MORPHOLOGY, MERISTIC COUNTS, AND MELANOPHORE DESCRIPTION FOR *DIONDA DIABOLI* (CYPRINIDAE) DURING DEVELOPMENT

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ABSTRACT

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Devils River minnow *Dionda diaboli*, listed as threatened (USA) and endangered (Mexico), coexists with at least three congeners throughout its south Texas and northern Mexico range. Monitoring of abundance, distribution, reproduction, and dispersion will be improved with the capability to distinguish larval and juvenile Devils River minnows from congeners and other cyprinids. I described and quantified various characteristics of Devils River minnow early life stages at intervals ranging from time of hatch to 128 days

to facilitate larval and juvenile identification. Distinguishing characteristics included mid-lateral melanophores separate from a rounded basicaudal spot by Day 8, lateral snout to eye melanophores by Day 16, initial coiling of intestine by Day 32, wedge-shaped basicaudal spot by Day 64, and mid-lateral double dashes along lateral line and scale borders by Day 128. Collectively, these characteristics and others described herein provided a detailed account of Devils River minnow development through the juvenile stage, but this information may be inadequate for confident identification until early development of other cyprinids is described.

INTRODUCTION

Devils River minnow *Dionda diaboli* is endemic to spring-influenced tributaries of the Rio Grande drainage including Devils River, San Felipe Creek, Sycamore Creek, Pinto Creek, and Las Moras Creek in Texas and 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 numerous other endemic minnows within the Rio Grande drainage, *D. diaboli* are declining in abundance and distribution attributed to modifications in habitat, water quantity and quality, and to 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 US Fish and Wildlife Service and Texas Parks and Wildlife Department (Hubbs et al., 1991; Garrett et al., 1992; USDI, 1999), and endangered in Mexico (Contreras-Balderas et al., 2002). In a conservation agreement among federal, state, and local agencies, *D. diaboli* abundance and distribution are monitored to ensure persistence of a viable population (USDI, 1999).

Distribution and potential distribution of *D. diaboli* overlap distributions of several congeners in the Rio Grande drainage including *D. argentosa* found along with *D. diaboli* in all Texas locations except Las Moras Creek, *D. episcopa* found in the Rio Grande and tributaries upstream from the Devils River, and *D. melanops* found along with *D. diaboli* in the Río Salado drainage of northern Mexico (Garrett et al., 1992;

Mayden at al., 1992). Adult *D. diaboli* differs morphometrically from these congeners by having a wedge-shaped basicaudal spot, dark pigments on scale margins (scale borders) that are most prominent dorsally, and doubled dashes along the lateral line (Girard, 1856; Hubbs and Brown, 1956). Numerous other morphometric, meristic, and genetic differences exist (Girard, 1856; Hubbs and Brown, 1956; Contreras-Balderas and Verduzco-Martínez, 1977; Hubbs et al., 1991; Gold et al., 1992; Mayden et al., 1992). However, larval and juvenile morphologies have not been described and are needed to identify early life stages of *D. diaboli* from those of congenera and other cyprinids.

Objectives of this study were to determine age and size of fish at which wedge-shaped caudal spot, scale margin pigments, and doubled dashes along the lateral line became apparent. Additional objectives were to describe other morphometric and meristic characters of protolarvae, mesolarvae, metalarvae, and juvenile *D. diaboli* from Day 2 to Day 128 including time of yolk sac absorption, myomere counts, loss of the median and preanal finfolds, development of median and paired fins, emergence of the lateral line, and basic patterns in pigmentation. Information from this study will aid in the identification of larval and juvenile *D. diaboli* in order to more accurately monitor and assess the abundance, distribution, and dispersion of the *D. diaboli* population.

MATERIALS AND METHODS

Brood fish were obtained from the National Fish Hatchery and Technology Center (NFHTC), San Marcos, Texas. Brood fish were wild and first generation descendents of wild fish captured from the Devils River and its tributaries in August 2000 (Val Verde County, Texas). In the NFHTC laboratory, breeding adults ($N = 300$) were maintained in a 719-L and two 830-L fiberglass tanks (Living Stream; Frigid Unit, Toledo, Ohio), and two 16.5-L, flow-through aquaria placed on top of the fiberglass tanks. Plastic trays (14 x 14 x 4 cm) filled with gravel were used for spawning substrate (Gibson et al., 2004) and placed in each fiberglass tank and aquarium. Well water was pumped into each tank at a rate of 15 L/h and into each aquarium at a rate of 5.2 L/h. Water was circulated by a pump (Model SP 125J, Hayward Pool Products, Inc., Elizabeth, New Jersey). Temperature was maintained between 22° and 24°C by a heater-chiller unit (Model UTCH-3, Universal Marine Industries, Inc., San Leandro, California). Photoperiod was 12-h light and 12-h dark. Dissolved oxygen (mg/l), conductivity (uS), water temperature (°C), and pH were measured twice a week, and ammonia concentrations (mg/l) were measured once a week.

Eggs were removed daily from the gravel substrate. Eggs with fungus were discarded. Once the number of eggs from all breeding tanks and aquaria exceeded 100 within a 24-h period, all eggs were placed into one of five 16.5-L, flow-through rearing

aquaria located on top of a 830-L fiberglass tank. Water from the fiberglass tank was pumped into the rearing aquaria at a rate of 5.2 L/h. Source of water, temperature, photoperiod, and schedule for water testing were the same as for the breeding adults.

Five groups of eggs in five separate aquaria were produced from December 2002 through January 2003. Eggs generally hatched within 5 days after being placed in rearing aquaria. Once hatched, fish (N = 5/d) were removed on days 2, 4, 8, 16, 32, 64, and 128 and exposed to a lethal dose of tricaine methanesulfonate (>80 mg/L). Fish were 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, larval fish were fed liberal amounts of brine shrimp several times weekly and Gold Fry-3 daily (Aurum Aquaculture Ltd, Kirkland, WA). After Day 16, fish were fed Spirulina flakes and worm flakes (Aquatic Eco-systems, Inc., Apopka, Florida) three times weekly. Dead fish and debris were siphoned from aquaria as needed.

Using a dissecting scope and with the aid of digital photographs, the following measurements were taken, when available, from each fish following methods described by Hubbs and Brown (1956), Snyder and Muth (1990), and Trautman (1981): pectoral fin length (distance from origin of pectoral fin to distal end of longest fin ray), snout to pectoral fin length (distance from snout to origin of pectoral fin), body depth (greatest depth excluding fleshy or scaly structures), orbital length (greatest distance between free orbital rims), postorbital length (posterior portion of orbital rim to posterior margin operculum), head length (distance from snout to posterior margin of operculum), caudal peduncle depth (narrowest region of the body anterior to caudal fin), depressed dorsal fin length (distance from origin of depressed dorsal fin to distal end of longest fin ray),

depressed anal fin length (distance from origin of depressed anal fin to distal end of longest fin ray), caudal peduncle length (oblique distance between posterior end of anal fin to base of the middle caudal ray), pelvic fin length (distance from origin of pelvic fin to distal end of longest fin ray), and standard length (distance from snout to end of notochord in larvae; distance from snout to end of hypural plate or structural base of the caudal fin in larger fish). Presence of yolk sack and fin folds, position of the notochord and hypural plate, development of an emarginated tail, emergence of the horizontal septum, formation of lateral line, shape of intestines, and length of intestines (straightened and measured from esophagus to anus) were described. Preanal, postanal, and total myomere counts and caudal, dorsal, anal, and pelvic fin ray counts were reported on a subsample of fish (total $N = 3 - 10$) following the methods described by Contreras-Balderas and Verduzco-Martinez (1977) and Snyder and Muth (1990). Pigmentation appearance and shape of basicaudal spot, appearance of dorsal pigments around scales, appearance of double dash pigments on the lateral line, and other melanophores were described for all larval and juvenile fish.

Morphometric, meristic, and pigmentation characteristics were described for protolarvae, mesolarvae, metalarvae, and juveniles (Cooper, 1980; Snyder and Muth, 1990). Protolarvae had no dorsal, anal, and caudal rays. Mesolarvae had at least one dorsal, anal, or caudal fin ray and the absence of fully developed fin rays or pelvic fin buds or fins. Metalarvae had fully developed caudal, dorsal, and anal fin rays, or pelvic fin buds or fins. Juveniles had fully developed fins and fin rays, and completely absorbed preanal fin fold. Morphological characteristics (mean ± SD) expressed as percent of SL, counts (mode; range) or range if mode was not observed, and pigmentation (%

occurrence) were calculated for Day 2 and Day 4 protolarvae, Day 8 and days 16 and 32 mesolarvae, days 32 and 64 metalarvae, and days 64 and 128 juveniles.

RESULTS

Protolarvae (4.3 to 5.8 mm SL; Day 2 and Day 4)

Standard length of Day 2 protolarvae ($N = 18$) ranged from 4.3 to 5.1 mm. Fish had preanal and median fin folds, straight notochord, round eyes, and yolk sac (Figure 1a). Myomere count [mode (range) or range only when no mode was present] was 21 - 22 for preanal, 15 (13 - 15) for postanal, and 37 (34 - 37) for total (Table 1). Pectoral buds formed in 58% of the fish and pectoral fins developed in 42%. Mean distances (expressed as a % of SL; ±SD) were 5.0 (0.68) for pectoral fin length, 16.8 (0.60) for snout to pectoral fin length, 16.1 (1.41) for body depth including yolk sac, 6.8 (0.45) for orbital length (Table 2). Dorsal melanophores were visible on occipital region in 11% of the fish (Table 3). Ventral melanophores were visible posterior to vent in 50%.

Standard length of Day 4 protolarvae ($N = 25$) ranged from 5.1 to 5.8 mm. Fish had preanal and median fin folds, straight notochord, and yolk sac (Figure 1b). Myomere count [mode (range) or range only when no mode was present] was 21 (20 - 22) for preanal, 13 - 15 for postanal, and 34 (34 - 36) for total (Table 1). Pectoral fins formed in all fish. Mean distances were 8.5 (1.26) for pectoral fin length, 18.6 (0.80) for snout to pectoral fin length, 12.6 (0.85) for body depth, less than that observed for Day 2 fish attributed to yolk absorption, 8.4 (0.76) for orbital length, 8.1 (0.63) for postorbital length, 18.4 (0.60) for head length, and 3.7 (0.69) for caudal peduncle depth (Table 2). Dorsal melanophores were visible on occipital region of head in 92% of fish, and on body

in 8%. Ventral melanophores were visible between yolk sac and end of notochord, between pectoral fins in 96%, and on the isthmus in 52%. Lateral melanophores were visible on the opercle in all fish, extending to mid-body posteriorly in 48%. Preanal and median fin fold melanophores were visible in 4% (Table 3).

Mesolarvae (5.8 to 8.1 mm, Day 8, 16, and 32)

Standard length of Day 8 mesolarvae ($N = 24$) ranged from 5.8 to 6.4 mm. Fish had preanal and median fin folds, slightly upturned notochord, and nearly absorbed yolk sac (Figure 1c). Myomere count [mode (range) or range only when no mode was present] was 21 - 22 for preanal, 14 - 15 for postanal, and 36 (35 - 37) for total (Table 1). Mean distances (±SD) were 12.7 (1.14) for pectoral fin length, 20.3 (1.34) for snout to pectoral length, 11.9 (2.28) for body depth with yolk sac, 9.0 (0.73) for orbital length, 9.1 (0.68) for postorbital length, 20.4 (1.07) for head length, and 3.7 (0.6) for caudal peduncle depth (Table 2). Elements of caudal fin rays were visible but not fully developed.

Dorsal melanophores were visible on occipital region of head on all fish, and on body in 71% in Day 8 fish. Ventral melanophores were visible from vent to notochord flexion and from vent anterior to yolk sac. Melanophores extended obliquely from posterior ventral margin of yolk sac to opercle. Ventral melanophores were visible on isthmus and between pectoral fins where ventral melanophores extended laterally, forming a U-shaped pattern that opened posteriorly. Lateral melanophores extended across opercle posteriorly to caudal fin region in all fish and were separated from basicaudal melanophores near area of notochord flexion in 96% of the fish. Melanophores were visible in interradial members of caudal fin (Table 3).

Standard length of Day 16 ($N = 21$) and Day 32 ($N = 6$) mesolarvae ranged from 5.8 to 8.1 mm. Fish had preanal and median fin folds, highly upturned notochord, and fully absorbed yolk sac (Figure 1d). Horizontal septum was visible in Day 32 mesolarvae. Caudal fin rays were visible in all fish. Caudal fin ray count [mode (range) or range only when no mode was present] was 18 and 19 (15 – 20). Emarginated caudal fins observed in 83% of Day 32 mesolarvae. Dorsal fins were observed in all Day 32 mesolarvae, and elements of dorsal fin rays were visible in 29% of Day 16 mesolarvae. Dorsal fin ray count was 8 (5 - 8). A membrane outline of anal fins was visible in 48% of Day 16 fish and 33% of Day 32 mesolarvae. Anal fins and fin rays were visible in 67% of Day 32 fish although not fully developed. Anal fin ray count was 5 (2 - 5). Myomere count [mode (range) or range only when no mode was present] was 21 (20 - 21) for preanal, 14 (13 - 15) for postanal, and 35 (33 - 36) for total. Intestine was straight in Day 16 fish ($N = 3$; Figure 2); mean intestinal length to SL ratio (±SD) was 0.41 (0.013).

Mean distances (±SD) were 13.6 (1.12) for pectoral fin length, 23.8 (2.51) for snout to pectoral fin length, 15.9 (3.64) for body depth, 10.0 (0.78) for orbital length, 11.0 (1.41) for postorbital length, 24.2 (2.64) for head length, and 4.8 (1.34) for caudal peduncle depth (Table 2). Depressed dorsal fin length was 16.4 (3.16), depressed anal fin length 6.7 (4.76), and caudal peduncle length 23.8 (1.81).

Dorsal melanophores were visible on occipital region of head and concentrated anteriorly on body in all fish; dorsal melanophores on snout in 48%; and between orbitals in all Day 32 mesolarvae. Ventral melanophores were visible from vent to base of caudal fin, and obliquely from anal opening to mid-gut region. Oblique melanophores appeared to be fewer than those in Day 8 mesolarvae. Ventral melanophores were visible between

pectoral fins on 81% of the fish and isthmus on 96%. Ventral melanophores extended laterally from the pectoral fins in a U-shaped pattern that opened posteriorly on Day 16 fish. Ventral melanophores extended laterally from the pectoral fins onto the lower portion of the gut on Day 32 fish. Lateral melanophores were visible on snout, forming a partially developed lateral stripe from eye to snout, and across the opercle posteriorly towards caudal fin, where they were separated from basicaudal melanophores. Basicaudal melanophores were visible in 96% of the 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 (6.8 to 12.0 mm, 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. Fish had preanal fin folds, and median fin folds at the caudal fin were observed in 95%. A horizontal septum was visible in all fish (Figure 1e, f). Caudal fin ray count [mode (range) or range only when no mode was present] was 20 (19 - 20) (Table 1). Hypural plates were visible in all fish, and emarginated caudal fins in 95%. Dorsal fins fully developed with 8 fin rays. Anal fin was formed in all fish; 14% of fish were without fully developed anal fin rays. Anal fin ray count was 8 (7 - 8). Pelvic fin buds were anterior to dorsal fin and formed in 29%. Pelvic fins were opposite to dorsal fin and formed in 71%. Pelvic fin ray count was 4 (4 - 7). Pectoral fin rays formed in 62%. Myomere count [mode (range) or range only when no mode was present] was 21 (19 - 21) for preanal, 15 (14 - 15) for postanal, and 36 (34 - 36) for total. Intestines in Day 32 fish (N = 3) looped once (Figure 2); mean intestinal length to SL ratio (±SD) was 0.58 (0.008).

Mean distances (±SD) were 14.8 (2.42) for pectoral fin length, 29.4 (2.03) for snout to pectoral length, 22.2 (3.20) for body depth, 10.9 (0.78) for orbital length, 13.1 (1.06) for postorbital length, 28.9 (2.23) for head length, and 8.2 (0.90) for caudal peduncle depth (Table 2). Depressed dorsal fin length was 19.0 (2.36); depressed anal fin length 12.7 (2.55); caudal peduncle length 22.0 (2.11); and pelvic fin length 9.3 (2.40).

Dorsal melanophores were visible on occipital region of head (forming a heart-shaped pattern), body, and snout between orbitals (Table 3). Ventral melanophores were visible from the vent toward base of caudal fin and from vent toward pelvic fins or buds in all fish. Ventral melanophores were sparse between pectoral fins, at isthmus, and obliquely on lower gut. Lateral melanophores were more numerous and intense, forming a mid-lateral stripe from eye to snout and extending posteriorly across opercle towards base of the caudal fin. Basicaudal melanophores formed a rounded pattern in 81% and a wedge-shaped pattern in 19% fish, all of which were Day 64 fish. Melanophores were visible on caudal fin interradial membranes and on the radials of all fins.

Juvenile (10.0 to 29.0 mm, Day 64 and 128)

Standard length of Day 64 (N=16) and Day 128 (N=22) juveniles ranged from 10 to 29 mm. Preanal fin fold was absent. Median fin fold was visible at the base of the caudal fin in 8, Day 64 fish and 2, Day 128 fish (Figure 1f, g). Lateral lines were visible in 50%. Fish had emarginated caudal fins and fully developed dorsal, anal, and pelvic fins. Fin ray count [mode (range) or range only when no mode was present] was 20 (19 – 20) for caudal fin rays, 8 (8 – 9) for dorsal fin rays, 8 for anal fin rays, and 7 (6 - 8) for pelvic fin rays (Table 1). Pectoral fin rays were developed in all fish. Myomere count [mode (range) or range only when no mode was present] was 19 – 21 for preanal, 14 for

postanal, and 33 – 35 for total. Myomeres were difficult to count because of intense pigmentation. Intestines in Day 64 fish ($N = 3$) looped twice and were more convoluted than Day 32 fish (Figure 2); mean intestinal length to SL ratio (±SD) was 1.41 (0.19). Intestines of Day 128 fish ($N = 3$) looped several times and were highly convoluted; mean intestinal length to SL ratio (±SD) was 2.8 (0.44).

Mean distances (± SD) were 16.6 (1.38) for pectoral fin length, 28.6 (2.03) for snout to pectoral length, 24.1 (3.11) for body depth, 10.0 (1.09) for orbital length, 11.7 (1.11) for postorbital length, 26.3 (2.12) for head length, 10.2 (0.69) for caudal peduncle depth, and 22.9 (1.71) for caudal peduncle length (Table 2). Depressed dorsal fin length was 22.3 (1.36), depressed anal fin length was 16.6 (1.07), and pelvic fin length was 13.0 (1.53).

Dorsal melanophores were visible on occipital region of head forming a heart-shaped pattern, on snout between eyes, and anterior and posterior areas of the body in all fish (Table 3). Lateral melanophores formed a solid stripe from the eye to snout, extending across opercles towards base of the caudal fin, and distinct double dashes in 89% of the fish ventral to mid-lateral stripe. Lateral melanophores outlined scales, forming scale borders and were most prominent dorsal to septum in 66% of fish. Basicaudal melanophores formed wedge-shaped pattern in 92%. Ventral melanophores formed a continuous black stripe posterior to vent and at base of the anal fin. Melanophores were visible on caudal fin interradial membranes and on the radials of all fins.

DISCUSSION

Intestine morphology and melanophore distributions are primary distinguishing features for adult identification among *Dionda* and other cyprinids in the Rio Grande drainage (Hubbs et al., 1991). Intestines of juvenile and adult *D. diaboli*, congenera, *Hybognathus*, and *Campostoma* are long and coiled, reflecting adaptation for consuming algae and diatoms (Kraatz, 1924; Kafuku, 1958; Hubbs and Miller, 1977; Contreras-Balderas and Verduzco-Martinez, 1977). For *D. diaboli*, indications of a coiled intestine were observed with a single loop by Day 32 to a multi-looped, convoluted intestine by Day 128 (see also Contreras-Balderas and Verduzco-Martinez, 1977) whereas intestines of insectivorous cyprinids generally are straight throughout the larval stage and eventually develop a simple loop during juvenile stage (Junger et al., 1989). Similar to *D. diaboli*, the intestine of *Campostoma anomalum* forms a single loop before reaching a TL of 13 mm (age unknown), but differs from *D. diaboli* by extending dorsally and coiling around the air bladder, which is evident at TL of 16 mm (Kraatz, 1924). Basicaudal and snout to eye melanophores are useful features separating *Dionda* from many familial genera including *Hybognathus* with similar intestine morphology (Hubbs and Miller, 1977). In *D. diaboli*, basicaudal melanophores were visible from Day 8 to Day 128, and snout to eye melanophores were visible from Day 16 to Day 128. Wedge-shape basicaudal spot, lateral double dashes, and scale borders distinguish *D. diaboli* from *D. argentosa*, the congener with a distribution most similar to that of *D. diaboli*

(Hubbs and Brown, 1956). However, these features were prominent in less than half of *D. diaboli* until Day 64 and thus not useful for distinguishing between these two species during the larval stage. One caveat in using melanophore distributions for distinguishing larval fishes is that pigmentation occurrence and intensity will vary with food availability and habitat conditions (Fuiman et al., 1983; Snyder and Muth, 1990).

Adult *D. diaboli* has a narrower body depth than *D. episcopa*, *D. argentosa*, and *D. melanops* (Girard, 1856; Hubbs and Brown, 1956), a shorter caudal peduncle depth and postorbital length than *D. episcopa*, and a shorter orbital length than *D. argentosa* (Hubbs and Brown, 1956). Applicability of these relative morphological characters to larval fishes is unknown until descriptions of congeners are completed for comparisons to *D. diaboli*. Until this is done, their inclusion here may simply provide a higher level of confidence for larval *D. diaboli* identification. As determined in this study, *D. diaboli* mean body depth ranged from 11.9 to 24.1 of SL (Day 2 – Day 128), mean caudal peduncle depth ranged from 3.7 to 10.2 of SL (Day 4 – Day 128), mean postorbital length ranged from 8.1 to 13.1 of SL (Day 4 – Day 128), and mean orbital length ranged from 6.8 to 10.9 of SL (Day 2 – Day 128).

Potentially useful morphological characteristics in identifying larval *D. diaboli* from those of familial genera and other fishes common to the Rio Grande drainage include formation of spinous rays, larval fish eye shape, and relative length of pectoral fins. Larval perciforms (e.g., *Lepomis* and *Etheostoma*) form detectable spinous rays in dorsal and anal fins at total lengths of 8.6 mm in *L. cyanellus* and >7 mm in *E. grahami* (Taubert, 1977; Aguilera et al., 1999) whereas *Dionda* and most other cyprinids lack spinous rays. Larval eye shape is round in *D. diaboli*, but flattened dorsally and ventrally

in *Macrhybopsis aestivalis*, *Notropis stramineus*, and *Phenacobius mirabilis* (Fuiman et al., 1983). In this study, relative pectoral fin length of *D. diaboli* was shorter (mean = 7% TL and 7.6% SL for Day 2 and 4 fish) than that of *Notemigonus crysoleucus, N. spilopterus, P. pimephales,* and *C. lutrensis* (range of means = 11 – 14% TL) during the protolarval stage, but was similar to relative size of these cyprinids by the mesolarval stage with the exception of *C. lutrensis*, which had longer relative pectoral fin lengths (Saksena, 1962; Snyder et al., 1977).

Numbers of preanal, postanal, and total myomeres are useful in larval fish identification because extensive pigmentation does not obscure myomere counts (Yeager and Semmens, 1987; Snyder and Muth, 1990). *Dionda diaboli* and cyprinids in general differ from catostomids (*Ictiobus, Carpiodes, Cycleptus, Moxostoma*) by having a larger number of postanal myomeres (Fuiman, 1978; Yeager and Baker, 1982; Bosley and Conner, 1984; Yeager and Semmens 1987); *D. diaboli* has 13 – 15 postanal myomeres whereas catostomids range from 5 – 10. Conversely, *D. diaboli* and cyprinids in general differ from centrarchids (*Lepomis* and *Micropterus*) and *Etheostoma* by having fewer postanal myomeres (Kramer and Smith, Jr., 1960; Taubert, 1977; Bosley and Conner, 1984; Aguilera et al., 1999); *Lepomis cyanellus* has 16 – 17, *L. macrochirus* 15-18, *M. salmoides* 24 – 25, and *E. grahami* 18 – 19 postanal myomeres. Myomere counts are similar among most cyprinids (Snyder 1979); however, preanal myomeres of *D. diaboli* (19 – 22) were lower than those for *Campostoma anomalum* (26 – 28), *Semotilus atromaculatus* (25 – 26), and *Rhinichthys cataractae* (25 – 27) (Fish, 1932; Hogue et al., 1976; Fuiman and Loos, 1977; Fuiman and Loos, 1978; Snyder, 1979; Fuiman et al., 1983).

Spawning season of *Dionda* varies with *D. nigrotaeniata* reproducing from February through September (Wayne and Whiteside, 1985), *Dionda* sp. reproducing late fall to late spring (Hubbs and Miller, 1977), *D. serena* reproducing in April (Hubbs, 1951), and *D. diaboli* spawning year round in wild and hatchery-reared populations (G. Garrett, pers. com.; R. Gibson pers. com.). Protracted spawning season or year-round spawning is common among fishes associated with spring outflows (Schenck and Whiteside, 1977; Cantu and Winemiller, 1997; Gibson et al., 2004). In contrast, *Campostoma*, *Notropis*, *Cyprinella*, and *Ictiobus* spawn from late spring to late summer and have a shorter reproductive season (Farringer et al., 1979; Ross, 2001). Consequently, knowledge of larval fish availability may facilitate identification of *Dionda* and possibly *D. diaboli*.

Although quantifying distinguishable characteristics in melanophores, meristics, and morphology 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 two-days post hatch (4.3 – 5.1 mm SL; 4.5 – 5.4 mm TL) was similar to that described for *Pimephales promelas, Notemigonus crysoleucas*, *Notropis spilopterus*, *Notropis cornutus*, *N. rubellus*, and *Cyprinella lutrensis* at time of hatch (Reed, 1958; Saksena, 1962; Snyder et al., 1977; Buynak and Mohr, 1980a; Fuiman et al., 1983), but shorter than those of *C. anomalum* (5.8- 6 mm SL), *R. cataractae* (4.5-5.9 mm TL), and *S. atromaculatus* (5.3 to 6.2 mm TL) (Buynak and Mohr, 1979; Buynak and Mohr, 1980b; 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 *Rhinichthys cataractae* (6 - 7 days), and longer than that of *Cyprinella lutrensis* (4 - 5 days) (Saksena,

1962; Cooper, 1980). Length of *D. diaboli* in this study (5.2 to 6.4 mm SL, and 5.4 to 7.0 mm TL) at time of absorption was similar to that described for *C. lutrensis*, *N. crysoleucas*, and *N. rubellus*, but shorter than *S. atromaculatus*, *R. cataractae*, and *N. cornutus* at 8 to 9.5 mm TL (Saksena, 1962; Snyder et al., 1977; Buynak and Mohr, 1979; Buynak and Mohr, 1980a; Cooper, 1980; Heufelder and Fuiman, 1982; Fuiman et al., 1983).

In summary, distinguishing characteristics included mid-lateral melanophores separate from a rounded basicaudal spot by Day 8, lateral snout to eye melanophores by Day 16, initial coiling of intestine by Day 32, wedge-shaped basicaudal spot by Day 64, and mid-lateral double dashes along lateral line and scale borders by Day 128. Collectively, these characteristics and other morphometric, meristic, and melanophore attributes described herein provided a detailed account of Devils River minnow development through the juvenile stage, but this information may be inadequate for confident identification until early developments of other cyprinids common to the Rio Grande drainage, especially congeners, are described.

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TABLES AND FIGURES

Table 1. Meristic characteristics (modes and ranges) for Devils River minnow *Dionda diaboli* through early development. Number of specimens examined is in parentheses. N/A indicates a count without a mode.

	Protolarvae				Mesolarvae				Metalarvae		Juvenile	
	Day 2		Day 4		Day 8		Day 16 & 32		Day 32 & 64		Day 64 & 128	
Counts	mode	range	mode	range	mode	range	mode	range	mode	range	mode	range
Preanal myomere	N/A (4)	21-22	21 (4)	20-22	N/A (4)	21-22	21 (5)	20-21	21 (4)	19-21	N/A (3)	19-21
Postanal myomere	15 (4)	13-15	N/A (4)	13-15	N/A (4)	14-15	14 (5)	13-15	15 (4)	14-15	N/A (3)	14
Total myomere	37 (4)	34-37	34 (4)	34-36	36 (4)	35-37	35 (5)	33-36	36 (4)	34-36	N/A (3)	33-35
Caudal fin ray							18,19 (9)	15-20	20 (5)	19-20	20 (8)	19-20
Dorsal fin ray							8 (4)	5-8	N/A (6)	8	8 (10)	8-9
Anal fin ray							5 (4)	2-5	8 (6)	7-8	N/A (10)	8
Pelvic fin ray									4 (4)	4-7	7 (9)	6-8

Table 2. Morphometric characteristics through early development (mean ± SD) for Devils River minnow *Dionda diaboli*. Number of specimens examined is in parentheses.

	Protolarvae				Mesolarvae				Metalarvae		Juvenile	
	Day 2		Day 4		Day 8		Day 16 & 32		Day 32 & 64		Day 64 & 128	
Lengths (% SL)	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD	mean	SD
Pectoral fin	5.0	0.68	8.5	1.26	12.7	1.14	13.6	1.12	14.8	2.42	16.6	1.38
	(8)		(25)		(23)		(27)		(21)		(38)	
Snout-pectoral fin	16.8	0.60	18.6	0.80	20.3	1.34	23.8	2.51	29.4	2.03	28.6	2.03
	(10)		(25)		(23)		(27)		(21)		(38)	
Body depth	16.1	1.41	12.6	0.85	11.9	2.28	15.9	3.64	22.2	3.2	24.1	3.11
	(18)		(25)		(24)		(27)		(21)		(38)	
Orbital	6.8	0.45	8.0	0.76	9.0	0.73	10.0	0.78	10.9	0.78	10	1.09
	(17)		(25)		(24)		(27)		(21)		(38)	
Postorbital			8.1	0.63	9.1	0.68	11.0	1.41	13.1	1.06	11.7	1.11
			(6)		(24)		(27)		(21)		(38)	
Head			18.4	0.60	20.4	1.07	24.2	2.64	28.9	2.23	26.3	2.12
			(6)		(24)		(27)		(21)		(38)	
Caudal peduncle depth			3.7	0.69	3.7	0.60	4.8	1.34	8.2	0.90	10.2	0.69
			(10)		(24)		(27)		(21)		(38)	
Depressed dorsal fin							16.4	3.16	19.0	2.36	22.3	1.36
							(6)		(21)		(38)	
Depressed anal fin							6.7	4.76	12.7	2.55	16.6	1.07
							(4)		(21)		(38)	
Caudal peduncle							23.8	1.81	22	2.11	22.9	1.71
							(3)		(21)		(38)	
Pelvic fin									9.3	2.4	13.0	1.53
									(15)		(38)	

Table 3. Percentage of fish with head and body melanophores for Devils River minnow *Dionda diaboli* through early development in order of first appearance.

	%					
	Protolarvae		Mesolarvae		Metalarvae	Juveniles
Melanophores:	Day 2	Day 4	Day 8	Day 16 & 32	Day 32 & 64	Day 64 & 128
Occiptal area	11	92	100	100	100	100
Ventral	50	96	100	100	100	100
Dorsum of body		8	71	100	100	100
Mid-lateral stripe		48	100	100	100	100
Interradial and fin rays		4	100	100	100	100
Basicaudal spot			96	96	100	100
Dorsum of snout				48	100	100
Lateral: snout to eye				100	100	100
Wedge-shape basicaudal spot					19	92
Mid-lateral double dashes						89
Scale borders						66

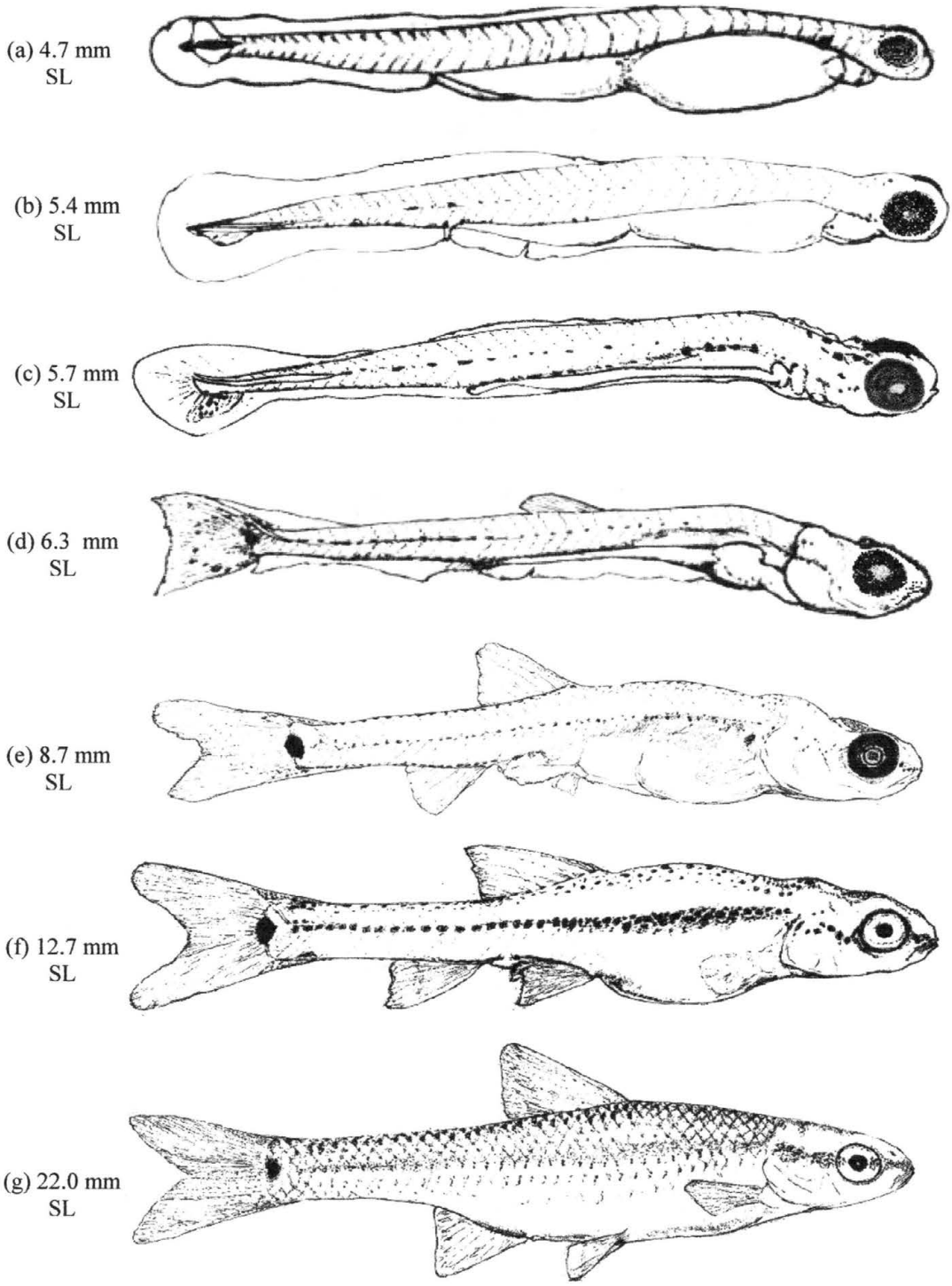

Figure 1. Illustrations of Devils River minnow and mean SL (mm) for (a) Day 2 protolarvae; (b) Day 4 protolarvae; (c) Day 8 mesolarvae; (d) Day 16 mesolarvae; (e) Day 32 metalarvae and juvenile; (f) Day 64 metalarvae and juvenile; and (g) Day 128 juvenile.

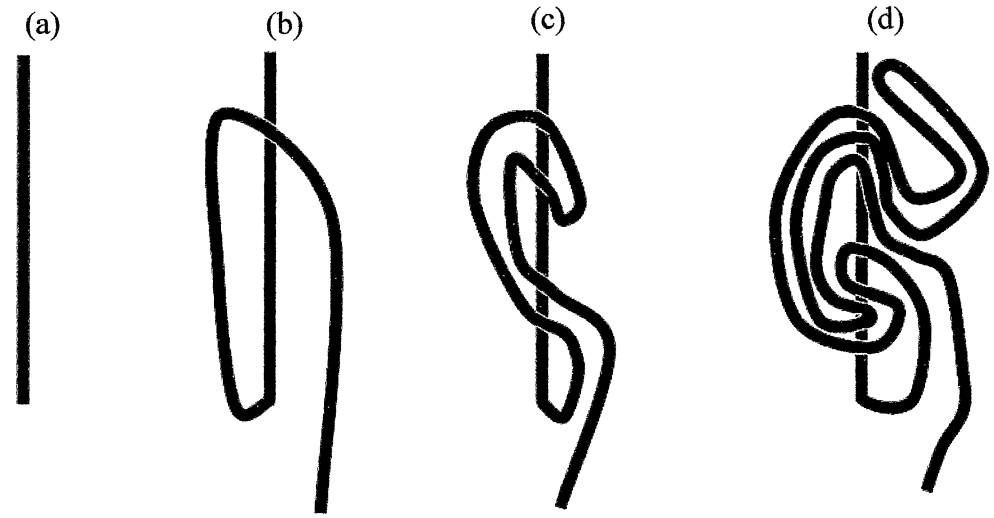

Figure 2. Schematic illustration depicting ventral view of Devils River minnow intestinal coiling for (a) Day 16 mesolarvae; (b) Day 32 metalarvae; (c) Day 64 juvenile; and (d) Day 128 juvenile. Esophagus is at top and anus at bottom of the illustration.

VITA

Julie Hulbert was born in Boston, Massachusetts, on 13 August 1973. She completed a Bachelor of Science in Biology from Trinity University in San Antonio, Texas, between 1991 and 1995. During this time she also completed courses at New York University and the University of New South Wales in Sydney, Australia. After this, Julie spent some time in Crested Butte, Colorado, pursuing adventures in the outdoors and exploring various careers. During the last 4 years, she was employed by the City of San Marcos as a Resource Conservation Biologist, began graduate school, taught biology labs, interned at Texas Parks and Wildlife, and participated in various volunteer activities and field data collections.

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