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Cover Photograph: Collection site for *Neoheterandria tridentiger* at the headwaters of Río Antón, El Valle de Antón, Panamá, on May 18, 2015. Photograph © Bruce Stallsmith.

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Bruce Stallsmith[1*]

Abstract - *Neoheterandria tridentiger* (Garman) is a poeciliid fish endemic to freshwater drainages in Panamá. This species utilizes superfetation, the carrying of broods of different ages. Life history parameters including incidence of superfetation, number of embryos, and size at maturity are presented for a population of *N. tridentiger*. Fish were collected in May, 2015, at the end of the dry season from Río Antón in El Valle de Antón in central Panamá at an elevation of 700 m. Female size at maturity was 22 mm SL. 75% of mature females were found to be carrying at least one developing embryo, the mean number of embryos was five, and the mean embryo dry mass was 1.42 mg. Superfetation was found in 23% of gravid females. Earlier research findings from two different populations in August, 1973, during the rainy season found that gravid female size was smaller at (17 mm SL), with superfetation rates of 70% and 80%. Males collected in May, 2015, were 16–27 mm SL, whereas those collected in August, 1973, were 10–16 mm SL. Shifting size and fecundity could be driven by seasonal food web changes affecting both adults and offspring.

Introduction. *Neoheterandria tridentiger* (Garman) is endemic to freshwater drainages in central Panamá on both the Atlantic and Pacific versants. The life history of livebearing poeciliid species is known to vary in response to fluctuating abiotic and biotic factors (Grether et al. 2001; Jennions et al. 2006; Reznick et al. 1990; Stallsmith 2018). A major abiotic factor influencing the life history of freshwater fishes in Panamá is the pronounced seasonality of precipitation, with a dry season from late December to early May and a wet season from late May to early December. In general, poeciliids native to tropical areas with dry and wet seasons have higher fecundity in the wet season (Magurran 2005; Stallsmith 2018; Turner 1938; Winemiller 1993).

A single published study addresses the reproductive life history of *N. tridentiger* (Stearns 1978). The species exhibits superfetation, "the ability of females to simultaneously gestate multiple broods of offspring at different stages of development" (Furness et al. 2021). Superfetation is found in some but not all poeciliids (Frías-Alvarez et al. 2015). Recent studies have led to a clear picture of the phylogenetic history of the trait within Poeciliidae (Furness et al. 2021). Two other species of *Neoheterandria* have been described, *N. cana* (Meek and Hildebrand) from eastern Panamá and *N. elegans* (Henn) from northwestern Colombia. *Neoheterandria cana* have been reported to exhibit superfetation (Wischnath 1993). *Neoheterandria elegans* also appears to exhibit superfetation based on the description in Wischnath (1993), "One to two young are born at intervals of 2 to 4 days."

Little is known of the life history of *N. tridentiger* in nature, including whether fecundity and superfetation may vary seasonally. *Neoheterandria tridentiger* has been reported to be lecithotrophic, meaning that developing embryos consume nutrients provided in the egg with little or no direct maternal support after fertilization (Reznick and Miles 1989). Here

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I present life history parameters including incidence of superfetation, calculation of the matrotrophy index to assess possible maternal feeding of embryos, number of embryos, and size at maturity of a population of *N. tridentiger* from a collection made in early May, 2015, at the end of the well-defined dry season in central Panamá at a high elevation site (700 m). Findings from this May population are compared to those reported from two low elevation (<100 m) populations collected in central Panamá in August, 1973, when the rainy season is well underway in Panamá (Stearns 1978). These August data appear to provide a comparison of the reproductive parameters between the dry season and rainy season, although I acknowledge that the comparison is confounded by the elevational differences between the May and August samples. Since the life history parameters of many poeciliid fishes vary seasonally, I expect to find seasonal differences in these parameters.

Methods. Fish were collected on 8 May 2015 from the headwaters of Río Antón on the northeast corner of the caldera encircling El Valle de Antón, Coclé Province, Panamá. Coordinates for the site are 8° 37' 12"N, 80° 6' 21"W at an elevation of 700 m. Fish were caught with a Perfect Dipnet Model 1P (Jonah's Aquarium, Ohio, USA) with a 1.3 m handle and a 3 mm mesh. The stream was clear, flowing water, no more than 30 cm deep, over a mixed substrate of sand, gravel, cobble, and boulder. Water temperature was 24°C. Fish were most easily caught along stream banks. Specimens were euthanized on site by adding two ml of a solution of 90% ethanol and 10% clove oil to a jar with approximately 250 ml of stream water holding the fish. Euthanized specimens were transferred to a solution of 95% ethanol for tissue fixation and storage.

Following Johnson and Belk (2001) and Stallsmith (2018), seven life-history traits for females were measured: size at maturity, brood size, size of offspring as dry mass, reproductive allotment, percentage of gravid females, incidence of superfetation, and matrotrophy index. Gravid was defined as carrying stage 4 or higher embryos (Haynes 1995). The standard length (SL) of the smallest gravid females and sexually mature males was used to determine size of sexual maturity. If a majority of individuals at a given SL were respectively gravid or possessed a gonopodium, this SL was used to define minimum size for maturity. The gonopodium is used in male poeciliids to transfer sperm to females. Because poeciliid males typically stop growing at reproductive maturity, the SL of the smallest male was used to define minimum size at maturity (Johnson and Belk 2001). The mean and range of SL were based on caliper measurements.

Brood size of females was determined by dissecting, removing, and counting all embryos. The developmental stage of embryos was determined using the scheme of Haynes (1995). Superfetation was identified in a female if embryos of more than one developmental stage were found. Collected embryos along with the bodies of dissected females with viscera removed were desiccated in an oven at 55° for 24 hours. Mass of offspring was determined by dividing the dry mass of a brood by the number of embryos. An Ohaus Explorer balance was used to determine mass.

Reproductive allotment (RA) in females was calculated as the mass of desiccated embryos divided by the sum of the mass of desiccated embryos and the mother's desiccated mass. This is the same calculation used by Stallsmith (2018) and Reznick and Endler (1982), but different from the RA reported by Johnson and Belk (2001) which simply reported desiccated embryo mass. Reporting RA as a percentage of maternal mass enables direct comparison between species. The RA was calculated the same way for those females exhibiting superfetation and those carrying only one brood.

The matrotrophy index (MI) indicates if a species is matrotrophic, with a pregnant female providing regular nourishment to developing embryos beyond what was present in

the fertilized egg (Reznick and Miles 1989). MI was calculated using a linear regression of embryonic development stage and log10 transformed mean embryo dry mass. From this regression, MI was determined to be the ratio between predicted dry mass of the last developmental stage, and predicted dry mass of a recently fertilized egg. A value greater than one indicates matrotrophy, and a value less than one indicates lecithotrophy in which developing embryos utilize only what was provided in the fertilized egg, and later developmental stage embryos are smaller than early-stage embryos (Marsh-Matthews 2011).

All data manipulations and data analysis were performed using Statistics Kingdom 2017.

Results. One hundred six individuals were collected, comprising 55 females, 41 males, and 10 juveniles. Mean male SL was 19 mm with a range of 16–27 mm. Mean female size was 24 mm SL with a range of 18–35 mm. A two-tailed Welch's t-test was used to test the null hypothesis that male and female SL are not significantly different. The null hypothesis was rejected, female SL was significantly larger than male SL by the test results of $t_{(86)}$ = 9.70, p < 0.001.

Table 1 shows phenotypic and reproductive status collected in this study along with equivalent data reported by Stearns (1978) if available. A majority of females from the May collection were found to be gravid at 22 mm SL which was used as size of reproductive maturity. Forty of the females were 22 mm SL or larger, and all were dissected to determine if they were carrying developing embryos. All of the following results are presented in Table 1. For these reproductively mature females the mean SL was 26 mm. Mean maternal dry mass was 73.2 mg (SD 0.04) with a range of 28.8–196.9 mg. Thirty of these females (75%) were found to be carrying at least one developing embryo. The mean brood size was five, with a range of 1–13 (SD 3.1). Mean embryo dry mass was 1.4 mg (SD 0.26) with a range of 0.9–1.9 mg and mean brood dry mass was 7 mg (SD 4.7) with a range of 1.1–17.9 mg. Mean RA was 0.11 (SD 0.06) with a range of 0.02–0.25. Superfetation was found in seven of the 30 females carrying developing embryos, a 23% rate, and all of those seven carried two broods.

Table 2 shows the mean dry mass of embryos in the eight different developmental stages, separated into embryos from non-superfetating and superfetating mothers. Figure 1 shows mean embryo dry mass after log10 transformation regressed against embryonic development stage to determine MI. Superfetating and non-superfetating embryos were all used for this regression. Because some of the embryo dry mass values were less than one and would result in negative log values, one was added to all of the brood dry mass values before log10 transformation. The linear regression found a statistically significant relationship between development stage and individual embryo mass, $F(1,28)$ = 4.35, p =0.046, with a negative slope, y = 0.4316 - 0.009315 x. The R^2 for the regression line was 0.13. MI was calculated as 1.20/1.46 = 0.82 indicating lecithotrophy.

There were differences in reproductive output between what was found for two populations of *N. tridentiger* collected in August during the wet season (Stearns 1978) and the population collected in May during the dry season. Poeciliids have been generally found to have lower reproductive output in the dry season (Reznick and Endler 1982; Reznick et al. 1990; Reznick et al. 1997; Stallsmith 2018). One notable difference between the wet and dry season findings is that the smallest pregnant female in May was 22 mm SL, while in August the lengths at which 50% of females were found to be pregnant was 17.3 and 17.6 mm SL at the two respective sites. The fish sampled in May differed slightly from one of the August populations in several measures, but more sharply compared to the other. In the May population compared to the August populations a slightly higher percentage of mature females was found to be pregnant (75% vs. 72% and 56%) but a much lower percentage was superfetating (23% vs. 80% and 70%). Females collected in May carried fewer, larger

embryos than those collected in August with a mean number of 5 embryos compared to 5.6 and 8.1, and a mean dry mass of 1.4 mg compared to 1.3 and 0.6 mg. There was no equivalent of the calculation of mean RA of 11% found in May. Findings for the August collections only reported observations of females (Stearns 1978), so there is no basis for comparison of male size or number to the May collection.

Table 1. Life history data found in May and August in *N. tridentiger*. August data contain fewer measurements than May.

Females

	May	August (2 Sites)
Total Collected	55	
Mean Size (Range)	24 mm SL (19–35)	
Smallest Gravid	22 mm SL	
Number of Mature Females	40	
Number of Gravid Females	30	
Number Superfetating Females	7	
Mean Size (Range):		
Non-superfetating	26 (22–35) mm SL	
Superfetating	27 (25-30) mm SL	
Mature Mean Size (Range)	26 (22–35) mm SL	14 mm SL
Superfetation Rate	23%	86% or 70%
Mean Dry Mass	73.2 mg (SD 36.2)	
Mean Embryo Dry Mass	1.4 mg (SD 0.25)	
Mean Embryo Brood Dry Mass	7.0 mg (SD 4.7)	
Mean Embryo Number (Range):		
Non-superfetating	3.9 (1–8)	N/A
Superfetating	9.1 (6–13)	N/A
All	5.0 (SD 3.1)	5.6 or 8.1
"Reproductive Effort"/Reproductive Allocation, Mean (Range):		
Non-superfetating	0.08 (0.03–0.17)	
Superfetating	0.18 (0.12–0.25)	
All	0.11 (SD 0.06)	0.17 or 0.12
Males		
Total Collected	41	N/A
Mean Size (Range)	19 (16–27) mm SL	10–16 mm SL

Discussion. Direct comparison of fish collected in the dry season (May) versus rainy season (August) suggests seasonal influence in life history parameters. Pregnant females in May exhibited a lower rate of superfetation along with lower RA and carrying fewer embryos. It is however impossible to be certain of these differences given the limited observations.

Another possible influence on fecundity is elevation, with the May population found at higher elevation. Stallsmith (2018) found seasonal differences in the fecundity of three *Brachyrhaphis* species on an elevational gradient, with the lowland species breeding evenly year-round and the species at highest elevation showing little or no reproduction in the dry season. It is possible that the primary production of streams may vary more widely at higher elevations between the dry and wet seasons, influencing stream fish fecundity. Variable stream flow and discharge affect the flow of nutrients to primary producers, and thus affect the quality and quantity of food for stream fishes (Kohler et al. 2012). Seasonal variation in productivity is still poorly understood in tropical streams.

Ecological factors shape the observed differences between fecundity in dry and wet seasons. Seasonal availability of food resources may affect the observed fecundity pattern; however reported observations of seasonal variation in stream food resource avail-

Table 2. Mean embryo dry mass, mg per stage separated into non-superfetating and superfetating broods.

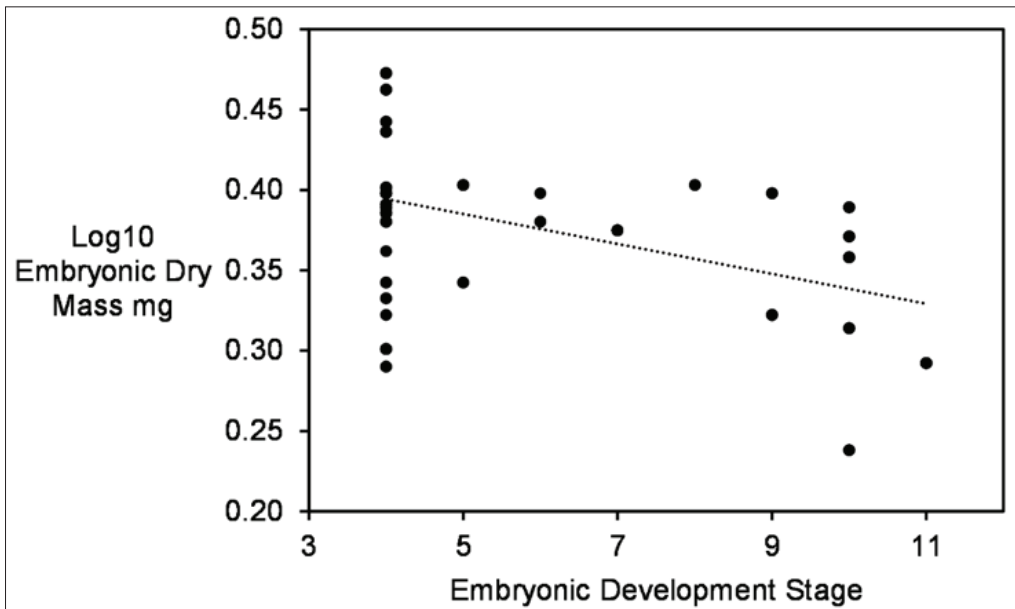|	Developmental Stage							
	4	5	6	7	8	9	10	11
Non-Superfetating (n = 80)	1.52	1.48	1.50	--	1.53	1.28	1.35	--
Superfetating (n = 65)	1.42	1.20	1.40	1.37	--	--	1.05	0.96

Figure 1. Mean dry mass of all individuals of each brood after log10 transformation regressed against embryonic development state. The negatively sloped regression line indicates lecithotrophic development in *N. tridentiger* embryos, with MI = 0.82.

ability in tropical streams have been ambiguous with complex interactions (Grether et al. 2001). Increased food availability during tropical wet seasons in some areas has been reported to support increased fecundity, as was found with *Poecilia gillii* (Kner) and *Phallicththys amates* (Miller) in Costa Rica (Winemiller 1993). Such a seasonal change in food availability may likely benefit juvenile growth and survival rather than maternal function (Winemiller 1993).

The fact that superfetation was lower in the dry season at the 700 m elevation of the collection site suggests diminished food resources as a possible influence on the potential advantage of superfetation. Superfetation has also been suggested to be an adaptation to factors such as unpredictable juvenile survival, high life expectancy of females, uncertain adult survival probabilities, or interactions between these factors (Burley 1980; Downhouser and Brown 1975; Frías-Alvarez and Zúñiga-Vega 2015; Travis et al. 1987). It was found that superfetation in *Poeciliopsis turrubarensis* (Meek) was more common in mountainous inland streams than along the coast, which was attributed to superfetation allowing a more fusiform female shape in faster mountain streams than in slower coastal streams (Zúñiga-Vega et al. 2007). Such a relationship may exist in *N. tridentiger* but would require finding and testing populations from different environments using the methods of Zúñiga-Vega et al. (2007).

For *N. tridentiger,* the survival of all life stages may be diminished in the wet season by increased predation as predators reproduce and need more food, even as food resources become more available to both adults and juveniles of *N. tridentiger*. This is an untested hypothesis that should be investigated. For a population of a superfetating species in the dry season, the better strategy may be to reduce superfetation and produce fewer, larger young at longer intervals.

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