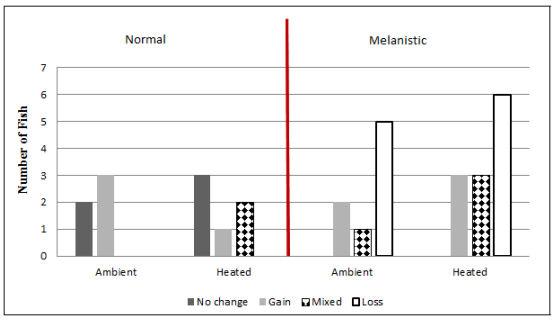

Figure 2. Changes in surface area of spots in normal and melanistic smallmouth bass grouped by water temperature across all tanks. Fish were considered to gain or have a loss of spots if the difference in surface area from first and last photograph was greater than 1 cm^2. Mixed fish were considered to have gained surface area of spots on one side and lost surface area of spots on the opposite side.

After analyzing the surface area of blotchy spots on bass from photographs taken on 1 October 2015, changes in the 11 normal fish were identified. Five normal fish in tanks A, B, and E did not add more than 1cm^2 in blotchy bass spots (Table 2). Four normal fish in tanks C and D gained spots on each side (Figure 2) (Figure 4). Two normal fish in tanks B and C gained spots on the right side but did not gain spots on the left side (Figure 2). Five melanistic fish gained surface area of spots on each side (Figure 2). Eleven smallmouth bass lost surface area of spots on both sides (Figure 2). Four fish gained area of spots on one side and lost spots on one side (Figure 2).

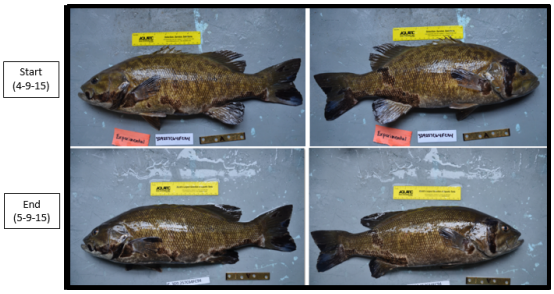

Figure 3. A melanistic smallmouth bass showing loss of surface area of spots on left and right sides from the first photograph day (top), 9 April 2015, and the last photograph day (bottom), 9 May 2015.

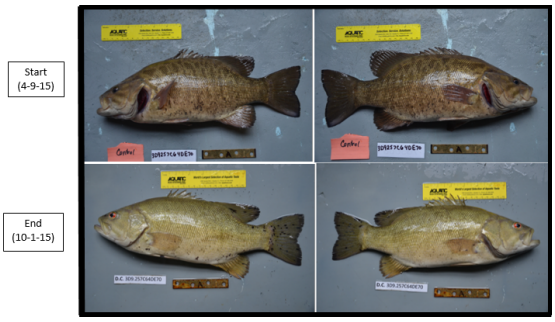

Figure 4. Left and right sides of a normal smallmouth bass from first photograph day (top), 9 April 2015, to last photograph day (bottom), 1 October 2015. The top photographs show that there was no appearance of blotchy bass spots on either side. The bottom photographs show that the normal fish gained spots at the end of the study.

DISCUSSION

The changes in blotchy bass spots do not appear to be the cause of early death of smallmouth bass. Twenty six of the 31 bass exhibited a change in the surface area of the melanistic spots during our study with the number of fish categorized as gain, mixed, or loss of spots at 9, 6, and 11, respectively (Figure 2). The surface area of the spots in several of the bass that died early showed a decrease while only one fish that died gained area of spots on both sides. One fish showed a considerable loss in area of spots on each side but still died before the end of the experiment (Figure 3). With six out of seven fish that died before October 1 showing a decrease in surface area of spots, we do not believe blotchy bass spots were the cause of death for these fish unless the loss of spot area is indirectly connected to an underlying physiological stressor in these bass. Further histological studies would be necessary (see below) to assess if the fish that died exhibit signs of stress. The high proportion of fish dying in tank B cannot easily be attributed to the higher water temperature because tank A and tank C were also heated and fish deaths did not happen in these tanks after water was heated; each tank had a single mortality that occurred at ambient temperature.

Although our results do not strongly support the hypothesis that temperature influences spot changes, they are suggestive of a higher prevalence of blotchy bass spots on fish in colder months compared to summer months. Thirteen smallmouth bass in the heated tanks showed no changes or loss in surface area in spots, while only five fish gained in spot surface area. Therefore, out of the 18 fish in the heated tanks only 27.7% of the fish gained spots. In ambient temperature tanks, seven fish had a loss or no change in surface area spots and five fish had a gain in surface area, meaning 46.2% of fish gained in surface area. We recommend a systematic survey of wild smallmouth bass populations across seasons/water temperatures to verify whether spot prevalence is higher during

colder months. This study does not indicate a clear pattern to the changing surface area of blotchy bass spots. Each of our tanks contained a smallmouth bass that either lost area of spots on one or both sides and a bass that gained spots on one or both sides (Figure 2). Tanks A and C contained normal fish that remained spotless lending some support to the hypothesis that the cause of spots is not contagious (Figure 2). To the contrary, tanks B, D, and E each contained at least one normal smallmouth bass that gained spots by the end of the experiment (Figure 2). The cause of blotchy bass syndrome is still unknown; however, our experiment documents that the blotchy bass spots can change over time: blotchy bass spots spontaneously arise in fish that did not previously show signs of spots and fish with blotchy bass spots showed increases or decreases in surface area. Our study was limited by the logistics of collecting and transporting fish and the number of tanks to hold the fish; future laboratory based studies are being planned to include more fish and a broader temperature range to better determine how temperature might influence blotchy spot development.

Changes in spot size can be indicative of many different physiological responses. The production of melanin, called melanogenesis, is extensively controlled by genetics and the neuroendocrine system. Hormonal imbalances such as hyperthyroidism, hypoadrenalism, and elevated levels of estrogen can upregulate the rate limiting proteins of melanogenesis causing hyperpigmentation (Blanton 2000, Jegstrup and Rosenkilde 2003, Slominski et al. 2004). The unique structure of melanin gives it the ability to bind to xenobiotics, pathogens, and parasites (Riley 1997). Researchers have been using melanin producing cells as biomarkers for detecting heavy metals and pesticides in fish (Allen et al. 2004, Dierksen et al. 2004, Singh et al. 2015, Luga et al. 2008). Pathogens and parasites like *Flavobacterium psychrophilum* and *Neascus* (black spot disease) have also been known to cause hyperpigmentated areas (Steedman 1991, LaFrentz et al. 2002). Further research will be needed to learn if and how individual fish physiology or pathogens contribute to blotchy bass spots in smallmouth bass.

Future Research

The remaining fish were sent to the U.S Geological Survey, Fish Health Branch, Leetown Science Center, WV. Complete necropsies will be performed to collect tissue samples for a broad suite of analyses to determine contributing factors. Skin samples will be collected for histopathological examination and gene expression analysis. Depending on the findings, further analyses such as chemical contaminant concentrations in skin tissue or measuring various plasma hormone concentrations may be necessary.

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