

# Spatial and temporal variation in superfoetation and related life history traits of two viviparous fishes: *Poeciliopsis gracilis* and *P. infans*

Patricia Frías-Alvarez · Constantino Macías García ·  
Luis F. Vázquez-Vega · J. Jaime Zúñiga-Vega

Received: 17 July 2014 / Revised: 23 September 2014 / Accepted: 25 September 2014 / Published online: 5 October 2014  
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**Abstract** Superfoetation is the ability of females to simultaneously bear multiple broods of embryos at different developmental stages. Most studies on the phylogenetic distribution of superfoetation and on the factors that potentially promote superfoetation ignore variation within species. Here, we studied 11 populations of two species of viviparous fishes of the family Poeciliidae (*Poeciliopsis gracilis* and *Poeciliopsis infans*) and document wide variation in superfoetation and in three related life history traits: brood size, individual embryo mass and total reproductive allotment. We found significant differences in the average number of simultaneous broods among populations of *P. gracilis* but not among populations of *P. infans*. In addition, we found even greater variation between months within populations for both species, although no specific pattern of temporal variation was evident. Instead of the expected consistency of seasonal differences in superfoetation across populations, we found that large variation among months within seasons and the amount and

direction of this monthly variation differed widely between populations. Our results emphasize the importance of including intraspecific variation in superfoetation and other life history traits in studies that aimed at finding general explanations of life history trait evolution.

**Keywords** Life histories · Mexico · Poeciliidae · Reproduction · Simultaneous broods

## Introduction

Superfoetation is the ability of females to simultaneously bear multiple broods of offspring at different developmental stages (Turner 1937; Scrimshaw 1944; Roellig et al. 2011). This reproductive strategy has been documented in many taxa, including angiosperm plants (Kennedy 1978), viviparous fishes (Scrimshaw 1944; Reznick et al. 2007) and in at least ten different orders of mammals (Roellig et al. 2011), including humans (Pape et al. 2008; Lantieri et al. 2010). In altricial birds, clutch overlap represents a similar phenomenon because the parents provide care to distinct sets of offspring in different stages of development (Burley 1980). Superfoetation is common in viviparous fishes (Turner 1937; Scrimshaw 1944; Thibault and Schultz 1978) and has been reported in at least three unrelated families: Clinidae (Gunn and Thresher 1991), Zenarchopteridae and Poeciliidae (Reznick and Miles 1989; Reznick et al. 2007). Within the Poeciliidae, superfoetation occurs in several genera such as *Poeciliopsis*, *Heterandria*, *Neoheterandria* (Reznick and Miles 1989; Reznick et al. 1992; Pollux et al. 2009) and *Poecilia* (subgenus *Micropoecilia*; Pires et al. 2010), whereas other genera do not include superfoetating species (e.g. *Belonesox*, *Brachyrhaphis* and *Xiphophorus*; Reznick and Miles 1989; Pollux et al. 2009). This phylogenetic distribution suggests that, in fishes and particularly within

Communicated by: Sven Thatje

**Electronic supplementary material** The online version of this article (doi:10.1007/s00114-014-1247-2) contains supplementary material, which is available to authorized users.

P. Frías-Alvarez (✉) · J. J. Zúñiga-Vega  
Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria 04510, Distrito Federal, México  
e-mail: pfríasalvarez@ciencias.unam.mx

C. Macías García  
Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México, Apartado Postal 70-275, Ciudad Universitaria 04510, Distrito Federal, México

L. F. Vázquez-Vega  
Museo de Zoología, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria 04510, Distrito Federal, México

Poeciliidae, superfoetation has evolved multiple times independently (Reznick and Miles 1989; Reznick et al. 2007; Pollux et al. 2009).

The number of broods present within a female (i.e. degree of superfoetation) varies substantially among species. For instance, female *Poeciliopsis baenschi* bear two to three simultaneous broods (Molina-Moctezuma 2011), whereas female *Heterandria formosa* can bear up to eight broods in different stages of development (Travis et al. 1987). Additionally, a few studies have documented intraspecific variation in superfoetation (Johnson and Bagley 2011). In the laboratory, Travis et al. (1987) found that female *H. formosa* experimentally given high food levels had a significantly greater incidence of superfoetation than females given restricted amounts of food, whereas Pires et al. (2007) found differences in the degree of superfoetation exhibited by two distinct populations of *Poeciliopsis prolifica* in captivity. In the field, population differences in superfoetation have been reported in *P. baenschi* (Molina-Moctezuma 2011), *Poecilia branneri* (Pires et al. 2010) and *Poeciliopsis turrubarensis*, which produce more simultaneous broods (and are thus more streamlined) in fast- than in slow-flowing streams (Zúñiga-Vega et al. 2007), whereas seasonal intrapopulation differences in superfoetation have been documented only in *H. formosa* (Travis et al. 1987; Leips and Travis 1999). As the limited number of the above examples indicates, evidence of intraspecific variation in superfoetation is scarce (Johnson and Bagley 2011).

The comparative method has been used to investigate the evolution of superfoetation (Meredith et al. 2011; Pollux et al. 2009, 2014). However, sampling errors in classification have led to erroneous mapping of the trait onto phylogenies. For example, Pires et al. (2010) recently found superfoetation in the lineage composed of *Poecilia bifurca*, *P. branneri* and *P. parae*, and this represents an additional independent origin of superfoetation that was previously unknown. In addition, more information is needed to clarify the spatial and temporal variation in this reproductive trait. A simple classification of species as either having or not having superfoetation, without assessment of intraspecific variation, may also obscure our inferences about the mode by which superfoetation may respond to the environment or its relation to other phenotypic traits.

Indeed, theoretical models posit that superfoetation should be selected for in particular environments/conditions. Several hypotheses have been proposed to explain the adaptive significance of this reproductive strategy (reviewed in Zúñiga-Vega et al. 2010). One hypothesis contends that superfoetation is beneficial in environments where a streamlined body shape is needed, such as in fast-flowing streams or in habitats where fish must swim fast to escape from predators. Under these situations, superfoetation may allow females to produce a relatively high number of offspring without large increases

in body mass or volume (Thibault and Schultz 1978; Zúñiga-Vega et al. 2007). A second hypothesis suggests that superfoetation reduces peak reproductive demand and, therefore, should be favoured in environments where reproduction is costly and resources are scarce. The reasoning behind this hypothesis is that superfoetation may spread reproduction more evenly over time (e.g. superfoetating females produce two or more small broods spaced in time instead of a single large brood), reducing the total reproductive investment made by the female at any particular time (Downhower and Brown 1975; Thibault and Schultz 1978). A third hypothesis proposes that superfoetation increases the rate of offspring production because females overlap different broods (Burley 1980; Travis et al. 1987). For example, during a certain time period, a female without superfoetation may produce a single brood of, say, four newborns, whereas a superfoetating female may overlap two smaller broods of three embryos each, which results in a total of six newborns. According to this hypothesis, if natural selection favours higher fecundity, then females will use any additional amount of resources to produce more newborns, presumably by means of increased superfoetation (Travis et al. 1987).

Given the marked seasonal changes in water flow—and thus in ecology—of streams and rivers (Allan and Castillo 2007), we predict substantial variation in the incidence of superfoetation associated with seasonal changes in the fluvial regime. The predicted changes in superfoetation could result from at least one of the mechanisms proposed by the three hypotheses mentioned above. The first hypothesis predicts that superfoetation should increase during the rainy season, because water flow increases dramatically, and therefore, more superfoetation may result in smaller body mass and volume and improved ability to deal with fast currents. The second hypothesis predicts that superfoetation should decrease when reproduction becomes less costly, which in rivers coincides with those months when primary productivity, and thus food availability, are higher. In subtropical latitudes, during the late dry season, temperatures are warmer, and water volume is low (Allan and Castillo 2007). These circumstances promote productivity (Moss 2013), making reproduction a less costly process and, hence, superfoetation less necessary. We inferred productivity from water physicochemical parameters, since productivity is positively correlated with temperature, and with the concentration of nitrogen in the form of nitrites and nitrates and phosphorous in the form of phosphates. Finally, and contrary to the second hypothesis, the third hypothesis predicts higher superfoetation during the late dry season, because greater amounts of food should be used to increase the number of offspring.

In this study, we examine temporal (monthly) and spatial (among 11 populations) variation in superfoetation and related life history traits (brood size, individual embryo mass and reproductive allotment [RA]) of *Poeciliopsis gracilis* and

*Poeciliopsis infans*, two native Mexican poeciliids (Miller et al. 2005). We aim to find seasonal patterns, consistent across populations, which could provide support for one of the hypotheses that attempt to explain the adaptive significance of superfoetation.

## Material and methods

### Study species

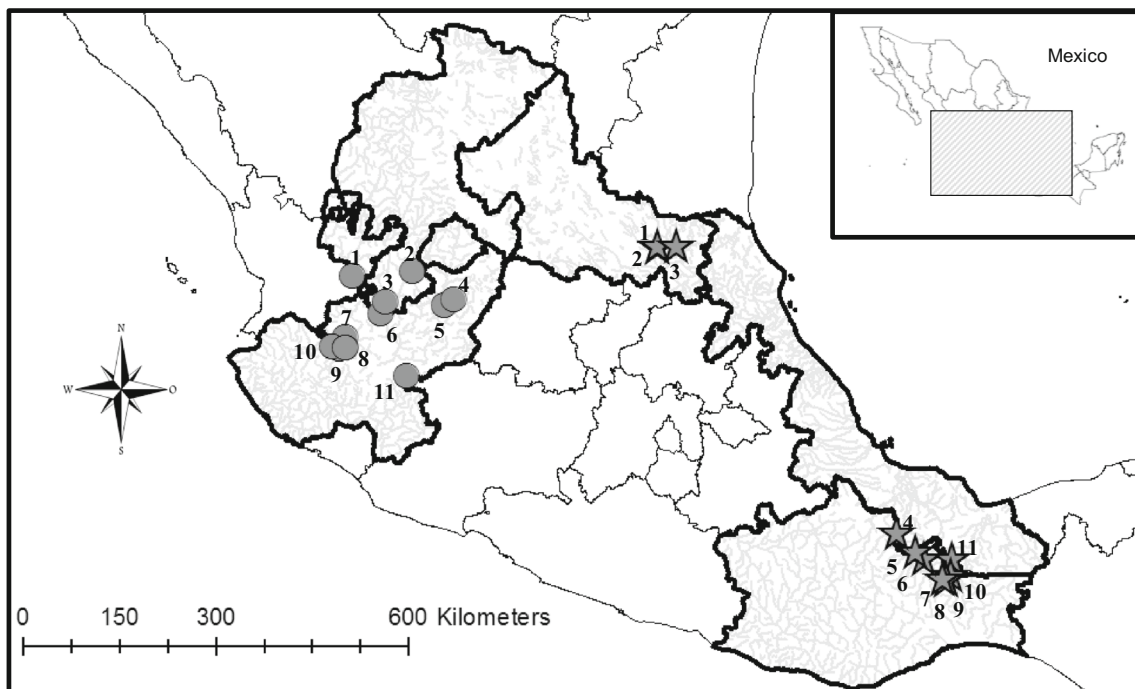
*P. gracilis* is native to basins in the Atlantic (Gulf) slope of Mexico, mainly those of rivers Coatzacoalcos and Papaloapan, in the Mexican states of Veracruz and Oaxaca, although it has been introduced and successfully colonized some basins of rivers in western and central Mexico (Gutiérrez-Cabrera et al. 2005; Miller et al. 2005). It is found in most types of water bodies of varying turbidity and water flow (Miller et al. 2005). In one of the localities where it has been introduced, Gómez-Márquez et al. (2008) found that only 25 % of the females bore simultaneous broods. We collected female *P. gracilis* from eight different localities within its original geographic range and from three newly colonized localities north of its native range (sites 1–3; Fig. 1; Table 1).

*Poeciliopsis infans* is native to basins of the Pacific slope of Mexico, mostly found in the Lerma-Santiago catchment, but also in the basins of the Ameca, Armeria, Coahuayana and

Balsas rivers (Mateos et al. 2002; Galindo-Villegas and Sosa-Lima 2002; Miller et al. 2005). It is also found in a variety of water bodies of varying turbidity and water flow. It has been reported that females can bear two simultaneous broods (Turner 1937). Females were collected from 11 different populations throughout the states of Jalisco and Zacatecas (Fig. 1; Table 1).

### Field methods

Collections of both species and of water chemistry were made during the dry (November–May) and rainy (June–October) seasons (see Table 1). Every effort was made to collect at least 20 mature females per locality on each visit using seine nets (1.3-m depth×5-m length, 8-mm mesh). Captured fish were anaesthetized with 3-aminobenzoic acid ethyl ester (MS-222™), sacrificed by immersion in 95 % ethanol and taken to the laboratory, where they were stored in 70 % ethanol. We did not assess productivity directly, but at each site collected data on water physicochemistry which are correlated with it (Moss 2013). At each visit, we measured in situ temperature (°C), pH, salinity (g/Kg), acidity (mg/L) and the concentration of phosphorus, phosphates, nitrites, nitrates and ammonium (mg/L) using a multiparameter “HI 83200”, (Hanna Instruments). All field and laboratory procedures were approved by the Mexican fisheries and environmental agencies (Comisión Nacional de Acuacultura y Pesca and Secretaría de Medio Ambiente y Recursos Naturales). Sample sizes are



**Fig. 1** Sampled populations of *Poeciliopsis gracilis* (stars) and *P. infans* (circles). Black lines correspond to state boundaries; the thickest lines indicate the Mexican states where samples were conducted. Grey lines represent rivers

**Table 1** Sampling locations of *Poecilopsis gracilis* and *P. infans*

Population number	Geographic references	Altitude (m)	State	March 2012	April 2012	May 2012	June 2012	September 2012	November 2012	January 2013	March 2013	September 2013
<i>P. gracilis</i>												
1	21° 58' N 99° 15' W	295.6	SLP	–	6/4/67	–	23/0/0	20/11/55	20/8/40	–	–	–
2	21° 59' N 99° 15' W	284.3	SLP	–	3/0/0	–	30/9/30	5/0/0	20/18/90	18/16/89	19/15/79	–
3	21° 58' N 98° 57' W	101	SLP	–	32/3/97	–	20/15/75	18/10/56	20/8/40	20/0/0	20/9/45	–
4	17° 25' N 95° 01' W	32.1	Veracruz	30/27/90	31/31/100	–	30/28/93	–	5/4/80	20/9/45	20/19/95	–
5	17° 12' N 95° 03' W	58.2	Oaxaca	18/12/67	30/29/97	–	16/12/75	20/11/55	–	7/5/71	16/15/94	–
6	17° 08' N 95° 07' W	89.7	Oaxaca	27/15/56	24/21/88	–	30/28/93	29/21/72	28/14/50	35/33/94	17/5/29	–
7	17° 08' N 95° 07' W	97.7	Oaxaca	31/22/71	24/19/79	–	18/0/0	–	4/4/100	11/11/100	19/15/79	–
8	17° 09' N 95° 10' W	121.8	Oaxaca	27/5/19	9/5/56	–	32/7/22	16/0/0	12/10/83	–	18/7/39	–
9	17° 26' N 95° 26' W	83.1	Oaxaca	30/30/100	30/29/97	–	13/13/100	6/6/100	8/7/88	20/20/100	19/19/100	–
10	17° 33' N 95° 32' W	56.4	Oaxaca	33/33/100	–	–	28/12/43	20/16/80	20/11/55	20/20/100	15/13/87	–
11	17° 50' N 95° 49' W	35.2	Veracruz	27/25/93	28/28/100	–	25/15/60	–	20/7/35	20/4/20	23/22/96	–
<i>P. infans</i>												
1	20° 34' N 103° 51' W	1250	Jalisco	–	–	20/17/85	20/20/100	–	19/18/95	12/4/33	19/18/95	–
2	21° 39' N 102° 57' W	1371	Zacatecas	–	–	20/19/95	20/20/100	1/0/0	19/13/68	20/20/100	21/20/95	1/0/0
3	21° 12' N 103° 22' W	1199	Zacatecas	–	–	20/17/85	20/19/95	–	31/31/100	30/27/90	32/31/97	20/20/100
4	21° 14' N 102° 20' W	1709	Jalisco	–	–	20/12/60	20/20/100	15/8/53	30/8/27	20/14/70	–	–
5	21° 09' N 102° 28' W	1736	Jalisco	–	–	14/11/79	5/5/100	36/36/100	20/0/0	–	–	–
6	21° 02' N 103° 25' W	1196	Jalisco	–	–	20/14/70	20/6/30	21/16/76	2/0/0	9/7/78	21/21/100	–
7	20° 33' N 103° 57' W	1259	Jalisco	–	–	13/10/77	20/10/50	30/20/67	9/0/0	8/8/100	5/4/80	–
8	20° 33' N 103° 57' W	1252	Jalisco	–	–	17/0/0	20/17/85	19/5/26	29/8/28	29/9/31	20/19/95	–
9	20° 32' N 104° 03' W	1243	Jalisco	–	–	20/12/60	20/15/75	22/19/86	30/17/57	19/0/0	–	–
10	20° 34' N 104° 09' W	1210	Jalisco	–	–	19/0/0	24/8/33	24/24/100	28/7/25	26/18/69	20/20/100	–
11	20° 09' N 103° 02' W	1537	Jalisco	–	–	19/9/47	23/19/83	–	20/6/30	20/16/80	29/23/79	20/20/100

Site numbers are used throughout the text. For each month, we show the following: number of females larger than the minimum size at maturity that were dissected/number of females with at least one brood/percentage of gravid females. Empty cells indicate months in which no individuals were collected

SLP San Luis Potosí, State

shown in Table 1. Lack of collections in some months in particular populations was due to logistical constraints such as lack of field crew or flooded (inaccessible) rivers after hurricanes (e.g. most rivers in September 2013; Table 1).

### Quantifying life history traits

All females were dissected, and if found pregnant, we quantified superfoetation (number of broods in different developmental stages) and brood size (number of developing embryos per brood), measured individual embryo mass and calculated RA following Reznick and Endler (1982) and Zúñiga-Vega et al. (2007). Embryos which shared developmental stage (as per Haynes 1995) were counted to obtain the number of embryos per brood. Individual embryo mass was measured by drying the entire brood for 24–48 h at 55 °C, weighing it (Sartorius™ LA120S,  $\pm 0.05$  mg) and dividing brood dry mass by the number of embryos. RA was calculated as the total dry weight of all the broods borne by the female. Additionally, we measured the female dry mass (24–48 h at 55 °C) excluding the digestive tract.

### Statistical analyses

To estimate variation among populations and between months within populations in life history traits, we applied general linear models with “population” and “month” (nested within population) as the two main factors and number of simultaneous broods (superfoetation), brood size, individual embryo mass and RA as response variables. We conducted one model per response variable per species using STATISTICA™ 7.0 (StatSoft). All models included female dry mass as a covariate, and the models to evaluate variation in embryo mass had as an additional covariate the stage of development. In addition, we included in all models the interaction between “month” (nested within “population”) and female dry mass to account for temporal and spatial differences in the way that the studied life history traits covary with female size. Since estimates from simultaneous broods are not independent, we randomly choose one brood from each superfoetating female, thus ensuring that each female was represented only once in the analyses of brood size and individual embryo mass. Only data from pregnant females were used.

Number of simultaneous broods (superfoetation) and brood size were square-root transformed, and individual embryo mass and RA were log-transformed to meet assumptions of normality and homogeneity of variances. An additional set of equivalent linear models were conducted on untransformed data to generate graphs and least-square means in the original scale of the variables in order to facilitate interpretation. Least-square means derived from general linear models represent values adjusted for the effect of the covariates (Sokal and Rohlf 2012). Hence, hereafter, we report mean values per

month, population and species adjusted for the effect of female mass (all traits) and developmental stage (individual embryo mass).

Finally, we searched for statistical associations between superfoetation and physicochemical parameters of the rivers by means of Spearman rank correlation coefficients. We used the mean value per population of superfoetation (adjusted for female mass) and of each physicochemical parameter. Correlation coefficients were calculated using JMP™ 7.0 (SAS Institute Inc.).

## Results

Both species had similar overall incidence of superfoetation (pregnant *P. gracilis*, means adjusted for female mass  $\pm$  SE =  $1.9 \pm 0.02$ ; range 1–4; *P. infans*,  $1.8 \pm 0.02$ ; range 1–4 simultaneous broods across all months and populations). The mean percentages of pregnant females bearing two or more simultaneous broods were 68, 73 and 65 % in non-native populations of *P. gracilis* (sites 1–3), native populations of *P. gracilis* and all populations of *P. infans*, respectively. Pregnant females of both species were found in all months, but the proportion of gravid females varied between months within populations (Table 1). The standard length (SL) of the smallest gravid females was 18.7 (*P. gracilis*) and 15.4 mm (*P. infans*).

### Spatial and temporal variation in superfoetation

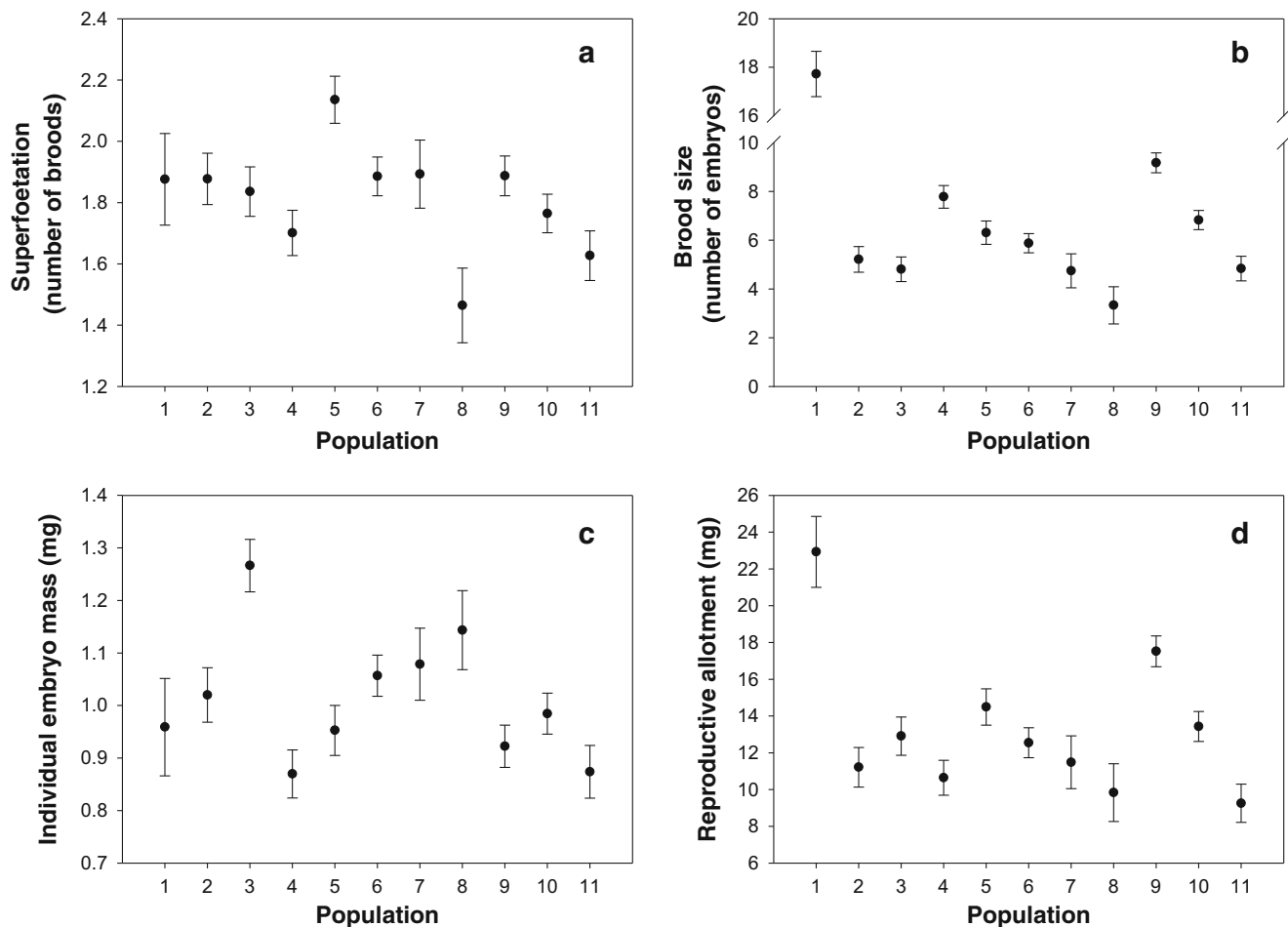
Variation in mean degree of superfoetation between populations was large and substantial for *P. gracilis* ( $F_{10,861} = 3.53$ ,  $P = 0.0001$ ; Table 2), with population means ranging from (means adjusted for female mass  $\pm$  SE)  $1.46 \pm 0.12$  broods per female in population 8 to  $2.14 \pm 0.08$  broods in population 5 (Fig. 2a). Although significant, the effect size of population was weak (partial  $\eta^2 = 0.04$ ; Table 2). In contrast, for *P. infans*, variation in superfoetation between populations was not significant ( $F_{10,774} = 1.75$ ,  $P = 0.07$ ; Table 2), although mean values varied from  $1.39 \pm 0.11$  broods in population 8 to  $1.97 \pm 0.10$  broods in population 2 (Fig. 3a). Female dry mass covaried positively with superfoetation (*P. gracilis*,  $\beta = 3.44 \pm 0.66$ ; *P. infans*,  $\beta = 14.65 \pm 2.51$ ).

Superfoetation also varied temporally within populations of *P. gracilis* ( $F_{49,861} = 3.51$ ,  $P < 0.0001$ ; Table 2), but we cannot discern any clear temporal pattern in this variation. Neither during particular months nor during the rainy (June–October) or late dry (Mar–May) season was superfoetation consistently higher or lower across populations (Fig. 4). The smallest number of simultaneous broods ( $1.03 \pm 0.25$ ) was found in June among females from population 8, whereas the highest occurred at population 6 in November ( $2.55 \pm$

**Table 2** Results of the general linear models that examined variation in superfoetation and life history traits of *Poeciliopsis gracilis* and *P. infans*

Effect	SS	df	MS	F	P	Partial $\eta^2$ *
<i>Poeciliopsis gracilis</i>						
Superfoetation						
Female dry mass	4.69	1	4.69	105.62	<0.0001	0.11
Population	1.56	10	0.16	3.53	0.0001	0.04
Month (population)	7.63	49	0.16	3.51	<0.0001	0.17
Female dry mass $\times$ month (population)	0.46	6	0.08	1.73	0.11	0.01
Error	38.22	861	0.04			
Brood size						
Female dry mass	59.59	1	59.59	148.00	<0.0001	0.15
Population	52.47	10	5.25	13.03	<0.0001	0.13
Month (population)	208.13	49	4.25	10.55	<0.0001	0.37
Female dry mass $\times$ month (population)	6.19	6	1.03	2.56	0.02	0.02
Error	346.69	861	0.40			
Individual embryo mass						
Developmental stage	3.21	1	3.21	21.02	<0.0001	0.02
Female dry mass	3.75	1	3.75	24.57	<0.0001	0.03
Population	6.46	10	0.65	4.24	<0.0001	0.05
Month (population)	26.82	49	0.55	3.59	<0.0001	0.17
Female dry mass $\times$ month (population)	2.25	6	0.37	2.45	0.02	0.02
Error	131.25	860	0.15			
Reproductive allotment						
Female dry mass	151.35	1	151.35	457.54	<0.0001	0.35
Population	31.79	10	3.18	9.61	<0.0001	0.10
Month (population)	192.87	49	3.94	11.90	<0.0001	0.40
Female dry mass $\times$ month (population)	10.31	6	1.72	5.19	<0.0001	0.03
Error	284.82	861	0.33			
<i>Poeciliopsis infans</i>						
Superfoetation						
Female dry mass	1.40	1	1.40	33.38	<0.0001	0.41
Population	0.73	10	0.07	1.75	0.07	0.02
Month (population)	8.61	43	0.20	4.79	<0.0001	0.21
Female dry mass $\times$ month (population)	0.56	6	0.09	2.21	0.04	0.02
Error	32.39	774	0.04			
Brood size						
Female dry mass	19.22	1	19.21	33.64	<0.0001	0.04
Population	44.76	10	4.48	7.84	<0.0001	0.09
Month (population)	155.65	43	3.62	6.34	<0.0001	0.26
Female dry mass $\times$ month (population)	4.01	6	0.67	1.17	0.32	0.01
Error	442.06	774	0.57			
Individual embryo mass						
Developmental stage	4.98	1	4.98	29.77	<0.0001	0.04
Female dry mass	3.96	1	3.96	23.70	<0.0001	0.03
Population	7	10	0.70	4.18	<0.0001	0.05
Month (population)	24.52	43	0.57	3.41	<0.0001	0.16
Female dry mass $\times$ month (population)	0.45	6	0.08	0.45	0.84	0.003
Error	129.32	773	0.17			
Reproductive allotment						
Female dry mass	61.67	1	61.67	202.09	<0.0001	0.21
Population	16.12	10	1.61	5.28	<0.0001	0.06
Month (population)	139.88	43	3.25	10.66	<0.0001	0.37
Female dry mass $\times$ month (population)	3.37	6	0.56	1.84	0.09	0.01
Error	236.19	774	0.30			

Superfoetation was measured as the number of simultaneous broods present within each female. The factor “month” was nested within the factor “population”



**Fig. 2** Interpopulation variation in superfoetation and life history traits of *Poeciliopsis gracilis*. Population means were adjusted for female mass (all traits) and stage of development (individual embryo mass). **a**

Superfoetation, **b** brood size, **c** individual embryo mass, **d** reproductive allotment. Population numbers as in Table 1. Error bars represent  $\pm 1$  SE

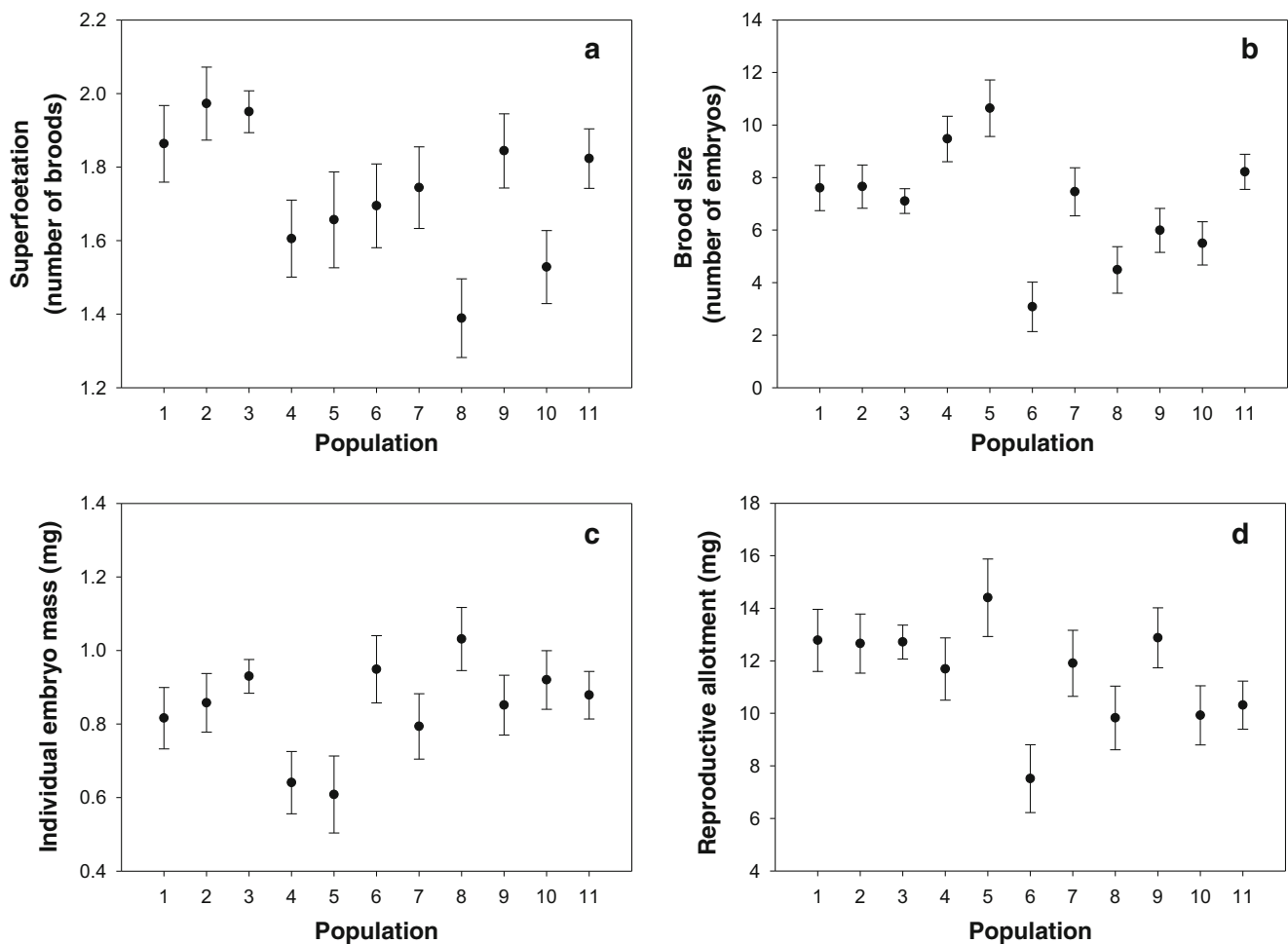
0.16; Fig. 4). The effect size of month was the largest among those of all the factors tested ( $\eta^2=0.17$ ; Table 2).

Temporal variation in superfoetation within populations was also significant in *P. infans* ( $F_{43,774}=4.79$ ,  $P<0.0001$ ; Table 2), but again, variation did not seem to be associated with seasons or with rainfall patterns (Fig. 4). The smallest number of simultaneous broods ( $1.02\pm 0.16$ ) was found in January among females from population 11, whereas the highest occurred at population 10 in September ( $2.50\pm 0.13$ ; Fig. 4). The effect size of month was the second largest ( $\eta^2=0.21$ ), only after that of female mass ( $\eta^2=0.41$ ; Table 2).

The interaction between month (nested within populations) and female mass had a significant effect on superfoetation of *P. infans* ( $F_{6,774}=2.21$ ,  $P=0.04$ ) but not *P. gracilis* ( $F_{6,861}=1.73$ ,  $P=0.11$ ; Table 2). The effect size of this significant interaction affecting superfoetation of *P. infans* was weak ( $\eta^2=0.02$ ). Variation among rivers in psychochemical parameters could not explain the observed variation in superfoetation as indicated by non-significant correlation coefficients (Table S1).

Spatial and temporal variation in additional life history traits

There was substantial interpopulation variation in brood size, individual embryo mass and RA of *P. gracilis* (Table 2; Fig. 2b–d), and female dry mass was positively correlated with these three variables (brood size,  $\beta=32.91\pm 4.14$ ; individual embryo mass,  $\beta=0.003\pm 0.0004$ ; RA,  $\beta=0.21\pm 0.01$ ). Females from population 8 produced the smallest broods (means adjusted for female mass  $\pm$  SE  $3.33\pm 0.76$  embryos), whereas females from population 1 had the largest ( $17.72\pm 0.94$  embryos; Fig. 2b). Such a large mean brood size was due to the presence in our April sample of three particularly large females ( $>46$  mm SL) bearing broods with more than 50 embryos. Variation in individual embryo mass among populations ranged between (means adjusted for female mass and stage of development)  $0.87\pm 0.05$  mg in population 4 and  $1.27\pm 0.05$  mg in population 3 (Fig. 2c). RA ranged between (means adjusted for female mass)  $9.26\pm 1.04$  mg in population 11 and  $22.93\pm 1.93$  mg in population 1, again due to the presence of those three very large females (Fig. 2d).



**Fig. 3** Interpopulation variation in superfoetation and life history traits of *Poecilopsia infans*. Population means were adjusted for female mass (all traits) and stage of development (individual embryo mass). **a**

**b** Superfoetation, **b** brood size, **c** individual embryo mass, **d** reproductive allotment. Population numbers as in Table 1. Error bars represent  $\pm 1$  SE

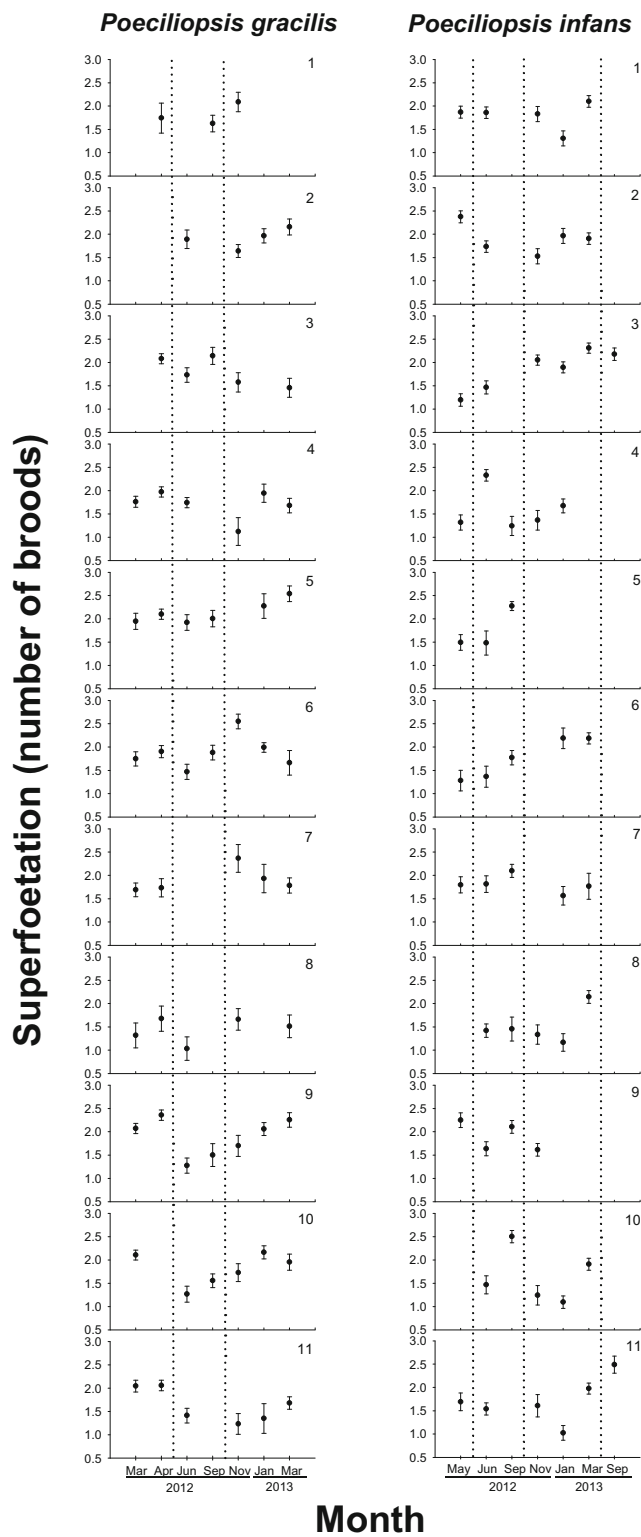
Interpopulation variation in brood size, embryo mass and RA of *P. infans* was also large (Table 2; Fig. 3b–d), and as with *P. gracilis*, all three variables were positive functions of female dry mass (brood size,  $\beta=92.28\pm 20.73$ ; individual embryo mass,  $\beta=0.002\pm 0.0002$ ; RA,  $\beta=0.23\pm 0.03$ ). Variation in brood size among populations ranged between  $3.08\pm 0.94$  embryos in population 6 and  $10.64\pm 1.08$  embryos in population 5 (Fig. 3b), whereas embryo mass ranged from  $0.61\pm 0.11$  mg in population 5 to  $1.03\pm 0.09$  mg in population 8 (Fig. 3c). Females from population 6 made the smallest RA ( $7.51\pm 1.29$  mg), whereas those from population 5 made the largest ( $14.40\pm 1.48$  mg; Fig. 3d). We found a trade-off between number and size of embryos in both species as evidenced by significant negative correlations between average values per population of brood size and individual embryo mass (Fig. 5).

Life history traits of *P. gracilis* also varied between months within populations (Table 2), but again, this variation was neither consistent across populations nor linked with seasons in any obvious way (Figs. 6 and 7). Mean brood size and RA

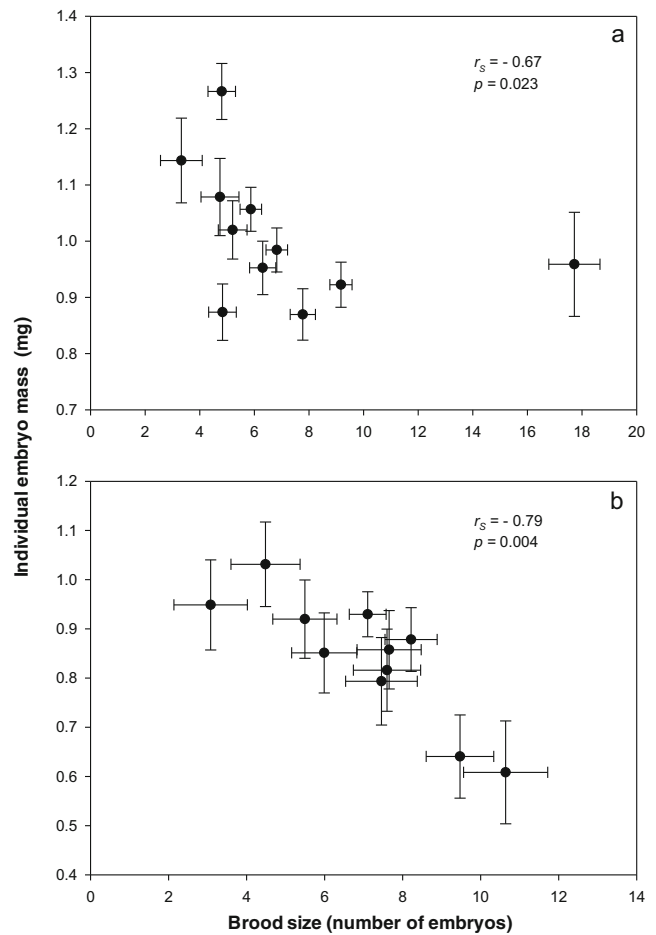
of females from population 1 were notably larger in April 2012 (Fig. 7), when the three large females were collected. As with its congener, life history traits of *P. infans* varied between months within populations (Table 2), and once more, no consistent pattern of temporal variation was evident (Figs. 6 and 7). Month (nested within population) was the strongest predictor of the three traits for both *P. gracilis* (brood size, partial  $\eta^2=0.37$ ; individual embryo mass, partial  $\eta^2=0.17$ ; RA, partial  $\eta^2=0.40$ ) and *P. infans* (brood size, partial  $\eta^2=0.26$ ; individual embryo mass, partial  $\eta^2=0.16$ ; RA, partial  $\eta^2=0.37$ ; Table 2).

The relationships between female size and life history traits varied between months within populations for *P. gracilis* as indicated by significant female dry mass  $\times$  month interactions (Table 2). However, the differences between months in the slopes of these relationships were small (brood size, partial  $\eta^2=0.02$ ; individual embryo mass, partial  $\eta^2=0.02$ ; RA, partial  $\eta^2=0.03$ ). In contrast, these interactions were not significant for *P. infans*, revealing that the effect of female mass on life history traits was consistent across months.





**Fig. 4** Temporal variation in superfoetation of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass. Population numbers are shown at the top right corner of each panel. Dotted lines indicate rainy months. Error bars represent  $\pm 1$  SE. Sampled months for *P. gracilis* March (Mar), April (Apr), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar) in 2013. Sampled months for *P. infans* May (May), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar), September (Sep) in 2013

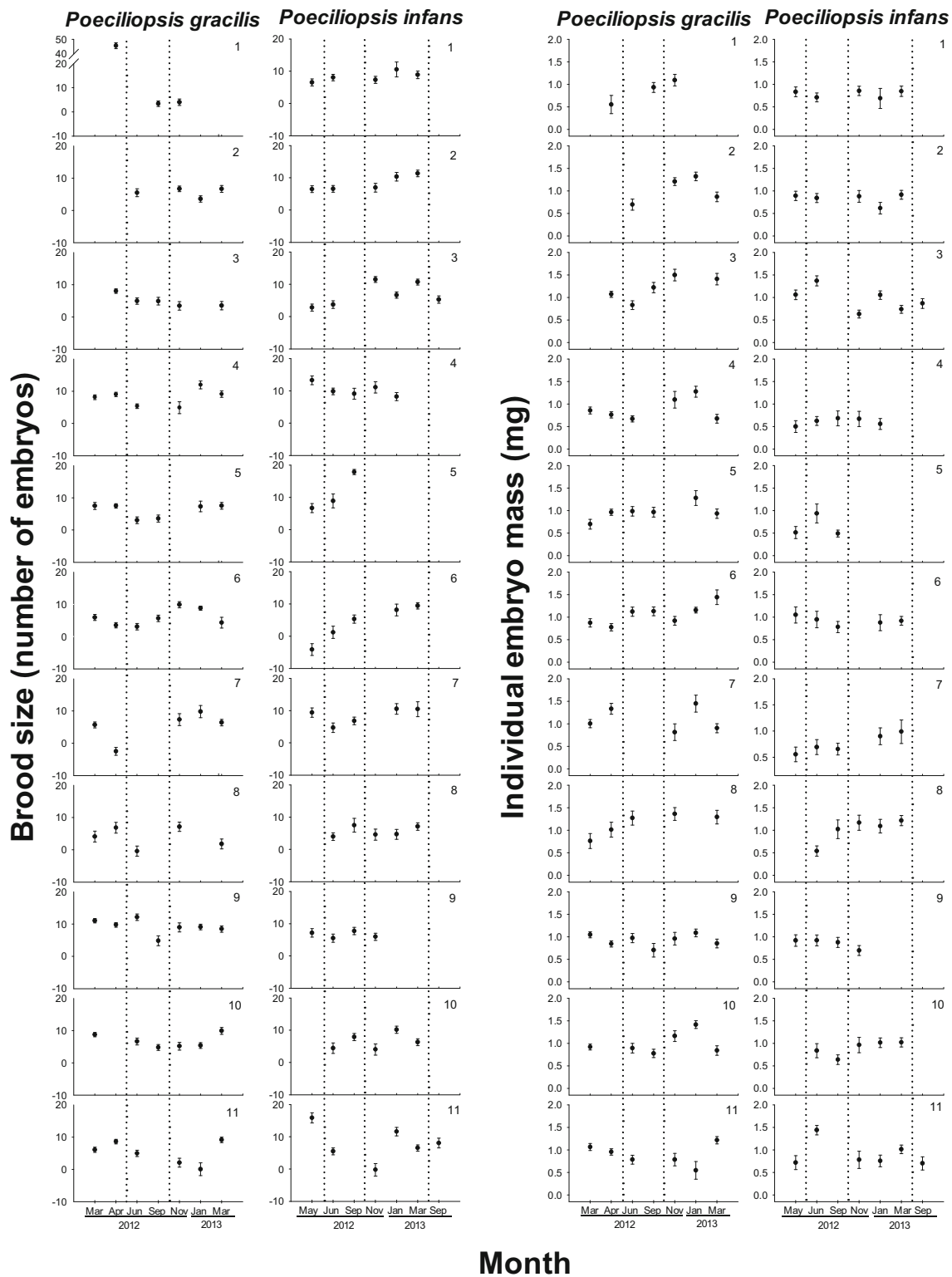


**Fig. 5** Relationships between brood size and individual embryo mass for **a** *Poeciliopsis gracilis* and **b** *P. infans*. Data points represent average values per population adjusted for female mass (both traits) and stage of development (individual embryo mass). Error bars represent  $\pm 1$  SE

## Discussion

### Spatial and temporal variation in superfoetation

Our data reveal a substantial amount of variation in the degree of superfoetation among and within populations of *P. gracilis* as well as within populations of *P. infans*. This adds to the still small number of studies reporting population variation in the number of simultaneous broods that females bear (Johnson and Bagley 2011). In spite of substantial spatial variation in water physicochemistry, we did not find any association between the correlates of water productivity (temperature, phosphorous, phosphates, nitrites, nitrates) and superfoetation in either species. This is unlikely to indicate that productivity is irrelevant for superfoetation and may instead be consequence of individual variation/plasticity in the strategic reproductive responses of females. In addition, spatial variation in water



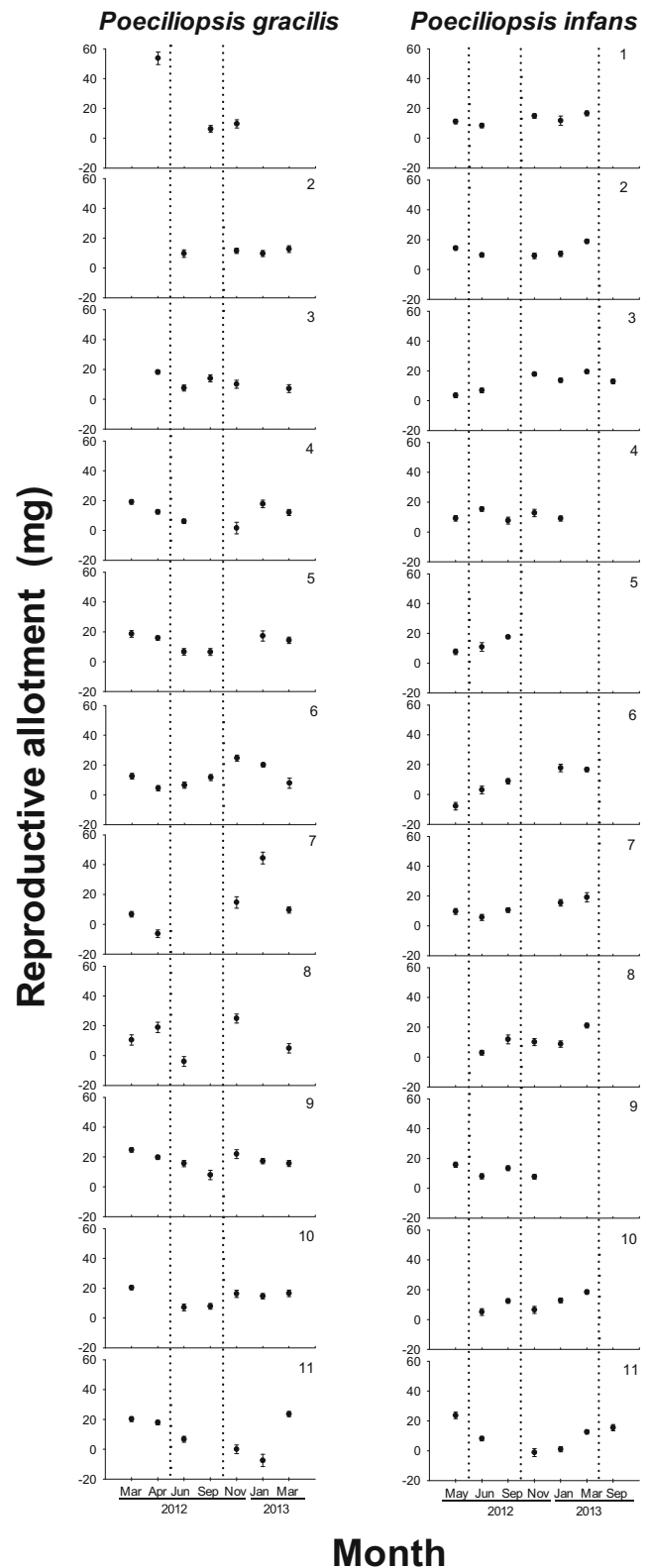
**Fig. 6** Temporal variation in brood size and individual embryo mass of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass (both traits) and stage of development (individual embryo mass). Population numbers are shown at the top right corner of each panel. Dotted lines indicate rainy months. Error bars represent  $\pm 1$  SE. Non-visible error bars are

contained within the symbols. Sampled months for *P. gracilis* March (Mar), April (Apr), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar) in 2013. Sampled months for *P. infans* May (May), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar), September (Sep) in 2013

**Fig. 7** Temporal variation in reproductive allotment of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass. Population numbers are shown at the top right corner of each panel. Dotted lines indicate rainy months. Error bars represent  $\pm 1$  SE. Non-visible error bars are contained within the symbols. Sampled months for *P. gracilis* March (Mar), April (Apr), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar) in 2013. Sampled months for *P. infans* May (May), June (Jun), September (Sep), November (Nov) in 2012, January (Jan), March (Mar), September (Sep) in 2013

temperature could promote the observed interpopulation differences in superfoetation not only through its effect on primary productivity but also through its potential effect on the average size of adult females (Vondracek et al. 1988). In other words, warmer rivers may result in larger females, and as our results indicate, larger females of both species bear more simultaneous broods. However, variation among populations in female mass was not statistically associated with variation among rivers in water temperature (Fig. S1). Further work should evaluate the possibility that the observed local variation in superfoetation is linked to differences among rivers in water velocity (e.g. Zúñiga-Vega et al. 2007), in food availability (e.g. Travis et al. 1987) or in age-specific mortality rates (e.g. due to differences in predation; Downhower and Brown 1975).

Monthly differences in superfoetation—and in the additional life history traits—were larger in both species than those observed among populations (Table 2). We anticipated that temporal consistence between populations would help inferring the underlying causes of variation in superfoetation. According to the first hypothesis that contends that superfoetation is beneficial in environments where a streamlined body shape is needed (Thibault and Schultz 1978; Zúñiga-Vega et al. 2007), increased superfoetation was expected during the rainy season. However, a detailed examination of Fig. 4 reveals that, although rivers carried a greater water volume in the rainy months, females collected in this season did not bear, on average, more broods simultaneously than females captured during the dry season. The second hypothesis suggests that superfoetation reduces peak reproductive demand for pregnant females (Downhower and Brown 1975; Thibault and Schultz 1978). Hence, less superfoetation was expected during the late dry season when food availability is highest and reproductive costs are lowest. Our data did not support this hypothesis either because females did not bear, on average, less simultaneous broods during these dry months when reproduction is presumably less costly (Fig. 4). Thus, superfoetation does not appear to be the result of reproductive costs. The third hypothesis suggests that superfoetation increases the rate of offspring production (Burley 1980; Travis et al. 1987). Therefore, given the higher food availability during the late dry season, females should use these additional resources to produce more offspring by means of increased superfoetation. Again here, we



found no support for this hypothesis. We did not find consistently higher superfoetation during these late dry months (Fig. 4).

Our (rather standard) methods to quantify temporal variation in superfoetation being destructive, we are unable to address the question of whether this lack of temporal consistency across populations is the result of (1) phenotypic plasticity, (2) maternal effects priming the breeding strategy of females of the subsequent cohorts or (3) different genotypes breeding in different seasons. The lifespan of similar-sized poeciliids (up to 5 years in captivity) seems long enough to allow the same females to breed in different seasons (even years), but the estimates are from laboratory (e.g. Carey and Judge 2000; Tacutu et al. 2013) and should be taken with caution. If wild females live throughout a single year, our results would indicate that they produce different numbers of simultaneous broods in different seasons (i.e. the degree of superfoetation would be a plastic response to the environmental conditions). In addition, genotype  $\times$  environment interactions could be expected, with individual females differing in their reaction norms. The complex variation that we observed among months within populations is likely the result of a complex interaction between phenotypic plasticity and genetic differences among individuals.

#### Variability in superfoetation and comparative studies

The comparative studies that have attempted to explain the evolution and maintenance of superfoetation and its relationships with other phenotypic traits have assumed time invariance or lack of variation among populations (Pires et al. 2007; Zúñiga-Vega et al. 2007; Johnson and Bagley 2011). For instance, in their comprehensive summary of life histories within the family Poeciliidae, Reznick and Miles (1989) classified species as either superfoetating or non-superfoetating. Yet, ignoring intraspecific variation did lead to incorrect classifications; these authors put down *Poecilia parae* as non-superfoetating, but a subsequent study showed that females sometimes bear simultaneous broods (Pires et al. 2010), a finding that revealed an additional independent evolutionary origin of superfoetation (Meredith et al. 2011). Even repeated sampling, if limited, can lead to an underestimate of the number of species that undergo superfoetation. As shown by our data, no superfoetating females were found in our June 2012 sample of populations 1 and 7 of *P. gracilis*, or in the May 2012 and January 2013 samples of populations 8 and 9 of *P. infans*, respectively. If these were our only samples, we should have concluded that these species are non-superfoetating (Table 1; Fig. 4).

Adaptive explanations have also ignored temporal variation in superfoetation. Zúñiga-Vega et al. (2007) demonstrated that differences in the degree of superfoetation among populations of *P. turrubarensis* are partially due to differences in water flow between rivers, as females inhabiting fast-flowing waters produce more simultaneous broods and are more streamlined—and hence their swimming is more energetically

efficient—than females inhabiting slow-flowing waters. That study was based, however, on samples taken only in the dry season. As our results of congeneric *P. infans* and *P. gracilis* demonstrate, the number of simultaneous broods can vary substantially between months. Thus, the association found between stream flow, body shape and superfoetation in *P. turrubarensis* might not hold during the wet season, when water flow is greatest. Indeed, physical constraints for reproduction should increase during rainy months also in more lentic habitats, such as the sites that Zúñiga-Vega et al. (2007) classified as “slow-water environments” during the dry season. Therefore, we recommend investigating the evolution and possible adaptive consequences of superfoetation by repeated sampling encompassing all seasons, preferably in different localities. In addition, controlled experiments, in which putative selective agents (e.g. water flow or food availability) are modified, would also provide insight on the adaptive significance of superfoetation.

#### Spatial and temporal variation in additional life history traits

Substantial intraspecific variation in brood size, individual embryo mass and RA has been reported in several poeciliid species (Reznick et al. 1992; Zúñiga-Vega et al. 2007; Johnson and Bagley 2011), a list to which we add *P. gracilis* and *P. infans*. Ultimate causal factors of such variation may include differences in temperature (McManus and Travis 1998; Karayucel et al. 2008), predation (Reznick and Endler 1982; Jennions and Telford 2002), population density (Leips and Travis 1999; Soucy and Travis 2003; Schrader and Travis 2012) and the physico-chemical composition of the water bodies (Riesch et al. 2010). We cannot assign the observed variation in our samples to fish density (as patterns of variation are unrelated to season, a major correlate of density), and we are currently assessing the possible role of predation. The above ecological factors may have led through selection to different, relatively stable phenotypes or combinations of phenotypes in each population (Plath et al. 2010), or to the evolution of different reaction norms within and among populations (Green 2008; Aubin-Horth and Renn 2009). It is also possible that the observed variation is solely due to the same genotypes responding plastically to spatial and temporal variation in ecology (Reznick and Yang 1993), but we find this possibility unlikely given the diversity of strategies shown by females within the same locality and in the same month.

The several life history traits measured here are likely to be intercorrelated. For instance, Reznick and Miles (1989) and Pollux et al. (2009) suggested that more superfoetation could entail the production of smaller broods. Hence, we expected smaller broods in those populations with the highest degree of superfoetation, a prediction that was not borne by our data (Figs. 2a, b and 3a, b). Instead, we found evidence of a trade-off between number and size of embryos in both *P. gracilis*

and *P. infans* that apparently is independent of the degree of superfoetation (Fig. 5). Indeed, such negative association can be found in taxa where there is no superfoetation or an equivalent (Charnov et al. 1995; Jennions and Telford 2002). A potential association between superfoetation and RA was not evident either (Figs. 2a, b and 3a, b).

Superfoetation may also be associated with larger embryos given the proposed relationship between matrotrophy and superfoetation (Reznick and Miles 1989; Pires et al. 2011) and the potential effect of matrotrophy on offspring size (Schrader and Travis 2009). Matrotrophy is defined as the mode of reproduction in which females transfer nutrients to embryos during development as opposed to lecithotrophy in which females provide nutrients to embryos before fertilization in the form of yolk (Wourms 1981). Matrotrophy may result in a conflict between mother and embryos with respect to the amount of nutrients that must be transferred, and this in turn may promote competition between embryos, high abortion rates and fewer larger embryos per brood (Schrader and Travis 2009). Given that a large number of species with superfoetation are matrotrophic (Pires et al. 2011), we expected larger embryos in those populations where females produce on average more simultaneous broods. This hypothesis was not supported in our data as can be seen in (Figs. 2a, c and 3a, c). The likely reason for this lack of association between superfoetation and mean embryo mass is that both *P. gracilis* and *P. infans* are predominantly lecithotrophic (Reznick et al. 2002), and hence, the conflict between mother and embryos in the amount of nutrient transfer must be small or even inexistent because females provide most nutrients before fertilization.

Given that our samples are substantial and encompass a variety of habitats and seasons and are essentially the same for two allopatric yet widely distributed species, we are confident that our finding that brood size, individual embryo mass and RA do not covary with superfoetation is robust. This suggests that genetic integration of those traits is weak, permitting the evolution of a wider variety of life history traits than might have been expected.

**Acknowledgments** This study was supported by the Mexican Research Council (Consejo Nacional de Ciencia y Tecnología, CONACYT) through the grant no. 129675 and through a doctorate scholarship awarded to PFA and is a partial fulfillment of the requirements for the doctoral degree (Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México) of PFA under the supervision of JJZV. Fieldwork was conducted under permits SDPA/DGVS/03492, DGOPA. 07010.210612.1749 and PPF/DGOPA-223/2013. D. Piñero gave extensive advice, and logistic assistance was provided by E. Ávila-Luna, J. L. Bortolini-Rosales, H. Espinosa-Pérez, M. Hernández-Quiroz, I. A. Morales-Salas, P. Mendoza-Hernández, M. E. Muñoz-Díaz de León, M. E. Pérez-Cruz and B. Zúñiga-Ruiz. We also thank the following people for field and laboratory assistance: P. García-Avilés, A. Hernández-Rosas, A. Molina-Moctezuma, C. Olivera-Tlahuel, H. Pérez-Mendoza, D. Robledo, N. Saleh-Bubaie, H. Salinas-Matus, T. Sandoval, R. Vega-Trejo, D. Villa-Meza and I. Zapata-Morán.

**Ethical standards** The study reported in this paper conform to the laws in the country in which they were performed.

**Conflict of interest** We have no conflict of interest

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