Reproductive differences between three species of Brachyrhaphis livebearing fishes in Panamá

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ABSTRACT

Both theory and the published literature support the importance of demographic selection for differences in life history traits between populations as well as between related species. Much attention in this area has been paid to species in the livebearing fish family Poeciliidae. I studied populations of three species of poeciliid fishes of the genus Brachyrhaphis (episcopi, roswithae, terrabensis) found from low to high elevation in western and central Panamá for reproductive life history phenotypes. To determine how reproductive life history phenotypic characters subject to both selection and environmental influence may vary between these species, field collections were made at the end of the wet and dry seasons over several years. Work with other poeciliid species suggested that these three stream species, found in different environments largely defined along an elevational gradient, may have evolved different responses in their reproductive timing and effort in response to differences in abiotic and biotic environmental factors. Differences were found between female wet and dry season reproductive output in all three species. Brachyrhaphis episcopi and B. roswithae showed similar responses to seasonal changes, with larger reproductive allotment (RA) and brood size but smaller embryo size in the wet season and a higher percentage of gravid females found in the wet season compared to the dry, 86% to 49% for B. episcopi and 71% to 45% for B. roswithae. Female B. terrabensis showed different responses to seasonal changes. RA, brood size and embryo size were all larger in the wet season than in the dry season. The percentage of gravid females among those of reproductive size was 37% in the wet season compared to 10% in the dry season. Patterns of male size varied between the three species, with more muted seasonal differences than was found with females of all three species. Brachyrhaphis terrabensis males showed a much wider size range, possibly indicative of two male reproductive strategies in the species. There is a gradient in several measures from lowland to highland species such as highland reproduction apparently stopping entirely in the dry season.

RESUMEN

Tanto la teoría como la literatura publicada apoyan la importancia de la selección demográfica sobre las diferencias en los rasgos de historia de vida entre poblaciones, así como entre especies relacionadas. Estudie los fenotipos de historia de vida reproductiva de las poblaciones de tres especies de peces del género poeciliido Brachyrhaphis (episcopi, roswithae, terrabensis) que se encuentran de baja a alta elevación en el oeste y centro de Panamá. Para determinar que los caracteres fenotípicos de historia de vida reproductiva, los cuales están sujetos tanto a la selección como a la influencia del medio ambiente, puede variar entre estas especies, recolectaron muestras al final de las estaciones secas y húmedas de estas zonas durante varios años. Mi hipótesis es que estas tres especies de río angosto, que se encuentran en ambientes ligeramente diferentes en gran medida definidos a lo largo de un gradiente altitudinal, han desarrollado diferentes mecanismos en su tiempo y esfuerzo reproductivo en respuesta a diferentes factores ambientales abióticos y bióticos. Se encontraron diferencias significativas entre la capacidad reproductiva de las hembras de las tres especies tanto en la estación húmeda como en la estación seca. Brachyrhaphis episcopi y B. roswithae mostraron respuestas similares a los cambios estacionales, con mayor asignación reproductiva (AR) y tamaño de la camada, pero generando embriones más pequeños en la estación húmeda y mostrando un mayor porcentaje de hembras grávidas en la estación húmeda que en la estación seca. B. terrabensis hembras mostraron diferentes respuestas a los cambios estacionales. El AR, el tamaño de la camada y el tamaño del embrión fueron todos más grandes en la estación húmeda que en la estación seca. El porcentaje de hembras grávidas entre los individuos de tamaño reproductivo fue del 37% en la estación húmeda en comparación con 10% en la estación seca. Los patrones de tamaño de los machos variaron entre las tres especies, con diferencias estacionales moderadas que aquellas observadas para las hembras de las tres especies. Hay un claro gradiente en varias medidas de las especies de peces que van de las tierras bajas hasta las tierras altas.
Introduction

Both theory and the published literature support the importance of demographic selection for differences in life history traits between populations as well as between related species [1–3]. Such selection has been found in response to biotic and abiotic factors including predation, density, parasitism, and climate patterns such as seasonal precipitation. Much attention in this area has been paid to species in the livebearing fish family Poeciliidae [4–8]. The Poeciliidae are found across the tropical and subtropical Americas in a wide range of habitats from montane streams to estuaries. This habitat diversity along with small body size and short generation time makes poeciliids good subjects for life history studies. Poecilia reticulata, the guppy, is the most well studied species in the family showing great phenotypic and behavioral plasticity at the population level in response to a wide variety of biotic and abiotic factors [9]. Of particular interest to many investigators has been any difference between species and populations for size and number of offspring that may be shaped by abiotic factors such as elevation and seasonal variation. A major abiotic shaper of lotic fish habitat is flow variability which affects habitat structure, historically better studied in temperate habitats [10] but with recent work in tropical habitats [11].

Studies have shown phenotypic plasticity such as increased reproductive allotment in several Central American poeciliid species in response to varying levels of seasonal food availability in the wet season. One such study examined Alfaro cultratus, Pelichthys amates, and Poecilia gilli in Costa Rica [12]. But guppy populations in the Northern Range of Trinidad have lower fecundity and reduce their reproductive investment in the wet season of May to December [9]. The presence or absence of piscine predators also shapes reproductive differences between Costa Rican populations of Brachyrhaphis rhabdophora [6] as well as in Panamanian populations of B. episcopi [13]. A study of another genus of poeciliid fishes in Mexico, Xiphophorus, tested whether speciation was associated with abiotic niche conservatism or niche divergence with niche in their data-set primarily defined by patterns of precipitation and air temperature. The authors found that niche evolution and modern niches were most closely associated with the biogeographic barrier of the Trans-Mexican Volcanic Belt, with some clades being much more evolutionarily labile than others [14].

Panamá has been a good study site of factors affecting the reproductive biology of several poeciliid species, with the seasonal variation of well-defined dry and wet seasons shaping stream flow and food availability. Panamá is also topographically complex and offers a variety of environments within which to examine life history within a range of poeciliid species and their populations. For instance, more than half of the country is higher than 300 m in elevation. The Caribbean versant is low elevation rain forest and the Pacific versant contains the remnants of dry tropical forest [15,16].

I studied populations of three species of poeciliid fishes of the genus Brachyrhaphis (episcopi, roswithae, terrabensis) in western and central Panamá for reproductive life history phenotypes. These species are found in discrete allopatric ranges so there is no competition between them, and their phenotypes are apparently shaped by different biotic and abiotic environments. Given what appear to be different environments, the three species are likely to exhibit differences in reproductive timing, reproductive investment, and size at reproduction.

Brachyrhaphis episcopi is endemic to Panamá and has been the subject of many studies of its ecology and life history dating back to the 1930s [17,18], but no study has addressed possible reproductive life history differences between populations. Brachyrhaphis roswithae is also endemic to Panamá and was described from populations of what had previously been considered B. episcopi in El Valle de Antón in central Panamá [19]. Recent DNA-based phylogenetic work found B. roswithae to be paraphyletic, and in the authors’ opinion part of an as-yet unresolved B. episcopi species complex [20]. Based on previous observations of B. roswithae populations in El Valle de Antón I feel that they are distinct at least in that area, so I retain the name B. roswithae. Brachyrhaphis terrabensis has been studied for divergence from its sister species, B. roseni, based on living in different predation environments due to B. terrabensis living in the headwaters of stream systems where B. roseni is found in lower reaches of the same streams [8].

To determine how reproductive life history phenotypic characters subject to selection and environmental influence may vary between these species, field collections were made at the end Panamá’s wet and dry seasons in December and May, respectively, over several years. I wished to observe whether the three species of interest, all stream species found in different environments largely defined along an altitudinal gradient, have evolved different responses in their reproductive timing and effort in response to differences in both abiotic and biotic environmental factors. I looked for differences in the following parameters. Females of different species and in different seasons should vary in the percentage of gravid individuals reflecting seasonal fluctuations in food resources. Gravid is defined as the presence of embryos at stage 3 or higher in the poeciliid developmental classification of Haynes [21]. The minimum size of gravid females should also fluctuate seasonally with varying resources and predation threat. The number of offspring and offspring size may respond in varying ways to resource fluctuations, with low resource availability possibly curtailing most female production or producing a compromise such as fewer but larger offspring.
compared to high resource seasons. Reproductive allotment, the ratio of dry brood mass to dry maternal mass, can be interpreted as a summary statement of maternal condition since it would be expected to increase in times of higher resource availability. The reproductive condition of males can be measured as mean standard length, size at reproductive maturity, and the size range of adults. These male measurements should vary with environmental pressures such as resource availability and predation. Under stressful conditions such as high predation risk males would benefit by becoming reproductive at smaller sizes, foregoing possible greater reproductive success later at a larger size [6].

Materials and methods

Biological collections

Collection sites are listed in Table 1. Collections were made either in May, the end of Panamá’s dry season, or December, the end of the wet season so as to accentuate any seasonal variation. 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. My intention was to collect 40 individuals from each site’s population, as stated in my collecting permits. All collections were made from similar stream environments of clear, flowing water no more than 20 cm deep over mixed substrates of sand, gravel, cobble, and boulder. All three species were most easily caught along stream banks. Specimens were euthanized with 10% clove oil to a jar with ~250 ml of stream water held on site by adding two ml of a solution of 90% ethanol and water.

Table 1. Brachyrhaphis collection sites in 2011, 2012, 2013, and 2015 arranged by month, year, and species with site elevation.

| Species, Date | Site, Latitude, Longitude | Elevation |
|---------------|---------------------------|-----------|
| B. episcopi  | Quebrada Sardinilla N 9° 4.941’, W 79° 39.89’ | 98 m |
| May 2011     | Río Mendoza N 9° 0.527’, W 79° 29.824’ | 75 m |
| May 2011     | Río Frijolito N 9° 9.685’, W 79° 43.482’ | 75 m |
| May 2011     | Río Macho N 9° 10.921’, W 79° 49.000’ | 75 m |
| December 2013 | Río Mendoza N 9° 0.527’, W 79° 29.824’ | 75 m |
| December 2013 | Río Frijolito N 9° 9.685’, W 79° 43.482’ | 75 m |
| May 2011     | Río Mata Ahogado N 8° 37.213’, W 80° 5.845’ | 715 m |
| December 2013 | Río Mata Ahogado’ | 715 m |
| December 2013 | Quebrada Embalsadero N 8° 36.240’, W 80° 7.66’ | 590 m |
| December 2013 | Río Antón N 8° 37.233’, W 80° 6.122’ | 730 m |
| May 2015     | Río La Estancia N 8° 35.473’, W 80° 10.398’ | 527 m |
| May 2015     | Quebrada La Pita N 8° 36.733’, W 80° 12.633’ | 300 m |
| May 2015     | Quebrada Desconocido N 8° 36.328’, W 80° 8.020’ | 588 m |
| May 2015     | Río Mata Ahogado N 8° 37.213’, W 80° 5.845’ | 715 m |
| B. roswithae | May 2012 | Río Caldera, N 8° 48.241’, W 82° 27.118’ | 1265 m |
| May 2012     | Río Cascada, N 8° 42.354’, W 82° 13.956’ | 475 m |
| May 2012     | Río Chiriquito, N 8° 39.940’, W 82° 15.402’ | 544 m |
| May 2012     | Río Chiriquito, N 8° 40.060’, W 82° 14.670’ | 613 m |
| May 2012     | Río Chiriqui, N 8° 42.354’, W 82° 13.956’ | 398 m |
| May 2012     | Río La Esperanza, N 8° 35.135’, W 82° 19.774’ | 180 m |
| May 2012     | Río La Parada, N 8° 41.498’, W 82° 19.129’ | 354 m |
| December 2013 | Río Cascada, N 8° 42.354’, W 82° 13.956’ | 475 m |
| December 2013 | Río Chiriquito, N 8° 40.060’, W 82° 14.670’ | 613 m |
| December 2013 | Río Caldera, N 8° 48.241’, W 82° 27.118’ | 1265 m |

Measurement of life history characteristics

Following Johnson & Belk [6], I measured five life history traits for females in each population. These were size at maturity, brood size, size of offspring as dry mass, and reproductive allotment. I also calculated the percentage of gravid females in each population, with gravid defined as carrying stage 3 or higher embryos [21]. The standard length (SL) of the smallest gravid female was used to define size at maturity for a given population. Brood size of females was determined through dissection and removing all embryos of stage 3 or higher. Collected embryos along with the bodies of dissected females with viscera removed were dessicated in an oven at 55° for 24 h. Size of offspring was determined by dividing the dry mass of a brood by the number of embryos.

Reproductive allotment (RA) in females was calculated as the mass of dessicated embryos divided by the sum of the mass of dessicated embryos and the mother’s dessicated mass. This is different from the RA reported by Johnson & Belk [6] who simply reported dessicated embryo mass in their study of only one species, but is the same calculation used by Reznick et al. [4]. Because I studied three species who differ in size, reporting only dessicated embryo mass would inflate the value in the largest species, B. terrabensis with maximum SL of 60 mm [16] or larger (B. Stallsmith, pers. obs.) compared to 50 mm SL for both B. episcopi [22] and B. roswithae [23]. Reporting RA as a percentage of maternal mass enables direct comparison between species.

Males were determined by the presence of a well-developed gonopodium, used in male poeciliids to transfer sperm. Because poeciliid males typically stop growing at reproductive maturity, the SL of the smallest male was...
used to define size at maturity for a given population [6]. The average and range of male SL in each population was calculated as was the coefficient of variation (CV), which describes the amount of variability relative to the mean. All calculations were performed using Microsoft Excel 2010.

Results

Female life history

Strong differences were found between female wet and dry season reproductive output in all three species (Table 2). *Brachyrhaphis episcoli* and *B. roswithae* showed similar responses to seasonal changes, with larger RA and brood size but smaller embryo size in the wet season compared to the dry. The minimum size of gravid females was larger in the dry season, by a mean of 4 mm SL for *B. episcoli* and 9 mm SL for *B. roswithae*. In both species, a higher percentage of gravid females among females of reproductive size was found in the wet season compared to the dry, 86–49% for *B. episcoli* and 71–45% for *B. roswithae*.

The females of *B. terrabensis* populations showed different responses to seasonal changes compared to the other two species. RA, brood size, and embryo size were all larger in the wet season than in the dry season. The average minimum size of gravid females was larger in the wet season than in the dry season, 42 mm compared to 36 mm. The percentage of gravid females among those of reproductive size was 37% in the wet season compared to 10% in the dry season. In the dry season, no gravid females were found in the four populations from the highest elevation streams: Río Caldera, Río Chiriquicitos (El Valle), Río Chiriquicitos (Entre Ríos), and Río Cascada (Tables 1 and 2).

Male life history

Patterns of male size varied between the three species, with more muted seasonal differences than was found with females of all three species (Table 3). Male *B. episcoli* had a mean SL of 22 mm in both the wet and dry seasons, while the mean SL of the smallest males was 17 mm in the wet season and 25 mm in the dry season. Male *B. roswithae* had a mean SL of 20 mm in the wet season and 25 mm in the dry season, while the mean SL of the smallest males was 17 mm in the wet season and 22 mm in the dry season. The range of values for the CV of adult SL of both *B. episcoli* and *B. roswithae* varied little between seasons, from 0.10 to 0.19.

Table 2. Reproductive parameters for females. Results for all three species grouped by wet and dry season.

| Species     | n gravid females | Minimum size of gravid females, mm | Reproductive allotment, % | Mean number of offspring, size, mg |
|-------------|------------------|-----------------------------------|---------------------------|------------------------------------|
| *B. episcoli* |                  |                                   |                           |                                    |
| Wet 2013    | Río Mendoza      | 27 of 31 (87%)                    | 22                        | 8.4                                | 6.4 1.80                   |
| Wet 2013    | Río Friojolito   | 16 of 19 (84%)                    | 15                        | 15.1                               | 4.6 1.41                   |
| Mean        |                  | 86%                               | 19                        | 11.8                               | 5.5 1.60                   |
| Dry 2011    | Río Mendoza      | 7 of 22 (32%)                     | 29                        | 5.0                                | 4.7 2.55                   |
| Dry 2011    | Río Friojolito   | 6 of 21 (29%)                     | 19                        | 8.1                                | 3.2 2.09                   |
| Dry 2011    | Río Macho        | 6 of 12 (50%)                     | 25                        | 9.1                                | 5.2 1.96                   |
| Dry 2011    | Quebrada Sardínilla | 10 of 12 (83%)              | 20                        | 8.2                                | 5.4 1.81                   |
| Mean        |                  | 49%                               | 23                        | 7.6                                | 4.6 2.10                   |
| *B. roswithae* |                 |                                    |                           |                                    |
| Wet 2013    | Río Mata Ahogado | 9 of 10 (90%)                    | 23                        | 13.7                               | 7.7 2.62                   |
| Wet 2013    | Quebrada Embalsadero | 12 of 17 (71%)             | 28                        | 12.6                               | 19.9 2.55                  |
| Mean        |                  | 71%                               | 26                        | 16.5                               | 15.5 2.29                  |
| Dry 2011    | Río Mata Ahogado | 3 of 19 (16%)                    | 35                        | 5                                  | 4.0 2.89                   |
| Dry 2015    | Río Mata Ahogado | 7 of 13 (54%)                    | 32                        | 7                                  | 3.3 3.31                   |
| Dry 2015    | Río La Estancia  | 5 of 8 (63%)                     | 44                        | 10                                 | 13.0 3.29                  |
| Dry 2015    | Quebrada Desconocido | 9 of 13 (69%)              | 30                        | 12.3                               | 8.1 2.89                   |
| Mean        |                  | 45%                               | 34                        | 5.0                                | 4.0 1.01                   |
| *B. terrabensis* |              |                                    |                           |                                    |
| Wet 2013    | Río Caldera      | 5 of 17 (29%)                    | 47                        | 18.8                               | 26.8 4.86                  |
| Wet 2013    | Río Chiriquitcos (Valle) | 4 of 9 (44%)                  | 32                        | 14.0                               | 17.2 3.95                  |
| Mean        |                  | 37%                               | 42                        | 14.7                               | 30.0 2.53                  |
| Dry 2012    | Río Caldera      | 0 of 10 n.a.                     | n.a.                      | n.a.                               | n.a. n.a.                  |
| Dry 2012    | Río Chiriquitcos (Valle) | 0 of 14 n.a.                  | n.a.                      | n.a.                               | n.a. n.a.                  |
| Dry 2012    | Río Cascada      | 0 of 9 n.a.                      | n.a.                      | n.a.                               | n.a. n.a.                  |
| Dry 2012    | R. Chiriquitcos (Entre Ríos) | 0 of 9 n.a.              | n.a.                      | n.a.                               | n.a. n.a.                  |
| Dry 2012    | Río Parada       | 4 of 6 (67%)                     | 36                        | 5.0                                | 8.8 1.81                   |
| Dry 2012    | Río La Esperanza | 1 of 9 (11%)                    | 36                        | 6.1                                | 5.0 2.75                   |
| Mean        |                  | 10%                               | 36                        | 5.6                                | 6.9 2.28                   |
30 mm, compared to 28 mm in the dry season, and the mean SL of the smallest males was 21 mm in both seasons. In both seasons, the range of CV of SL was larger than that of either *B. episcopi* or *B. roswithae*, 0.15–0.36 in the wet season and 0.14–0.26 in the dry season.

**Discussion**

I found differences in reproductive biology between three species of *Brachyrhaphis*, specifically in seasonality, patterns of female reproductive effort, and male size at reproductive maturity. The wet season (December) is the peak breeding season for all three species, most especially for those *B. terrabensis* populations at higher elevation. The seasonal responses of females of *B. episcopi* and *B. roswithae* were similar to each other. In the wet season, females of both species reproduced at a smaller size, and produced larger broods of smaller offspring compared to the dry season. This is similar to the pattern seen in *Poecilia reticulata* between upstream and downstream populations of a stream in which downstream populations have higher food availability and females produce larger broods of smaller offspring [4, 24, 25]. Also in the wet season, in both *B. episcopi* and *B. roswithae* a majority of females of reproductive size were found to be gravid while the fraction of gravid females in both species in the dry season dropped below half. Turner [18] found *B. episcopi* to reproduce year round, and Jennions et al. [13] found a similar pattern to my findings for *B. episcopi* which they attributed both to fluctuations in predation pressure as well as food availability.

Seasonal response by female *B. terrabensis* was very stark. Three high elevation populations of Ríos Caldera, Chiriquicitos (El Valle), and Cascada in the wet season had a lower combined percentage of gravid females in the wet season, 37%, compared to the other two species. But in the dry season that percentage dropped to zero. The mean values in the wet season for reproductive allotment (15.8%), brood size (24.7), and offspring size (3.78 mg) of the three high elevation populations do not uniformly vary compared to those of *B. episcopi* and *B. roswithae*. Because reproductive allotment is reported as a percentage, it’s directly comparable, and it’s similar to the 16.5% found for *B. roswithae* and the 11.1% found for *B. episcopi*. The strongest difference between the three species is in brood size. *Brachyrhaphis terrabensis* had a mean brood size of 24.7, *B. roswithae* had 15.5 and *B. episcopi* had 5.5 Mean offspring size followed a similar pattern with *B. terrabensis* having the largest mean size followed in order by *B. roswithae* and *B. episcopi*. The general pattern in measures of wet season reproductive effort is a gradient of increasing effort from a species living at low elevation, *B. episcopi*, to *B. roswithae* at higher elevation and *B. terrabensis* living at the highest elevation. It is also apparent that for high elevation *B. terrabensis*, the wet season is the entire reproductive

### Table 3. Reproductive parameters for males. Results for all three species grouped by wet and dry season.

| Date   | Length, n males | Mean length of adults (range) | CV for adult |
|--------|-----------------|-----------------------------|-------------|
| Wet 2013 | 11              | 16 (16–27)                  | 0.19        |
| Wet 2013 | 2               | 18 (18–25)                  | n.a.        |
| Mean   | 17              | 22 (18–29)                  | 0.15        |
| Dry 2011 | 11              | 19 (19–25)                  | 0.11        |
| Dry 2011 | 10              | 20 (24–27)                  | 0.11        |
| Dry 2011 | 6               | 18 (21–26)                  | 0.19        |
| Mean   | 19              | 22 (21–28)                  | 0.13        |
| Wet 2013 | 14              | 17 (17–28)                  | 0.15        |
| Wet 2013 | 13              | 21 (21–33)                  | 0.15        |
| Wet 2013 | 13              | 14 (14–19)                  | 0.11        |
| Mean   | 17              | 20 (n.a.)                   | n.a.        |
| Dry 2011 | 1               | 24 (24–32)                  | n.a.        |
| Dry 2015 | 15              | 18 (23–27)                  | 0.10        |
| Dry 2015 | 2               | 24 (24–32)                  | n.a.        |
| Dry 2015 | 14              | 21 (25–30)                  | 0.13        |
| Dry 2015 | 2               | 25 (25–n.a.)                | n.a.        |
| Mean   | 22              | 25 (n.a.)                   | n.a.        |
| Wet 2013 | 9               | 24 (24–42)                  | 0.15        |
| Wet 2013 | 9               | 20 (31–47)                  | 0.32        |
| Wet 2013 | 4               | 18 (24–37)                  | 0.36        |
| Mean   | 21              | 30 (25–42)                  | 0.14        |
| Dry 2012 | 11              | 26 (23–33)                  | 0.15        |
| Dry 2012 | 13              | 20 (30–40)                  | 0.24        |
| Dry 2012 | 6               | 18 (25–32)                  | 0.26        |
| Dry 2012 | 2               | 22 (28–33)                  | n.a.        |
| Dry 2012 | 0               | n.a.                       | n.a.        |
| Mean   | 21              | 28 (n.a.)                   | n.a.        |
season. It is impossible to directly compare the influence of season on specific measures of reproductive effort in high elevation *B. terrabensis* females because of the total lack of gravid females in the dry season.

What ecological factors shaped the observed gradient of differences between these three species? Higher elevation tropical streams typically have lower predation, so with that reduced pressure it seems plausible that reproduction would be relatively equal year round. But that is very different from what was observed. Would available food resources somehow shape the observed reproduction pattern? Reported observations of seasonal variation in stream food resource availability have been ambiguous. Availability has been reported to increase during tropical wet seasons in some areas and to support increased fecundity, as was found with *Poecilia gilli* and *Phallicthys amates* in Costa Rica [12]. Such a seasonal change in stream productivity may likely benefit juvenile growth and survival rather than maternal function [12]. But *Poecilia reticulata* populations in the Northern Range of Trinidad have lower fecundity in the same autumnal wet season for unknown reasons [9]. Grether et al. [26] suggest that *P. reticulata* in Trinidad has evolved different life history traits between upstream and downstream populations in response to differential food availability independent of predation pressures. Such variations in food availability between highland and lowland *Brachyrhaphis* populations may well drive both the evolution of life history traits and speciation.

Work by Kohler et al. [11] in six streams in the Northern Range of Trinidad focused on the abundance and quality of epilithon in response to variable stream flow rate. Dry season flow rates are more stable allowing the production of higher quality epilithon. Epilithon is a biofilm on stream substrate surfaces, composed of algae, bacteria, fungi, and detritus, and is an important food resource for *P. reticulata*. Seasonal shifts in the quality of epilithon may induce habitat shifts in *P. reticulata*, from pools to riffles with higher quality epilithon. This spatial and temporal variation in epilithon quality is likely key to understanding how bottom-up processes can influence food quality in tropical streams as well as responses by vertebrates such as stream fishes. To my knowledge, there has never been any research on the importance of epilithon to *Brachyrhaphis* species, all considered to be insectivorous. Even if *Brachyrhaphis* species don’t feed directly on epilithon it is likely to influence the availability of preferred food such as insects. Stream scour from flood events in the wet season was observed to reduce epilithon in Trinidad, which is likely to be the case in Panamá. Given the observed pattern of increased wet season fecundity in the three *Brachyrhaphis* species in this study, it may well be the case that young are preferentially produced at a time just before stream productivity increases. Higher stream flow in the late wet season may also help juveniles escape predation through a dilution effect. This differs from the observations of Winemiller [12] that food availability for poeciliid species was higher during the wet season in a low lying area of Costa Rica The likely root of all of these poorly understood responses to seasonal variation is our poor understanding of how landscape influences the ecology of streams. Exactly how water moves across a landscape shapes plant and animal communities in a stream, and thus local productivity [10].

Male size patterns in *B. episcopi* and *B. roswithae* were more similar to each other than to *B. terrabensis*. In streams tributary to the Panamá Canal, *B. episcopi* males were observed in the size range 15–36 mm SL in monthly collections over a year by Turner [18], while Jennions et al. [13] in the same area found males in the size range 14–31 mm SL in collections made in both the wet and dry seasons. This compares to a size range of 16–29 mm SL over both seasons found in this study. Jennions et al. [13] also reported CV values of 0.09–0.16 for their collections compared to 0.11–0.19 in this study. The only comparable data for *B. roswithae* come from Jennions et al. [13], who as I did collected fish from Río Mata Ahogado and Río Antón in El Valle de Antón but called the fish *B. episcopi* rather than *B. roswithae*, as done in this study. Males in those collections were 20–31 mm SL with a CV range of 0.07–0.11. My size range for a larger number of *B. roswithae* populations across both seasons was 14–32 mm SL with a CV range of 0.10–0.15. Male size range and CV in these two species are comparable to that found for *B. rhabdophora* in Costa Rica [5,6]. By contrast, male size patterns in *B. terrabensis* are characterized by a wider range with the largest males often more than twice the size of the smallest, and larger CV values ranging from 0.14–0.36. One possible explanation for this greater size range in *B. terrabensis* is well-defined different male reproductive strategies. Smaller males may function as less successful ‘sneakers,’ maturing at a younger age to have an earlier start to their reproductive lives. Males that mature later at larger sizes may use a reproductive strategy based on display to attract females. Poeciliid females are often attracted to larger males (for *P. reticulata*, see [27–29]; but see [30]).

Differences in predation pressure have been observed to shape life history parameters differentially between different populations of *Poecilia reticulata* in Trinidad [4,24,25] and in *B. episcopi* [13]. I did not test for the possible influence of variable predation pressures between and within the three species I observed. What I observed while collecting fish was that *B. terrabensis* was either the only fish species present (Río Caldera) or coexisted with the small cichlid *Aequidens pulcher* that is unlikely to be a threat to adults (other collection sites). *Brachyrhaphis roswithae* sites were also observed to have *A. pulcher* present, while one site also had the small catfish *Trichomycterus striatus* (Río Antón). *Brachyrhaphis episcopi* sites were often observed to contain larger
predatory species such as the characiform Hoplias malarbaricus especially in the wet season with elevated stream flow. My experience was that B. episcopi were typically found in very shallow water along the bank, or in small tributaries just off of the main stream, both areas free of large predatory fish. The two Brachyrhaphis species at higher elevations are likely to have less predatory pressure from other species of fish than B. episcopi.

The factors shaping the reproductive differences I found between the three Brachyrhaphis species remain to be fully explained. Brachyrhaphis episcopi and B. roswithae were similar but possibly not identical in observed life history traits; sample sizes for many collection sites in this study are too small to allow robust statistical analysis. Larger collections in the future would allow well-supported testing for life history differences between B. episcopi and B. roswithae. There is a gradient in several measures from lowland to highland species including highland reproduction apparently stopping entirely in the dry season, highland males showing more variance in size, and likely a reduction in predation pressure at highland sites [6,8].

The central question is how the interaction of many factors shapes seasonal differences in tropical stream productivity and why these differences may vary between apparently similar streams regionally. To my knowledge, no such investigation has been done into stream productivity in Panamá. Such an investigation would likely strengthen our understanding of the observed differences between the three Brachyrhaphis species.

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