

EARLY DEVELOPMENT OF THE DEVILS RIVER MINNOW,
DIONDA DIABOLI (CYPRINIDAE)

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ABSTRACT—The Devils River minnow (*Dionda diaboli*) coexists with at least 2 congeners and several other cyprinids throughout its range in southern Texas and northern Mexico. Larval and juvenile descriptions are needed to monitor *D. diaboli* larvae and juveniles as part of recovery efforts for this species of conservation concern. The purpose of this study was to describe and quantify characteristics of early life stages of *D. diaboli* from hatching to 128 d post hatch to facilitate larval and juvenile identification. Descriptive characters include mid-lateral band of melanophores by Day 8 (>5.1 mm SL; >5.4 mm TL), mid-lateral band of melanophores separate from a rounded caudal spot and lateral snout-to-eye melanophores by Day 16 (>5.8 mm SL; >6.3 mm TL), initial coiling of intestine by Day 32 (>6.2 mm SL; >7.2 mm TL), wedge-shaped caudal spot by Day 64 (>8.7 mm SL; >10.0 mm TL), and melanophores around scale margins and mid-lateral double dashes along lateral line by Day 128 (>13.5 mm SL; >16.0 mm TL). However, reliable separation among sympatric *Dionda* might not be possible until larvae and juveniles of congeners are described.

RESUMEN—*Dionda diaboli* coexiste con por lo menos dos otras especies del mismo género y unos cuantos ciprinos a través de la región sureña de Texas y la región norteña de México. Disponibles se encuentran conceptos y estudios publicados acerca de cómo identificar especies ciprinas adultas. Sin embargo, no hay descripciones larvales ni juveniles disponibles las cuales son necesitadas para poder monitorear el pez *D. diaboli* durante su etapa larval y juvenil. Estas descripciones funcionarían como parte de los esfuerzos de recuperación de esta especie que se encuentra amenazada en los Estados Unidos y en peligro de extinción en México. Es por eso que el propósito de este estudio es describir y cuantificar las características de la especie en cuestión desde su nacimiento hasta 128 días después de su nacimiento lo cual facilitará el poder identificar a esta especie durante su etapa larval y juvenil. Las características descriptivas de *D. diaboli* incluyen melanocitos laterales medios separados de una mancha circular en la región caudal al octavo día. Para el día 16, melanocitos en la región lateral de la boca que se estiran hasta los ojos comienzan a aparecer. Para el día 32, se nota el inicio del enroscamiento de los intestinos. Para el día 64, una mancha triangular en la región caudal es aparente. Para el día 128, guiones dobles a través de la línea lateral y márgenes de escamas son evidentes. Colectivamente, estas y otras características descritas en este estudio proveen un acontecimiento detallado del desarrollo de *D. diaboli* a través de su etapa juvenil. Sin embargo, identificación segura y precisa entre especies del mismo género será escasa hasta que descripciones larvales y juveniles de diferentes especies en el mismo género sean realizadas.

Devils River minnow (*Dionda diaboli*) is endemic to spring-fed tributaries of the Rio Grande drainage, including the Devils River, San Felipe Creek, Sycamore Creek, Pinto Creek, and Las Moras Creek in Texas and in Río San Carlos and Río Sabinas in Mexico (Miller, 1978; Smith and Miller, 1986; Hubbs and Garrett, 1990; Garrett et al., 1992; Garrett et al., 2004). Similar to other endemic minnows within the Rio Grande drain-

age, declines in abundance and distribution of *D. diaboli* have been attributed to modifications of habitat, water quantity and quality, and introduced species (Davis, 1980; Williams et al., 1989; Garrett et al., 1992; Anderson et al., 1995; Robertson and Winemiller, 2001). Currently, *D. diaboli* is listed as threatened by the United States Fish and Wildlife Service and Texas Parks and Wildlife Department (Hubbs et al., 1991; United

States Department of the Interior, 1999; Garrett et al., 2004) and an endangered species in Mexico (Contreras-Balderas et al., 2002).

Distribution of *D. diaboli* and 2 congeners overlap in the Rio Grande drainage. Manantial roundnose minnow (*Dionda argentosa*) occurs with *D. diaboli* in all Texas locations, except Las Moras Creek, and spotted minnow (*Dionda melanops*) occurs with *D. diaboli* in the Río Salado drainage of northern Mexico (Garrett et al., 1992; Mayden et al., 1992). In addition, roundnose minnow (*Dionda episcopa*) occurs in the Rio Grande and tributaries upstream from the Devils River, and although its distribution currently does not overlap with that of *D. diaboli*, it has the potential to occur with *D. diaboli*. Adult *D. diaboli* differ morphologically from all 3 congeners by having a wedge-shaped caudal spot, dorsal and dorsal-lateral scales outlined with dark pigments, and double dashes along the lateral line (Girard, 1856; Hubbs and Brown, 1956). Numerous other morphological, meristic, and genetic differences are given in Girard (1856), Hubbs and Brown (1956), Contreras-Balderas and Verduzco-Martinez (1977), Hubbs et al. (1991), Gold et al. (1992), and Mayden et al. (1992). However, morphology of larvae and juveniles is not described and is needed to distinguish early life stages of *D. diaboli* from congeners and other cyprinids. Objectives of this study were to describe basic pigmentation patterns and to quantify morphological and meristic characters at early developmental phases of *D. diaboli* from hatching to Day 128.

METHODS—Brood fish consisted of individuals taken in August 2000 from the Devils River and its tributaries (Val Verde County, Texas) and their first generation descendants reared at the National Fish Hatchery and Technology Center-San Marcos. Breeding adults ($n = 300$) were maintained in one 719-L and two 830-L fiberglass tanks (Living Stream; Frigid Unit, Toledo, Ohio), and two 16.5-L flow-through aquaria. Gravel placed in plastic trays ($14 \times 14 \times 4$ cm) was used for spawning substrate (Gibson et al., 2004) in each fiberglass tank and aquarium. Eggs were removed daily from the gravel substrate, and those with fungus were discarded. When the number of eggs from all breeding tanks and aquaria exceeded 100 within a 24-h period, they were placed into 16.5-L flow-through rearing aquaria. Freshwater was pumped into the rearing aquaria at a rate of 5.2 L/h. Water temperatures were maintained at 22–24°C.

Five groups of eggs were produced from December 2002 through January 2003, with each group being placed into a separate aquarium. After allowing 5 d for most of the fish to hatch, 5 fish were removed from

each aquarium on days 2, 4, 8, 16, 32, 64, and 128 post-hatch and exposed to a lethal dose of tricaine methanesulfonate (>80 mg/L). Fish were then fixed in 10% formalin and preserved in 70% ethanol. Collectively, a total of 175 fish were preserved; however, only 153 were suitable for measurements. Throughout the study, larvae of *D. diaboli* were fed liberal amounts of *Artemia* several times weekly and Gold Fry-3 daily (Aurum Aquaculture Ltd., Kirkland, Washington). After Day 16, fish were fed *Spirulina* flakes and worm flakes (Aquatic Eco-systems, Inc., Apopka, Florida) 3 times weekly. Dead fish were removed and debris was siphoned from aquaria as needed.

Using digital photographs and a stereomicroscope with an ocular micrometer, the following measurements were taken, when applicable, from each fish by following methods described by Hubbs and Brown (1956), Trautman (1981), and Snyder and Muth (1990, unpublished report, Colorado Division of Wildlife, Fort Collins): length of right pectoral fin (distance from origin of pectoral fin to distal end of longest fin ray), length of snout to pectoral fin (distance from right side of snout to origin of right pectoral fin), depth of body (greatest depth excluding finfolds or fins), orbital length (greatest distance between free orbital rims), postorbital length (posterior portion of orbital rim to posterior margin operculum), length of head (distance from snout to posterior margin of operculum), depth of caudal peduncle (shallowest depth of the body anterior to caudal fin), length of depressed dorsal fin (distance from origin of depressed dorsal fin to distal end of longest fin ray), length of depressed anal fin (distance from origin of depressed anal fin to distal end of longest fin ray), length of caudal peduncle (oblique distance between posterior end of anal fin base to base of the middle of caudal ray), length of left pelvic fin (distance from origin of pelvic fin to distal end of longest fin ray), standard length (SL; distance from snout to end of notochord in Day-2 and Day-4 protolarvae and Day-8 and Day-16 mesolarvae; distance from snout to end of hypural plate or structural base of the caudal fin in Day-32, Day-64, and Day-128 fish), and total length (TL; distance from snout to end of caudal fin).

Morphological, meristic, and melanophore characteristics were described for protolarvae, mesolarvae, metalarvae, and juveniles following Cooper (1980) and Snyder and Muth (1990, in litt.). Presence of yolk sac and fin folds, shape of eye (round or flattened along dorsal and ventral rim; Fuiman et al., 1983), position of notochord and hypural plate, development of an emarginated tail, formation of lateral line, and shape, coiling pattern, and length of intestine were described. Preanal, postanal, and total myomere counts and caudal (principal rays only), dorsal, anal, and pelvic fin ray counts were reported on a subsample of fish ranging from 3 to 10 individuals (Contreras-Balderas and Verduzco-Martinez, 1977; Snyder and Muth, 1990, in litt.). The appearance and form of selected pigmentation patterns, including the caudal spot, melanophores along the posterior margins of dorsal scales, and double dashes along the lateral line, were documented. Morphological characteristics (mean \pm SD) expressed as percentage of SL, counts (mode and range, or range only if mode was not available), and

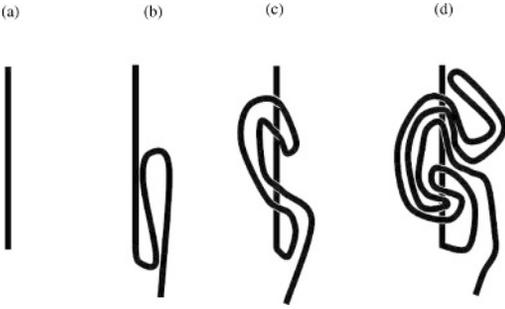


FIG. 2.—Schematic illustration depicting ventral view of intestine of Devils River Minnow (*Dianda diaboli*) for (a) Day-16 mesolarva, (b) Day-32 metalarva, (c) Day-64 juvenile, and (d) Day-128 juvenile. Esophagus is at top and anus is at bottom of illustration.

rays developed. Emarginated caudal fins were observed in none of the Day-16 mesolarvae and 83% of Day-32 mesolarvae. Elements of principal dorsal fin rays were visible in 29% of Day-16 mesolarvae. Principal dorsal fin rays were present in all Day-32 mesolarvae. A membrane outline of the anal fin was visible in 48% of Day-16 mesolarvae and 33% of Day-32 mesolarvae. Anal fin and fin rays were visible in 67% of Day-32 mesolarvae, although not all principal rays were present. Intestine was straight in Day-16 mesolarvae ($n = 3$; Fig. 2); mean intestinal length to SL ratio ($\pm SD$) was 0.41 (0.013).

Dorsal melanophores of Day-16 and Day-32 mesolarvae were visible in a scattered pattern on the occipital region of the head and concentrated anteriorly on the body in a slightly scattered pattern 2 melanophores wide in all fish. Dorsal melanophores were scattered on the snout in 48% of fish, and between orbitals in all Day-32 mesolarvae. Ventral melanophores were visible in a row, one melanophore wide, from vent to base of caudal fin and obliquely from the anal opening dorsally to mid-gut region in all fish. Ventral melanophores were visible between pectoral fins on 81% and isthmus on 96% of all fish. Ventral melanophores extended laterally in a row, one melanophore wide, from the pectoral fins in a U-shaped pattern that opened posteriorly on Day-16 mesolarvae. Ventral melanophores extended laterally from the pectoral fins onto the lower portion of the gut in all Day-32 mesolarvae. Lateral melanophores were visible on the snout, forming a partially developed lateral stripe from eye to snout, and across the opercle posteriorly toward the caudal fin, where

they were separated from caudal melanophores. Caudal melanophores were visible in 96% of all fish, fusing to form a distinct round spot in Day-32 mesolarvae only. Interradial melanophores were visible on the caudal fin and radial melanophores were visible on all fins.

Metalarvae (Day 32 and Day 64)—Standard length of Day-32 ($n = 15$) and Day-64 ($n = 6$) metalarvae ranged from 6.8 to 12.0 mm; TL ranged from 9.0 to 14.0 mm. Median fin folds anterior to the caudal fin were observed in 95% of fish. The horizontal septum was prominent in all fish (Fig. 1e). Hypural plates were visible and caudal fins were emarginated in all fish. Dorsal fins were fully developed with 8 rays. Anal fin was formed in all fish; however, 14% of metalarvae were without the typical adult complement of rays. Pelvic fin buds were anterior to dorsal fin in 29% of fish and opposite of dorsal fin in 71% of fish. Pectoral fin rays formed in 62% of fish. Intestines in Day-32 metalarvae ($n = 3$) looped twice; mean intestinal length to SL ratio ($\pm SD$) was 0.58 (0.008).

Dorsal melanophores in all Day-32 and Day-64 metalarvae were visible on the body, on the snout between the orbitals, and in a heart-shaped pattern on the occipital region of the head. Ventral melanophores were visible from the vent toward the base of the caudal fin and from vent around the anal fin base toward pelvic fins or buds in all fish. Ventral melanophores were sparse between pectoral fins, at the isthmus, and obliquely and dorsal to the lower gut. Lateral melanophores were numerous, forming a mid-lateral stripe from eye to snout and extending posteriorly across the opercle toward base of the caudal fin. Melanophores were visible on caudal fin interradian membranes and on the radials of all fins. Caudal melanophores formed a rounded spot in 81% and formed a wedge-shaped spot in 19% of the fish. Four of the 6 Day-64 metalarvae had wedge-shaped spot.

Juvenile (Day 64 and 128)—Standard length of Day-64 ($n = 16$) and Day-128 ($n = 22$) juveniles ranged from 10 to 29 mm; TL ranged from 13.5 to 35.0 mm. Median fin fold was visible at the base of the caudal fin in 50% of Day-64 juveniles and 9% of Day-128 juveniles (Figs. 1f, 1g). Lateral lines were visible in 50% of fish. Intestines in Day-64 juveniles ($n = 3$) looped twice and were more convoluted than Day-32 metalarvae; mean intestinal length to SL ratio ($\pm SD$) of Day-64 juveniles was 1.41 (0.19). Intestines

of Day-128 juveniles ($n = 3$) looped several times and were highly convoluted; mean intestinal length to SL ratio ($\pm SD$) was 2.8 (0.44).

Dorsal melanophores of all Day-64 and Day-128 juveniles were visible on the snout between orbitals, on anterior and posterior areas of body, and in a heart-shaped pattern on the occipital region of head in all fish. Lateral melanophores formed a solid stripe from eye to snout, extending across opercles towards the base of the caudal fin, and distinct double dashes ventral to mid-lateral stripe in 89% of fish. Lateral melanophores outlined scales and were most prominent on the dorsum in 66% of fish. Caudal melanophores formed a wedge-shaped spot in 92% of fish. Ventral melanophores formed a continuous black, midventral stripe posterior to vent and around the base of anal fin toward the gut. Melanophores were visible on caudal fin intraradial membranes and on radials of all fins.

DISCUSSION—Shape of intestines distinguishes adult *Dionda* and other herbivores from familial genera. For *D. diaboli*, intestinal coiling developed from a single loop by Day 32 (>6.2 mm SL; >7.2 mm TL), to a multi-looped, convoluted intestine by Day 128 (>13.5 mm; >16.0 mm TL; see also Contreras-Balderas and Verduzco-Martinez, 1977), whereas intestines of insectivorous cyprinids generally are straight throughout most of the larval period and eventually develop an S-shaped loop by or during the juvenile period (D. E. Snyder, pers. comm.; Junger et al., 1989). Configuration differences among fishes with long and coiled intestines are used to distinguish *Dionda* and *Hybognathus* from *Campostoma*. The intestine of the central stoneroller (*Campostoma anomalum*) forms a single loop before individuals reach a TL of 13 mm (age unknown), whereas the intestine of *D. diaboli* forms a more convoluted loop in fish >8.7 mm SL (>10.0 mm TL). Also, intestines of *Campostoma* differ from *Dionda* and *Hybognathus* by extending dorsally and coiling around the air bladder at 16 mm TL (Kraatz, 1924).

Caudal and snout-to-eye melanophores are useful features separating adult *Dionda* from *Hybognathus* (Hubbs and Miller, 1977; Hubbs et al., 1991) and likewise can be used to facilitate identification of mesolarvae through juvenile *D. diaboli*. In *D. diaboli*, caudal melanophores were visible from Day 16 to Day 128, and snout-to-eye melanophores were visible from Day 16 to Day

128. Wedge-shaped caudal spot, lateral double dashes, and scale margins distinguish *D. diaboli* from *D. argentosa*, the congener with a distribution most similar to that of *D. diaboli* (reported as *D. episcopa*—Hubbs and Brown, 1956). However, these features were not prominent in *D. diaboli* until Day 64 (fish >9 mm SL) and, thus, were not useful for distinguishing between these 2 species during the larval period.

Potentially useful morphological characteristics in identifying larval *D. diaboli* from familial genera that are common to the Rio Grande drainage include larval eye shape and relative length of pectoral fins. Larval eye shape is round in *D. diaboli*, but horizontally oval (or flattened) in the earlier larvae of the speckled chub (*Macrhybopsis aestivalis*) and the sand shiner (*Notropis stramineus*—Fuiman et al., 1983; Smith and Miller, 1986). In this study, relative length of pectoral fin bud of *D. diaboli* was shorter (mean = 7.2% TL and 7.6% SL; range = 0.19 to 0.58 mm for Day-2 and Day-4 protolarvae) than that of the golden shiner (*Notemigonus crysoleucas*), fathead minnow (*Pimephales promelas*), and red shiner (*Cyprinella lutrensis*) (range of means = 11 to 12.5% TL) during the protolarval stage, but becomes similar by the mesolarval stage (Saksena, 1962; Snyder et al., 1977).

Numbers of postanal, preanal, and total myomeres are useful in protolarval and mesolarval fish identification when extensive pigmentation does not obscure myomere counts (Yeager and Semmens, 1987; Snyder and Muth, 1990, in litt.). *Dionda diaboli* and other cyprinids have a greater number of postanal myomeres than catostomids (*Ictiobus*, *Carpionodes*, *Cycleptus*, *Moxostoma*) occurring in the Rio Grande drainage; *D. diaboli* has 13–15 postanal myomeres, whereas catostomids range from 5 to 10 (Fuiman, 1978; Yeager and Baker, 1982; Bosley and Conner, 1984; Yeager and Semmens, 1987). Although myomere counts are similar between *Dionda* and *Notropis*, these counts might be useful in distinguishing *D. diaboli* from other genera of cyprinids; preanal myomeres of *D. diaboli* (19–22) are notably fewer than those for *Campostoma anomalum* (26–28), and longnose dace (*Rhinichthys cataractae*, 25–27—Fish, 1932; Hogue et al., 1976, unpublished report, Tennessee Valley Authority, Norris, Tennessee; Fuiman and Loos, 1977, 1978; Snyder, 1979; Fuiman et al., 1983).

Although quantifying distinguishable characteristics in melanophores, meristics, and mor-

phology were primary objectives of this study, it is worth noting similarities in early development between *D. diaboli* and other cyprinids. In this study, size of *D. diaboli* protolarvae 2 d post hatch (4.3 to 5.1 mm SL; 4.5 to 5.4 mm TL) was similar to that described for *Pimephales promelas*, *Notemigonus crysoleucas*, and *Cyprinella lutrensis* at hatching (Saksena, 1962; Snyder et al., 1977; Fuiman et al., 1983), but shorter than those of *Campostoma anomalum* (5.8 to 6.0 mm SL) and *Rhinichthys cataractae* (4.5 to 5.9 mm TL—Buynak and Mohr, 1980; Cooper, 1980; Fuiman et al., 1983; Ross, 2001). Likewise, timing of yolk sac absorption, occurring by Day 8 in *D. diaboli*, was similar to that of *R. cataractae* (6 or 7 d) and longer than that of *C. lutrensis* (4 or 5 d) (Saksena, 1962; Cooper, 1980). Length of *D. diaboli* in this study (5.2–6.4 mm SL, and 5.4–7.0 mm TL) at time of yolk absorption was similar to that described for the *C. lutrensis* and *N. crysoleucas*, but shorter than that for *R. cataractae* at 9–9.5 mm TL (Saksena, 1962; Snyder et al., 1977; Cooper, 1980; Heufelder and Fuiman, 1982; Fuiman et al., 1983).

In summary, distinguishing characteristics for *D. diaboli* included mid-lateral melanophores separate from a rounded caudal spot and lateral snout-to-eye melanophores by Day 16, initial coiling of intestine by Day 32, wedge-shaped caudal spot by Day 64, and mid-lateral double dashes along the lateral line and scale borders by Day 128. Collectively, these characteristics and other morphological, meristic, and melanophore attributes described herein provided a descriptive account of development of *D. diaboli* through the juvenile stage. We encourage additional studies that describe characteristics of early life stages of congeners and other cyprinids common to the Rio Grande Basin to facilitate identification of early life stages of *D. diaboli*.

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