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Tests of Adaptive Coloration Hypotheses for Madtom (Notorus) Catfishes (Siluriformes: Ictaluridae)

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TESTS OF ADAPTIVE COLORATION HYPOTHESES FOR MADTOM (*NOTURUS*)
CATFISHES (SILURIFORMES: ICTALURIDAE)

A Thesis

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Abstract

Predators select for defensive adaptations, such as stings, toxins, and camouflage color patterns. Madtoms, *Noturus,* are diminutive catfishes with dorsal and pectoral stings. Thirteen of the 25 nominal species have serrated spines in the pectoral sting and a contrasting pigment pattern. Behavior of two saddled species, *N. miurus* and *N. hildebrandi*, and one uniformly colored species, *N. leptacanthus,* was investigated to test if the pigment pattern is camouflage. Saddle spacing and crypticity of the saddled species were measured against various substrates and were found to be unevenly spaced, which could be camouflage when viewed against gravel. Given substrate choices, madtoms preferred gravel during daylight conditions. In subsequent experiments, all species were given colored gravel to test color vs. texture-based substrate choice and preferred dark substrates. In the presence of a predator stimulus, madtoms preferred gravel at night and dawn. The pigment pattern likely is camouflage when viewed against gravel substrates.

Introduction

Predation influences morphological, behavioral, physiological, and biochemical evolution in prey and is such a powerful selection pressure that most animals have anti-predator adaptations (Cott, 1940; Edmunds, 1974; Malcolm, 1992). Bony fishes, with more species than any other vertebrate group, have diverse defensive adaptations, including spines, toxic stings and skin, cryptic color patterns, and behaviors such as intimidation, flight, and retreat (Cott, 1940; Hoogland, 1957; Hinton, 1962; Breder, 1963; Edmunds, 1974; Endler, 1988; Guilford, 1992; Marshall, 2000). For example, sticklebacks (Gasterosteidae) have sharp spines (Hoogland, 1957), pufferfish (Tetraodontidae) have toxic tissues (Landau, 1997), and some darters (Percidae) have cryptic color patterns (Armbruster and Page, 1996). Predation also influences multiple trait evolution in fishes so that many species have complex anti-predator strategies (Phoon and Alfred, 1965; Marshall, 2000). These include, stonefishes with a cryptic color pattern and poisonous dorsal spines (Phoon and Alfred, 1965), and some coral reef fishes have cryptic color patterns (Marshall, 2000) and escape behaviors, such as hiding among corals or associating with stinging anemones (Edmunds, 1974).

Despite the diversity of putative anti-predator adaptations among prey species, very few traits have been demonstrated to provide an adaptive benefit. Among those anti-predator adaptations that have been examined experimentally, tests of predicted benefits have yielded ambiguous results (Hoogland, 1957; Hinton, 1962; Breder, 1963; Gilbert, 1967) as illustrated by work with sticklebacks that have spines that presumably deter predators (Hoogland, 1957). Under the assumption of anti-predator benefit, the number of spines should be inversely related to susceptibility to predation. However, three-spined sticklebacks survive attack more often than ten-spined sticklebacks (Hoogland, 1957). Apparently, the predicted relationship between spine number and susceptibility to predation is confounded by spine length; three-spined sticklebacks have fewer but longer spines (Hoogland, 1957).

Assessing the adaptive value of any defensive trait is difficult because often traits have more than one characteristic that could be defensive, such as, length and number of spines.

Particularly challenging is this regard are species with multiple, complex adaptations. Among freshwater fishes, nocturnal species are likely to have defense strategies composed of multiple antipredator adaptations due to a dual predation threat. Nocturnal species face visually orienting diurnal predators (wading birds, bass, sunfish, trout) and nocturnal predators (snakes), which typically hunt by chemo- or electroreception. Although diurnal species also face both types of predation, night active species are more susceptible to nocturnal predation than diurnal predation because they are active at night, and diurnal predation, because they are resting on the substrate during the day. Not surprisingly, many nocturnal species have multiple anti-predator traits. Porcupine fishes (Diodontidae) have toxic skin as well as sharp spines covering much of the body (Malpezzi et al., 1997). Madtom catfishes (*Noturus*) have a disruptive color pattern and toxic fin stings (Reed, 1900; Reed, 1907; Taylor, 1969; Clark, 1978; Chan and Parsons, 2000).

When viewed from above, a madtom on gravel substrate is inconspicuous, at least to human eyes. Disturbances that alter stream substrate may contribute to greater predation in freshwater fishes that rely on a color pattern as an anti-predator trait. Anthropogenic alterations of stream substrates, especially through siltation, might be increasing the risk of extirpation for madtoms and other freshwater fishes with a similar camouflage pattern. Siltation, a common byproduct of environmental disturbance, covers the natural substrate with a fine layer of sand or other material (Ross et al., 1992) and could negate the benefit of a camouflage color pattern. Thirteen species with contrasting light and dark dorsal color patterns, including four madtom catfishes, eight darters (Percidae), and one sculpin (Cottidae), are on the Federal Endangered Species list. Also, several madtom species with a saddle pattern are locally protected or threatened. For example, *N. munitus* is protected in Louisiana, Mississippi, and Tennessee (Johnson, 1987), and *N. furiosus* is protected in North Carolina (Bailey, 1977). These species likely could be at a greater risk of extinction or extirpation due to the negation of an anti-predator adaptation by environmental conditions. Protection of endangered species, particularly through habitat preservation or restoration, requires an understanding of the adaptive value of all anti-predator defenses, including color patterns.

Common species are used as surrogates for rare or endangered species to study reproductive strategies, diet, habitat preference, habitat use, anti-predator adaptations, and a variety of other characteristics that might contribute to their persistence (Davidson et al., 1999). For example, rainbow trout (*Oncorhynchus mykiss*) were used as surrogates of the rare kahawai (*Arripis trutta*) to determine the effect of tagging on swimming performance (Davidson et al., 1999). Although choice of surrogates for endangered species is influenced largely by availability of critical biological information, sister or closely related species should be preferred. (Include a

reference to support this claim.) Allozymic, chromosomal, morphological, and mtDNA data (Etnier and Jenkins, 1980; Grady and LeGrande, 1992, and Hardman 2003) suggest a close relationship between two of my study species, which are common, and two protected species. Determining the potential effects of habitat alterations on the predation threat to endangered species, as measured through surrogates, contributes to the development of effective management strategies for the two threatened madtom species.

Thirteen of the 25 nominal madtom species have concentrations of pigment that form four dark bands that extend across the dorsal surface and onto the lateral body surface. Lightly pigmented regions separate the dark bands, producing dorsal saddles. Presumably, the saddle pattern is camouflage against a mottled background or substrate, such as gravel (Armbruster and Page, 1996).

Possible adaptive values of a contrasting pigment pattern, such as seen in madtoms, include predator confusion, conspecific signaling for mating, aposematic (warning) coloration, and camouflage by disruptive coloration (Cott, 1940; Keune and Barbour, 1983; Mboko and Kohda, 1995; Armbruster and Page, 1996; Guilford, 1988). A pigment pattern that confuses predators is typical of schooling fishes, which often have a barred color pattern that extends across the lateral body surface (Leal and Rotman, 1993). Deep-bodied schooling fishes have lateral banded color patterns to appear larger (Barlow, 1972). Madtoms, however, are solitary, dorsoventrally flattened fishes in which the pigment often does not extend below the dorsal third of the body. Therefore, the color pattern is not likely for predator confusion.

A common use for a conspecific signaling color pattern is mate recognition. If the contrasting saddle pattern functions in mate recognition, as in many darter species (Keuhne and Barbour, 1983), madtoms should be sexually dimorphic for color pattern; however, sexual dimorphism for this trait has not been documented among madtom catfishes (Taylor, 1969).

Some fishes warn potential predators of their unpalability or toxicity with a bright aposematic (warning) color pattern (Guilford, 1988). For a color pattern to serve as aposematic coloration, the fish must be unpalatable due to traits such as sharp spines or toxins. Also, the color pattern should be conspicuous (Cott, 1940). Madtoms have both sharp spines and toxins; however, according to experimental observations they spend the day resting on matching substrate and are likely inconspicuous.

A fourth potential benefit of a contrasting pigment pattern in fishes is camouflage by disruptive coloration. Disruptive coloration, as defined by Cott (1940), is "a superimposed pattern of contrasted colours and tones serving to blur the outline and to break up the real surface form, which is replaced by an apparent but unreal configuration." A disruptive pattern renders

an individual's outline inconspicuous against its substrate and should be accompanied by inconspicuous behavior (Cott, 1940).

Study Species

      The three study species are common in the southeastern United States. *Noturus miurus*, the brindled madtom, is widely distributed in eastern and central North America, extending from the Great Lakes drainages (Lake Erie and Lake Ontario only) through the Ohio and Mississippi River basins south to the Gulf Coastal drainages (Rohde, 1978*c*). *Noturus hildebrandi*, the least madtom, occurs in western tributaries of the Mississippi River, from the Hatchie River south to the Homochitto River (Taylor, 1969; Rohde, 1978*a*). The speckled madtom, *N. leptacanthu*s, ranges from the Amite and Comite Rivers east along the Gulf Coastal Plain to the Edisto River in South Carolina (Taylor, 1969) and south to the upper St. John's River system in peninsular Florida (Rohde, 1978*b*).

      Noturus miurus and *N. hildebrandi* have serrated dorsal and pectoral fin spines (Taylor, 1969) and a contrasting dorsal pigment pattern. Both species are frequently collected in gravel riffles (Rohde, 1978*a*, Mayden and Walsh, 1984, Pflieger, 1991), where the contrasting color pattern could be camouflage (Armbruster and Page, 1996). However, *N. miurus* also occurs in pools with soft bottom such as sand or mud (Rohde, 1978*c*, Burr and Mayden, 1982), and *N. hildebrandi* is occasionally taken over sandy substrate (Rohde, 1978*a*, Mayden and Walsh, 1984). Both *N. miurus* and *N. hildebrandi* are members of the subgenus *Rabida*, which includes all madtoms with a contrasting dorsal pigment pattern and serrated spines (Taylor, 1969). *Noturus miurus* is a surrogate for *N. taylori* to which it is closely related (Grady and LeGrande, 1992; Hardman 2003), and *N. hildebrandi* is a surrogate for *N. stanauli*, its sister species (Etnier and Jenkins, 1980; Grady and LeGrande, 1992).

      Noturus leptacanthus (subgenus *Schilbeodes*) (LeGrande, 1981) was included in this study to test the potential benefits of dorsal saddles in madtoms by investigating the behavior of a non-saddled species. Unlike *N. miurus* and *N. hildebrandi*, the speckled madtom lacks both the contrasting dorsal pigment pattern (Knopf, 1995) and serrations on the toxin delivering spines. *Noturus leptacanthus* is commonly taken in vegetated gravel or coarse sand riffles (Taylor, 1969; Rohde, 1978*b*).

      Armbruster and Page (1996) hypothesized that saddle spacing is predictive of function and examined the saddle spacing in six stream fishes, including the checkered madtom (*N. flavater)* by measuring the intervals between the saddles and comparing the interval lengths within a species. They suggested that unevenly spaced dorsal saddles on a fish that occurs over an

unevenly colored background, such as gravel, which is composed of rocks and gravel of different sizes, would be disruptive coloration. Applying Armbruster and Page's (1996) methods to two of my study species, *N. miurus* and *N. hildebrandi,* offers some indication of the potential benefit of the saddles as camouflage. Both species have unevenly spaced saddle patterns that should, according to the criteria of Armbruster and Page (1996), be disruptive coloration.

For the saddle pattern to be effective disruptive coloration, saddled madtoms should be sedentary diurnally to remain inconspicuous against the substrate (Cott, 1940). Also, saddled madtoms should use substrates against which they are camouflaged. These predictions were tested, first by measuring the effectiveness of the color pattern as camouflage against various substrates, second by observing the substrate choice and nocturnal behavior of *N. miurus* and *N. hildebrandi*, and finally by studying the behavior of *N. leptacanthus*. *Noturus leptacanthus* lacks dorsal saddles and the contrasting dorsal pigmentation pattern of *N. hildebrandi* and *N. miurus* and should show no bias when offered alternative substrates.

Materials and Methods

To determine if saddle pattern is a passive antipredator defense, saddle spacing was measured along the dorsal surface of two saddled madtom species, *N. hildebrandi* and *N. miurus*. Crypticity of pigmentation patterns for each species was measured across several natural substrates. Finally, behavioral experiments investigated the effects of substrate texture and color, water flow, and predators on substrate choice.

Saddle Pattern Measurements

Armbruster and Page (1996) suggested that saddle spacing distinguishes a dorsal saddle pattern that is aposematic from a camouflaging pattern. Camouflaging saddles are irregularly spaced, whereas aposematic saddles should be distributed uniformly. Armbruster and Page's (1996) methods were applied to determine saddle spacing in *N. hildebrandi* and *N. miurus*. To standardize for saddle width between species, the distance from the origin of the anterior most saddle (saddle 1) to the anterior and posterior edge of each more posterior saddle was recorded for 60 individuals of each species. The distances from the origin of saddle 1 to the midpoint of each more posterior saddle (saddle distances) (S2D, S3D, S4D; Figure 1) were calculated by averaging the distance from the origin of saddle 1 to the anterior and posterior edge of each saddle and are referred to as saddle distances. Interval lengths (Figure 2) were calculated (using the equations included in Figure 2) to determine saddle spacing. Equal interval lengths indicate evenly spaced saddles, while unequal intervals indicate unevenly spaced saddles. The ratio of interval length (Figure 2) to entire saddled length (Figure 1, SL) was calculated to standardize for length variation among species (J. Armbruster, pers. comm.) and were arc-sine transformed. An ANOVA was performed to determine whether dorsal saddles are evenly spaced (Sokal and Rohlf, 1995), and a post-hoc Tukey-Kramer test was run to determine if interval lengths differed significantly within each species, which would indicate an unevenly spaced saddle pattern (Sokal and Rohlf, 1995).

Crypticity Estimates

Cott (1940) and Endler (1984) suggested that camouflaged organisms should choose habitats that match the organism's color pattern, which can be determined from a correlation table relating color pattern and substrate. Endler's (1984) methods were adapted to measure the correlation between the color pattern of each species and the substrates offered in substrate-choice experiments (see below). A wooden frame (0.3048 m X 0.3048 m) was placed on a black plexi-glass sheet and filled with one substrate, either sand or natural, white, or black gravel. Natural gravel was uniformly spray painted either black or white because of the contrast of these two colors. The box and substrate were photographed from a height of one meter with a Nikon CoolPix 990 Digital Camera. The pictures were transferred to an overhead transparency and projected onto white poster board from a distance of one meter. Three transects were made through the substrate photograph and color patch boundaries were marked. Sand has naturally varying colors and the painted gravel had shadows which were measured. The number of colors along the transect was recorded and the length of each color patch was measured. Color patch lengths were summed across transects and a percentage of total transect length was calculated for each color in each substrate.

Three individuals of each species were also photographed to measure specific color of each species (Endler, 1984). One individual was placed on a gray plexi-glass sheet and photographed from a height of one meter with a Nikon CoolPix 990 Digital Camera. The photographs were transferred to an overhead transparency and projected onto white poster board from a distance of one meter. One transect was drawn along the longitudinal axis of the fish and the number of colors and length of each color patch were measured as above. Color patch lengths were summed for each individual and the totals for each species were summed. A percentage of total transect length was calculated for each color (Endler, 1984). Correlation coefficients, *r*, were calculated between color patch lengths of each species' color pattern and each substrate to determine matching of species' color pattern and substrate (Endler, 1984; Sokal and Rolhf, 1995).

Behavioral Experiments

Specimen Acquisition

Specimens for behavioral experiments were collected by seining at the field sites listed in Appendix 1 and transported to the laboratory, where they were held in 10 or 20-gallon tanks for a minimum of one week before being used in experiments. Madtoms were fed brine shrimp once daily and were initially kept on a constant 12:12 L:D photoperiod, which was later changed to an
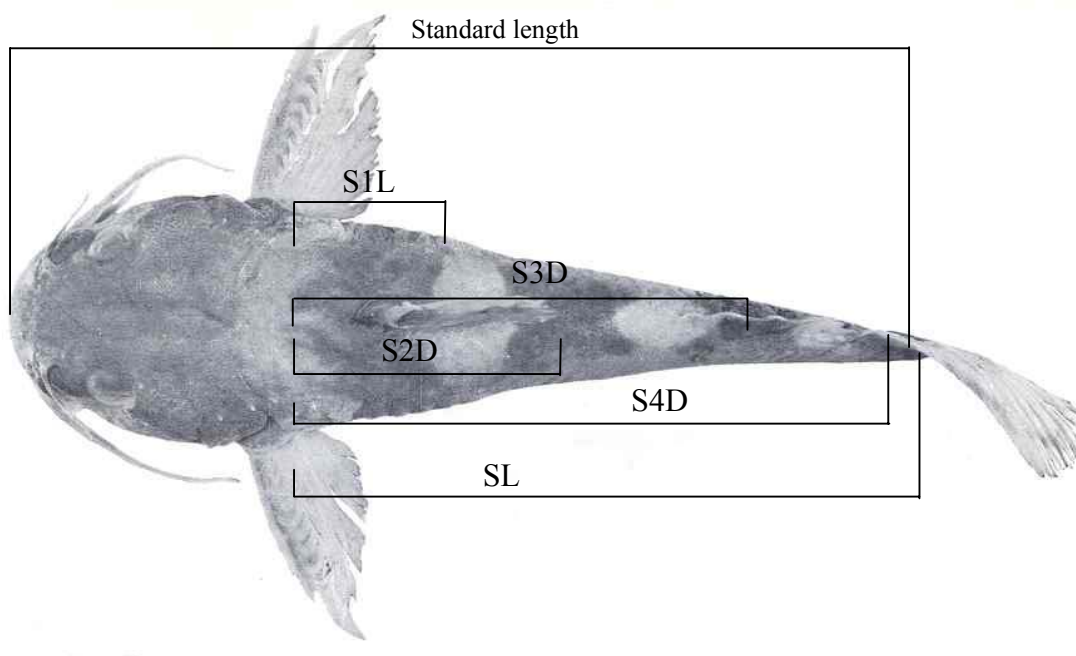
Figure 1. Saddle measurements, following Armbruster and Page (1996). S1L = Length of saddle 1; S2D = saddle 2 distance; S3D = saddle 3 distance; S4D = saddle 4 distance; SL = saddle length.
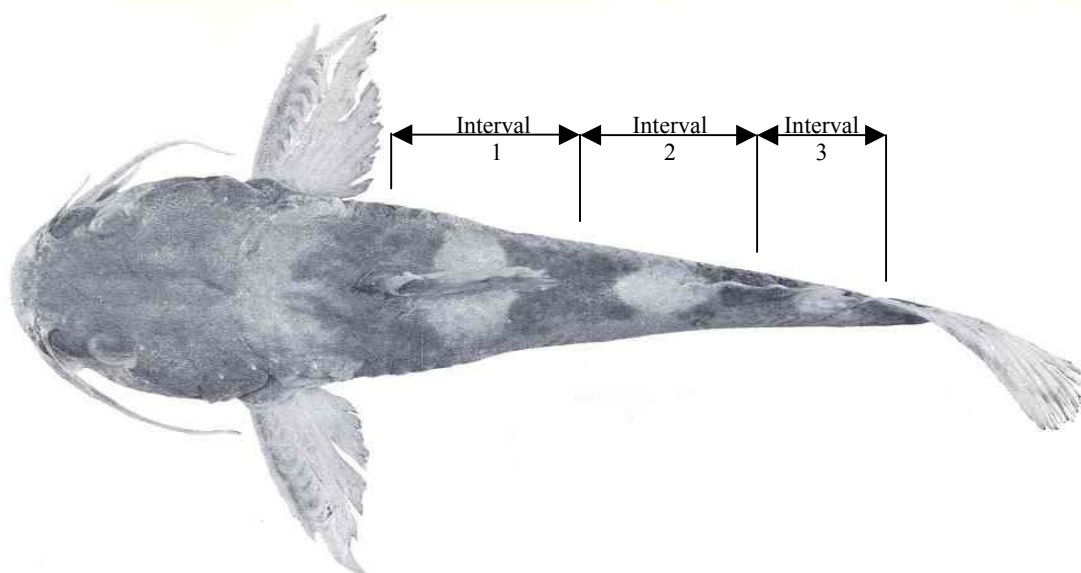
Figure 2. Saddle interval measurements, following Armbruster and Page (1996).
Interval 1 = S2D – ½ S1L, interval 2 = S3D – S2D, and interval 3 = S4D – S3D.

8:16 L:D photoperiod to accommodate experiments. Sample size for each experiment was 60 fish of each species, and fish were used in multiple experiments. All substrates used in experiments were autoclaved or thoroughly cleaned. Substrates chosen for experiments are often associated with madtom habitat and are naturally occurring in areas where madtoms were collected.

Experimental Design –Substrate Choice

For the color pattern to be camouflaging, saddled madtoms should choose mottled substrates, which best match their color pattern (Cott, 1940; Endler, 1984, Armbruster and Page, 1996). To determine which substrate madtoms use under varying laboratory conditions, substrate choice experiments were conducted, using wading pools (diameter: 1.1 m, depth: .15 m) and several natural substrates, such as gravel and sand. The bottom of each pool was divided into six sections (0.38 m X 0.14 m X .5 m), with an open rectangle in the center (0.1 m X 0.14 mm), and one substrate, either sand or gravel, was placed into three alternating sections (Figure 3).

Substrate Texture Preference

Madtoms tended to make an immediate substrate choice upon introduction to the pool. If introduced during daylight, they did not move from initial substrate choice. To control for an initial flee response, experimental animals were allowed to acclimate overnight. Fifteen madtoms were placed in the pool at least one hour before sunset and allowed to acclimate overnight. Pools were placed near a window so that substrate choice would be influenced by natural light cycles and the fright response to sudden bright light would be minimized. Substrate choice was noted one hour after sunrise and was recorded once an hour for five hours to determine if individuals would move after the initial choice. Madtoms did not move and the substrate chosen initially was recorded as substrate choice. Chi-Square tests were used to assess bias in substrate choice. To control for position preferences unrelated to substrate type, such as orientation to light, pools were rotated every two days.

Substrate Color Choice

According to Armbruster and Page (1996), a species with an unevenly spaced saddle pattern should choose an unevenly colored substrate. Therefore, to test the effect of substrate color on substrate choice, the bottom of the pools were divided into six sections (0.38 m X 0.14 m X .5 m) and three substrates, natural colored gravel (unpainted), white gravel (spray-painted with a non-toxic waterproof paint), and black gravel (spray-painted with a non-toxic waterproof paint), were each placed into two opposite sections (Figure 4). White and black were chosen as uniform

colors because of the contrast between dark and light. Fifteen fish were introduced into each pool at least one hour before sunset and allowed to acclimate overnight. As described above, substrate choice was recorded one hour after dawn and once an hour for the following five hours. The results were analyzed using a Chi-Square test to determine if substrate color choice was random.

Flow Effect

Because the three species of madtoms used in this research are stream species, lack of flow could have affected substrate choice in the pools. Therefore, a rectangular plexi-glass artificial stream (.325 m X 1.92 m) was constructed to test the effect of flow on substrate preference. Low flow (1.5 m/s) was produced with an Ehiem pump (model 2213). The stream floor was divided into six sections (0.325 m X 0.32 m), and three nonadjacent sections were filled with gravel and three with sand. Fifteen individuals were introduced into the stream at least one hour before sunset and allowed to acclimate overnight. Substrate choice was recorded for each fish one hour after sunrise, and once an hour for five hours. Sixty-three percent of the madtoms preferred the corners of the stream, regardless of the substrate. The stream setup was abandoned due to a corner effect, and flow was included in the pool setup by using an Eheim pump to draw water from the center of the pool and return it to the margin via a tube. Fifteen individuals were introduced into the pool at least one hour prior to sunset and allowed to acclimate overnight. Substrate choice was recorded one hour after sunrise, and once an hour for five hours. Each species' substrate choice was analyzed using a Chi-Square test to determine if substrate was randomly chosen for each species. To test the potential effect of flow, the results from the flow experiments and the substrate texture experiments were compared using a Linear Log-likelihood test to determine if the results from these experiments differed.

Predator Effects

Predators, acting as a selective agent, determine the adaptive value of a trait. Previous experiments tested the behavior of a species with a putative anti-predator trait (the saddle pattern), but did not address the behavior in the presence of a predator. Experiments to test the behavior of a saddled species in the presence of a predator were necessary to determine whether behavior differed due to a predator. Including a predator in the pool experiments was logistically challenging. Therefore, effects of a predator on madtom behavior was tested directly by exposing the specimens to a predator and indirectly by introducing water from a predator holding tank into the madtom tank. Because the reaction to both stimuli was similar, "predator water" (water taken from a 5-gallon aquarium in which a large-mouth bass (*Micropterus salmoides*) had been held for
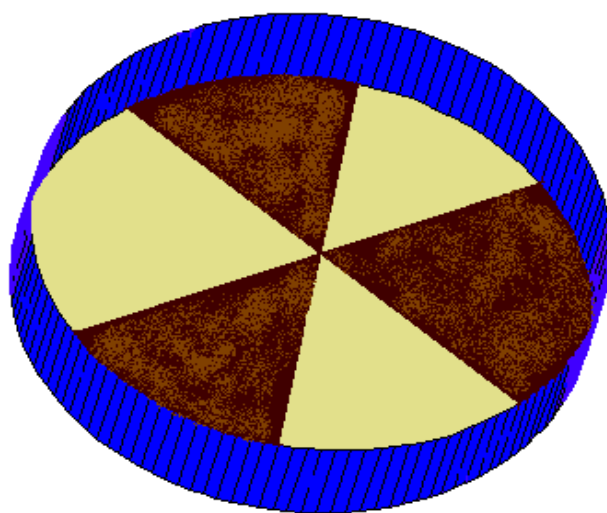
Figure 3. Substrate presentation in substrate texture experiments. Light areas are sand; dark areas are gravel.
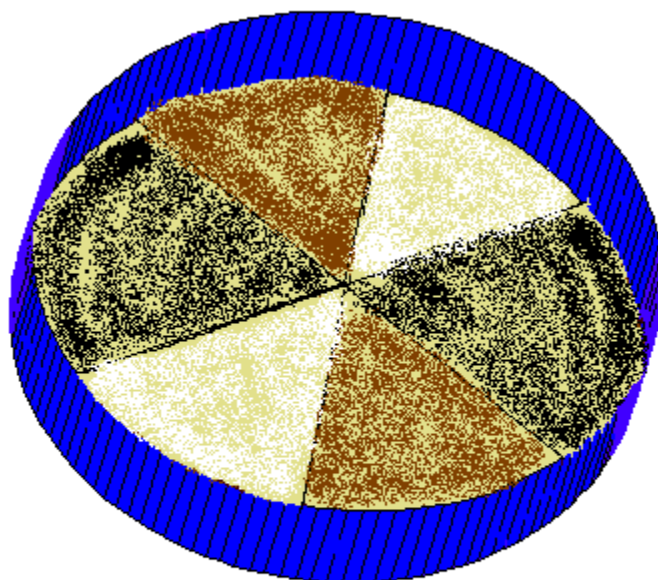
Figure 4. Substrate presentation in substrate color choice experiments. Fish were offered white, black, and natural gravel.

24 hours) was used as a stimulus to determine how predators influence substrate choice. Fifteen madtoms were introduced into a pool one hour before sunset and allowed to acclimate. One hour after sunset, individuals were observed and activity was recorded (using a red flashlight to facilitate observations). One hundred milliliters of predator water, taken from a 5-gallon aquarium in which a large-mouth bass had been held for 24 hours, was added to the center of the pool via a tube. The predator stimulus was introduced from behind a blind to minimize a possible fright reaction. Thirty seconds after the stimulus reached the pool, madtom activity and substrate choice were recorded. This experiment was repeated at 0500 and again two hours after sunrise to determine if time of day had an effect on substrate choice. These data were analyzed using a Chi-Square test to determine if substrate choice was random, in the presence and absence of a predator, and a log likelihood to test the null hypothesis that madtom behavior did not change due to a predator and response to a predator did not differ due to time of day.

Activity Periods

Cott (1940) suggested that camouflaged organisms should remain inconspicuous diurnally. To determine if madtoms rest on the substrate during the day, three individuals were placed in a pool at least one hour before sunset and allowed to acclimate overnight.   Each individual's activity was recorded every five minutes for one hour beginning at 0900 the following morning. Observations were repeated one hour after dark the same evening, using a red flashlight to facilitate nocturnal observations. Twenty-one trials with three individuals per trial were conducted and a G-test of Independence was used to test the null hypothesis that diurnal and nocturnal behaviors are not different.

Results

Saddle Pattern Measurements

Armbruster and Page (1996) suggest that a saddle pattern that consists of evenly spaced saddles would be more conspicuous than an unevenly spaced saddle pattern when viewed over a gravel substrate. To determine the saddle pattern spacing in *N. hildebrandi* and *N. miurus*, the ratios of interval length to saddled length (referred to as interval) for each species were compared within a species. According to the Tukey-Kramer tests, each interval was significantly different in length in *N. hildebrandi* (n = 60, F = 6.03, df = 1, P < 0.05); however, in *N. miurus*, intervals 2 and 3 do not differ significantly in length (n = 60, F = 2.01, df = 1, P > 0.05). Despite the apparent similarity of interval lengths in *N. miurus*, saddles were unevenly spaced in *N. hildebrandi* and *N. miurus* based on ANOVA (P < 0.05; Figure 5).

Crypticity Estimates

Endler (1984) indicates that a strong correlation between an organism's color pattern and chosen substrate color suggests a high level of crypticity. A correlation coefficient of 1 implies complete camouflage, while a coefficient of −1 would imply complete visibility. Both saddled species' color patterns are highly correlated with natural gravel (*N. miurus,* r = 0.6415; *N. hildebrandi,* r = 0.5755; Table 1); however, color is negatively correlated with natural gravel for *N. leptacanthus* (r = -0.2404; Table 1). Therefore, *N. miurus* and *N. hildebrandi* should prefer natural gravel as a substrate, and *N. leptacanthus* should prefer black gravel (r =0.9639; Table 1), strictly based on color pattern correlation. Although color pattern in each species is positively correlated with black gravel (*N. miurus*, r = 0.3842; *N. hildebrandi*, r = 0.4767), this relationship is strongest for *N. leptacanthus* (r = 0.9639; Table 1). Neither the color patterns of *N. leptacanthus*, *N. miurus*, or *N. hildebrandi* were positively correlated with white gravel or sand (Table 1).

Behavioral Experiments

Substrate Texture Preference

During the substrate texture experiments, *N. hildebrandi* exclusively used gravel substrates, and only two individuals of *N. miurus* and one individual of *N. leptacanthus* used sand as a substrate. Based on Chi-Square analysis of observed substrate choice versus expected substrate choice, *N. miurus*, *N. hildebrandi*, and *N. leptacanthus* preferred gravel to sand substrate (*N. miurus*: X^2 = 52.3,

df = 1, P < 0.001; *N. hildebrandi*: X^2 = 60, df = 1, P < 0.001; *N. leptacanthus:* X2 = 56.06, df = 1, P < 0.001; Figure 6).

Substrate Color Choice

Only fifteen individuals (five *N. miurus*, four *N. leptacanthus,* and six *N. hildebrandi*), of the 180 included in this experiment, used the white gravel as substrate. Chi-Square analysis indicate that species did not discriminate between natural and black gravel (*N. miurus*: X^2 = 5.85, df = 2, P > 0.05; *N. hildebrandi*: X^2 = 0.01, df = 2, P > 0.05; *N. leptacanthus*: X^2 = 0.01, df = 2, P > 0.05; Figure 7) but avoided white gravel (*N. miurus*: X^2 = 17.1, df = 2, P < 0.001; *N. hildebrandi*: X^2 = 14.7, df = 2, P < 0.001; *N. leptacanthus*: X^2 = 19.2, df = 2, P < 0.001; Figure 7). All three madtom species preferred darker colored substrates and did not distinguish between black and natural gravel.

Flow Effects

To test the effects of flow on substrate choice, experiments were conducted in pools that included flow. G-tests of Independence indicate that flow did not affect substrate choice in pools (G = 0.15, df = 1, P > 0.05), and the Chi-Square results indicate that all three species significantly preferred gravel (*N. miurus*: X^2 = 52.3, df = 1, P < 0.001; *N. hildebrandi*: X^2 = 60, df = 1, P < 0.001; *N. leptacanthus*: X^2 = 56.06, df = 1, P < 0.001) in the presence or absence of flow.

Predator Effects

In the presence of a predation threat, madtoms again preferred gravel to sand. At night, most madtoms, except one *N. leptacanthus* individual, were swimming and immediately moved to the substrate and selected gravel (Figure 8) when exposed to a predator stimulus. Three *N. miurus* and one *N. leptacanthus* chose sand substrate during the nighttime experiments. Chi-Square results indicated a significant preference (*N. miurus*: X^2 = 102.9, df = 2, P < 0.001; *N. hildebrandi*: X^2 = 120, df = 1, P < 0.001; *N. leptacanthus*: X^2 = 114.1, df = 1, P < 0.001; Figure 8) for gravel when madtoms were exposed to a predator at night. Near dawn, seven madtoms (three *N. miurus* and four *N. hildebrandi*) had already chosen gravel substrate. One hundred seventy, of the 180 individuals included in this trial, selected gravel and three (*N. leptacanthus*) remained active when exposed to a predator at 0500 (Figure 9). Individuals of each species selected gravel over sand at or near sunrise (*N. miurus*: X^2 = 120, df = 2, P < 0.001; *N. hildebrandi*: X^2 = 97.6, df = 1, P < 0.001; *N. leptacanthus*: X^2 = 102.9, df = 1, P < 0.001).
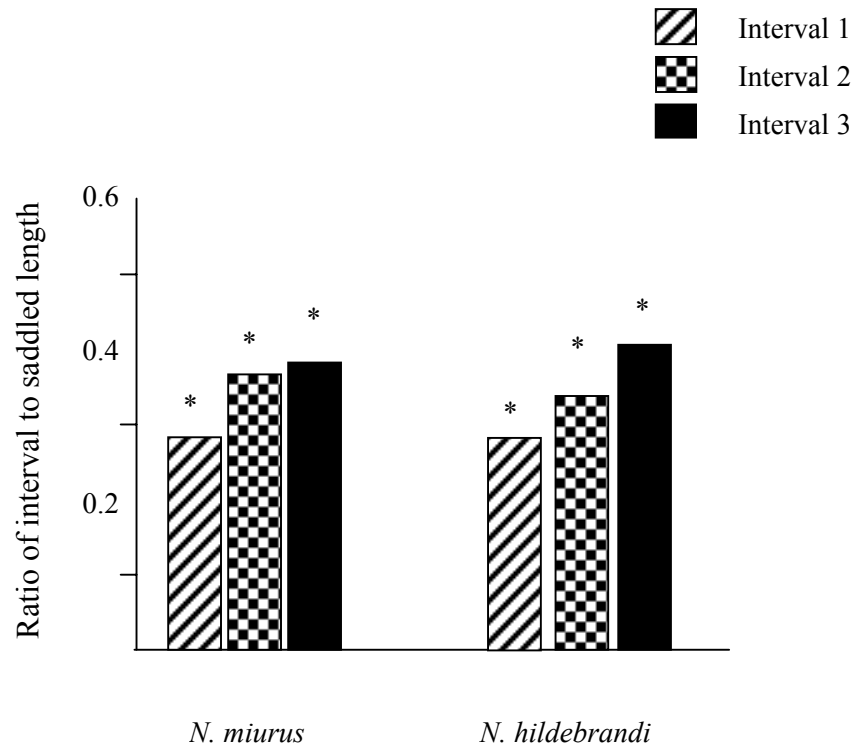
Figure 5. Saddle intervals in *N. hildebrandi* and *N. miurus*. * Designates significantly different intervals (Tukey-Kramer tests) (P<0.05).

Table 1. Substrate and color pattern correlations (r) for three species of *Noturus*.

	Black	White	Sand	Natural
N. hildebrandi	0.4776	-0.2555	-.01701	0.5755
N. leptacanthus	0.9639	-0.1692	-0.1527	-0.2404
N. miurus	0.3842	-0.2499	-0.1612	0.6415
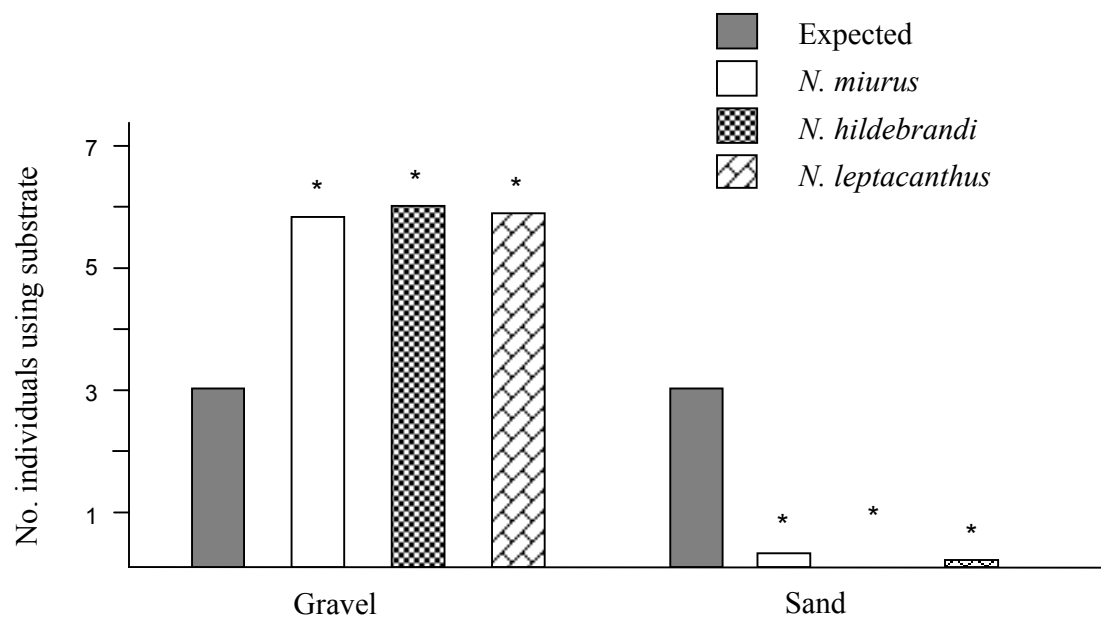
Figure 6. Substrate texture preference by species. * Significant deviation from Chi-square expectations (P < 0.001).

The predator experiments were repeated during the day, and only seven of the 180 individuals included in these trials used sand. The remaining 173 madtoms had previously chosen gravel as a substrate. Introduction of a predator stimulus did not trigger madtom movement from the previously chosen substrate, and the results of the Chi-Square indicate a preference for gravel (*N. miurus*: $X^2 = 97.6$, df = 2, P < 0.001; *N. hildebrandi*: $X^2 = 120$, df = 1, P < 0.001; *N. leptacanthus*: $X^2 = 102.9$, df = 1, P < 0.001; Figure 10).

In addition to a Chi-Square test for substrate choice at each time of day, a G-test of Independence was run for each time of day to test the null hypothesis that behavior is consistent regardless of presence of a predator. At night, when madtoms were actively swimming, the predator stimulus caused an immediate reduction in activity (*N. miurus*: G = 22.4, df = 1, P < 0.001; *N. hildebrandi*: G = 22.4, df = 1, P < 0.001; *N. leptacanthus*: G = 16.2, df = 1, P < 0.001). Near dawn, the madtoms became inactive, again significantly (*N. miurus*: G = 19.5, df = 1, P < 0.001; *N. hildebrandi*: G = 17.6, df = 1, P < 0.001; *N. leptacanthus*: G = 19.5, df = 1, P < 0.001); however, during the day, the behavior of all species did not change (*N. miurus*: G = 0.01, df = 1, P > 0.05; *N. hildebrandi*: G = 0.01, df = 1, P > 0.05; *N. leptacanthus*: G = 0.01, df = 1, P > 0.05).

When offered a choice of colored gravel in the presence of a predator, madtoms avoided white gravel, but did not distinguish between black and natural gravel at night (*N. miurus*: $X^2 = 5.85$, df = 2, P > 0.05; *N. hildebrandi*: $X^2 = 0.01$, df = 2, P > 0.05; *N. leptacanthus*: $X^2 = 0.01$, df = 2, P > 0.05), near sunrise (*N. miurus*: $X^2 = 5.85$, df = 2, P > 0.05; *N. hildebrandi*: $X^2 = 0.0$, df = 2, P > 0.05; *N. leptacanthus*: $X^2 = 0.01$, df = 2, P > 0.05) , or during the day (*N. miurus*: $X^2 = 5.85$, df = 2, P > 0.05; *N. hildebrandi*: $X^2 = 0.01$, df = 2, P > 0.05; *N. leptacanthus*: $X^2 = 0.01$, df = 2, P > 0.05).

Activity Periods

Twelve madtoms, four *N. miurus*, three *N. hildebrandi*, and five *N. leptacanthus*, were inactive, resting on the substrate, during the nocturnal observations. During the diurnal observation periods only eight madtoms, five *N. miurus* and three *N. leptacanthus,* were swimming, all others were at rest on a substrate. No *N. hildebrandi* were swimming during the day. A G-test of Independence indicated a significant difference in activity during the diurnal and nocturnal observations (*N. miurus*: G = 30.2, df = 1, P < 0.001; *N. hildebrandi*: G = 44.3, df = 1, P < 0.001; *N. leptacanthus*: G = 16.8, df = 1, P < 0.001).
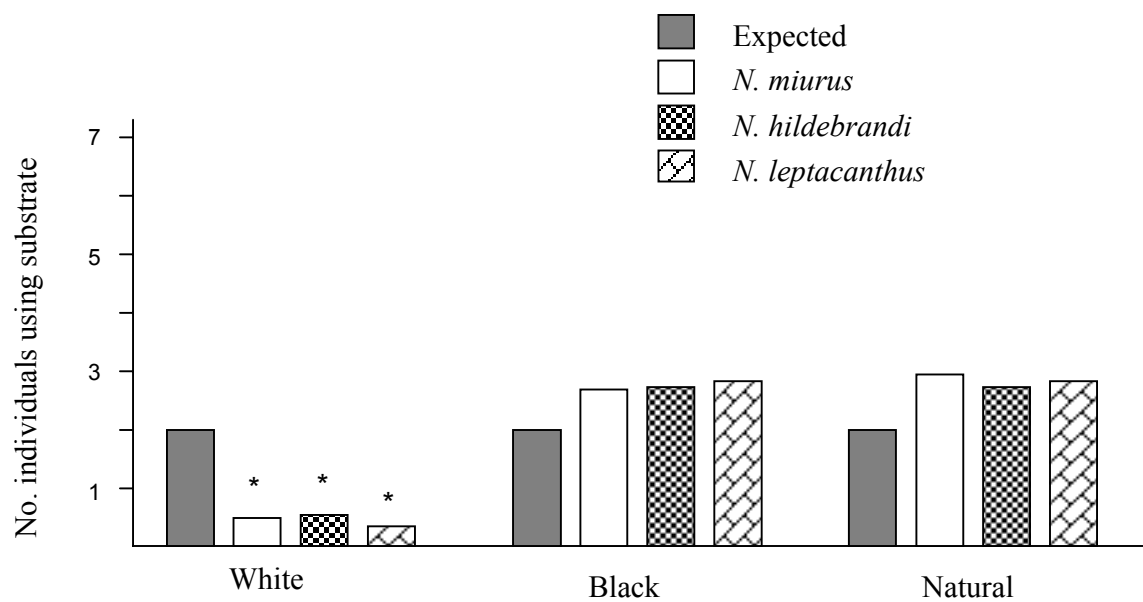
Figure 7. Substrate color choice by species. *Significant deviation from Chi-square expectations (P > 0.05).
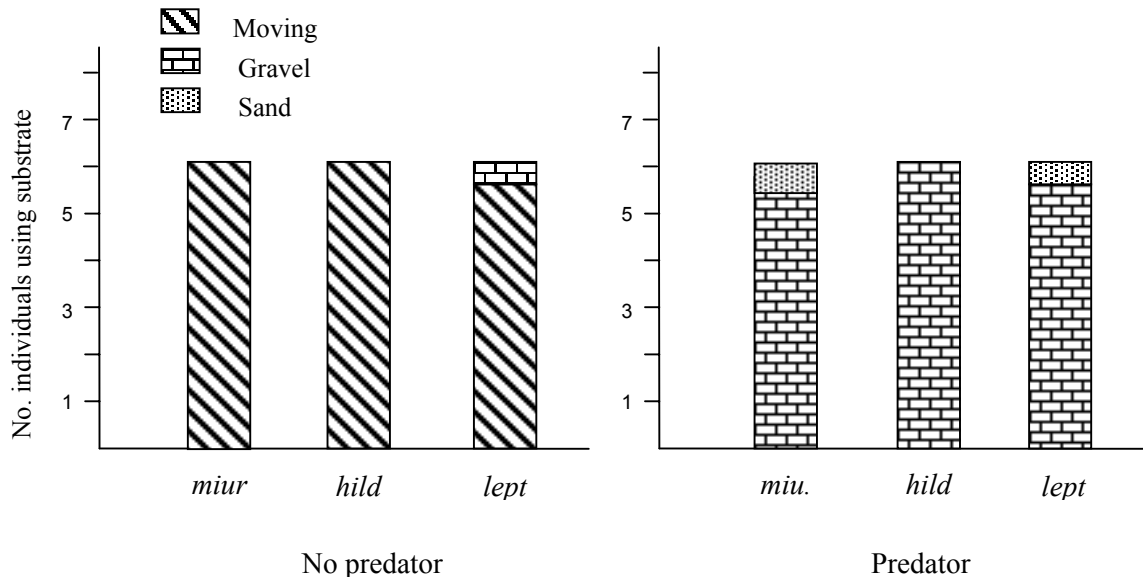
Figure 8. Nocturnal substrate choice with and without a predator (*miur = N. miurus, hild = N. hildebrandi, lept = N. leptacanthus).*
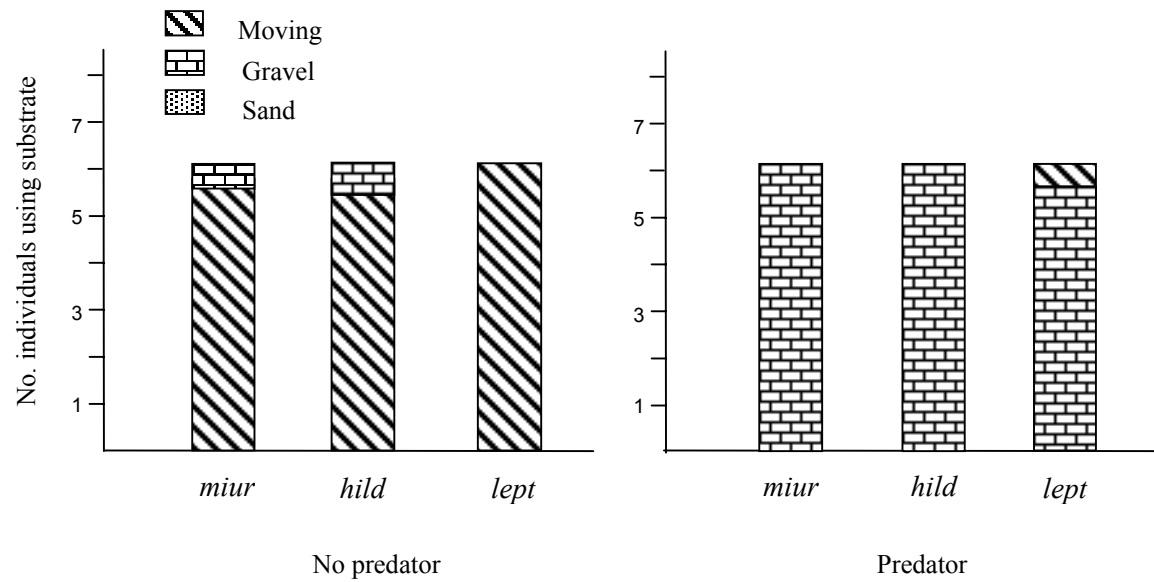
Figure 9. Substrate choice with and without a predator at sunrise (*miur = N. miurus, hild = N. hildebrandi, lept = N. leptacanthus).*
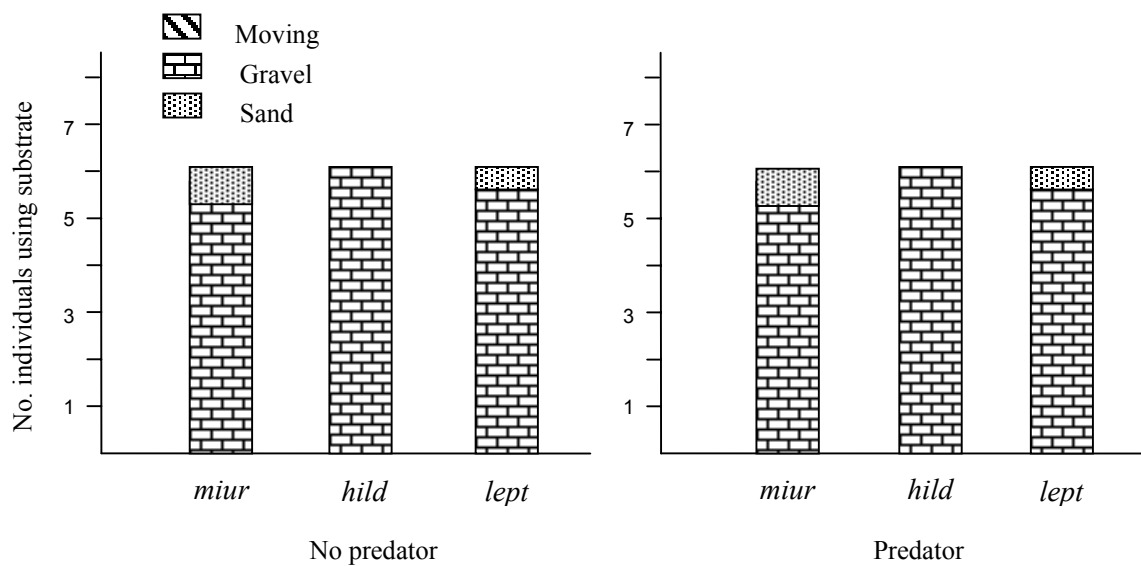
Figure 10. Diurnal substrate choice without and with a predator (*miur = N. miurus, hild = N. hildebrandi, lept = N. leptacanthus).*

Discussion

The selection pressure exerted by predators is reflected in the myriad of anti-predator defense mechanisms in potential prey. Individually, predator defense adaptations range from simple to complex, as illustrated by spines in sticklebacks, which are defensive in both length and number of spines (Hoogland, 1957). Selection by predators also contributes to multiple anti-predator adaptations within species, such as the covariation between serrated spines and saddle patterns in madtoms. Anti-predator adaptations are especially complex and numerous among nocturnal species, such as madtom catfishes, due to increased susceptibility to both nocturnal and diurnal predators. Madtoms catfishes and other nocturnal fish species, such as stonefishes (Cameron and Endean, 1966) have spines with toxins, exhibit cryptic behavior, and appear to be camouflaged. Multiple anti-predator traits may be advantageous due to multiple predation threats.

Among fishes, camouflage pigmentation is a common adaptive response to predation in diurnal and nocturnal species. Members of the families Ictaluridae (catfishes, including madtom catfishes), Catostomidae (suckers), Percidae (darters), and Cottidae (sculpins) have a dorsal pigmentation pattern consisting of alternating dark and light areas (dark saddles against a lighter background) that Armbruster and Page (1996) suggested is camouflage. Most of the camouflaged species alluded to above use gravel substrates predominantly, i.e. when foraging, resting, mating, etc.

My data on spacing and activity support the camouflage hypothesis, but substrate choice under various conditions could refute the hypothesis. Saddled species should demonstrate a higher association with gravel (a matching substrate), even in the absence of a predator, for camouflage to be effective. Non-saddled species, such as *N. leptacanthus*, should exhibit no substrate preference (sand versus gravel) because there is no camouflage benefit associated with either substrate. The coloration of *N. leptacanthus* does not closely match either sand or gravel (Table 1); however, speckled madtoms preferred gravel. Of the sand and gravel substrates offered in the first experiment, gravel provides more structure and refuge than sand. Therefore, *N. leptacanthus* may have been selecting substrate that offered more structure and protection, or may have been selecting gravel for other factors, i.e. foraging, nest building, etc, although these

factors did not appear to play an important role in substrate choice. An appropriate test of substrate choice based on structure versus color is to control for structure by using a common substrate type (gravel) of various colors. Experiments, in which individuals of the saddled species (*N. hildebrandi* and *N. miurus*) and the unsaddled species (*N. leptacanthus*) were offered various colors of gravel, controlled for the effects of texture. Based on the camouflage hypothesis, saddled madtoms should select natural-colored gravel, which is more highly correlated with their saddle pattern than either black or white gravel or sand. Conversely, uniformly pigmented madtoms should choose solid black gravel (Table 1). Interestingly, all madtom species avoided white gravel, but did not differentiate between black and natural gravel. Madtoms may choose dark substrates because they can differentiate between strongly contrasting backgrounds (light vs. dark) but do not appear to distinguish among dark substrates. Selection for visual capacity to distinguish among dark substrates may be weak, because uniformly colored gravel substrates are rare in habitats where the study species occur.

The unsaddled species *(N. leptacanthus)* also did not distinguish between black or natural gravel. Over an unevenly colored substrate, predation pressure should be lower for *N. miurus* and *N. hildebrandi* than *N. leptacanthus*, which could be tested by comparing survival over evenly and unevenly colored substrates. Survival experiments were not done due to limitations of study specimens.

Since madtoms are predominately benthic and stationary diurnally, predators probably strongly influence substrate use. Determining the behavior of individuals in a species with a contrasting pigment pattern in the presence of a predator could determine the adaptive value of this trait. Substrates provide refuge or camouflage against predators as well as providing diurnal habitats for inactive madtoms, and determining how madtoms use the substrate when threatened by a predator might distinguish between substrate uses, i.e. inactivity during the day versus avoiding predation via camouflage. Presumably, madtoms using substrate simply as a resting place would select for texture, not color; however, the results of the color gravel substrate experiments are not consistent with this expectation. Predators might test the madtom's ability to discriminate between natural and uniformly dark substrates. Therefore, experimental conditions were modified to simulate the presence of a predator by adding predator water to the substrate texture experiment. Saddled and unsaddled species responded similarly to the simulated predation threat by moving to a dark textured substrate. Addition of a predator stimulus at night, when the madtoms were active, prompted individuals to move to the substrate and become stationary. Addition of a predator stimulus at sunrise prompted madtoms to move to gravel substrate quickly, and during the day, madtoms did not move from previously chosen substrates

when threatened. Regardless of time, all species used gravel when threatened. Madtoms did not distinguish between dark substrates in the presence of a predator. Low light, as at night, dusk, or dawn, would render them camouflaged against most substrates, except the white gravel. Therefore, substrate choice at night and dawn probably reflects preference for structure and for substrates where they can hide from a predator. Predation likely affects substrate choice by encouraging individuals to choose dark structured substrate.

The contrasting dorsal pigment pattern found in madtoms could be an anti-predator adaptation that reduces predation in dark habitats with textured substrates. Color pattern and habitat choice probably lower the risk of diurnal predation to saddled madtoms; however, the experiments in this project were not designed to provide data on reduced predation.   Experiments in which the color pattern is manipulated or survivorship on various substrates is measured would be a useful extension of this research to provide more evidence of the adaptive value of a saddle color pattern by directly measuring the effect of predation on saddled species.

Although there is no direct evidence of lower predation, the substrate choice experiments and the correlation between color pattern and gravel indicate that saddles contribute to a camouflage color pattern that matches gravel. Interestingly, thirteen fish species recognized as endangered or threatened in the U.S. and many other locally protected fish species have dorsal saddles. Habitat alterations could be contributing to the declining abundance of saddled species, including madtoms. Siltation, a common byproduct of environmental disturbance, could negate the benefit of a camouflage color pattern (Ross et al., 1992), increasing predation pressure. Silt is usually more uniformly pigmented and lighter than natural substrates, particularly gravel. Dark bands against a light background contrast sharply with a uniform substrate. Interestingly, the three study species avoided the uniformly pigmented light substrates, which are similar to the substrate homogenization caused by siltation.

Obtaining a comprehensive biological understanding of endangered and threatened species, including the role of pigmentation in predator avoidance, is important to the development of effective conservation strategies (Weis et al., 1999). Freshwater streams are often physically and chemically disturbed (Rogers et al., 2002), which can negate predator defense adaptations, such as camouflage pigmentation, and alter behavioral patterns (Fialkowski et al., 2003). Both effects can increase predation pressure and ultimately reduce individual survival and population persistence (Weis et al., 1999). An appropriate conservation strategy to maintain populations of these fishes is to reduce anthropogenic sources of habitat degradation and siltation in impacted streams and rivers and to restrict siltation in unimpacted areas.

Literature Cited

Armbruster, J.W. and L. M. Page. 1996. Convergence of a cryptic saddle pattern in benthic freshwater fishes. Environmental Biology of Fishes 45:249-257.

Bailey, J.R. and Committee. 1977. Freshwater fishes. Species list, pages 278-280. *In*: Endangered and Threatened Plants and Animals of North Carolina. J.E. Cooper, S.S. Robinson, and J.B. Funderburg (eds). North Carolina State Museum, Raleigh.

Barlow, G.W. 1972. The attitude of fish eye-lines in relation to body shape and to stripes and bars. Copeia 1972:4-12.

Breder, C.M. 1963. Defensive behaviour and venom in *Scorpaena* and *Dactylopterus*. Copeia. 1963:237-242.

Burr, B.M. and R.L. Mayden. 982. Life history of the brindled madtom, *Noturus miurus*, in Mill Creek, Illinois (Pisces: Ictaluridae). American Midland Naturalist 107:25-41.

Cameron, A. and R. Endean. 1966. The venom apparatus of the scorpion fish *Notesthes robusta*. Toxicon 4:111-121.

Clark, Kathleen. 1978. Ecology and life history of the speckled madtom, *Noturus leptacanthus* (Ictaluridae). MS Thesis. University of Southern Mississippi. 134 pages.

Cott, H.B. 1957. Adaptive coloration in animals. Methuen and Co., London. 508 pages.

Davidson, G.W., M.K. Sheehan, P.S. Davie. 1999. The effect of tagging on the swimming performance of rainbow trout as a surrogate for kahawai *Arripis trutta*. Transactions of the American Fisheries Society 128(5):971-973.

Edmunds, M. 1974. Defence in Animals. Longman Inc., New York. 357 pages.

Endler, J.A. 1986. Natural Selection in the Wild. Princeton University Press, Princeton, NJ. 336 pages.

Endler, J.A. 1984. Progressive background in moths, and a quantitative measure of crypsis. Biological Journal of the Linnean Society 22:187-231.

Etnier, D.A. and R. E. Jenkins. 1980. *Noturus stanauli*, a new madtom catfish (Ictaluridae) from the Clinch and Duck Rivers, Tennessee. Bulletin of the Alabama Museum of Natural History 5:17-22

Fialkowski, W., M. Klonowska-Olejnik, B.D. Smith, and P.S. Rainbow. 2003. Mayfly larvae (*Baetis rhodani* and *B. vernus*) as biomonitors of trace metal pollution in streams of a catchment draining a zinc and lead mining area of Upper Silesia, Poland. Environmental Pollution 121(2):253-267.

Grady, J.M., and W.H. LeGrande. 1992. Phylogenetic relationships, modes of speciation, and historical biogeography of the madtom catfishes, genus *Noturus Rafinesque* (Siluriformes: Ictaluridae), pages 747-777. *In*: Systematics, historical ecology, and North American freshwater fishes. R.L. Mayden (ed.). Stanford University Press, Stanford, CA

Guilford, T. 1988. The evolution of conspicuous coloration. American Naturalist 131:S7-S21.

Guilford, T. 1992. Predator psychology and the evolution of prey coloration, pages 450-456. *In*: natural enemies. The population biology of predators, parasites and diseases. N.J. Crawley (ed.). Blackwell Scientific Publications, Oxford.

Hinton, S. 1962. Unusual defense movements in *Scorpaena plumieri mystes*. Copeia 1962:842.

Hoogland, R., D. Morris, and N. Tinbergen. 1957. The spines of sticklebacks (*Gasterosteus* and *Pygosteus*) as means of defence against predators (*Perca* and *Esox*). Behaviour 10:205-236.

Johnson, J.E. 1987. Protected fishes of the United States and Canada. American Fisheries Society, Bethesda, MD. 42 pages.

Keuhne, R.A. and R.W. Barbour. 1983. The American Darters. Lexington, University Press of Kentucky, Lexington, KY. 177 pages.

Knopf, A.A. 1995. National Audubon Society Field Guide to North American Fishes, Whales and Dolphins. Chanticleer Press, New York, NY. 845 pages.

Landau, M. 1997. Poisonous, Venomous, and Electric Marine Organisms of the Atlantic Coast, Gulf of Mexico, and the Caribbean. Plexus Publishing, Inc., Medford, NJ. 217 pages.

LeGrande, W.H. 1981. Chromosomal evolution in North American catfishes (Siluriformes: Ictaluridae) with particular emphasis on the madtoms, *Noturus*. Copeia.1981:33-52.

Malcolm, S.B. 1992. Prey defence and predator foraging, pages 103-127. *In*: Natural enemies. the population biology of predators, parasites and diseases. N.J. Crawley (ed.). Blackwell Scientific Publications, Oxford.

Malpezzi, E.L., J.C. de Freitas, and F.T. Rantin. 1997. Occurrence of toxins, other than paralysing type, in the skin of Tetraodontiformes fish. Toxicon 35(1):57-65.

Marshall, N.J. 2000. Communication and camouflage with the same 'bright' colours in reef fishes. Philosophical Transactions of the Royal Society of London. Series B, Biological Sciences 355(1401):1243-1248.

Mayden, R.L. and S.J. Walsh. 1984. Life history of the least madtom, *Noturus hildebrandi* (Siluriformes: Ictaluridae) with comparisons to related species. American Midland Naturalist 112:349-368.

Mboko, S.K. and M. Kohda. 1995. Pale and dark dichromatism related to microhabitats in a herbivorous Tanganyikan cichlid fish, *Telmatochromis temporalis*. Journal of Ethology 13:77-83.

Pflieger, W.L. 1991. The Fishes of Missouri. Missouri Department of Conservation. Missouri. 372 pages.

Phoon, W.O. and E.R. Alfred. 1965. A study of stonefish (*Synanceja*) stings in Singapore with a review of the venomous fishes of Malaysia. Singapore Medical Journal 6(3):158-163.

Reed, H.D. 1900. The structure of the poison glands of *Schilbeodes gyrinus*. Proceedings of the American Association for the Advancement of Science 49:232-233.

Reed, H.D. 1907. The poison glands of *Noturus* and *Schilbeodes*. The American Naturalist 39: 553-566.

Rogers, C.E., D.J. Brabander, M.T. Barbour, and H.F. Hemond. 2002. Use of physical, chemical, and biological indices to assess impacts of contaminants and physical habitat alteration in urban streams. Environmental Toxicology Chemistry 21(6):1156-1167.

Rohde, F.C. 1978 *a*. *Noturus hildebrandi*, catfish, p 460. *In:* Atlas of North American freshwater fishes. D.S. Lee, et al. (eds.) N.C. State Museum of Natural History, Raleigh, NC. 854 pages.

Rohde, F.C. 1978*b*. *Noturus leptacanthus*, catfish, p 463. *In:* Atlas of North American freshwater fishes. D.S. Lee, et al. (eds.) N.C. State Museum of Natural History, Raleigh, NC. 854 pages.

Rohde, F.C. 1978*c*. *Noturus miurus*, catfish, p 464. *In:* Atlas of North American freshwater fishes. D.S. Lee, et al. (eds.) N.C. State Museum of Natural History, Raleigh, NC. 854 pages.

Ross, S.T., J.G. Knight, and S.D. Wilkins. 1992. Distribution and microhabitat dynamics of the threatened Bayou Darter, *Etheostoma rubrum*. Copeia 1992: 658-671.

Sokal, R. R. and Rohlf, F. J. 1995. Biometry. 3rd ed. W.H. Freeman & Co., New York, NY. 887 pages.

Taylor, W.R. 1969. Revision of the catfish genus *Noturus* Rafinesque with an analysis of higher
    groups in the Ictaluridae. U.S. National Museum Bulletin. 315 pages.

Weis, J.S., G.M. Smith, and T. Zhou. 1999. Altered predator/prey behavior in polluted
    environments: Implications for fish conservation. Environmental Biology of Fishes
    55(1-2):43-51.

Appendix 1

Field sites and species collected for behavioral experiments

Species	River Drainage	Locality
N. hildebrandi	Bayou Pierre	Claiborne Co., MS, 2 mi NW of Port Gibson, MS on Anthony Street.
N. leptacanthus	Amite	East Feliciana Parish, LA, 10.9 mi. E of Clinton, LA Hwy 10.
	Bogue Chitto	Walthal Co., MS, from jct. MS hwy 27 and MS Hwy 48 in Tylertown, MS, 6.52 mi. W on MS hwy 48, .5 mi. S on Walker Bridge Water Park Rd.
	Bogue Chitto	Lincoln Co., MS, from jct. MS Hwy 51 and Fox Rd in Norfield, MS, 1.49 mi. E on Fox Rd.
	Bogue Falaya	St. Tammany Parish, LA, LA Hwy 25, approximately 5.6 mi SSE of Folsom.
N. miurus	Amite	East Feliciana Parish, LA, 10.9 mi. E of Clinton, LA Hwy 10.
	Bogue Chitto	Walthal Co., MS, from jct. MS Hwy 27 and MS Hwy 48 in Tylertown, MS, 6.52 mi. W on MS Hwy 48, .5 mi. S on Walker Bridge Water Park Rd.
	Bogue Chitto	Lincoln Co., MS, from jct. MS Hwy 51 and Fox Rd in Norfield, MS, 1.49 mi. E on Fox Rd.

Vita

Amanda Grace Stokes was born 11 Nov 1975 in Stuttgart, Arkansas. She is the daughter of Charlotte K. and Thomas E. Stokes. She moved to Memphis, Tennessee in 1979 and graduated from Memphis Harding Academy in 1994. She received a Bachelor of Science degree with a major in Biological Sciences in 1998 from Mississippi State University.