

**ARTICULO ORIGINAL**

**PARTIAL TROPHIC SEGREGATION IN CO-OCCURRING GAMBUSIA SPECIES (CYPRINODONTIFORMES: POECILIIDAE) IN A NATURAL WETLAND OF CUBA**

*Segregación trófica parcial entre especies de Gambusia (Cyprinodontiformes: Poeciliidae) en un humedal natural de Cuba*

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**ABSTRACT**

*Gambusia punctata* and *Gambusia puncticulata* show a distinctive distribution pattern in Cuba: *G. punctata* dominates mountain streams, whereas *G. puncticulata* is more abundant in lowland wetlands. Nevertheless, there are some transition zones along few freshwater basins in which these species co-exist in similar abundance. Recent studies in wild populations revealed similar use of food resources for both *G. punctata* and *G. puncticulata*. However, little is known about possible evolutionary mechanisms in the feeding strategies of these species allowing them to co-exist. We assessed the diet composition, feeding strategy, niche width, and dietary overlap between species and between sexes of these two *Gambusia* species in a natural wetland of Cuba. Our results showed partial segregation in the consumption of available resources between *G. punctata* and *G. puncticulata*. Such incomplete trophic niche segregation may be facilitated by their generalist trophic niche and their specific feeding strategies. *G. punctata* showed a generalist strategy, given that several individuals shared a high number of food items. Whereas, *G. puncticulata* showed a specialist strategy explained by the variation amongst individual feeding on different food items. The wide spectra of consumed food items could explain the low trophic segregation found between sexes of both species. Sex-specific reproductive roles known for this fish family, seem not to induce differences in the diet between male and female individuals of *G. punctata* and *G. puncticulata*.

**KEY WORDS:** dietary overlap, feeding strategy, resource partitioning, stomach content, trophic niche width.

**RESUMEN**

*Gambusia punctata* y *Gambusia puncticulata* muestran un patrón de distribución muy característico: *G. punctata* domina arroyos de

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montaña, mientras que *G. puncticulata* es más abundante en humedales de zonas bajas. No obstante, hay algunas zonas de transición a lo largo de las cuencas de agua dulce en las cuales estas especies coexisten en similar abundancia. Estudios recientes en poblaciones naturales revelaron un uso similar de los recursos por *G. punctata* y *G. puncticulata*. Sin embargo, poco se sabe sobre los mecanismos evolutivos que permiten la coexistencia de estas especies. En este estudio, evaluamos la composición de la dieta, la estrategia alimentaria, la amplitud de nicho trófico y la superposición alimentaria entre especies y entre sexos de estos poeciliidos en un humedal natural de Cuba. Nuestros resultados mostraron una segregación parcial en el consumo de los recursos disponibles entre *G. punctata* y *G. puncticulata*. Dicha segregación trófica incompleta puede estar facilitada por sus nichos tróficos generalistas y por sus particulares estrategias alimentarias. *G. punctata* presentó una estrategia generalista, debido a la presencia de elementos compartidos entre un alto número de individuos. Mientras que *G. puncticulata* presentó una estrategia especializada reflejada en las variaciones entre individuos especializados en uno o pocos ítems alimentarios. El amplio espectro de consumo de alimentos pudiera explicar el bajo nivel de segregación trófica encontrada entre los sexos de ambas especies. Las diferencias de roles de cada sexo durante la reproducción de estas especies parecen no contribuir a diferencias en la dieta entre machos y hembras.

**PALABRAS CLAVE:** amplitud del nicho trófico, contenido estomacal, estrategia alimentaria, distribución de los recursos, superposición trófica.

## INTRODUCTION

Niche segregation is an evolutionary mechanism that allows avoiding and/or minimizing competition among co-occurring fish species (Moyle and Cech, 1996; Corrêa *et al.*, 2009). This mechanism gains special significance when two ecologically equivalent species from distinct ecosystems, co-occur in transitional or connecting habitats having to

compete for the available resources. However, trophic niche segregation among species and between sexes seems to play a minor role contributing to the coexistence of some poeciliid species, particularly among detritivores (Tobler, 2008; Scharnweber *et al.*, 2011a, b). But the evolutionary significance of such mechanism allowing the coexistence of polyphagous or carnivorous species has not been tested. Thus, gathering new information about diet and trophic relationships of potentially competing species may help to better understand underlying trophic mechanisms of coexistence.

In Cuba, the family Poeciliidae is represented with 12 species, 10 of which (83%) are endemic (Lara, *et al.*, 2010; Ponce de León & Rodríguez, 2010). *Gambusia punctata* and *Gambusia puncticulata* are the most common poeciliid species in Western Cuba. These two species show a distinctive distribution pattern: *G. punctata* dominates mountain streams, whereas *G. puncticulata* is more abundant in lowland wetlands (Ponce de León & Rodríguez, 2010). Nevertheless, there are some transition zones along few freshwater basins connecting northern mountain ranges with the southern swamps, in which these species coexist in similar abundance. Recent studies in wild populations revealed similar use of food resources for both *G. punctata* and *G. puncticulata* (Fong, *et al.*, 1996; Ponce de León & Rodríguez, 2013; S. Rodríguez-Machado & J. L. Ponce de León, unpublished). However, little is known about the diet, trophic relationships, or the mechanisms to avoid competition of these polyphagous species when co-occurring in the same habitat.

Here we investigated some aspects of the trophic ecology of co-occurring populations of *G. punctata* and *G. puncticulata* in

a natural wetland of Cuba. First, we expected that these two species would show different feeding strategies and/or use different food resources given that both are predatory species adapted to different habitats (Ponce de León & Rodríguez, 2010). *G. puncticulata* is highly abundant in more productive and biotope-variable habitats such as swamps and river mouths while *G. punctata* is the dominant species in mountain streams (Ponce de León, 2012; Ponce de León & Rodríguez, 2013). Secondly, we expected that sexual dimorphism would affect the type and amount of food items ingested by each sex within both species. These assumptions are based on a number of sex-specific traits found in Poeciliids: 1- males are smaller (Rosen & Bailey, 1963; Ponce de León & Rodríguez, 2010); 2- males show lower feeding activity compared to females, given that males spend more time harassing females and seeking copulation (Köhler *et al.*, 2011); and 3- female poeciliids need a higher energetic budget for reproduction than males given that egg formation as well as embryonic nourishment and development, are female-exclusive processes (Wourms, *et al.*, 1988; Meffe & Snelson, 1989).

## MATERIALS AND METHODS

### Study area and sampling

Fish were collected in July, 2013 at Guanímar channel (22°41'40.3" N, -82°39'05.7" W), a freshwater marsh located in the southern coast of Artemisa province, Cuba. Aquatic vegetation was dominated by *Nymphaea odorata*, *Eichornia crassipes* and *Thypha dominguensis*, whereas the banks were dominated mostly by grasses (Poaceae and Cyperaceae) and 5-7m tall *Casuarina equisetifolia* trees. Native fish fauna was composed by *Gambusia punctata*,

*Gambusia puncticulata*, *Limia vittata*, *Girardinus falcatus*, *Girardinus metalliscus*, *Alepidomus evermanni*, *Cubanichthys cubensis*, *Rivulus cylindraceus*, *Nandopsis tetracanthus* and the introduced species *Clarias gariepinus*, *Oreochromis aureus* and *Betta splendens*. Fish collection and identification were done from the banks or using nylon seines and hand nets.

We collected 20 adult individuals of both *G. punctata* and *G. puncticulata* (ten females and ten males of each species). All fish samplings were done between 11:00 and 14:00 hours, to guarantee the fullness of the digestive tract (see Pyke, 2005 for a review). In the field, every specimen was anaesthetized using MS 222 (Tricaine Methanesulphonate) prior to fixation in 70% ethanol to avoid regurgitation and losing of stomach content (Mansfield & Mcardle, 1998).

### Stomach content analyses

Individual fish were measured for total length (TL: the length between the snout and the distal extreme of the caudal fin), and once dissected total gastrointestinal tract length (GL), stomach length (SL) and intestine length (IL) were measured to the nearest 0.01 mm using a Vernier caliper. We considered as stomach the portion of digestive tube from the esophagus to where the tube bends ventrally (Daniels & Felley, 1992) and the intestine, from the end of the stomach to the anus. Each individual's stomach content was extracted, dried on towel paper during one minute at room temperature and weighed using an analytical balance (Startorius BS 124S) to the nearest 0.001 g. Food items were analyzed using a stereoscopic microscope 40x. Orders and families of insects were identified following Borror and DeLong (1970)

criteria. Non-insect invertebrates were classified to orders following Ruppert *et al.* (2000).

To analyze stomach contents, we followed the methodology proposed by Lima-Junior and Goitein (2001), which is an alternative of Hynes (1950) and Hyslop (1980) Points Methods. Hence, it is included Frequency of occurrence ( $F_i = 100n_i/n$ ) and Volumetric Analysis Index ( $V_i = 25 M_i; [M_i = \Sigma_i / n]$ ), where  $n_i$ : number of digestive tracts in which the  $i$  item is found;  $n$ : total number of digestive tracts with food in the sample;  $M_i$ : mean of the ascribed points for the  $i$  food item;  $\Sigma_i$ : sum of the ascribed points for the  $i$  food item; 25: multiplication constant to obtain a percentage. These two values lead to the calculation of the Importance Index  $AI_i = F_i * V_i$ , for each food item. We choose this method of stomach content analysis considering the suitability of volumetric methods to compare diet of polyphagous species (Mansfield & Mcardle, 1998).

### Feeding strategy

For feeding strategy diagrams construction, we followed the Costello's modified method (Amundsen *et al.*, 1996). This method gives the possibility to make an approach to the feeding strategy of analyzed taxa as well as the importance of a particular food item and its contribution to the niche width. In these two-dimension diagrams, each point represents the frequency of occurrence ( $F_i$ ) and the specific abundance of every food item,  $\%P_i = (\Sigma S_i / \Sigma St_i) * 100$ , where  $\Sigma S_i$  is the sum of the ascribed points for item  $i$ , and  $\Sigma St_i$  is the total stomach content in only those individuals with item  $i$  in their stomach. The software STATISTICA 7.0 (StatSoft Inc., Tulsa, OK, USA, 2004) was used for diagrams construction.

The feeding strategy diagrams allow qualitative analyses of individual contribution to total population trophic niche. That is, items with high specific abundance and low frequency (upper left corner) indicate high individual specialization, or a high within-individual contribution, whereas food items with low specific abundance and moderate to high frequency (lower right corner) suggest low dietary variation among individuals and thus more intra-population generalization.

### Trophic niche width

For statistical procedures, measurements of dietary niche breadth of each specimen were calculated using the inverse of Simpson's (1949) diversity measure  $\beta = 1/\Sigma p_i^2$ , where  $p$  is the proportional utilization of each trophic resource  $i$  (Levins, 1968). However, as niche breadth values are indicative of specific feeding strategies, with lower values suggesting more specialized feeding strategies and *vice-versa*, we used the Levins Standardized Niche Breadth  $\beta' = \beta - 1/n - 1$ , where  $\beta$  is the inverse of Simpson's (1949) diversity measure and  $n$  is the total number of items, to obtain values between 0 and 1 for the calculation of niche breadth of species overall and sexes (Hurlbert, 1978). In this case, values close to zero correspond to narrower niches and specialized diets whereas values close to 1 indicate wider niches and more generalized diets.

### Dietary overlap

To calculate dietary overlap between species and sexes we used Pianka's measure of overlap (1973),

$$O_{jk} = \Sigma_i^n p_{ij} p_{ik} / \left( \sqrt{\Sigma_i^n p_{ij}^2 \Sigma_i^n p_{ik}^2} \right)^{-1}$$

where  $O_{jk}$ : Pianka's measure of niche

overlap between species  $j$  and species  $k$ ,  $p_{ij}$ : proportion resource  $i$  of the total resources used by species  $j$ ,  $p_{ik}$ : proportion resource  $i$  of the total resources used by species  $k$ ,  $n$ : total number of food items.

To classify the degree of feeding similarity between both species and sexes, we followed the criterion of Hyslop (1980), which defines four categories: low (0 – 24%), moderate (25 – 49%), high (50 – 74%) and very high similarity (75 – 100%). As food items in our study are considered discrete variables (Smith, 1982), we made a Principal Component Analysis (PCA) to obtain a graphic representation of the contribution of each trophic item to the total variance and accordingly, to illustrate the trophic overlap of sexes by species.

### Statistical analysis

We performed two types of statistical analyses using permutational approaches with PRIMER v 6.1.15., PERMANOVA+ v 1.0.5 statistical package (Anderson *et al.*, 2008). Firstly, we made two separate multivariate analyses of variance testing the null hypothesis of no significant qualitative and quantitative differences in stomach composition (dependent variables), between species and between sexes. The qualitative analysis was based on the type of trophic resource ingested, while the quantitative analysis was based on the proportion of each resource in each stomach. Secondly, we made three univariate analyses of variance testing the null hypothesis of no significant differences in 1- trophic niche breadth between species and sexes and 2- stomach length and 3- intestine length between species and sexes as additional morphological indicators of trophic niche partitioning. These types of analyses are proven to be appropriate for

studies on trophic niche strategies of poeciliids (Scharnweber *et al.*, 2011a, b) and other fishes (Smith, *et al.*, 2011; Carniatto, *et al.*, 2016).

The above analyses were performed using a design of two factors: Factor Species (2 levels, fixed) and Factor Sex (2 levels, fixed), based on Bray-Curtis similarity (calculated from presence-absence transformed data for the qualitative analysis and from four root-transformed data for the remaining analyses) and with 9999 permutations of residuals under a reduced model, as suggested by Anderson *et al.* (2008). We used TL as covariate in all analyses given that stomach content and stomach size vary with fish size.

### RESULTS

From a qualitative point of view, 57% of food resources were present exclusively in the stomachs of one of the two species (Table 1). Although both species consumed terrestrial invertebrates, there was a clear differentiation in the composition of resources used by the two species. *G. punctata* showed a high preference by terrestrial invertebrates while *G. puncticulata* additionally consumed a variety of aquatic invertebrates not found in *G. punctata* stomachs.

The highest diversity of consumed resources by *G. puncticulata* resulted in significant statistical differences in stomach content between species (Table 2). Also, there were significant differences in the type of trophic items by TL and in the interaction of the latter and the factor Species. On the contrary, there were no significant differences using “Sex” as main factor. Main effects (“TL”, “Species” and “Sex”) did not show significant differences comparing the volumetric proportion of

**Table 1.** Frequency of occurrence ( $F_i$ ), Volumetric Index ( $V_i$ ) and Importance Index ( $AI_i$ ) of trophic resources of females and males of *Gambusia punctata* and *Gambusia puncticulata*.

Trophic Resources	<i>Gambusia punctata</i>						<i>Gambusia puncticulata</i>					
	Females			Males			Females			Males		
	$F_i$	$V_i$	$AI_i$	$F_i$	$V_i$	$AI_i$	$F_i$	$V_i$	$AI_i$	$F_i$	$V_i$	$AI_i$
Ephemeroptera (L) <sup>a</sup>							10	2.5	25			
Chironomidae (L) <sup>a</sup>										10	5	50
Chironomidae (P) <sup>a</sup>							10	2.5	25			
Trichoptera (L) <sup>a</sup>							10	7.5	75			
Acari <sup>a</sup>							20	3.75	75			
Odonata (L) <sup>a</sup>	30	18.8	562.5							10	1.25	12.5
Hemiptera <sup>a</sup>							40	13.8	550	10	7.5	75
Ephemeroptera (A) <sup>t</sup>	10	3.75	37.5	20	10	200	10	8.75	88			
Hymenoptera <sup>t</sup>	10	3.75	37.5	10								
Formicidae <sup>t</sup>	70	31.3	2188	50	32.5	1625	40	17.5	700	10	15	150
Coleoptera <sup>t</sup>	20	1.25	25	10	3.75	37.5	20	10	200			
Dipteran parts				60	32.5	1950	20	12.5	250			
Chironomidae (A) <sup>t</sup>										30	21.3	638
Psocoptera <sup>t</sup>	10	15	150									
Araneae <sup>t</sup>				10	3.75	37.5						
Vegetable matter	20	7.5	150	20	3.75	75	20	8.75	175	40	22.5	900
Detritus										10	8.75	87.5
Mollusca <sup>a</sup>										10	2.5	25
Insect parts	40	18.8	750	40	23.8	950	30	12.5	375	10	3.75	37.5
Fish fry	10	3.75	37.5							10	2.5	25
Scales	50						70			20		

L: larvae, P: pupae, A: adult. a: aquatic invertebrates, t: terrestrial invertebrates

stomach contents (quantitative analysis). Only the interaction “TL x Species” reflected a significant effect (Table 3), with the largest species (*G. punctata*) having the highest total proportion of dietary items (see Table I).

Overall, both *Gambusia* species showed generalized feeding strategies. However, the way in which resources were used was different between the two of them. *Gambusia punctata* showed a more mixed feeding strategy, with varying degrees of

generalization and individual specialization on different dietary items (Fig. 1A). For example, prey types as Formicidae (ants), were found in high abundance in most of the individuals, which indicates a generalized use of this item in the species diet. On the other hand, Psocoptera and Odonata were found only in a low number of individuals and they were the only or the most important items in those individual's diet, indicating individual specialization in particular food items. However,

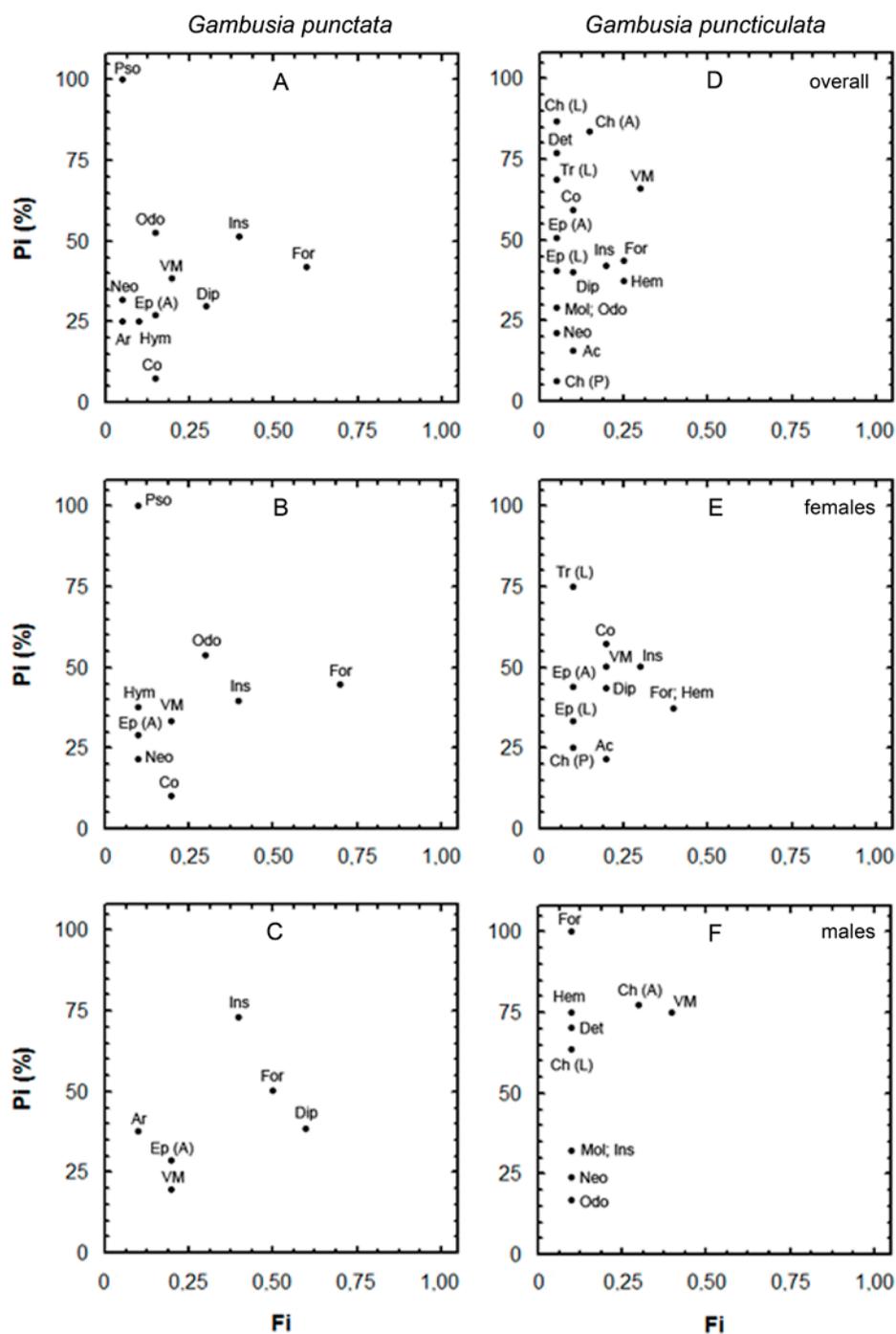


Fig. 1. Feeding strategy plots of *Gambusia punctata* overall (A), females (B) and males (C), and *Gambusia puncticulata* overall (D), females (E) and males (F). Fi: Frequency of occurrence, Pi: Specific abundance of each food item. Ep (L): Ephemeroptera (L), Ch (L): Chironomidae (L), Ch (P): Chironomidae (P), Tr (L): Trichoptera (L), Ac: Acari, Mol: Mollusca, Ep (A): Ephemeroptera (A), Hym: Hymenoptera, For: Formicidae, Co: Coleoptera, Dip: Dipteran parts, Ch (A): Chironomidae (A), Hem: Hemiptera, Odo: Odonata, Pso: Psocoptera, Ar: Araneae, Ins: Insect parts, VM: Vegetable matter, Det: Detritus, FF: Fish fry.

**Table 2.** Results of permutational MANCOVA testing for qualitative differences in stomach content between species (*Gambusia punctata* and *Gambusia puncticulata*) and between sexes. Values in bold are significant at  $\alpha = 0.05$ .

Source	df	SS	Pseudo-F	P (perm)
TL	1	3574.9	2.4722	<b>0.0332</b>
Species	1	3870.1	2.6763	<b>0.0232</b>
Sex	1	2262	1.5643	0.1705
TL x Species	1	4605.9	3.1852	<b>0.0068</b>
TL x Sex	1	1500.6	1.0377	0.4127
Species x Sex	1	2257.7	1.5613	0.1708

TL: total length

*Gambusia puncticulata* showed a generalized trophic niche with most members of the population specialized on different resources. That is. Each food item was consumed by a small fraction of the population, indicating a strong individual specialization (Fig. 1D). The overall species strategies matched well with the strategies of both males and females in both *G. punctata* (Figs. 1A- 1C) and *G. puncticulata* (Figs. 1D- 1F).

Both species showed relatively wide overall trophic niche breadth. However, niche breadth of *G. punctata* ( $\beta' = 0.73$ ) was slightly narrower than that of *G.*

**Table 3.** Results of permutational MANCOVA testing for quantitative differences in stomach content between species (*Gambusia punctata* and *Gambusia puncticulata*) and between sexes. Values in bold are significant at  $\alpha = 0.05$ .

Source	df	SS	Pseudo-F	P (perm)
TL	1	4849.3	1.8153	0.1012
Species	1	5056.9	1.8930	0.0892
Sex	1	2839.8	1.0630	0.3768
TL x Species	1	7021.7	2.6285	<b>0.0236</b>
TL x Sex	1	2576.8	0.9646	0.4563
Species x Sex	1	4250.3	1.5911	0.1491

TL: total length

**Table 4.** Results of permutational ANCOVA testing for differences in niche width between species (*Gambusia punctata* and *Gambusia puncticulata*) and between sexes. Values in bold are significant at  $\alpha = 0.05$ .

Source	df	SS	Pseudo-F	P (perm)
TL	1	7.8291	1.3729	0.2512
Species	1	4.0355	0.7076	0.4114
Sex	1	16.079	2.8196	0.1057
TL x Species	1	1.8102	0.3174	0.5763
TL x Sex	1	8.2109	1.4399	0.2342
Species x Sex	1	1.4801	0.2595	0.6109

TL: total length

*puncticulata* ( $\beta' = 0.79$ ). This pattern was also found when analyzing sexes separately, *G. punctata* females ( $\beta' = 0.72$ ) and males ( $\beta' = 0.70$ ) showed narrower niche breadth than *G. puncticulata* females ( $\beta' = 0.83$ ) and males ( $\beta' = 0.79$ ). In both species, females showed wider niche breadth than males. Despite some differences in those values, there were no significant effects of “Species” or “Sex” as main effects when comparing niche breadth (Table 4).

Overall, we found a moderate similarity (34%) in diet composition between *G. punctata* and *G. puncticulata*. This was also found when comparing intra-specific dietary overlap between the sexes of *G. punctata* (40%) and *G. puncticulata* (36%). Also, sex-specific comparisons between species showed moderate similarity in the composition of stomach content for males (31%) and females (34%). Convex hulls from PCA plot based on the volumetric proportion of all food items showed a similar pattern of diet overlap between sexes (Fig. 2). This analysis also showed that insect parts and vegetable matter were the items that contributed the most to the variability of the first principal component.

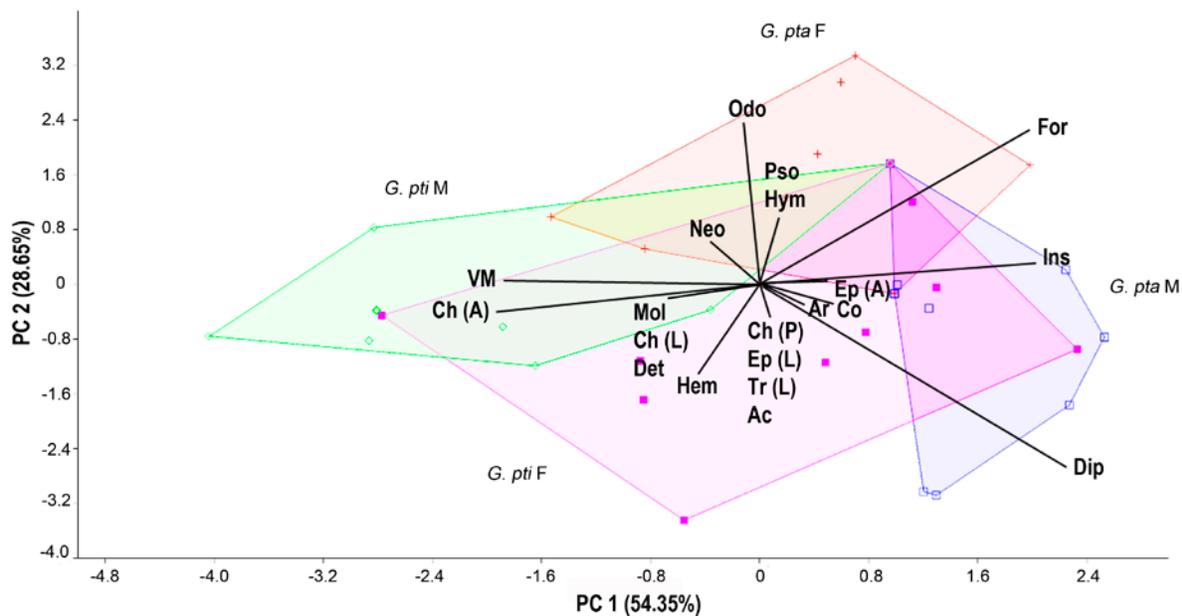


Fig. 2. Principal component analysis based on volumetric proportion of food items. The percentage of explained variance by the two first PCs: 83 %. Polygons represent species and sexes. *G. pta*: *Gambusia punctata*, *G. pti*: *G. puncticulata*, F: females, M: males. Abbreviations as in Fig. 1.

As expected, TL was a good predictor of differences in the length of the gastrointestinal tract and its sections (Table 5). We found significant differences in total GL between species: the largest species (*G. punctata*) showed largest GL. In the comparisons between females and males, there was a significant effect of “Sex” as main factor: females of both species had larger GL than males. When we compared SL, we found significant differences between sexes, as well in the interaction “TL x Species” as factors. However, we did not find significant effects of “Species” or “Sex” as main factors when comparing IL.

## DISCUSSION

Our results showed partial trophic segregation between *G. punctata* and *G. puncticulata* at Guanímar channel. Although having generalist niches and forage on several items in common, both species

consumed a high proportion of exclusive food items. Thus, certain resource partitioning was present at both inter and intra-specific levels, but not very marked between sexes.

Invertebrates were the most important food resources for both species. This finding matches with those of Fong *et al.* (1996), Fong and Garcés (1997), Rodríguez-Machado (2012) and Ponce de León and Rodríguez (2013) for these species in other Cuban ecosystems. Likewise, Gluckman and Hartney (2000) and Pyke (2005) described similar diet for *Gambusia* species from the Bahamas and other regions of the world, respectively. In fact, they found that invertebrates are the main components in the diet of *Gambusia* fishes. On the other hand, the inclusion of vegetable matter in the diet of both species confirmed their polyphagous behavior and it partially

**Table 5.** Results of permutational ANCOVA testing for differences in total gastrointestinal tract length, and stomach and intestine length between species (*Gambusia punctata* and *Gambusia puncticulata*) and between sexes. Values in bold are significant at  $\alpha = 0.05$ .

Source	df	SS	Pseudo-F	P (perm)
<i>Gastrointestinal tract length</i>				
TL	1	252.65	90.970	<b>0.0001</b>
Species	1	12.783	4.6024	<b>0.0385</b>
Sex	1	102.91	37.054	<b>0.0001</b>
TL x Species	1	11.559	4.1620	0.0531
TL x Sex	1	0.6950	0.2502	0.6187
Species x Sex	1	1.7988	0.6476	0.4243
<i>Stomach length</i>				
TL	1	224.48	68.526	<b>0.0001</b>
Species	1	13.211	4.0328	0.0518
Sex	1	128.43	39.205	<b>0.0001</b>
TL x Species	1	23.209	7.0848	<b>0.0137</b>
TL x Sex	1	1.2457	0.3802	0.5309
Species x Sex	1	1.2614	0.3850	0.5355
<i>Intestine length</i>				
TL	1	230.02	34.125	<b>0.0001</b>
Species	1	14.283	2.1190	0.1473
Sex	1	19.507	2.8940	0.0985
TL x Species	1	1.2162	0.1804	0.6737
TL x Sex	1	0.77638	0.1151	0.7322
Species x Sex	1	2.4183	0.3587	0.5637

TL: total length

explains their wide trophic niche. A previous study also showed that *G. punctata* consumed pollen and flowers parts fallen from the riparian vegetation (Ponce de León & Rodríguez, 2013).

Despite the overall consumption of invertebrates, exclusive sets of these food items were found in the stomachs of both species. *Gambusia punctata* feed mostly on terrestrial invertebrates whilst *G. puncticulata*

additionally included aquatic, mainly benthic invertebrates in its diet. This result suggests a differential use of microhabitats by these two species. Spatial segregation is one of the mechanisms potentially explaining the coexistence of these two aggressive polyphagous *Gambusia* species in the location of this study. Such mechanism has been described previously in other fish assemblages of Cuba. *Gambusia punctata* is a very aggressive surface-swimmer (Ponce de León & Rodríguez, 2013), whereas *G. puncticulata* is often found foraging in the middle of the water column or near the bottom (Ponce de León & Rodríguez, 2010). Interestingly, our results showed that *G. punctata* forages mainly in terrestrial insects, which are likely to be found in the surface while *G. puncticulata* feeds on a wider variety of items (See table 1), likely to be found at every level of the water column. Nonetheless, other studies indicate that fish species can switch their foraging behavior according to different selective pressures like competitors (Taniguchi & Nakano, 2000), the presence of predators (Werner *et al.*, 1983) and particular habitat conditions (Tobler, 2008).

Feeding strategy plots (Amundsen *et al.*, 1996) are useful to analyze the individual contribution to the population dietary strategy (*e.g.*, Mesa *et al.*, 2004; Specziár 2004; Leunda *et al.*, 2008; Smith *et al.*, 2011) since a population's niche is strictly related to individual feeding strategies (Bolnick *et al.*, 2010). In our study, both *Gambusia* species showed generalist niches, but the analysis of trophic strategies showed different proportion of individuals specialized in one or in a small number of items. *Gambusia punctata* showed a more marked generalist strategy, and this pattern was consistent in individuals of both

sexes. However, there were some individuals showing specialization in particular food items, indicating that the individuals within a population do not necessarily share identical feeding strategies (Bolnick *et al.*, 2003, 2007; Leunda, *et al.*, 2008). In contrast, the generalist niche exhibited by *G. puncticulata*, showed a high level of individual specialization, including most males and females, and was consistent with the findings of Mesa *et al.* (2004) and Smith *et al.* (2011) on other fish species and the analysis of Bolnick *et al.* (2007) for several vertebrate groups. This difference in individual contribution to population niche in co-existing populations of *G. punctata* and *G. puncticulata* suggests a divergence in trophic habits of both species and potentially in their function in the ecosystem. The above patterns of feeding strategies between both species could reflect another potential mechanism through which competition is avoided or minimized when *G. punctata* and *G. puncticulata* co-exist in the same ecosystems. Trophic segregation is considered a mechanism reducing inter-specific competition (Vanderkooy *et al.*, 2000; Oscoz *et al.*, 2006), therefore, allowing the competing species to use different resources. The broad trophic niche of *G. punctata* and *G. puncticulata* and the moderate similarity in their diets suggest reduced overlap in resource use (resource partitioning) and, consequently, reduced inter-specific competition.

Post maturation sex-specific levels of energy consumption are related to differential reproductive roles and vary drastically between female and male poeciliids. Females devote large amounts of energy in egg formation and often in embryonic nourishment (Wourms, *et al.*, 1988; Meffe and Snelson, 1989). Male poeciliids, in

contrast, need lower amounts of energy to accomplish their reproductive role which, in *Gambusia* fishes, is reduced to pursuing females and copulate (Pilastro, *et al.*, 1997; Deaton, 2008). However, contrary to predictions based on such dissimilarities, we found statistical differences neither in diet composition nor in niche width between sexes. Only differences in GL and SL were detected between sexes. Our findings regarding diet composition and digestive tract length between sexes are similar to those obtained by Scharnweber *et al.* (2011a, b) and Tobler (2008) for *Poecilia* species. The lack of significant differences in diet composition between sexes as well as the sex-specific significant differences in stomach and gut length could indicate that these phenomena seem to be independent of fish feeding habit (polyphagous-carnivorous or detritivorous) for Poeciliidae. However, a stronger assertion in this regard would come from future investigations including more species with different feeding habits. Additional analyses are required to understand how *Gambusia punctata* and *G. puncticulata* females and males compensate in terms of diet composition and differential energy demands resulting from sex-specific reproductive roles. Of special interest in this regard would be behavioral and metabolism studies.

Overall, our results demonstrate the usefulness of integrative analyses on the trophic dynamic of co-occurring fish species with similar feeding habits in providing a clearer picture of their interactions. This is the first study including qualitative and quantitative analyses of diet composition, feeding strategy, niche breadth and dietary overlap between species and between sexes of a poeciliid fish in Cuba. We found partial segregation in the consumption of

available resources between *G. punctata* and *G. puncticulata*. The incomplete trophic niche segregation between both species may be facilitated by their feeding strategies and broad trophic niches. This wide spectrum of food consumption seems to explain why we found a low trophic segregation between sexes in both species. Male and female individuals of the two *Gambusia* species analyzed in this study showed similar use of food items in spite of the sex-specific reproductive roles known for the family Poeciliidae.

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