UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
DOCTORADO EN CIENCIAS BIOMÉDICAS
INSTITUTO DE ECOLOGÍA

FACTORES QUE PROMUEVEN Y MANTIENEN LA VARIACIÓN EN LA SUPERFETACIÓN DE PECES VIVÍPAROS DE LA FAMILIA POECILIIDAE EN MÉXICO

TESIS

QUE PARA OBTENER EL GRADO DE:
DOCTORA EN CIENCIAS

PRESENTA:
PATRICIA DE LOURDES FRÍAS ÁLVAREZ

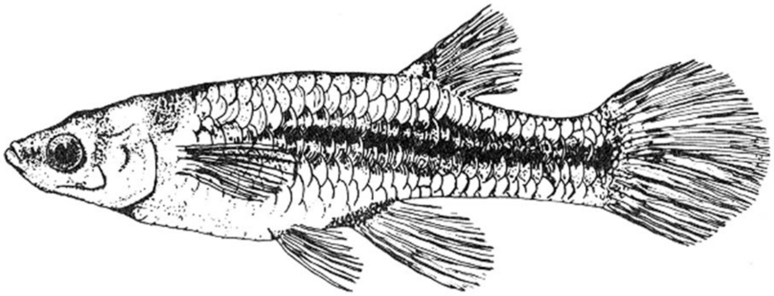
TUTOR PRINCIPAL:
DR. JOSÉ JAIME ZÚÑIGA VEGA,
FACULTAD DE CIENCIAS

COMITÉ TUTOR:
DR. CONSTANTINO MACÍAS GARCIA
INSTITUTO DE ECOLOGÍA
DR. DANIEL PIÑERO DALMAU
INSTITUTO DE ECOLOGÍA

MEXICO, D.F. AGOSTO 2015

La superfetación puede tener algunas ventajas evolutivas para los peces vivíparos, sin embargo, la fuerza selectiva que la causa, para mí, sigue siendo un enigma.

Thibault, 1975. Nature, 256



Poeciliopsis spp.

PARA MIS ALELES

ALEJANDRO FRÍAS GUILLOT

MARÍA DE LOURDES ÁLVAREZ CRAULES

AGRADECIMIENTOS ACADEMICOS

Esta tesis se realizó en cumplimiento para obtener el grado de Doctor en Ciencias Biomédicas de Patricia de Lourdes Frías Álvarez en la Universidad Nacional Autónoma de México, bajo la asesoría del Dr. José Jaime Zúñiga Vega, con el apoyo del Consejo Nacional de Ciencia y Tecnología (CONACyT) que financió el proyecto (no. 129675). ***"El significado adaptativo de la superfetación en peces vivíparos de la familia Poeciliidae"*****. Del cual se derivó esta tesis. Además del apoyo del Consejo Nacional de Ciencia y Tecnología (CONACyT) por otorgarme la beca para realizar este trabajo (no. 171465).**

AGRADECIMIENTOS ACADEMICOS

A mi alma máter, la Universidad Nacional Autónoma de México

A la Facultad de Ciencias

Al Instituto de Ecología

Al Doctorado en Ciencias Biomédicas

Al Consejo Nacional de Ciencia y Tecnología, CONACyT por otorgarme la beca para realizar este trabajo (no. 171465).

Al Consejo Nacional de Ciencia y Tecnología, CONACyT por financiar el proyecto de Investigación (no. 129675). El significado adaptativo de la superfetación en peces vivíparos de la familia Poeciliidae. Gracias a este proyecto fue posible la realización de este trabajo de investigación.

Al Dr. José Jaime Zúñiga Vega, asesor principal

Al Dr. Constantino Macías García y al Dr. Daniel Piñero Dalmau, comité tutor

A los miembros del jurado:

Dr. Constantino Macías Garcia
Dr. Carlos Rafael Cordero Macedo
Dr. Ernesto Maldonado Olvera
Dra. Mónica Vanessa Garduño Paz
Dra. Margarita Martínez Gómez

A todos los integrantes del Laboratorio Especializado en Ecología, especialmente a las siguientes personas: Dra. Irene Pisanty Baruch, Dra. Consuelo Bonfil Sanders, Dra. Teresa Valverde Valdés, Dr. Pedro Mendoza Hernández, Dra. Mariana Hernández Apolinar.

A los integrantes del laboratorio de Conducta Animal del IE, especialmente al Biol. Edgar G. Ávila Luna, Yolitzi Saldívar Lemus, Marcela Méndez Janovitz.

Al Acuario de la Facultad de Ciencias de la UNAM, especialmente a la M. en C. Ma. Estela Pérez Cruz y al M. en C. Ignacio Morales Salas.

Al Departamento de Biología Comparada de la Facultad de Ciencias, especialmente al M. en C. José Luis Bortolini Rosales y a la M. en C. Beatriz Zúñiga Ruiz.

Al Departamento de Ecología y Recursos Naturales, especialmente al M. en C. Manuel Hernández Quiroz.

A la Unidad Académica de Ciencia y Tecnología de la UNAM en SISAL, especialmente al Dr. Francisco Xavier Chiappa Carrara, a la M. en C. Maribel Badillo Alemán, y al M. en C. Alfredo Gallardo Torres.

Al laboratorio de Ecología y Fisiología de Algas en Ríos de la Facultad de Ciencias, especialmente al Dr. Javier Carmona Jiménez.

A la Colección Nacional de Peces del Instituto de Biología de la UNAM, especialmente al M. en C. Héctor Espinoza Pérez.

Quisiera agradecer y reconocer todo el esfuerzo que se realizó tanto en campo como en gabinete, para la obtención de los datos del presente trabajo de investigación, especialmente a las siguientes personas: Luis Felipe Vázquez Vega, Irene Zapata Moran, Claudia Olivera Tlahuel, Paulina García Avilés, Nabila Saleh Bubaie, Israel Solano, Oscar Olivares Loyola, Regina Vega Trejo, Diana Villa Meza, Hibraim Pérez Mendoza, Ana Hernández Rosas, Alejandro Molina Moctezuma, Hugo Salinas Matus, Tonantzi Sandoval, Miriam Barrientos Palacios, Gonzalo Ángel Ramírez Cruz.

El trabajo de campo fue realizado bajo los siguientes permisos SDPA/DGVS/03492, DGOPA.07010.210612.1749 y PPF/DGOPA-223/2013

AGRADECIMIENTOS PERSONALES

Gracias a mis padres, por su apoyo incondicional, por su fuerza, por su bondad y ayuda. GRACIAS A MIS ALELES por sus sabios consejos y sobre todo POR SU INFINITA PACIENCIA Y SU INMENSURABLE AMOR.

Gracias a mi amado Alejandro Frías Villegas. GRACIAS ALE por todo tu amor, tus consejos, tu guía, tu enseñanza, tu tolerancia, comprensión y paciencia en este tramo que hemos recorrido juntos. GRACIAS POR ESTAR EN MI CAMINO.

Gracias a mi hermano Alejandro y a mi Cucu Ángeles, y sobre todo a la luz de mi camino y a mi sonrisa de cada día a Rebeca, gracias Elaine por ser nuestra más bella espera.

Gracias a mi hermano Mauricio y a Claudia y también a mi fuente de inspiración Santi.

Gracias a mi Wensy, por tu amor y compañía.

Gracias a mi Prima Addy Rebeca, por tu ayuda y tus consejos, por tu amor y apoyo, gracias a mi Sergy y a Pili, gracias a mi Tia Addy y a mi muy querido Paninito por su amor y compañía.

Gracias a mi Tío Fernando, mi Tía Lucha, Lalo y Fer.

Gracias a toda la familia Pérez Álvarez a mis tíos, primos y sobrinos.

Gracias a mi querida Edna González, mi Flashh gracias por tus consejos, las horas de platica, por compartirme tu experiencia y tu apoyo.

Gracias Anibal Díaz de la Vega, gracias Chiquin por tus consejos, por compartir tus experiencias y por seguir en contacto conmigo a través de los años.

Gracias Mariana del Olmo, gracias Marianita por tu enseñanza, por tu ejemplo y tu amistad.

Gracias Noemí Matías, gracias Mimi por compartir los buenos momentos y los no tan buenos.

Gracias Rodolfo Pérez, gracias Rodo por hacerme reír y por tu ayuda y claro tus consejos.

Gracias Carlos Hernández, gracias Carlitos por estar en contacto y siempre ayudarme.

Gracias Alberto Mendoza, gracias Betito por estar ahí.

Gracias Noe Pacheco, porque siempre me alentaste a seguir adelante, por ser tan positivo y objetivo y ver en mi a una amiga.

Gracias Carolina Granados, gracias Caro por tu ayuda, tus sabias palabras y por impulsarme.

Quisiera decir **GRACIAS** a todos aquellos que estuvieron conmigo, me ayudaron, me guiaron y me brindaron su sincera amistad especialmente a las siguientes personas: Gracias **Marianita Hernández** por toda la sabiduría que me diste, Gracias **Pedro Eloy** por tus sabias y alentadoras palabras, Gracias **Irene Pisanty** por tu simpatía y tu presencia. Gracias **Tino** por tu apoyo y la confianza que depositaste en mi, Gracias **Daniel** por tus palabras tan alentadoras y tu apoyo. Gracias a todos los **lunáticos**, por sus increíbles reuniones de tanto aprendizaje y convivencia. Gracias **Clau**, **Irene**, **Pau** y **Nabi** por su sincera amistad, su apoyo, por caminar junto a mí en este proceso y por los mejores momentos. Gracias **José Luis Bortolini** por tantas platicas y apoyo. Gracias a **Mony Mildrette**, **Victor**, **Anain, Bruno**, **Jonathan Maceda**, **Vale** por la alegría compartida. Gracias a **Yoli**, **Marce** y **Gonzo**. En verdad no se que hubiera hecho sin ustedes y claro está sin ustedes no lo hubiera logrado.

Gracias José Ibarreche, me levantaste, me sacudiste, me limpiaste y me impulsaste.

Y sobre todo...

GRACIAS A MÉXICO Y A TODOS LOS EJEMPLARES DE *Poeciliopsis gracilis* y *P. infans* POR PERMITIRME ESTUDIARLOS.

Mi querido Ingeniero Maicol S. S. Atilano Ley Coati...

ÍNDICE

RESUMEN

La superfetación es la capacidad que tienen las hembras de llevar internamente y de manera simultánea múltiples camadas en diferentes estadios de desarrollo. La superfetación es más predominante en peces vivíparos de la familia Poeciliidae. El número total de camadas simultaneas presentes dentro de una hembra (i.e. grado de superfetación) varía substancialmente tanto dentro de una especie como también entre especies. En esta tesis se realizó una aproximación descriptiva espacial y temporal de la superfetación y se examinaron dos hipótesis que intentan explicar el significado adaptativo de la estrategia reproductora. Para ello, se estudiaron 11 poblaciones de dos especies de peces vivíparos nativos de México: *Poeciliopsis gracilis* y *P. infans.* La primera hipótesis menciona que la superfetación es el resultado de una restricción morfológica, en ambientes en los que se necesita un fenotipo delgado e hidrodinámico tal como ríos con rápida velocidad del agua. La siguiente hipótesis puesta a prueba menciona que la superfetación evolucionó debido a que reduce el costo pico de la reproducción y, por lo tanto, debería favorecerse en ambientes con baja disponibilidad de alimento. Se encontró una gran variación en el grado de superfetación entre diferentes meses del año y así como en distintas temporadas dentro de las poblaciones en ambas especies. No se encontró evidencia que soporte la hipótesis de restricción morfológica en ninguna de las dos especies. En ambas especies el grado de superfetación varió como una interacción compleja entre la población de origen y el tamaño de la hembra, sin encontrarse una relación entre la variación inter-poblacional y la velocidad del agua. Contrario a lo que se esperaba, las hembras de *P. gracilis* con un fenotipo más hidrodinámico habitan ríos de baja velocidad, mientras que para *P. infans* la velocidad del agua no predice la variación observada en la forma corporal. No se encontró

evidencia que soporte la hipótesis de reducción del costo pico reproductivo en *P. gracilis.* En contraste, en algunas de las poblaciones de *P. infans* se encontró evidencia para apoyar la hipótesis: Las hembras con menor ingesta de alimento presentaron un mayor grado de superfetación. Esta tesis demuestra que la asociación entre mayor superfetación y fenotipos hidrodinámicos en ambientes de rápido flujo de agua no es una regla general y que la superfetación no siempre está asociada a la cantidad de recursos que se encuentran en el ambiente. Por lo tanto, se puede concluir que el valor adaptativo de la superfetación puede diferir entre especies y que las fuerzas selectivas (e.g. velocidad del agua y cantidad de recursos) están actuando de diferente formas entre las distintas especies. Otras fuerzas selectivas que pueden estar relacionadas con la variación en historias de vida en peces pecílidos son la mortalidad, la selección de pareja y densidad poblacional.

ABSTRACT

Superfoetation is the ability of females to have simultaneously several broods in different developmental stages. Superfoetation is most common in fishes of the Poeciliidae Family. The total number of simultaneous broods present inside one female (i.e. superfoetation degree) varies greatly, not only in the same species but also between different species. In the present work, a spatial and temporal descriptive approximation of superfoetation was made and also two main hypothesis were examined trying to explain the adaptive significance of this reproductive strategy. To accomplish this, 11 populations of two native Mexican viviparous fishes were studied: *Poeciliopsis gracilis* and *P. infans. In* the first hypothesis we propose that superfoetation is the result of a morphological restriction, in environments such as fast water velocity rivers, where a thin and elongate body is favored. The next hypothesis mentions that superfoetation evolved because it reduces the cost of reproduction, therefore, superfoetation most be favored in environments with scarce food resources. We found a large variation in superfoetation among different months of the year and in the seasons, for populations in both species. No evidence was found to support the morphologic restriction hypothesis in any species. In both species the superfoetation degree varied as a complex interaction between the source population and female size and this inter-population variation was not associated with water velocity. In contrast, females of *P. gracilis* with thin and elongated bodies inhabit low velocity rivers, whereas for *P. infans* water velocity in rivers do not predict the variation observed in the corporal shape. No evidence was found which supports the reduction of pick cost hypothesis in *P. gracilis*. In contrast, in some populations of *P. infans* evidence was found to support the hypothesis: females with low food ingested showed a high superfoetation degree. This thesis

demonstrate that the association between superfoetation and hydrodynamic body phenotypes in environments of fast water flow it is not a general rule and the superfoetation is not always associated with the amount of resources presents in the environments. Thus, it can be concluded that the adaptive significance of superfoetation can differ among species and the selective forces (e.g. water velocity and resources availability) are acting in different ways among different species. Other selective forces that can be related with life history variation in poeciliids fishes are mortality, mate choice and population density.

CAPÍTULO I

INTRODUCCIÓN

INTRODUCCIÓN

Superfetación

La superfetación - del latín "*superfetatio"* (*super* = arriba, encima de; *fetus, fetura* = fértil, fertilizar, hijos) - es la capacidad que tienen las hembras de llevar internamente y al mismo tiempo varias camadas de embriones en diferentes estadios de desarrollo (Turner, 1937; Scrimshaw, 1944; Roellig et al. 2011). Los antiguos griegos como Aristóteles, Herodoto, Hipócrates y Plinio ya hacían mención al fenómeno (Roellig et al. 2011). En el siglo XIX surge la primera referencia básica de una definición clara del termino y no es sino hasta el siglo XX cuando se generaliza una definición estándar de superfetación (Rollhäuser, 1949; Roellig et al. 2011).

Este atributo reproductor ha sido documentado en plantas angiospermas (Kennedy, 1978), peces vivíparos (Scrimshaw, 1944; Reznick et al. 2007a), y en al menos 10 órdenes de mamíferos, entre los que se encuentran: carnívoros (*Meles meles*; Yamaguchi et al. 2006), diprotodontos (*Wallavia bicolor*; Paplinska et al. 2006), lagomorfos (*Lepus americanus*; Burton, 2002), quirópteros (*Peropteryx kappleri*; Rasweiler, 1982) , roedores (*Mus musculus*; Barnett y Munro, 1970) e inclusive humanos (Pape et al. 2008; Lantieri et al. 2010). En aves altriciales la superposición de puestas (i.e., superfetación) se presenta como la provisión simultánea de cuidado parental a grupos dependientes de crías en diferentes estadios de desarrollo (Burley, 1980; Hill, 1986; Hetmanski y Wolk, 2005).

La superfetación es más predominante en peces vivíparos (Turner, 1937; Scrimshaw, 1944; Thibault y Schultz, 1978), y se ha documentado en al menos tres familias pertenecientes a diferentes órdenes: Clinidae (Gunn y Thresher, 1991), Zenarchopteridae y

Poeciliidae (Reznick et al. 2007a; Fig. 1). La familia Clinidae (orden Peciformes) son peces pequeños que habitan el fondo marino (Gunn y Thresher, 1991; Fig. 1a). Se distribuyen del norte al sur del Pacifico, sureste del Atlántico y en el Mediterráneo (Springer, 1970). Dentro de las muy variadas estrategias reproductoras de los clínidos se encuentra la viviparidad, la cual está frecuentemente acompañada por superfetación (Gunn y Thresher, 1991). Esta última ha sido reportada en dos de sus 20 géneros (*Cristiceps, Heteroclinus*) y en nueve de sus 74 especies (Nelson, 2006; Gunn y Thresher, 1991; Tabla 1).

Zenarchopteridae es una familia perteneciente al orden de los Beloniformes (Lovejoy et al. 2004). La aleta anal en este grupo está modificada y conforma el andropodio (análogo al gonopodio encontrado en ciprinodontiformes), lo que facilita la fertilización interna (Nelson, 2006; Fig. 1b). Esta familia que se distribuye en el sureste de Asia y en el Archipiélago Malayo comprende cinco géneros que presentan diversas estrategias reproductoras (Nelson, 2006). Los géneros habitantes de cuerpos de agua dulce *Dermogenys* (10 spp.)*, Hemirhamphodon* (6 spp.)*, y Nomorhamphus* (13 spp.) son vivíparos (Reznick, 2007a). Se ha documentado superfetación en varias especies pertenecientes a estos tres géneros (Meisner y Burns, 1997; Reznick et al. 2007a; Tabla 1). *Hermirhamphodon tengah* es la única especie dentro de los géneros vivíparos que deposita huevos (Nelson, 2006; Dorn y Greven, 2007; Reznick et al. 2007a). Las especies del cuarto género, *Zenarchopterus* (20 spp.), depositan sus huevos en aguas marinas (Nelson, 2006; Reznick et al. 2007a). El modo reproductor del último género *Tondanichthys* (1 sp.) es aún desconocido (Nelson, 2006; Reznick et al. 2007a).

Tabla 1. Lista de especies de peces vivíparos de las familias Clinidae y Zenarchopteridae con presencia de superfetación.

Especies	**Bibliografía**
Peciformes	
Clinidae	
Cristiceps australis	Gunn y Thresher, 1991
Heteroclinus adelaide	Gunn y Thresher, 1991
Heteroclinus perspicillatus	Gunn y Thresher, 1991
Heteroclinus fosteri	Gunn y Thresher, 1991
Heteroclinus wilsoni	Gunn y Thresher, 1991
Heteroclinus heptaolus	Gunn y Thresher, 1991
Heteroclinus puellarum	Gunn y Thresher, 1991
Heteroclinus longicaudis	Gunn y Thresher, 1991
Heteroclinus sp. (Scott´s Weedfish)	Gunn y Thresher, 1991
Beloniformes	
Zenarchopteridae	
Nomorhamphus brembachii	Reznick et al. 2007a
Nomorhamphus manifesta	Reznick et al. 2007a
Nomorhamphus pectoralis	Reznick et al. 2007a
Nomorhamphus rossi	Reznick et al. 2007a
Hemirhamphodon pogonognathus	Reznick et al. 2007a
Hemirhamphodon kuekenthali	Reznick et al. 2007a
Hemirhamphodon kapuasensis	Reznick et al. 2007a
Dermogenys sumatrana	Reznick et al. 2007a
Dermogenys orientalis	Meisner y Burns, 1997; Reznick et al. 2007a
Dermogenys bispina	Reznick et al. 2007a
Dermogenys robertsi	Reznick et al. 2007a
Dermogenys pusilla	Meisner y Burns, 1997
Dermogenys viviparous	Meisner y Burns, 1997

Dentro del orden de los Cyprinodontiformes se encuentra la familia Poeciliidae (Lucinda y Ries, 2005). Los representantes de esta familia se distinguen por ser peces pequeños y lateralmente comprimidos que habitan cuerpos de agua dulce y salobre (Miller et al. 2005; Fig. 1c). Su distribución comprende desde el oriente de Estados Unidos de Norteamérica hasta el noroeste de Argentina, así como África y Madagascar (Miller et al. 2005). Ghedotti (2000) reconoce tres subfamilias: Aplocheilichthyinae, Procatopodinae, Poeciliinae. Aplocheilichthyinae contiene únicamente a la especie africana *Aplocheilichthys* *spilauchen* (Ghedotti, 2000). La subfamilia Procatopodinae con 78 especies es un grupo compuesto por el género habitante del sur de Ámerica *Fluviphylax,* además de los procatopodines de África (Ghedotti, 2000).

La subfamilia Poeciliinae se caracteriza por la modificación de la aleta anal del macho en un gonopodio, lo cual permite la fertilización interna (Parenti, 1981; Miller et al. 2005). Todos los miembros de Poeciliinae son endémicos de América, donde la mayoría de sus especies se encuentran en Centroamérica, las Antillas y México (Hrbek et al. 2007). La subfamilia Poeciliinae comprende 28 géneros y aproximadamente 225 especies (Lucina, 2003; Lucina y Reis, 2005). La estrategia reproductora de la subfamilia es la viviparidad y se ha documentado un amplio número de especies que presentan superfetación (Miller et al. 2005; Lucinda, 2003; Tabla 2). *Tomerus gracilis* es la única especie dentro del grupo que deposita sus huevos en el exterior (i.e. es la única especie ovípara; Rosen, 1964). Esta subfamilia es ampliamente reconocida por su importancia como peces de ornato para acuarios, algunas de las cuales son popularmente reconocidas como: gupis, peces mosquito, platis, molis, peces espada, guayacones y topotes (Miller et al. 2005; Lucinda y Reis, 2005).

La amplia distribución de la subfamilia en el nuevo mundo comprende otras especies que no se encuentran en México pero que también superfetan (Pollux et al. 2009). Entre éstas podemos mencionar a *Heterandria formosa, Gambusia hubbsi, Neoheterandria elegans, N. tridentiger, Phalloptychus januaris, Poecilia bifurca, P. branneri, P. parae, Pseudopoecilia festae, Poeciliopsis scarlii, P. retropina, P. elongata* y *P. paucimaculata* (Downhower et al. 2002; Pollux et al. 2009; Pires et al. 2010).

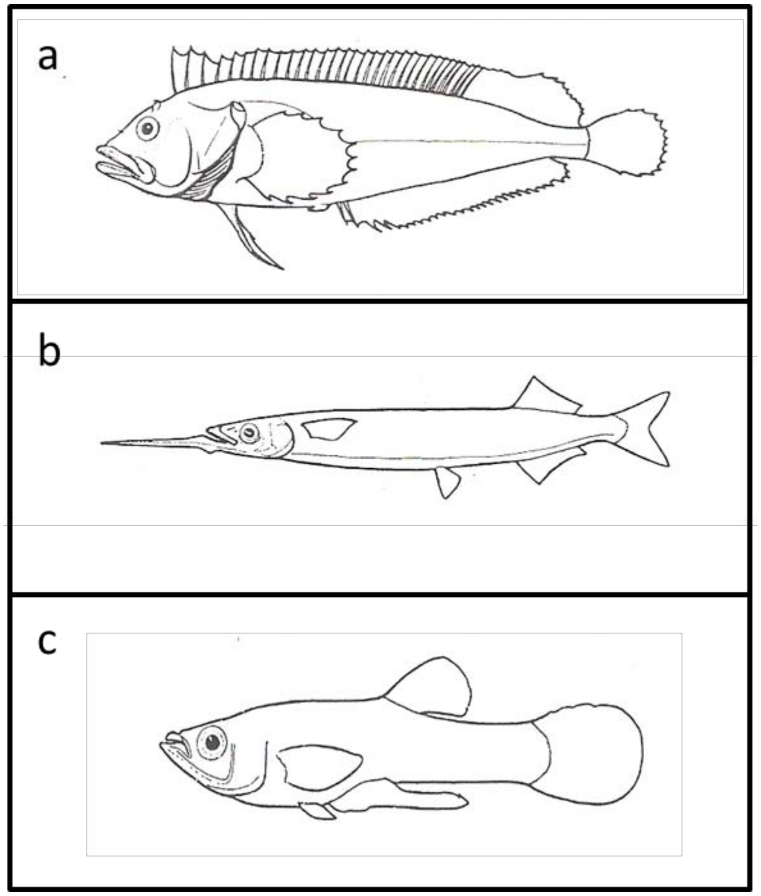


Fig. 1. Morfología característica de las tres familias de peces vivíparos que presentan superfetación. a) Clinidae b) Zenarchopteridae c) Poeciliidae. Ilustraciones tomadas de Nelson, (2006).

Tabla 2. Lista de las especies de la subfamilia Poeciliinae que habitan en México, señalando las especies endémicas y las especies reportadas con superfetación.

Especies	Endémica	Presencia de Superfetacion (SF)	Bibliografía
Cyprinodontiformes			
Poeciliidae			
Poeciliinae			
Belonesox belizanus			
Brachyrhaphis hartwegi			
Carlhubbsia kidderi			
Gambusia affinis			
Gambusia alvarezi	X		
Gambusia atrora	X		
Gambusia aurata	X		
Gambusia eurystoma	X		
Gambusia hurtadoi	X		
Gambusia krumholzi	X		
Gambusia marshi	X		
Gambusia regani	X		
Gambusia senilis			
Gambusia sexradiata			
Gambusia speciosa			
Gambusia vittata	X		
Gambusia yucatana			
Heterandria jonesii	X		
Heterophallus echeagarayi	X		
Heterophallus milleri	X		
Heterophallus rachovii	X		
Phallichthys fairweatheri			
Poecilia butleri			
Poecilia catemaconis	X		
Poecilia chica	X		
Poecilia formosa			
Poecilia latipinna			
Poecilia latipunctata	X		
Poecilia maylandi	X		
Poecilia mexicana			
Poecilia orri			
Poecilia petenensis			
Poecilia sphenops			
Poecilia sulphuraria	X		
Poecilia velifera	X		
Poeciliopsis baenschi	X	SF	Pollux et al. 2009
Poeciliopsis balsas	X		
Poeciliopsis catemaco	X	SF	Pollux et al. 2009

Poeciliopsis fasciata		SF	Pollux et al. 2009
Poeciliopsis gracilis	X	SF	Gómez-Márquez et al. 2008; Pollux et al. 2009
Poeciliopsis hnilickai		SF	Pollux et al. 2009
Poeciliopsis infans	X	SF	Pollux et al. 2009
Poeciliopsis latidens	X	SF	Pollux et al. 2009
Poeciliopsis lucida	X	SF	Thibault y Schultz, 1978; Pollux et al. 2009
Poeciliopsis monacha	X	SF	Thibault y Schultz, 1978; Pollux et al. 2009
Poeciliopsis occidentalis		SF	Scrimshaw, 1944; Pollux et al. 2009
Poeciliopsis pleurospilus		SF	Scrimshaw, 1944
Poeciliopsis presidionis	X	SF	Pollux et al. 2009
Poeciliopsis prolifica	X	SF	Thibault y Schultz, 1978; Pollux et al. 2009
Poeciliopsis sonoriensis			
Poeciliopsis turneri	X	SF	Thibault y Schultz, 1978; Pollux et al. 2009
Poeciliopsis turrubarensis		SF	Zúñiga-Vega et al. 2007; Pollux et al. 2009
Poeciliopsis viriosa	X	SF	Pollux et al. 2009
Priapella bonita	X	SF	Scrimshaw, 1944
Priapella compressa	X		
Priapella intermedia	X		
Priapella olmecae	X		
Xenodexia ctenolepis		SF	Reznick et al. 2007b; Pollux et al. 2009
Xiphophorus alvarezi			
Xiphophorus andersi	X		
Xiphophorus birchmanni	X		
Xiphophorus clemenciae	X		
Xiphophorus continens	X		
Xiphophorus cortezi	X		
Xiphophorus couchianus	X		
Xiphophorus evelynae	X		
Xiphophorus gordoni	X		
Xiphophorus hellerii			
Xiphophorus maculatus			
Xiphophorus malinche	X		
Xiphophorus meyeri	X		
Xiphophorus milleri	X		
Xiphophorus montezumae	X		
Xiphophorus multilineatus	X		
Xiphophorus nezahualcoyotl	X		
Xiphophorus nigrensis	X		
Xiphophorus pygmaeus	X		
Xiphophorus variatus	X		
Xiphophorus xiphidium	X		

Como se ha observado dentro de algunos géneros, hay especies que exhiben superfetación, mientras que otras no (Reznick et al. 2007a; Pires et al. 2010). La distribución filogenética según la estrategia reproductiva (Fig. 2) sugiere que la superfetación ha evolucionado independientemente y en múltiples ocasiones (Reznick y Miles, 1989; Reznick et al. 2002; Meredith et al. 2010; Pires et al. 2010; Meredith et al. 2011).

Más interesante es el hecho que diversos estudios han encontrado que el grado de superfetación, puede variar en tiempo y espacio dentro de una misma especie (Zúñiga-Vega et al. en prensa). Travis et al. (1987) documentó que hembras de *Heterandria formosa* criadas en laboratorio con diferentes cantidades de alimento, presentaron diferentes grados de superfetación. En las poblaciones de *Poeciliopsis turrubarensis* se ha observado una fuerte asociación entre el tipo del hábitat y el grado de superfetación (Zuñiga-Vega et al. 2007). Distintas poblaciones de *P. prolífica* en el noroeste de México exhiben notables diferencias en el nivel de superfetación (Pires et al. 2007). Esta variación intraespecífica observada en numerosas especies indica que la superfetación puede verse más favorecida en algunos ambientes que en otros (Zúñiga-Vega et al. 2010).

Zúñiga-Vega et al. (en prensa) expone que la evidencia de evolución múltiple e independiente aunada a la variación intraespecífica, sugiere que la superfetación debe proveer bajo algunas circunstancias ciertos beneficios para la adecuación de sus portadoras. Lo cual significaría que este atributo reproductor podría representar una adaptación a presiones selectivas particulares.

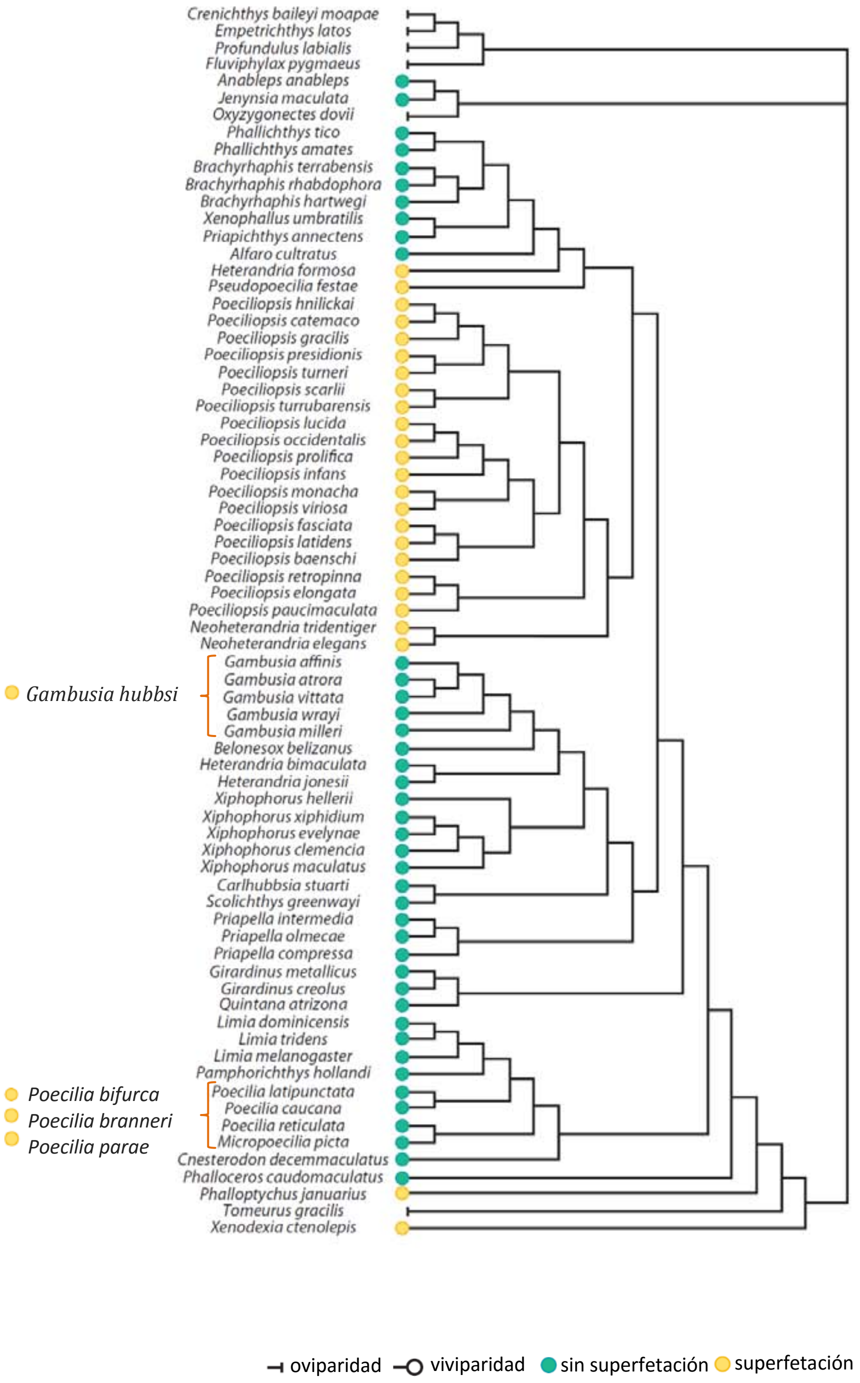


Figura 2. Árbol filogenético que muestra la distribución taxonómica de la superfetación en la familia Poeciliidae (tomado y modificado de Pollux et al. 2009). Se muestran frente a cada clado a las especies reportadas con superfetación que no fueron incluidas en el árbol. *Gambusia hubbsi* (Downhower et al. 2002), *Poecilia bifurcata, P. braneri, P. parae* (Pires et al. 2010).

Actualmente no existe un consenso sobre el significado adaptativo de la superfetación, a pesar del creciente interés en entender el papel que ha jugado la selección natural en el origen múltiple de esta estrategia reproductora (Travis et al. 1987; Downhower et al. 2002; Zúñiga-Vega et al. 2007).

La evidencia de este interés puede observarse en el desarrollo hasta el momento de seis hipótesis (revisadas de Zúñiga-Vega et al. 2010) que intentan explicar el origen y el significado adaptativo de la superfetación. Zúñiga-Vega et al. (2010) las clasifica en tres categorías: I) costo reproductor de la superfetación, la cual incluye dos hipótesis; II) ventajas demográficas de la superfetación, integrada por tres hipótesis; III) evolución de la superfetación para asegurar la variabilidad genética de la descendencia, comprendida por solo una hipótesis. A continuación se abordan cada una de las tres categorías y las hipótesis que las conforman.

Costo reproductor de la superfetación

Hipótesis 1. La superfetación disminuye el costo pico de la reproducción.

La superfetación podría reducir el pico de inversión reproductora que deben de hacer las hembras en un determinado momento (Downhower y Brown, 1975). El efecto neto de la superfetación es el de distribuir el esfuerzo reproductor a lo largo del tiempo, reduciendo la inversión máxima realizada por las madres en un determinado momento, sin afectar el total de crías que son producidas (Downhower y Brown, 1975). Por ejemplo, la especie no superfetadora *Poecilia reticula* a un tamaño de 30-35 mm, produce 24 crías en 21 días, en comparación con las especies superfetadoras *Poeciliopsis lucida* y *P.monacha,* que al mismo tamaño producen dos camadas de 11 crías en 22 días (Downhower y Brown, 1975). El número total de crías entre las especies (con y sin superfetación) es aproximadamente el mismo. Por lo tanto, el efecto neto de la superfetación es el de distribuir la reproducción de una manera más uniforme reduciendo la inversión total de recursos realizada por las hembras en un determinado momento sin afectar el rendimiento reproductor (Zúñiga-Vega et al, 2010). Bajo esta hipótesis se espera que la superfetación se vea favorecida en ambientes donde la reproducción sea costosa, como en ambientes inestables con baja disponibilidad en los recursos alimenticios (Downhower y Brown, 1975; Zúñiga-Vega et al. 2010).

Hipótesis 2. La superfetación resulta de una restricción morfológica.

No solo un ambiente con limitados recursos de comida puede producir altos costos en la reproducción (Zúñiga-Vega et al, 2010). En peces vivíparos, incrementar la asignación reproductora (i.e. la proporción de la masa de la hembra utilizada en el desarrollo de las crías), podría hacer a las hembras más vulnerables a depredadores, ya que el desarrollo interno de múltiples embriones impone una carga física que podría perjudicar el desempeño locomotor para escapar (Ghalambor et al. 2004). En ambientes acuosos con sistemas fluviales de alta velocidad, los peces con fenotipos delgados y alargados se ven favorecidos (Langerhans, 2008). En peces vivíparos un incremento en el volumen del cuerpo asociado a una inversión alta en la producción de crías podría implicar un costo alto (Zúñiga-Vega et al, 2010). Reduciendo el tamaño de ovarios y el número de embriones (i.e. superfetación) se puede mantener un fenotipo más hidrodinámico (Thibault y Schultz, 1978). Bajo esta hipótesis se espera que la superfetación incremente bajo presiones que favorezcan un fenotipo hidrodinámico (más alargado y esbelto). Específicamente se espera más superfetación en ambientes con alta velocidad en el flujo del agua y/o que concurran con depredadores (Thibault y Schultz, 1978; Zúñiga-Vega et al. 2010).

Zúñiga-Vega et al. (2007) compararon dos poblaciones de *Poeciliopsis turrubarensis* en Costa Rica. Algunas poblaciones habitan en lugares con bajas corrientes y pendientes moderadas, mientras que otras habitan en ríos de rápido afluente y altas pendientes. Los autores encontraron que las hembras habitantes de afluentes rápidos muestran un mayor número de camadas en diferentes estadios de desarrollo y tienen un fenotipo más alargado y esbelto a comparación de las hembras que se encuentran en afluentes lentos.

Ventajas demográficas de la superfetación

Hipótesis 3. La superfetación disminuye la tasa de mortalidad juvenil.

La teoría de historias de vida predice que cuando la mortalidad de los jóvenes es altamente impredecible en el tiempo y en el espacio, una tasa de producción de crías baja y constante debe ser selectivamente ventajosa (Roff, 2002; Einum y Fleming, 2004). El riesgo de perder a toda la progenie en algún evento ambiental desfavorable se verá disminuido si se reparte la inversión reproductora total en pequeños eventos. Thibault (1974) propone que en peces pecílidos, cuando la supervivencia de las crías es muy variable e impredecible, distribuir la producción de crías a través del tiempo dará como resultado a largo plazo menores tasas de mortalidad juvenil. Las hembras superfetadoras pueden producir el mismo número de crías que una hembra no superfetadora, sin embargo, si la superfetación permite la distribución de las crías en pequeñas camadas a lo largo del tiempo, la superfetación puede verse como una estrategia del tipo “mejor apostador” (i.e. esfuerzo reproductivo bajo y dispersado a través del tiempo; Zúñiga-Vega et al. 2010).

Bajo esta hipótesis se puede esperar que el nivel de superfetación sea mayor en poblaciones con tasas estocásticas de supervivencia juvenil en comparación con poblaciones que posean tasas constantes de supervivencia juvenil. Dicho de otro modo, la superfetación incrementará conforme la variabilidad en la mortalidad juvenil aumente (Thibault, 1974; Zúñiga-Vega et al. 2010).

Hipótesis 4. La superfetación incrementará en tanto disminuya la mortalidad en adultos.

Esta hipótesis reúne la teoría de historias de vida de iteroparidad y la hipótesis 1 anteriormente descrita. La teoría de historias de vida predice que la iteroparidad (capacidad de reproducirse varias veces en la vida) es favorecida cuando se experimentan tasas de mortalidad adulta bajas o constantes (Stearns, 1992; Roff, 2002). Las hembras superfetadoras distribuyen la demanda energética asignada a la producción de crías a través del tiempo (hipótesis 1; Downhower y Brown, 1975). Como consecuencia, presentarán costos reproductivos menores y, por lo tanto, una mayor probabilidad de sobrevivir y serán más iteróparas (Zúñiga-Vega et al. 2010). Bajo esta hipótesis la superfetación debe de verse favorecida en poblaciones en las que sea ventajoso ser longevo e iteróparo. Es decir, en poblaciones con tasas de supervivencia adulta alta o constante, en comparación de las poblaciones con tasas bajas o estocásticas de supervivencia adulta. Dicho de otro modo, la superfetación incrementará a medida que aumente la supervivencia adulta (Downhower y Brown, 1975; Zúñiga-Vega et al. 2010).

Hipótesis 5. La superfetación incrementa la tasa de producción de crías.

En aves se ha sugerido que la superposición de huevos (i.e. superfetación) podría resultar en tasas reproductoras más altas (Burley, 1980). En el mismo periodo de tiempo, las aves que superponen huevos a diferencia de las que no lo hacen, presentan un incremento en el número de polluelos que son criados, y además la energía total necesaria para criar a toda la descendencia es menor (Burley, 1980). Por ejemplo, Burley (1980) asume que una hembra que no exhiba superposición de puestas tendría el tiempo suficiente durante una temporada reproductora para criar, digamos, dos puestas de cuatro crías cada una. En este esfuerzo parental consumiría toda su energía. Alternativamente, una hembra que superponga sus puestas, podría criar tres puestas de tres polluelos en el mismo periodo de tiempo y gastar menor cantidad de energía. La última hembra habrá producido 9 crías en lugar de ocho, durante el mismo periodo de tiempo. Esta hipótesis considera a la superposición de huevos (i.e. superfetación) como eventos reproductores separados (en el ejemplo anterior cada puesta representa un evento reproductor por separado), al contrario de la hipótesis 1 en la cual se asume que la superfetación (i.e., superposición de huevos) es un esquema de redistribución de un solo evento reproductor pero en componentes pequeños y dispersos temporalmente (Zúñiga-Vega et al. 2010).

En síntesis, la superfetación, a través de la superposición de diferentes puestas, podría acelerar las tasas de producción de crías, bajo el costo potencial de perder futuras oportunidades reproductoras. De acuerdo con la teoría de historias de vida (Stearns, 1992), tasas altas de fecundidad deben favorecerse cuando las oportunidades de sobrevivir de los adultos son limitadas. Por lo tanto, si la superfetación aumenta el número de crías que son producidas, puede compensar el alto riesgo de morir de los padres y se vería favorecida en ambientes en los que las probabilidades de sobrevivir de los adultos son bajas o inciertas (Roff, 1992; Reznick et al. 1996). Bajo esta hipótesis ambientes o temporadas con supervivencia adulta baja o estocástica deben de estar asociados a mayores niveles de superfetación en comparación con los ambientes o temporadas con una supervivencia adulta estable (Zúñiga-Vega et al. 2010). Esta hipótesis discrepa con la hipótesis 4, en que la superfetación se verá favorecida en poblaciones con tasas de supervivencia adulta altas y constantes, en comparación de las poblaciones con tasas bajas o estocásticas de supervivencia adulta . Tomando en cuenta que bajo la hipótesis 4, los costos reproductivos de las hembras superfetadoras serán menores y con ello tendrán mayor posibilidad de sobrevivir (hipótesis 1).

Evolución de la superfetación para asegurar la viabilidad genética de la descendencia

Hipótesis 6.- La superfetación incrementa el control femenino sobre la paternidad.

Macías-García y González-Zuarth (2005) proponen que el conflicto sexual en peces puede causar la superfetación. Como consecuencia de una inversión reproductora asimétrica entre sexos, las hembras son seleccionadas para maximizar la calidad de sus parejas reproductoras (Sutherland y De Jong, 1991; Becher y Magurran, 2004). Evidencia sugiere que una hembra puede valorar la calidad de un macho cuando éste produce y mantiene caracteres sexuales secundarios costosos, como lo son cortejos prolongados, ornamentos grandes y colores vistosos (Zahavi, 1975, 1977). En la mayoría de las especies de pecílidos los machos no exhiben cortejos y no necesitan la presencia de los caracteres sexuales secundarios costosos, para que se lleve a cabo el apareamiento (Farr, 1989; Macías-García y González-Zuarth, 2005), además en estas especies los machos poseen un gonopodio grande (aleta anal modificada para llevar a cabo la fertilización interna), utilizado para inseminar a las hembras sin su consentimiento (Farr, 1989; Zúñiga-Vega et al. 2010). La imposibilidad de las hembras de valorar la calidad de los machos que las inseminan, podría ocasionar que éstas terminen cargando una camada de crías de una pareja de baja calidad (Macías-García y González-Zuarth, 2005). Estas hembras que no pueden controlar la calidad genética de sus crías, pueden promover la diversidad genética de sus camadas apareándose con diferentes machos y asignando un esfuerzo reproductor bajo a las camadas de cada una de sus parejas reproductoras (Eberhard, 1996; Macías-García y González-Zuarth, 2005, Pollux et al. 2014).

Bajo este escenario las hembras podrían utilizar la superfetación como un mecanismo que les permita disminuir el costo causado por la imposibilidad de seleccionar machos de calidad (baja calidad genética en crías), incrementando la variabilidad genética y asignando un esfuerzo reproductivo bajo a las camadas de cada pareja reproductora (Macías-García y González-Zuarth, 2005; Pollux et al. 2014). En otras palabras, si cada camada es fertilizada por un macho distinto, la superfetación podría ser un mecanismo para incrementar la variación de la progenie, al separarla en pequeños eventos de distintos padres. En consecuencia las hembras recuperan el control de la paternidad de sus crías. Bajo esta hipótesis la superfetación debe de verse favorecida en las hembras que tienen poco control en el apareamiento y que están imposibilitadas para valorar la calidad genética de los padres de sus crías (Zúñiga-Vega et al. 2010).

En el presente trabajo de tesis doctoral se da a conocer la variación intra e inter especifica de la superfetación en diferentes poblaciones de *Poeciliopsis gracilis* y *P. infans,* dos especies de pecílidos de amplia distribución en el territorio mexicano. Asimismo, se pretende poner a prueba dos de las seis hipótesis que intentan explicar el origen y el significado adaptativo de la superfetación. Las hipótesis que se pusieron a prueba en este trabajo de investigación son: (1) la superfetación disminuye el costo pico de la reproducción, y (2) la superfetación resulta de una restricción morfológica. Para poner a prueba las predicciones derivadas de estas dos hipótesis, se utilizó herramientas de análisis morfológico y estadísticas. A continuación se presentan en tres capítulos separados los resultados obtenidos de este trabajo de investigación.

BIBLIOGRAFÍA DEL CAPÍTULO I

Barnet SA, Munro KMH (1970) Superfoetation of mice. Nature 227:1343-1344.

Becher SA, Magurran AE (2004). Multiple mating and reproductive skew in Trinidadian guppies. Proceedings of the Royal Society B 271:1009-1014.

Burley N (1980) Clutch overlap and clutch size: alternative and complementary reproductive tactics. American Naturalist 115:223-246.

Burton C (2002) Microsatellite analysis of multiple paternity and male reproductive success in the promiscuous snowshoe hare. Canadian Journal of Zoology 80:1948-1956.

De la Vega-Salazar MY (2003) Situación de los Peces dulceacuíocolas en México. Ciencias 72:20-30.

Dorn A, Greven H (2007) Some observations on courtship and mating of *Hemirhamphodon tengah* Anderson & Collette, 1991 (Zenarchopteridae). Bulletin of Fish Biology 9:99-104.

Downhower JF, Brown L (1975) Superfoetation in fishes and the cost of reproduction. Nature 256:345.

Downhower JF, Brown LP, Matsui ML (2002) Litter overlap in *Gambusia hubbsi*: superfetation revisited. Enviromental Biology of Fishes 65:423-430.

Einum S, Fleming IA (2004) Enviromental unpredictability and offspring size: conservative versus diversified bet-hedging. Evolutionary Ecology Research 6:443-455.

Eberhard WG (1996) Female control: sexual selection by cryptic female choice. Princeton: Princeton University Press. p 501.

Farr JA (1989) Sexual selection and secondary sexual differentiation in poeciliids: determinants of male mating success and the evolution of female choice. En: Meffe GK., Snelson Jr. FF. (Editores). Ecology and Evolution of livebearing fishes (Poeciliidae). Pretice Hall, Englewood Cliffs, New Jersey. 91-123 pp.

Ghalambor CK, Reznick DN, Walker JA (2004) Constraints of adaptative evolution: the functional trade-off between reproduction and fast-start swimming performance in Trinidad guppy (*Poecilia reticulata*). The American Naturalist 164:38-50.

Gómez-Márquez JL, Peña-Mendoza B, Salgado-Ugarte IH, Sánchez-Herrera AK, Sastré-Baez L (2008) Reproduction of the fish *Poeciliopsis gracilis* (Cyprinodontiformes: Poeciliidae) in Coatetelco, a tropical shallow lake in Mexico. Revista de Biología Tropical 56:1801-1812.

Gunn JS, Thresher RE (1991) Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Environmental Biology of Fishes 31: 323-344.

Ghedotti MJ (2000) Phylogenetic analysis and taxonomy of the poecilioid fishes (Teleostei:Cyprinodontiformes). Zoological Journal of the Linnean Society 130:1-53.

Hill WE (1986) Clutch overlap in American coots. Condor 88:96-97

Hetmanski T, Wolk E (2005) The effect of environmental factors and nesting conditions on clutch overlap in the Federal Pigeon *Columba livia* f. urbana (Gm). Polish Journal of Ecology 53:523-534.

Hrbek T, Seckinger J, Meyer A (2007) A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Molecualr Phylogenetics and Evolution 43:986-998.

Langerhans RB (2008) Predactibility of phenotypic diferentation across flow regimes in fishes. Integrative and Comparative Biology 48: 750–768.

Lantieri T, Revelli A, Gaglioti P, Menato G, Gennarelli G, Delle Piane L, Massobrio M (2010) Superfetation after ovulation induction and intrauterine insemination performed during an unknown ectopic pregnancy. Reproductive BioMedicice Online 20:664-666.

Lovejoy NR, Iranpour M, Collette BB (2004) Phylogeny and jaw ontogeny of beloniform fishes. Integrative & Comparative Biology 44:366-377.

Lucinda PHF (2003) Family Poeciliidae. En: Reis RE., Kullander SO., Ferraris CJ. (Editores). Check list of the freshwater fishes of South and Central America. EDIPUCRS, Porto Alegre, Brazil. 555-581 pp.

Lucinda PHF, Reis RE (2005) Systematics of the subfamily Poeciliinae Bonaparte (Cyprinodontiformes: Poeciliidae), with an emphasis on the tribe Cnesterodontini Hubbs. Neotropical Ichthyology 3:1-60.

Kennedy H (1978) Systematics and pollination of the “closed-flowered” species of Calathea (Marantaceae). University of California Publications in Botany 71:1-90.

Nelson JS (2006) Fishes of the world. 4th edition. NY: John Wiley & Sons, Inc. Hoboken, New Jerey, USA. 601pp.

Macías-García C, González-Zuarth CA (2005) Reproductive behavior of viviparous fish and intrasexual conflict. En. Uribe MC., Grier HJ. (Editores). Viviparous fishes. Homestead: New Life Publications. 289-302 pp.

Meisner AD, Burns JR (1997) Viviparity in the halfbeaks genera *Dermogenys* and *Nomorhamphus* (Teleostei: Hemiramphidae). Journal of morphology 234:295-317.

Miller RR, Mincley WL, Norris SM (2005) Freshwater fishes of Mexico. University of Chicago Press, Illinois. 490 pp.

Meredith RW, Pires MN, Reznick DN, Springer MS (2010) Molecular phylogenetic relationships and the evolution of the placenta in Poecilia (Micropoecilia) (Poeciliidae: Cyprinodontiformes). Molecular Phylogenetics and Evolution 55: 631–639.

Meredith RW, Pires MN, Reznick DN, Springer MS (2011) Molecular phylogenetics relationship and the coevolution of placentotrophy and superfetation in *Poecilia* (Poeciliidae: Cyprinodontiformes). Molecular Phylogenetics and Evolution 59:148-157.

Nelson JS (2006) Fishes of the world. Fourth edition. Hoboken, New Jersey. 601 pp.

Pape O, Winer N, Paumier A, Philippe HJ, Flatrès B, Boog G (2008) Superfetation: case report and review of the literature. Journal de Gynécologie, Obstétrique et Biologie de la Reproduction 37:791-795.

Paplinska JZ, Moyle RL, Temple-Smith PD, Renfree MB (2006) Reproduction in female swamp wallabies. Reproduction Fertility and Development 18:735-743.

Parenti LR (1981) A phylogenetic and biogeographic analysis of cyprinodontiform fishes (Teleostei, Atherinomorpha). Bulletin of the American Museum of Natural History 168: 335-557.

Pires MN, McBride KE, Reznick DN (2007) Interpopulation variation in life-history traits of *Poeciliopsis prolifica*: implications for the study of placental evolution. Journal of Experimental Zoology 307A:113-125.

Pires M, Arendt J, Reznick DN (2010) The evolution of placentas and superfetation in the fish genus Poecilia (Cyprinodontiformes: Poeciliidae: subgenera *Micropoeciliia* and *Acanthophacelus*). Biological Journal of the Linnean Society 99:784-796.

Pollux BJA, Pires MN, Banet AI, Reznick DN (2009) Evolution of placentas in the family Poeciliidae: an empirical study of macroevolution. The Annual Review of Ecology, Evolution, and Systematics 40:271-289.

Rasweiler JJ IV (1982) The contribution of observations on early pregnancy on the little sac-winged bat, *Peropteryx kappleri* to an understanding of the evolution of reproductive mechanisms in monovular bats. Biology of reproduction 27:681-702.

Reznick DN, Miles DB (1989) Review of life history patterns in Poeciliid fish. En: Meffe GK., Snelson Jr. FF. (Editores). Ecology and Evolution of livebearing fishes (Poeciliidae). Pretice Hall, Englewood Cliffs, New Jersey. 125-148 pp.

Reznick DN, Butler MJ IV, Rodd FH, Ross P (1996) Life-history evolution in guppies (*Poecilia reticulate*) 6 Differential mortality as a mechanism for natural selection. Evolution 50:1651-1660.

Reznick D, Mateos M, Springer MS (2002) Independent origins and rapid evolution of the placenta in the fish genus *Poeciliiopsis*. Science 298: 1018-1020.

Reznick D, Meredith R, Collette BB (2007a) Independent evolution of complex life history adaptations in two failies of fishes, live-bearing halfbeaks (Zenarchopteridae, Beliformes) and Pociliidae (Cyprinodontiformes). Evolution 61:2570-2583.

Reznick D, Hrbek T, Caura S, De Greef J, Roff D (2007b) Life history of *Xenodexia ctenolepis* implications for life history evolution in the family Poeciliidae. Biological Journal of the Linnean Society 92:77-85.

Roellig K, Menzies BR, Hildebrandt TB, Goeritz F (2011) The concept of superfetation: a critical review on a ‘myth’ in mammalian reproduction. Biological Reviews 86:77–95.

Roff D (1992) The evolution of life histories: theory and analysis. New York: Chapman and Hall.

Roff D (2002) Life history evolution. Sunderland, Massachusetts USA. Sinauer Associates.

Rollhäuser H (1949) Superfetation in mouse. The Anatomical Record 105:657-663.

Rosen DE (1964) The relationships and taxonomic position of the halfbeaks, killifishes, silversides, and their relatives. Bulletin of the American Museum of Natural History 127:217-268.

Scrimshaw NS (1944) Superfetation in poeciliid fishes. Copeia 1944:180-183.

Stearns SC (1992) The evolution of life histories. Oxford University Press. 249pp

Springer VG (1970) The western south Atlantic clinid fish *Ribeiroclinus eigenmanni*, with discussion of the intrarelationships and zoogeography of the Clinidae. Copeia 3:430-436.

Sutherland WJ, De Jong MCM (1991) The evolutionarily stable strategy for secondary sexual characters. Behavioral Ecology 2:16-20.

Thibault RE (1974) Genetics of cannibalism in a viviparous fish and its relationship to population density. Nature 251:138-140.

Thibault RE, Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprynodontiformes: Poeciliidae). Evolution 32:320-333.

Travis J, Farr JA, Henrich S, Cheong RT (1987) Testing Theories of clutch overlap with the reproductive ecology of *Heterandria Formosa*. Ecology 68:611-623.

Turner CL (1937) Reproductive cycles and superfetation in poeciliid fishes. Biological Bulletin 72:145-164.

Yamaguchi N, Dugdale HL, MacDonald DW (2006) Female receptivity, embryonic diapause, and superfetation in the European badger (*Meles meles*) implications for the reproductive tactics of males and females. Quarterly Reviews of Biology 81:33-48.

Zahavi A (1975) Mate selection -a selection for a handicap. Journal of Theoretical Biology 53:205-214.

Zahavi A (1977) The cost of honesty (further remarks on the handicap principle). Journal of Theoretical Biology 67:603-605.

Zúñiga-Vega JJ, Reznick D, Johnson JB (2007) Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. Oikos 116:995-1005.

Zúñiga-Vega JJ, Macías-García C, Johnson JB (2010) Hypotheses to explain the evolution of superfetation in viviparous fishes. En: Uribe MC., Grier HJ. (Editores). Viviparous Fishes II. New life publications, Homestead, Florida. 13-30 pp.

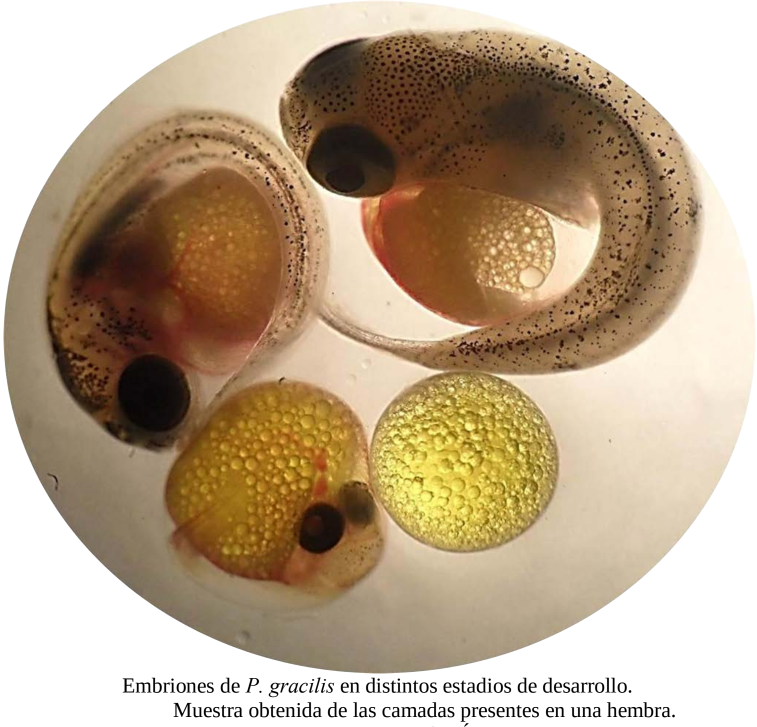
CAPÍTULO II

ARTÍCULO PUBLICADO

"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 Garcia, Luis F. Vázquez-Vega, J. Jaime Zúñiga-Vega.

Naturwissenschaften 101:1085–1098.
DOI 10.1007/s00114-014-1247-2



Embriones de *P. gracilis* en distintos estadios de desarrollo.
Muestra obtenida de las camadas presentes en una hembra.
Foto: Patricia Frías Álvarez.

Naturwissenschaften (2014) 101:1085–1098
DOI 10.1007/s00114-014-1247-2

ORIGINAL PAPER

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 Garcia · 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

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: pfriasalvarez@ciencias.unam.mx

C. Macías Garcia
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-amenobenzoic 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

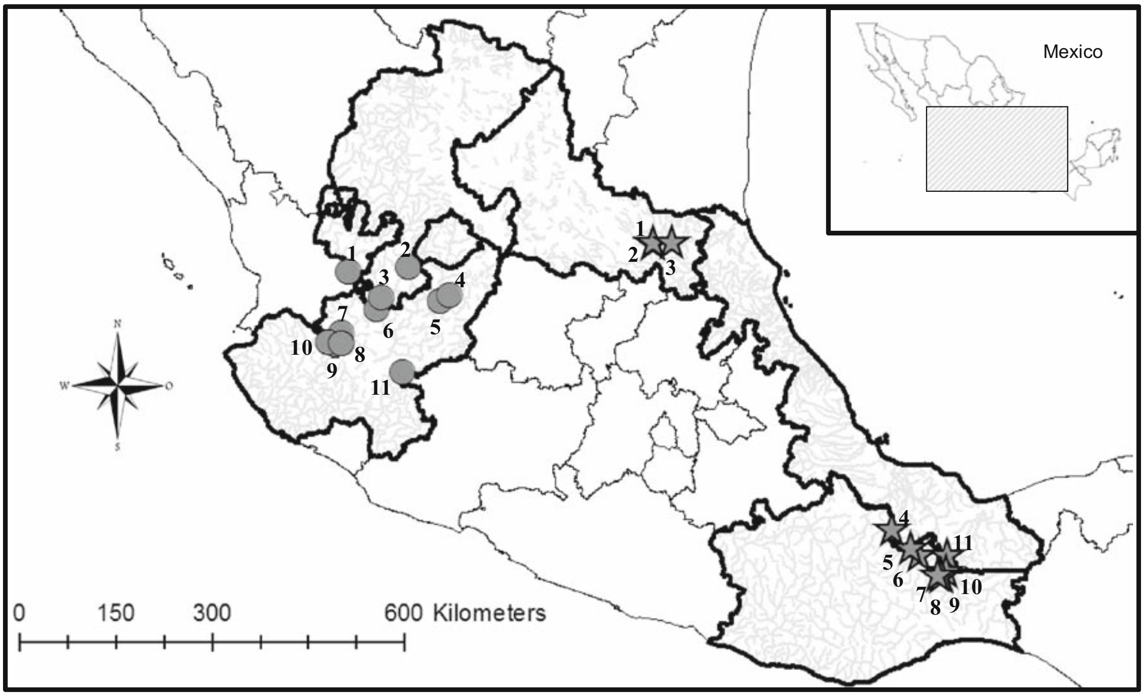


Fig. 1 Sampled populations of *Poeciliopsis gracilis* (*stars*) and *P. infans* (*circles*). *Black lines* correspond to state boundaries; the *thickest lines* indicate the Mexican states where samples were conducted. *Grey lines* represent rivers

Table 1 Sampling locations of *Poeciliopsis 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
							P. gracilis					
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	–	32/31/97	–	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	–
							P. infans					
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 Potosi, 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, ±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±SE= 1.9±0.02; range 1–4; *P. infans*, 1.8±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±SE) 1.46±0.12 broods per female in population 8 to 2.14±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±0.11 broods in population 8 to 1.97±0.10 broods in population 2 (Fig. 3a). Female dry mass covaried positively with superfoetation (*P. gracilis*, $\beta=3.44\pm0.66$; *P. infans*, $\beta=14.65\pm2.51$).

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±0.25) was found in June among females from population 8, whereas the highest occurred at population 6 in November (2.55±

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 η^2*
Poeciliopsis gracilis						
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 × 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 × 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 × 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 × month (population)	10.31	6	1.72	5.19	<0.0001	0.03
Error	284.82	861	0.33			
Poeciliopsis infans						
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 × 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 × 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 × 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 × 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"

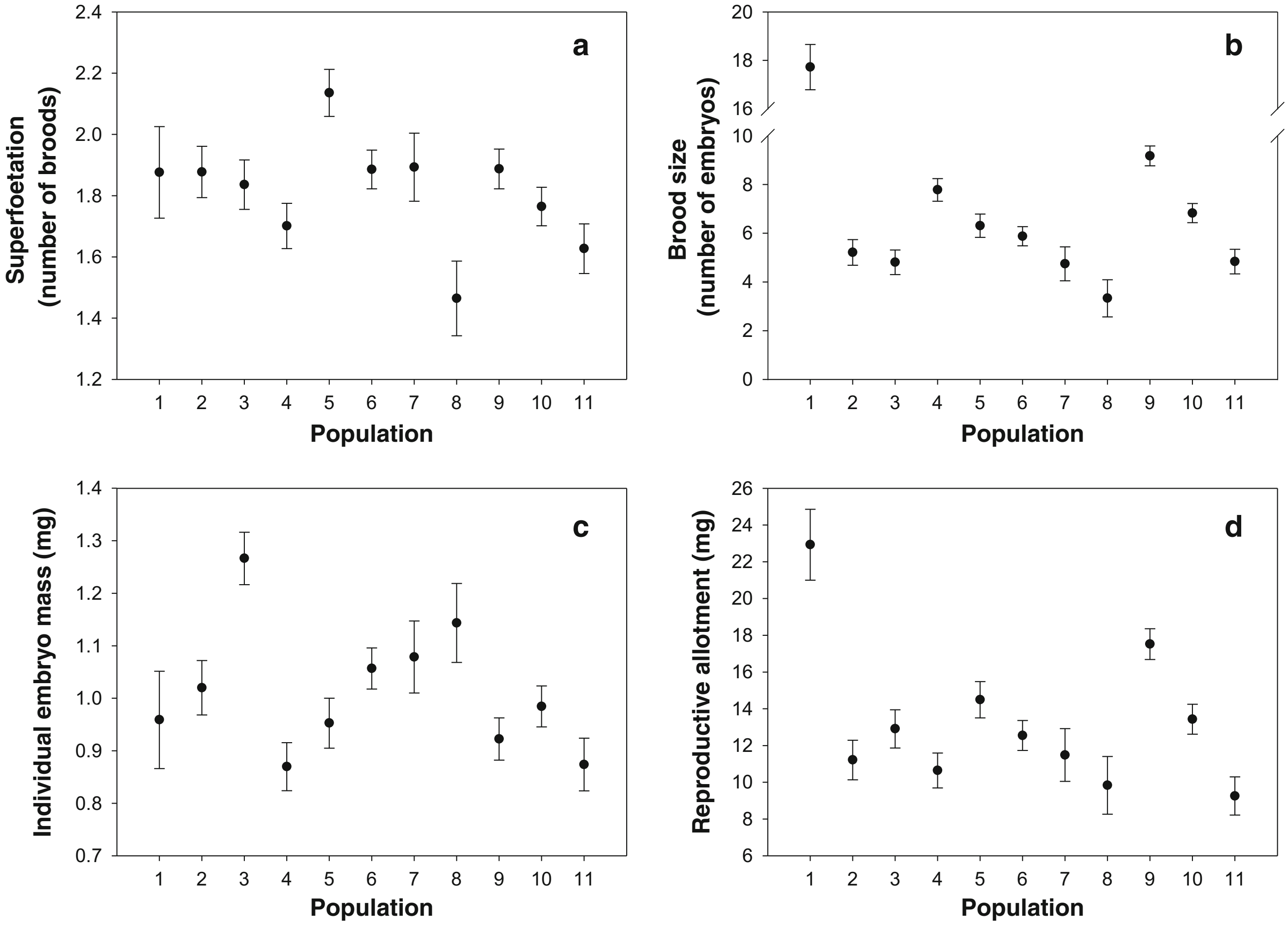


Fig. 2 Interpopulation variation in superfoetation and life history traits of *Poeciliopsis gracilis*. Population means were adjusted for female mass (all traits) and stage of development (individual embryo mass). **a** Superfoetation, **b** brood size, **c** individual embryo mass, **d** reproductive allotment. Population numbers as in Table 1. *Error bars* represent ±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 be associated with seasons or with rainfall patterns (Fig. 4). The smallest number of simultaneous broods (1.02±0.16) was found in January among females from population 11, whereas the highest occurred at population 10 in September (2.50±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, $\beta=32.91\pm4.14$; individual embryo mass, $\beta=0.003\pm0.0004$; RA, $\beta=0.21\pm0.01$). Females from population 8 produced the smallest broods (means adjusted for female mass±SE 3.33±0.76 embryos), whereas females from population 1 had the largest (17.72±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±0.05 mg in population 4 and 1.27±0.05 mg in population 3 (Fig. 2c). RA ranged between (means adjusted for female mass) 9.26±1.04 mg in population 11 and 22.93±1.93 mg in population 1, again due to the presence of those three very large females (Fig. 2d).

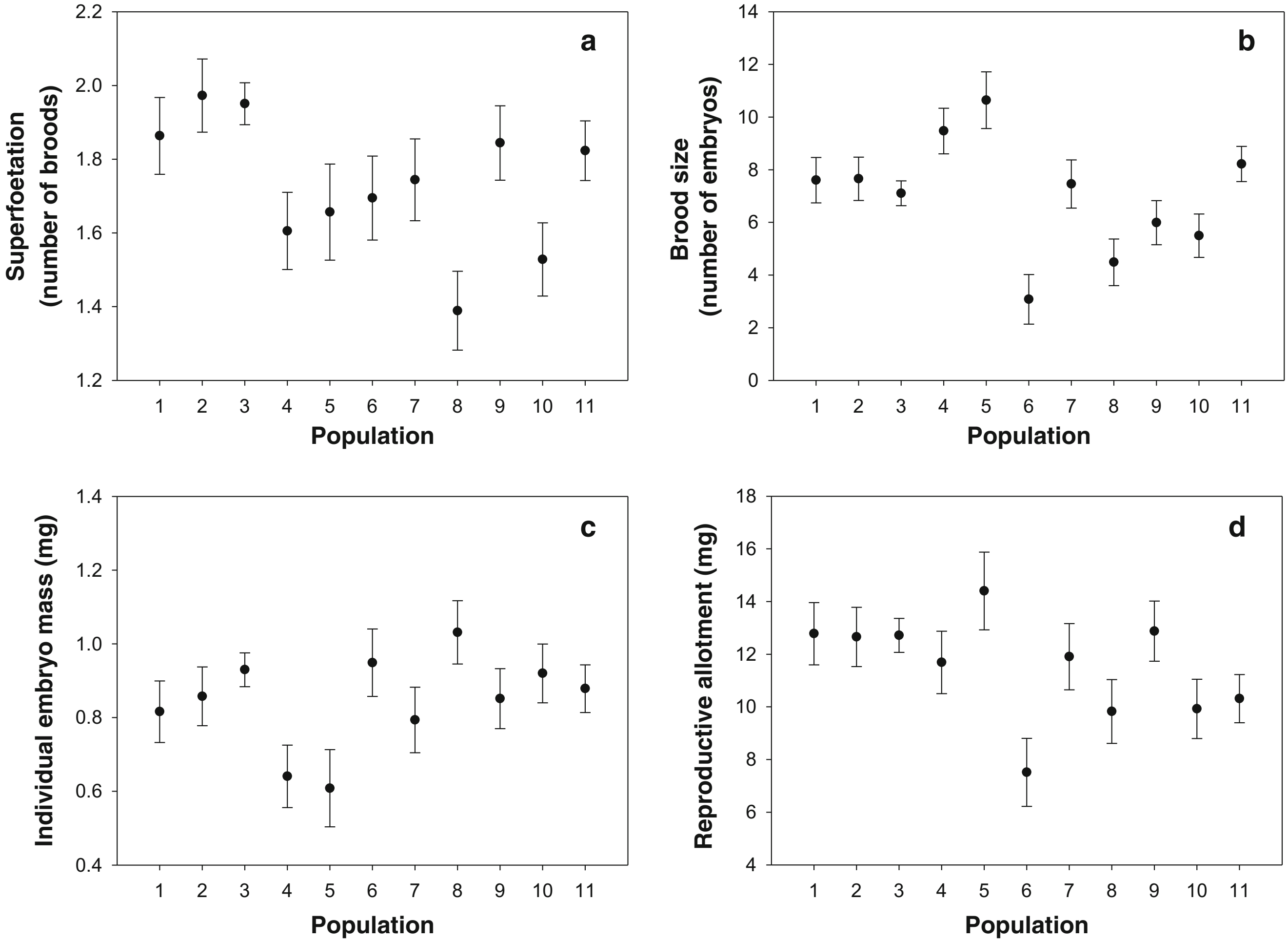


Fig. 3 Interpopulation variation in superfoetation and life history traits of *Poeciliopsis infans*. Population means were adjusted for female mass (all traits) and stage of development (individual embryo mass). **a** Superfoetation, **b** brood size, **c** individual embryo mass, **d** reproductive allotment. Population numbers as in Table 1. *Error bars* represent ±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, $\beta=92.28\pm20.73$; individual embryo mass, $\beta=0.002\pm0.0002$; RA, $\beta=0.23\pm0.03$). Variation in brood size among populations ranged between 3.08±0.94 embryos in population 6 and 10.64±1.08 embryos in population 5 (Fig. 3b), whereas embryo mass ranged from 0.61±0.11 mg in population 5 to 1.03±0.09 mg in population 8 (Fig. 3c). Females from population 6 made the smallest RA (7.51±1.29 mg), whereas those from population 5 made the largest (14.40±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 × 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.

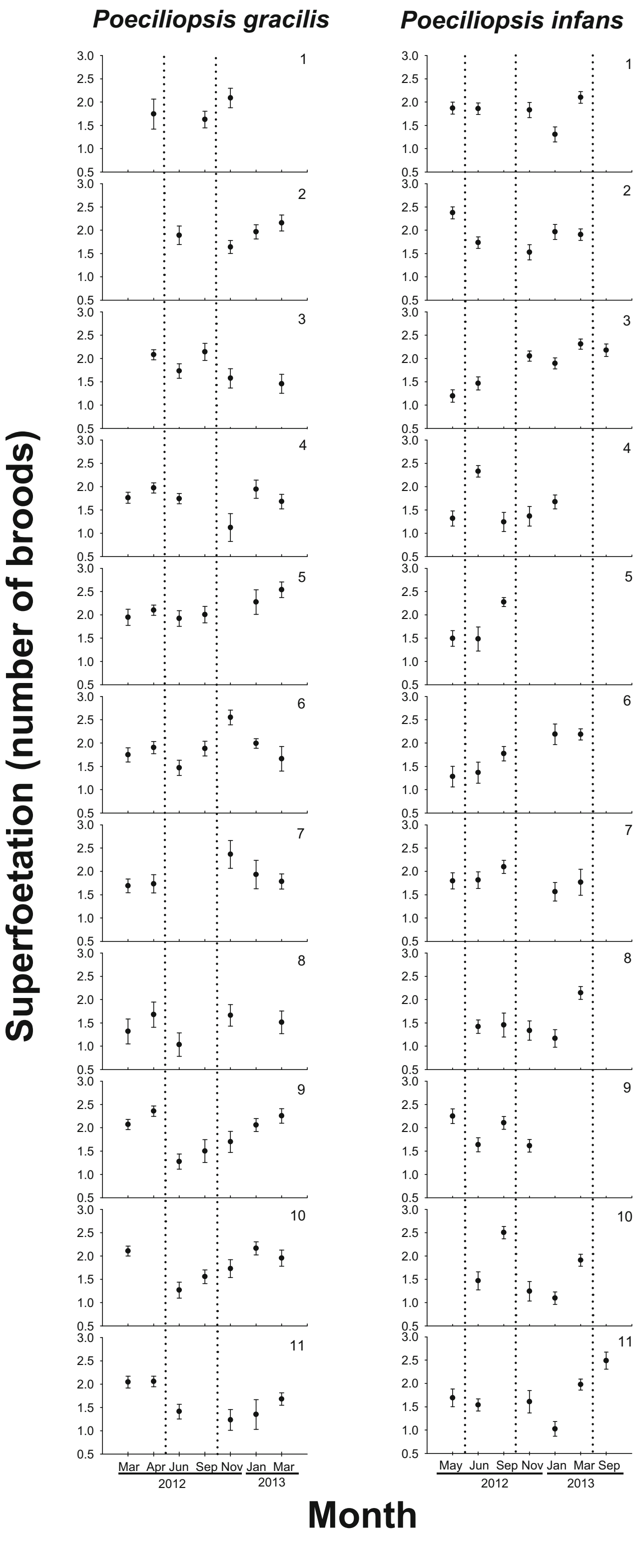


Fig. 4 Temporal variation in superfoetation of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass. Population numbers are shown at the *top right corner of each panel. Dotted lines* indicate rainy months. *Error bars* represent ±1 SE. Sampled months for *P. gracilis* 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

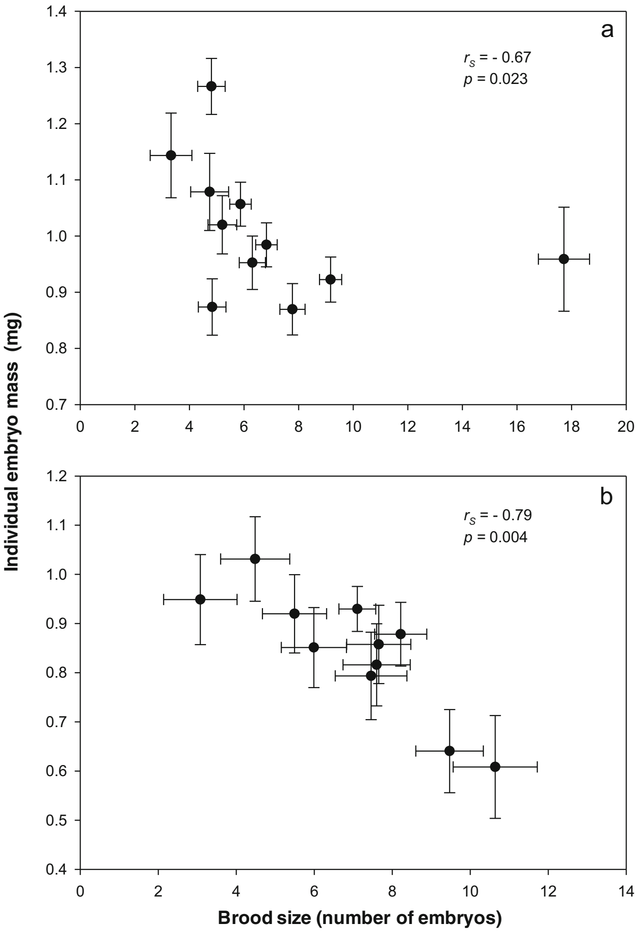


Fig. 5 Relationships between brood size and individual embryo mass for **a** *Poeciliopsis gracilis* and **b** *P. infans*. Data points represent average values per population adjusted for female mass (both traits) and stage of development (individual embryo mass). *Error bars* represent ±1 SE

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

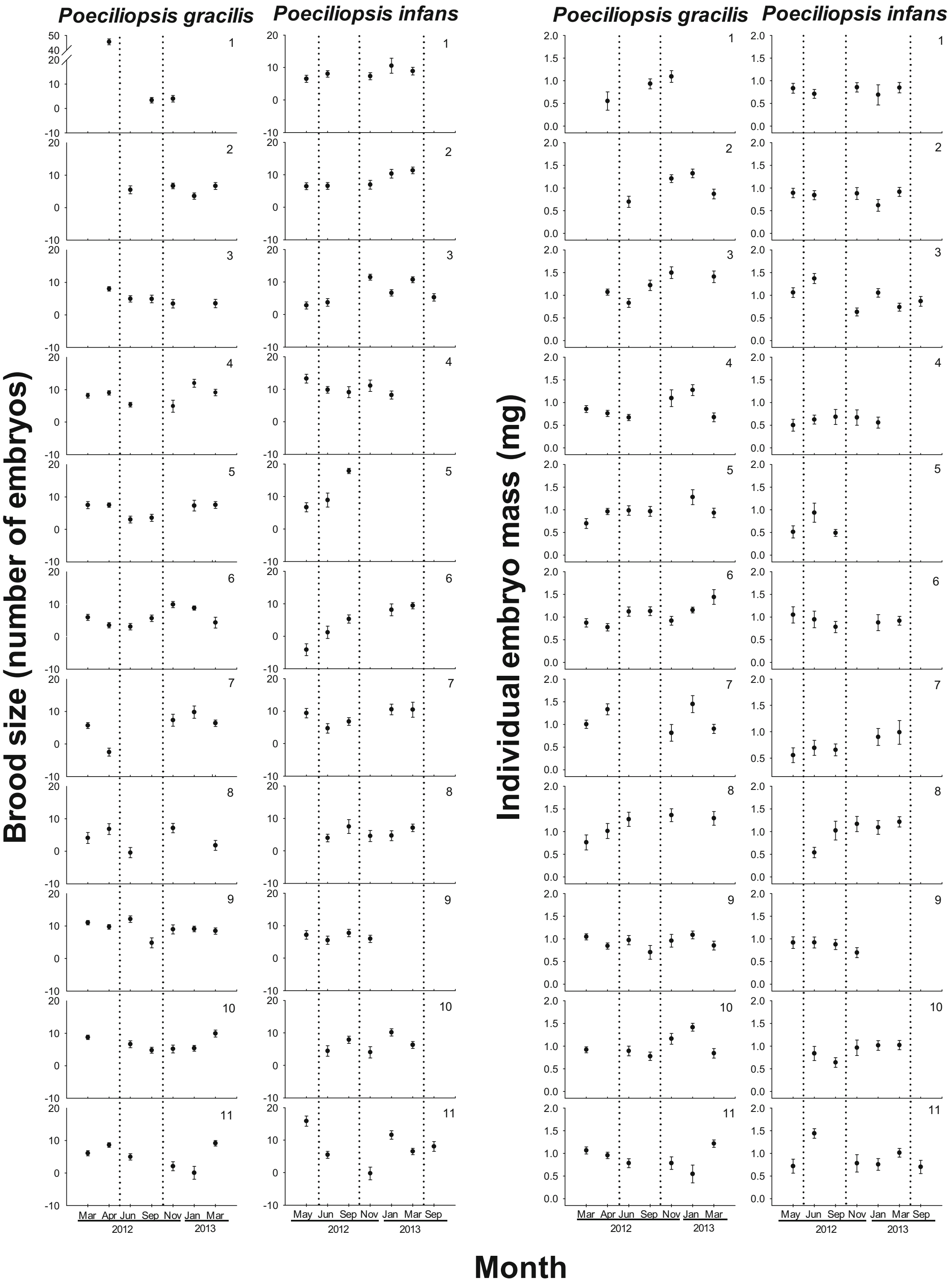


Fig. 6 Temporal variation in brood size and individual embryo mass of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass (both traits) and stage of development (individual embryo mass). Population numbers are shown at *the top right corner of each panel. Dotted lines*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

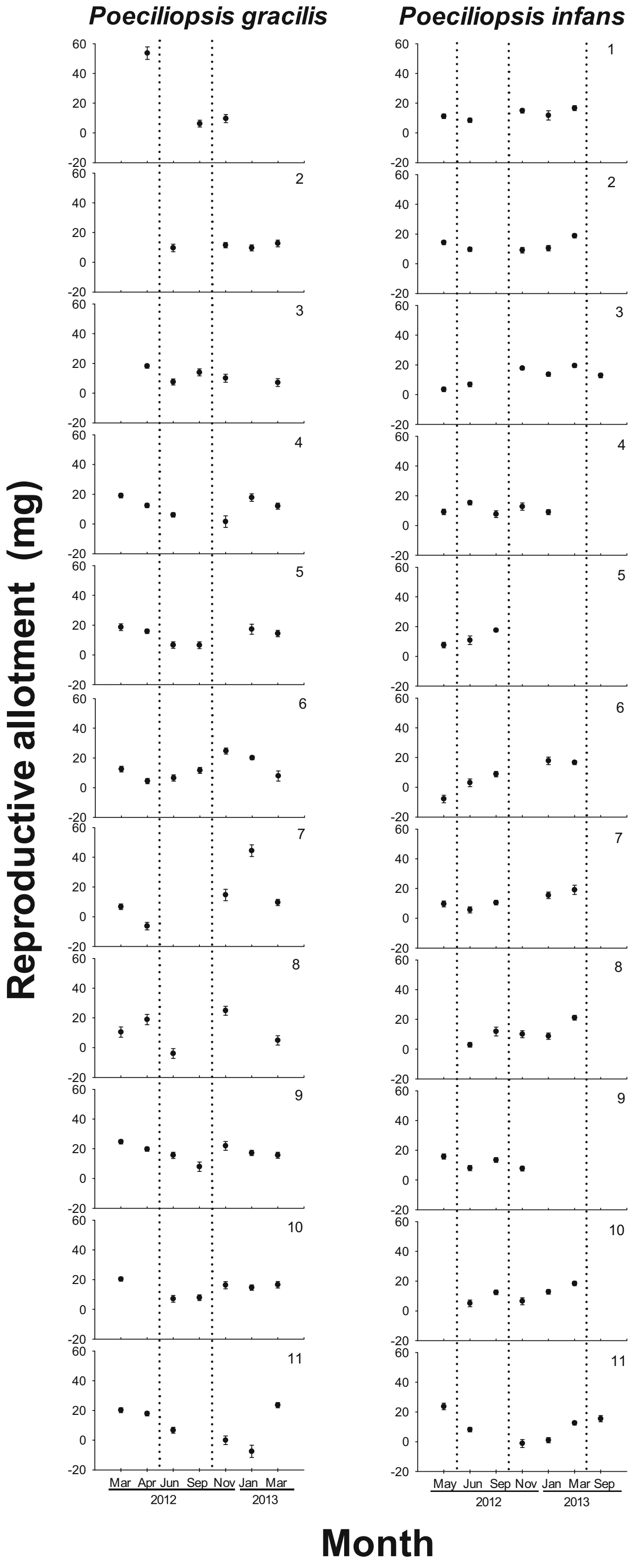


Fig. 7 Temporal variation in reproductive allotment of *Poeciliopsis gracilis* and *P. infans*. Monthly means were adjusted for female mass. Population numbers are shown at *the top right corner of each panel. Dotted lines* indicate rainy months. *Error bars* represent ±1 SE. *Non-visible error bars* 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 × 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. Muñiz-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

References

Allan JD, Castillo MM (2007) Stream ecology. Structure and function of running waters, 2nd edn. Spring, The Netherlands

Aubin-Horth N, Renn SCP (2009) Genomic reaction norms: using integrative biology to understand molecular mechanisms of phenotypic plasticity. Mol Ecol 18:3763–3780. doi:10.1111/j.1365-294X.2009.04313.x

Burley N (1980) Clutch overlap and clutch size: alternative and complementary reproductive tactics. Am Nat 115:223–246

Carey JR, Judge DS (2000) Longevity records: life spans of mammals, birds, amphibians, reptiles, and fish. Odense University Press, Odense Denmark

Charnov EL, Downhower JF, Brown LP (1995) Optimal offspring sizes in small litters. Evol Ecol 9:57–6.3. doi:10.1007/BF01237697

Downhower JF, Brown L (1975) Superfoetation in fishes and the cost of reproduction. Nature 256:345. doi:10.1038/256345a0

Galindo-Villegas J, Sosa-Lima E (2002) Gonopodial system review and a new fish record of *Poeciliopsis infans* (Cyprinodontiformes: Poeciliidae) for Lake Patzcuaro, Michoacan, central Mexico. Rev Biol Trop 50:1151–1157

Gómez-Márquez JL, Peña-Mendoza B, Salgado-Ugarte IH, Sánchez-Herrera AK, Sastré-Baez L (2008) Reproduction of the fish *Poeciliopsis gracilis* (Cyprinodontiformes: Poeciliidae) in Coatetelco, a tropical shallow lake in Mexico. Rev Biol Trop 56: 1801–1812

Green BS (2008) Maternal effects in fish populations. Adv Mar Biol 54: 1–105. doi:10.1016/S0065-2881(08)00001-1

Gunn JS, Thresher RE (1991) Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Environ Biol Fish 31:323–344. doi:10.1007/BF00002357

Gutiérrez-Cabrera AE, Pulido-Flores G, Monks S, Gaytán-Oyarzún JC (2005) Presencia de *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoidea: Bothriocephalidae) en peces de Metztitlán, Hidalgo, México. Hidrobiológica 15:283–288

Haynes JL (1995) Standardized classification of poeciliid development for life-history studies. Copeia 1995:147–154. doi:10.2307/1446809

Jennions M, Telford S (2002) Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. Oecologia 132:44–50. doi:10.1007/s00442-002-0942-4

Johnson JB, Bagley JC (2011) Ecological drivers of life-history divergence. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, pp 38–49

Karayucel I, Orhan AK, Karayucel S (2008) Effect of temperature on some reproductive parameters of gravid females and growth of newly hatched fry in guppy, *Poecilia reticulta* (Peters, 1860). J Anim Vet Adv 7:1261–1266

Kennedy H (1978) Systematics and pollination of the “closed-flowered” species of Calathea (Marantaceae). Univ Calif Publ Bot 71:1–90

Lantieri T, Revelli A, Gaglioti P, Menato G, Gennarelli G, Delle Piane L, Massobrio M (2010) Superfetation after ovulation induction and intrauterine insemination performed during an unknown ectopic pregnancy. Reprod Biomed Online 20:664–666. doi:10.1016/j.rbmo.2010.01.017

Leips J, Travis J (1999) The comparative expression of life-history traits and its relationship to the numerical dynamics of four populations of the least killifish. J Anim Ecol 68:595–616. doi:10.1046/j.1365-2656.1999.00311.x

Mateos M, Sanjur OI, Vrijenhoek RC (2002) Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). Evolution 56:972–984. doi:10.1111/j.0014-3820.2002.tb01409.x

McManus MG, Travis J (1998) Effects of temperature and salinity on the life history of the sailfin molly (Pisces: Poeciliidae): lipid storage and reproductive allocation. Oecologia 114:317–325. doi:10.1007/s004420050453

Meredith RW, Pires MN, Reznick DN, Springer MS (2011) Molecular phylogenetic relationships and the coevolution of placentotrophy and superfetation in *Poecilia* (Poeciliidae: Cyprinodontiformes). Mol Phylogenet Evol 59:148–157. doi:10.1016/j.ympev.2011.01.014

Miller RR, Mincley WL, Norris SM (2005) Freshwater fishes of Mexico. University of Chicago Press, Illinois

Molina-Moctezuma A (2011) Influencia de la depredación sobre las características de historias de vida y la dinámica poblacional de *Poeciliopsis baenschi.*. Dissertation, Facultad de Ciencias Universidad Nacional Autónoma de México

Moss BR (2013) Ecology of fresh waters: a view for the twenty-first century, 4th edn. Wiley-Blackwell, Oxford

Pape O, Winer N, Paumier A, Philippe HJ, Flatrès B, Boog G (2008) Superfetation: case report and review of the literature. J Gynecol Obstet Biol Reprod 37:791–795. doi:10.1016/j.jgyn.2008.06.004

Pires MN, McBride KE, Reznick DN (2007) Interpopulation variation in life-history traits of *Poeciliopsis prolifica*: implications for the study of placental evolution. J Exp Zool 307A:113–125. doi:10.1002/jez.a.356

Pires MN, Arendt J, Reznick DN (2010) The evolution of placentas and superfetation in the fish genus *Poecilia* (Cyprinodontiformes: Poeciliidae: subgenera *Micropoecilia* and *Acanthophacelus*). Biol J Linn Soc 99:784–796. doi:10.1111/j.1095-8312.2010.01391.x

Pires MN, Banet AI, Pollux BJA, Reznick DN (2011) Variation and evolution of reproductive strategies. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of Poeciliid fishes. The University of Chicago Press, Chicago, pp 28–37

Plath M, Hermann B, Schröder C, Riesch R, Tobler M, García de León FJ, Schlupp I, Tiedemann R (2010) Locally adapted fish populations maintain small-scale genetic differentiation despite perturbation by a catastrophic flood event. BMC Evol Biol 10:256. doi:10.1186/1471-2148-10-256

Pollux BJA, Pires MN, Banet AI, Reznick DN (2009) Evolution of placentas in the family Poeciliidae: an empirical study of macroevolution. Annu Rev Ecol Evol Syst 40:271–289. doi:10.1146/annurev.ecolsys.110308.120209

Pollux BJA, Meredith RW, Springer MS, Reznick DN (2014) The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature 513:233–236. doi:10.1038/nature13451

Reznick DN, Endler JA (1982) The impact of predation on life history evolution in trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177. doi:10.2307/2407978

Reznick DN, Miles DB (1989) Review of life history patterns in Poeciliid fish. In: Meffe GK, Snelson FF Jr (eds) Ecology and evolution of livebearing fishes (Poeciliidae). Pretice Hall, Englewood Cliffs, pp 125–148

Reznick DN, Yang AP (1993) The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. Ecology 74:2011–2019. doi:10.2307/1940844

Reznick DN, Miles BD, Winslow S (1992) Life History of *Poecilia picta* (Poeciliidae) from the Island of Trinidad. Copeia 1992:782–790

Reznick DN, Mateos M, Springer MS (2002) Independent origins and rapid evolution of the placenta in the fish genus *Poeciliiopsis*. Science 298:1018–1020. doi:10.1126/science.1076018

Reznick DN, Meredith R, Collette BB (2007) Independent evolution of complex life history adaptations in two families of fishes, livebearing halfbeaks (Zenarchopteridae, Beliformes) and Pociliidae (Cyprinodontiformes). Evolution 61:2570–2583. doi:10.1111/j.1558-5646.2007.00207.x

Riesch R, Plath M, García de Leon FJ, Schlupp I (2010) Convergent life-history shifts: toxic environments result in big babies in two clades of poeciliids. Naturwissenschaften 97:133–141. doi:10.1007/s00114-009-0613-y

Roellig K, Menzies BR, Hildebrandt TB, Goeritz F (2011) The concept of superfetation: a critical review on a 'myth'in mammalian reproduction. Biol Rev 86:77–95. doi:10.1111/j.1469-185X.2010.00135.x

Schrader M, Travis J (2009) Do embryos influence maternal investment? Evaluating maternal-fetal coadaptation and the potential for parent-offspring conflict in a placental fish. Evolution 63:2805–2815. doi:10.1111/j.1558-5646.2009.00763.x

Schrader M, Travis J (2012) Assessing the roles of population density and predation risk in the evolution of offspring size in populations of a placental fish. Ecol Evol 2:1480–1490. doi:10.1002/ece3.255

Scrimshaw NS (1944) Superfetation in poeciliid fishes. Copeia 1944:180–183

Sokal RR, Rohlf FJ (2012) Biometry, 4th edn. W.H. Freeman and Company, New York

Soucy S, Travis J (2003) Multiple paternity and population genetic structure in natural populations of the poeciliid fish, *Heterandria formosa*. J Evol Biol 16:1328–1336. doi:10.1046/j.1420-9101.2003.00608.x

Tacutu R, Craig T, Budovsky A., Wuttke D, Lehmann G, Taranukha D., Costa J, Fraifeld VE, de Magalhaes JP. (2013) Human Ageing Genomic Resources: Integrated databases and tools for the biology and genetics of ageing. Nucleic Acids Res gks:1155. doi: 10.1093/nar/gks1155

Thibault RE, Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprynodontiformes: Poeciliidae). Evolution 32:320–333

Travis J, Farr JA, Henrich S, Cheong RT (1987) Testing theories of clutch overlap with the reproductive ecology of *Heterandria formosa*. Ecology 68:611–623. doi:10.2307/1938466

Turner CL (1937) Reproductive cycles and superfetation in poeciliid fishes. Biol Bull 72:145–164

Vondracek B, Wurtsbaugh WA, Cech JJ (1988) Growth and reproduction of the mosquitofish, *Gambusia affinis*, in relation to temperature and ration level: consequences for life history. Environ Biol Fish 21:45–57. doi:10.1007/BF02984442

Wourms JP (1981) Viviparity: the maternal-fetal relationship in fishes. Am Zool 21:473–515

Zúñiga-Vega JJ, Reznick D, Johnson JB (2007) Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. Oikos 116:995–1005. doi:10.1111/j.0030-1299.2007.15763.x

Zúñiga-Vega JJ, Macias-Garcia C, Johnson JB (2010) Hypotheses to explain the evolution of superfetation in viviparous fishes. In: Urine MC, Grier HJ (eds) Viviparous fishes II. New Life Publications, Homestead, pp 241–253

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

Naturwissenschaften

Patricia Frías-Álvarez , Constantino Macías Garcia, Luis F. Vázquez-Vega and J. Jaime Zúñiga-Vega

Correspondence: pfriasalvarez@ciencias.unam.mx
Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad Universitaria, C.P. 04510, Distrito Federal, México.

Electronic Supplementary Material

Table S1 Spearman rank correlation coefficients (r_S) between superfoetation and physicochemical parameters of the rivers. p values are shown within parentheses.

	Ammonium mg/L	**Phosphorus mg/L**	**Nitrites mg/L**	**Nitrates mg/L**	**Phosphate mg/L**	**pH**	**Temperature °C**	**Salinity g/Kg (ppt)**	**Acidity mg/L (ppm) $CaCO_3$**
Poeciliopsis gracilis									
Superfoetation	0.39 (0.23)	-0.06 (0.85)	-0.04 (0.91)	0.21 (0.54)	0.12 (0.73)	0.14 (0.69)	0.06 (0.85)	-0.04 (0.89)	0.18 (0.59)
Poeciliopsis infans									
Superfoetation	-0.51 0.11)	-0.56 (0.07)	-0.28 (0.42)	-0.12 (0.73)	0.02 (0.96)	-0.04 (0.91)	0.22 (0.50)	0.38 (0.25)	- 0.08 (0.81)

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

Naturwissenschaften

Patricia Frías-Álvarez , Constantino Macías Garcia, Luis F. Vázquez-Vega and J. Jaime Zúñiga-Vega

Correspondence: pfriasalvarez@ciencias.unam.mx
Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México.
Ciudad Universitaria, C.P. 04510, Distrito Federal, México.

Electronic supplementary material

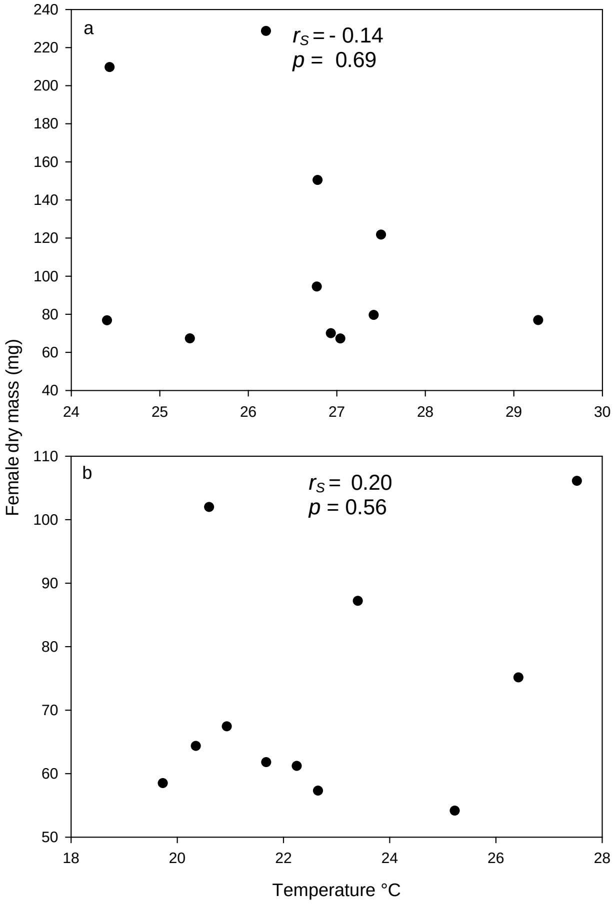


Fig. S1 Relationships between average temperature of the rivers and average female dry mass for **a** *Poeciliopsis gracilis* and **b** *P. infans*. Each data point represents one population

CAPÍTULO III

ARTÍCULO PUBLICADO

"Superfetation in livebearing fishes is not always the result of a morphological constraint"

Patricia Frías-Alvarez, Jaime Zúñiga-Vega.

Oecologia, Published on line 28 October 2015
DOI 10.1007/s00442-015-3477-1

Poeciliopsis infans

Fotos: Oscar Olivares Loyola

Oecologia
DOI 10.1007/s00442-015-3477-1

HIGHLIGHTED STUDENT RESEARCH

Superfetation in live-bearing fishes is not always the result of a morphological constraint

Patricia Frías-Alvarez[1] · **J. Jaime Zúñiga-Vega**[1]

Received: 2 March 2015 / Accepted: 4 October 2015

Abstract Superfetation is an unusual reproductive strategy that consists of the presence of multiple broods at different developmental stages within a single female. One hypothesis that was proposed to explain its adaptive significance suggests that, in fishes, superfetation is a response to selective pressures that promote a thin and streamlined body shape, such as high-velocity water systems. Superfetation may allow for reduction in ovary size and hence improve streamlining because superfetating females carry few large, full-term embryos at any given time. We tested this morphological constraint hypothesis using reproductive and morphological data from several populations of two viviparous fishes of the family Poeciliidae (*Poeciliopsis gracilis* and *Poeciliopsis infans*). We found no evidence to support the morphological constraint hypothesis. In both species the degree of superfetation varied as a function of a complex interaction between source population and female size, and this interpopulation variation was not associated with the velocity of the water current. Contrary to what we expected, females of *P. gracilis* with more streamlined bodies were observed in rivers where water velocity is slow or moderate. In *P. infans* the velocity of the water current did not predict variation in body shape. Our results are noteworthy because a previous study which focused on a congeneric species (*Poeciliopsis turrubarensis*) demonstrated strong support for this hypothesis. However, based on our evidence we conclude that the association among increased superfetation, streamlined morphologies, and fast-flowing environments is not a general rule and that the adaptive value of superfetation may differ among species.

Keywords Life histories · Poeciliidae · *Poeciliopsis* · Simultaneous broods · Water velocity

Introduction

Superfetation is the ability of females to simultaneously bear more than one brood of offspring, with each brood at a different developmental stage (Turner 1937; Scrimshaw 1944). This reproductive strategy is commonly found in viviparous fishes (Turner 1937; Scrimshaw 1944; Thibault and Schultz 1978) and has been reported in at least three families that belong to different orders: Clinidae (Gunn and Thresher 1991), Zenarchopteridae (Reznick et al. 2007), and Poeciliidae (Pires et al. 2011). Within the family Poeciliidae several genera include species with superfetation such as *Poeciliopsis*, *Heterandria,* and *Neoheterandria*. In contrast, superfetation is clearly absent in other poeciliid genera such as *Belonesox*, *Brachyrhaphis,* and *Xiphophorus* (Reznick and Miles 1989; Pollux et al. 2009). The phylogenetic distribution of superfetation in the family

Communicated by Joel Trexler.

Highlighted student paper This study tests the morphological constraint hypothesis proposed to explain the adaptive value of overlapping clutches in live-bearing fishes, a strategy termed "superfetation." The results contrast with those previously published for a different species suggesting that superfetation promotes thin and elongated phenotypes in fast-flowing rivers. This study illustrates the value of testing hypotheses about adaptation in multiple species and encourages the development of additional hypotheses to explain the adaptive value of superfetation.

✉ Patricia Frías-Alvarez
pfriasalvarez@ciencias.unam.mx

[1] Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México, Ciudad Universitaria, 04510 Mexico City, Distrito Federal, Mexico

Poeciliidae suggests that superfetation has evolved several times independently (Reznick and Miles 1989; Pollux et al. 2009; Pires et al. 2011).

The degree of superfetation (i.e., the number of simultaneous broods present within a single female) can vary among and within species. *Heterandria formosa* can bear up to eight broods at different developmental stages (Travis et al. 1987), whereas *Poeciliopsis prolifica* can bear up to four broods. Frías-Alvarez et al. (2014) found that the degree of superfetation in *Poeciliopsis gracilis* varied among 11 different populations, as well as among months within populations. The multiple independent origins and the observed inter- and intraspecific variation suggest that superfetation has adaptive benefits that depend on particular environmental conditions.

Given the interest in understanding the selective forces that promote superfetation in viviparous fishes, several hypotheses have been proposed (reviewed in Zúñiga-Vega et al. 2010). One of these suggests that superfetation results from a morphological constraint (Thibault and Schultz 1978; Reznick and Miles 1989). According to this model superfetation should be beneficial in environments where a streamlined phenotype is needed, such as fast-velocity water systems or high-predation habitats. In live-bearing females the physical space to bear offspring cannot increase indefinitely without some negative effect on swimming performance. High investment in the production of several full-term embryos is associated with a considerable increase in body volume, which is poorly adapted to fast-velocity waters and can increase mortality risk when predators are abundant (Plaut 2002; Ghalambor et al. 2004; Langerhans 2008). Thus, females are constrained to a body shape that restricts the space that they can allocate to developing embryos. Superfetation might provide an adaptive advantage in aqueous environments because superfetating females carry fewer large, full-term embryos at any given time without an apparent decrease in total number of offspring compared with non-superfetating females (Thibault and Schultz 1978; Zúñiga-Vega et al. 2010). Therefore, superfetation may allow females to produce a relatively high number of offspring while maintaining a slim and elongated phenotype.

Geographically widespread species are appropriate model systems to address the predictions derived from this hypothesis because different populations are likely to inhabit the selective environments that drive spatial differences in superfetation. According to the morphological constraint hypothesis we expect a higher degree of superfetation and a more streamlined phenotype in females inhabiting fast-velocity waters compared with those inhabiting slow-velocity waters. Zúñiga-Vega et al. (2007) provided the first formal test of these predictions using *Poeciliopsis turrubarensis* as a model system. They found that the differences among populations in the degree of superfetation were explained by differences among river drainages in the velocity of the water current. Females inhabiting fast-moving waters that drain from steep mountains produced more simultaneous broods and exhibited a more elongated phenotype than females inhabiting slow-moving coastal rivers near the ocean. Further tests with additional species should either support the generality of this association among velocity of the water flow, morphology, and superfetation or, alternatively, should demonstrate that intraspecific variation in superfetation is not always the result of a morphological constraint and that other factors can promote this reproductive strategy.

In the present study we used several populations of *Poeciliopsis gracilis* and *Poeciliopsis infans* to explore the association between the velocity of water flow, morphological constraints, and degree of superfetation. We tested the morphological constraint hypothesis by comparing the degree of superfetation and female body shape between populations inhabiting rivers that differ in the velocity of the water current. We expected a relatively high degree of superfetation associated with thin and streamlined body shapes in fast-flowing environments. In addition, we examined how variation among rivers in velocity of the water flow affected other life history traits [brood size, individual embryo mass, total number of embryos, and reproductive allotment (RA)] of these two live-bearing species.

Materials and methods

Study species

Poeciliopsis gracilis (Poeciliidae) inhabits rivers that drain the Atlantic slope of Mexico. This species is native to the Coatzacoalcos and Papaloapan river basins, in the Mexican states of Veracruz and Oaxaca. In addition, it has been introduced and successfully established in other river basins [e.g., the Pánuco river (Miller et al. 2005; Gutiérrez-Cabrera et al. 2005)]. *P. gracilis* inhabits most types of water bodies such as streams, lagoons, pools in creeks and rivers, with a wide range of turbidity and water flow velocity (Miller et al. 2005).

Poeciliopsis infans is native to western and central Mexico. This species occurs in the Lerma-Santiago river basin as well as in the basins of the Ameca, Armeria, Coahuayana, and Balsas rivers (Miller et al. 2005). *P. infans* is also found in a variety of freshwater habitats such as lakes, rivers, ponds, canals, ditches, and springs. Similar to *P. gracilis*, this species also inhabits environments that differ in turbidity and water flow velocity (Galindo-Villegas and Sosa-Lima 2002; Miller et al. 2005).

Collection sites and field methods

We collected females of *P. gracilis* from 11 distinct populations (Table 1). Eight of these were located within its original range of distribution (in the states of Veracruz and Oaxaca) and three in the newly colonized habitats (in the state of San Luis Potosí). We collected females of *P. infans* from 11 distinct populations, nine located in the

Table 1 Sampling locations and sample sizes (*n*) for *Poeciliopsis gracilis* and *Poeciliopsis infans*

Population number	Geographic coordinates	Altitude (m)	Water flow velocity (km h^{-1})	Category of water velocity	*n*
P. gracilis					
1	21°58′N 98°57′W	101	0.05 (0.05)	Slow	40
2	21°58′ N 99°15′W	295.6	0.13 (0.13)	Slow	17
3	21°59′N 99°15′W	284.3	0.55 (0.37)	Slow	48
4	17°09′N 95°10′W	121.8	1.25 (0.32)	Slow	22
5	17°08′N 95°07′W	97.7	1.64 (0.57)	Medium	41
6	17°08′N 95°07′W	89.7	1.66 (0.41)	Medium	64
7	17°50′N 95°49′W	35.2	1.88 (0.66)	Medium	30
8	17°25′N 95°01′W	32.1	2.05 (0.65)	Fast	40
9	17°12′N 95°03′W	58.2	2.43 (0.65)	Fast	42
10	17°26′N 95°26′W	83.1	3.43 (0.08)	Fast	30
11	17°33′N 95°32′W	56.4	3.83 (0.43)	Fast	50
P. infans					
1	21°14′N 102°20′W	1709	0.00 (0)	Slow	48
2	21°09′N 102°28′W	1736	0.00 (0)	Slow	32
3	21°12′N 103°22′W	1199	0.17 (0.07)	Slow	40
4	20°34′N 104°09′W	1210	0.23 (0.23)	Slow	35
5	20°33′N 103°57′W	1252	0.24 (0.13)	Slow	40
6	20°32′N 104°03′W	1243	0.25 (0.25)	Slow	34
7	20°09′N 103°02′W	1537	0.72 (0.32)	Medium	49
8	21°02′N 103°25′W	1196	0.88 (0.24)	Medium	30
9	21°39′N 102°57′W	1371	0.92 (0.35)	Medium	34
10	20°34′N 103°51′W	1250	1.07 (0.31)	Medium	34
11	20°33′N 103°57′W	1259	1.18 (0.50)	Medium	44

Populations are listed according to the velocity of the water flow; their order does not reflect geographic proximity. SEs for the average velocity of the water flow are shown within parentheses state of Jalisco and two in the state of Zacatecas (Table 1). All females were collected using seine nets (1.3 m depth × 5 m length, 8-mm mesh size) during repeated visits to the study sites that spanned both dry (November–May) and rainy (June–October) seasons, between 2012 and 2013. Captured fish were anesthetized with 3-amenobenzoic acid ethyl ester (MS-222$^{\mathrm{TM}}$), sacrificed by immersion in 95 % ethanol, and transported to the laboratory, where they were photographed (using a digital camera) and stored in 70 % ethanol. Sample sizes are shown in Table 1.

During every visit to the study sites we recorded the velocity of the water flow using a flow meter (Flowatch; JDC Electronic SA). Given that individuals of both species showed a preference for particular sections of the river (i.e., they avoid waterfalls or the fastest currents within a river), we only measured water velocity directly in those sections of the river where we observed and collected the fish. During each visit, we measured water velocity in three different spots where fish were observed. Then, we calculated an average, across replicates and months, of the water flow velocity per locality.

We observed wide variation in the velocity of the water current among the 11 water bodies where we collected *P. gracilis*. Originally, we attempted to categorize the habitats of this species into two categories: “slow” and “fast” water flow (as per Zúñiga-Vega et al. 2007). However, based on our observations and measurements of the water flow across several months, we realized that these two categories would underestimate the amount of environmental variation experienced by *P. gracilis*, in terms of water velocity. Instead, a more accurate classification was achieved by dividing *P. gracilis* habitats into three categories: slow (below average), medium (around average), and fast (above average) water flow (Table 1). Average water velocity in slow-flow habitats ranged from (mean ± SE) 0.05 ± 0.05 to 1.25 ± 0.32 km h^{-1}. Average water velocity in medium-flow habitats ranged from 1.64 ± 0.57 to 1.88 ± 0.66 km h^{-1}. Average water velocity in fast-flow habitats ranged from 2.05 ± 0.65 to 3.83 ± 0.43 km h^{-1} (Table 1).

Unlike the study sites of *P. gracilis*, the habitats of *P. infans* varied less in terms of velocity of the water current. Thus, we divided *P. infans* habitats into two categories: slow (below average) and medium (above average) water flow (Table 1). Average water velocity in slow-flow habitats ranged from 0.00 to 0.25 ± 0.25 km h^{-1}. Average water velocity in medium-flow habitats ranged from 0.72 ± 0.32 to 1.18 ± 0.50 km h^{-1}. We note here that the sites categorized as medium water flow for *P. infans* had on average slower water velocity than those categorized as medium water flow for *P. gracilis* (Table 1). However, the difference between slow- and medium-flow environments for *P. infans* was clear enough to reflect a change in water velocity that may promote differences in body shape and superfetation.

Quantifying superfetation and life history traits

We dissected preserved females to quantify superfetation (number of simultaneous broods per female), brood size (number of developing embryos per brood), individual embryo mass, total number of embryos (across all broods), and reproductive allotment (RA). We followed methods described by Reznick and Endler (1982) and Zúñiga-Vega et al. (2007). We defined a brood as all the embryos that shared the same developmental stage (as per Haynes 1995). Individual embryo mass was estimated by drying an entire brood of offspring in a desiccating oven for 24–48 h at 55 °C and dividing brood dry mass by the number of embryos in that brood. We also measured the dry mass (24–48 h at 55 °C) of reproductive females, excluding the digestive tract. RA was calculated as the proportion of the total dry mass of the female that is devoted to reproduction: total dry mass of all the broods found within a female/(dry mass of all broods + female somatic dry mass).

Quantifying morphological variation

To analyze variation in body shape among populations of *P. gracilis* and *P. infans* we followed two approaches. First, we applied geometric morphometric techniques (Zelditch et al. 2004). We used digital photographs of the lateral profile of preserved females. We only considered gravid females and all photographs were taken before dissection. We examined the lateral profile of each female by scoring 17 anatomical landmarks (as per Zúñiga-Vega et al. 2011) using the software tpsDig 2.17 (Rohlf 2013a; http://life.bio.sunysb.edu/morph). Based on these two-dimensional landmarks and using the thin-plate spline approach (Zelditch et al. 2004) implemented in the software tpsRelw 1.54 (Rohlf 2013b; http://life.bio.sunysb.edu/morph), we calculated centroid size (a geometric measure of body size) as well as two measures of shape variation for each gravid female: a set of uniform shape components, which describe changes in shape that are geometrically uniform across the entire body of the female (i.e., general increase in length or width with respect to a consensus shape across all females); a set of partial warps, which describe non-uniform changes in the position of a subset of landmarks with respect to other landmarks (Zelditch et al. 2004). Then, we calculated relative warp (RW) scores for each female by means of a principal component analysis conducted on the uniform and non-uniform shape variables. RWs are orthogonal axes of shape variation that summarize the morphological information into fewer variables. We used these RW scores as input variables in the statistical analyses that compared female morphology between water flow regimes. The thin-plate spline approach also allowed us to visualize differences in shape between water flow regimes by means of deformation grids, which are derived directly from the RW scores (Zelditch et al. 2004).

The second approach was based on calculating a fineness index (FI) for each female as follows: $FI = 1 - |1 - (FR/4.5)|$ (Langerhans and Reznick 2010). In this equation, FR indicates fineness ratio, which we calculated as body length/maximum body depth. An $FI = 1$ corresponds to a body shape that experiences the lowest drag with the maximum volume. Some authors have argued that this measure is a better predictor of swimming performance than simple thinness (Blake 2004; Langerhans and Reznick 2010; Haas et al. 2015). Therefore, we compared FI among water flow regimes, expecting FI values closer to 1 in relatively fast-moving waters.

Statistical analyses

We compared superfetation (i.e., number of simultaneous broods), brood size, individual embryo mass, total number of embryos, and RA among water flow regimes (among slow-, medium-, and fast-flow habitats in the case of *P. gracilis* and between slow- and medium-flow habitats in the case of *P. infans*) using general linear models. We used water flow regime and population (nested within water flow regime) as main factors. All models included female dry mass as a covariate. For superfetation, we also included total number of embryos as an additional covariate because we expected that females from relatively fast-flowing habitats partition their reproductive output into more broods without increasing total fecundity. Hence, for a fixed total number of embryos, females inhabiting fast- or medium-flow habitats should exhibit a higher degree of superfetation than those inhabiting slow-flow habitats. The models that examined variation in individual embryo mass and RA included stage of development as an additional covariate to make sure that all the estimated patterns in these two traits were not caused by regular changes in embryo mass throughout development. In particular, for RA we used an average developmental stage for those females that carried more than one brood (in both species the correlations between RA and average developmental stage were relatively low, but positive and significant; *P. gracilis*, $r = 0.15$, $P = 0.002$; *P. infans*, $r = 0.10$, $P = 0.03$).

We also included in all models the interaction between female dry mass and population (nested within water flow regime) to account for spatial differences in the way that life history traits covary with female size as well as to test the assumption of homogeneity of slopes. In those cases where this interaction was not significant we report and compare least-square means (i.e., adjusted for the effects of the covariates) among water flow regimes. When this interaction was significant, the mean values of the analyzed trait could not be compared directly because the direction and magnitude of the differences among populations and water flow regimes depend on the particular female size. Thus, we proceeded as suggested by Glantz and Slinker (2001) and plotted the estimated relationships (i.e., the estimated slopes) between the trait of interest and female mass for each population. Evidence for an effect of water flow regime would be provided by similar patterns among those populations that share the same category of water velocity (e.g., the slopes from all fast- or medium-flow habitats being higher than slopes from slow-flow habitats).

To avoid the problem of non-independence in brood size and individual embryo mass caused by the fact that some females may have more than one brood, we randomly chose one brood from each superfetating female. In this way, we ensured that each female was represented only once in the analyses of brood size and individual embryo mass. The number of simultaneous broods, the total number of embryos, and RA did not have this problem because for these traits only one data point was available per female.

We compared female shape between water flow regimes using RW scores and FI values as response variables in general linear models. We used water flow regime and population (nested within water flow regime) as main factors and centroid size as a covariate. The interaction between centroid size and population was also included to account for spatial differences in the way that shape covaries with size as well as to test the assumption of homogeneity of slopes. Here again, when this interaction was not significant we compared least-square means among water flow regimes. In contrast, when the interaction was significant, we plotted and compared among water flow regimes the estimated relationships between shape variables and centroid size for each population.

We conducted a separate model for each life history trait and for each shape variable using STATISTICA 7.0 (StatSoft). To meet assumptions of normality and homogeneity of variances we made the following data transformations: number of broods per female (i.e., superfetation), brood size, and total number of embryos were square-root transformed; individual embryo mass was log transformed; RA was transformed to the arcsine of its square root. The means (least-square means adjusted for the effects of the covariates) that we report here were obtained from equivalent analyses conducted on untransformed data to facilitate biological interpretation. However, in those cases where we plot relationships between phenotypic traits and female size, we used the transformed scale because we derived these graphs directly from the regression coefficients estimated from our linear models. When an additional covariate other than female mass or centroid size was included in the models, we kept this covariate fixed at its mean value.

Results

Water velocity and superfetation

For *P. gracilis* the main effect of water flow regime on superfetation was not significant ($F_{2,401} = 1.08$, $P = 0.34$; Table 2). However, the interaction between female mass and source population was significant ($F_{10,401} = 6.68$, $P < 0.0001$; Table 2). When examining the population slopes, the three localities where water velocity is intermediate between slower and faster rivers shared similar linear relationships between female mass and superfetation, with overall intermediate values and a slight decrease in the number of simultaneous broods as female mass increases (Fig. 1a). In contrast, wide interpopulation variation was observed in both slow- and fast-flow habitats in the relationship between superfetation and female mass. Total number of embryos had a positive and significant effect on superfetation ($F_{1,401} = 96.82$, $P < 0.0001$, $\beta = 0.01 \pm 0.001$ SE; Table 2). Females that produced more embryos also produced more simultaneous broods.

In contrast, for *P. infans* the effect of water flow regime on superfetation was significant ($F_{1,397} = 7.85$, $P = 0.005$; Table 3). In this case again, the interaction between female mass and source population was significant ($F_{10,397} = 2.85$, $P = 0.002$; Table 3). The slopes varied widely without a clear pattern in populations sharing the same category of water velocity (Fig. 2a). Total number of embryos had a positive and significant effect on superfetation ($F_{1,397} = 109.12$, $P < 0.0001$, $\beta = 0.01 \pm 0.001$ SE; Table 3). Similar to what we observed in *P. gracilis*, females of *P. infans* that produced more embryos also produced more simultaneous broods.

Water velocity and body shape

The first three RWs explained 49.41 % of the total morphological variation observed among females of *P. gracilis*. The first RW explained 24.9 % of the total morphological variance. We found a significant effect of water flow regime on this first RW ($F_{2,402} = 5.32$, $P = 0.005$; Table 2). However, the interaction between centroid size and source population significantly affected this RW1 ($F_{10,402} = 2.89$, $P = 0.002$; Table 2) and, therefore, the difference among populations and water flow regimes depended on the size of the females. In general, in all populations female thinness and the relative length of the caudal peduncle decreased with size, whereas the depth of the caudal peduncle increased (Fig. 1b). Populations from medium-flow habitats showed the most similar relationships between female size and this axis of shape variation. In contrast, wider interpopulation variation was observed in slow- and fast-flow habitats (Fig. 1b).

The second RW (14.01 % of explained variance) depicted an artifact generated by including photographs of bent females. Therefore, we did not analyze scores from this second RW. Scores from the third RW (10.5 % of explained variance) represented additional variation in body depth and length that did not differ among water flow regimes ($F_{2,402} = 1.78$, $P = 0.17$; Table 2; Fig. 1c). Centroid size and source population significantly affected this third RW, whereas their interaction did not (Table 2).

The main effect of water flow regime on FI values of *P. gracilis* was not significant ($F_{2,402} = 2.34$, $P = 0.10$; Table 2). However, the interaction between centroid size and source population was significant ($F_{10,402} = 2.44$, $P = 0.008$; Table 2). Contrary to what we expected, for small- and medium-sized females FI values were relatively lower in fast-flow habitats compared to those observed in medium- and slow-flow habitats (Fig. 1d). In addition, the slopes for all three medium-flow sites were fairly similar, with a decrease in fineness as size increased and intermediate values of the FI across all female sizes. Wider interpopulation variation was observed in slow-flow habitats in the linear relationship between fineness and centroid size (Fig. 1d).

The first three RWs explained 54.2 % of the total morphological variation observed among females of *P. infans.* The first RW explained 26.7 % of the total morphological variance. The main effect of water flow regime on this first RW was marginally significant ($F_{1,398} = 3.55$, $P = 0.06$; Table 3), whereas the interaction between centroid size and source population was quite significant ($F_{10,398} = 2.98$, $P = 0.001$; Table 3). In all populations, female thinness and the relative length of the caudal peduncle decreased as size increased (Fig. 2b). No clear differences between slow- and medium-flow habitats were evident in the relationships between this RW1 and female size.

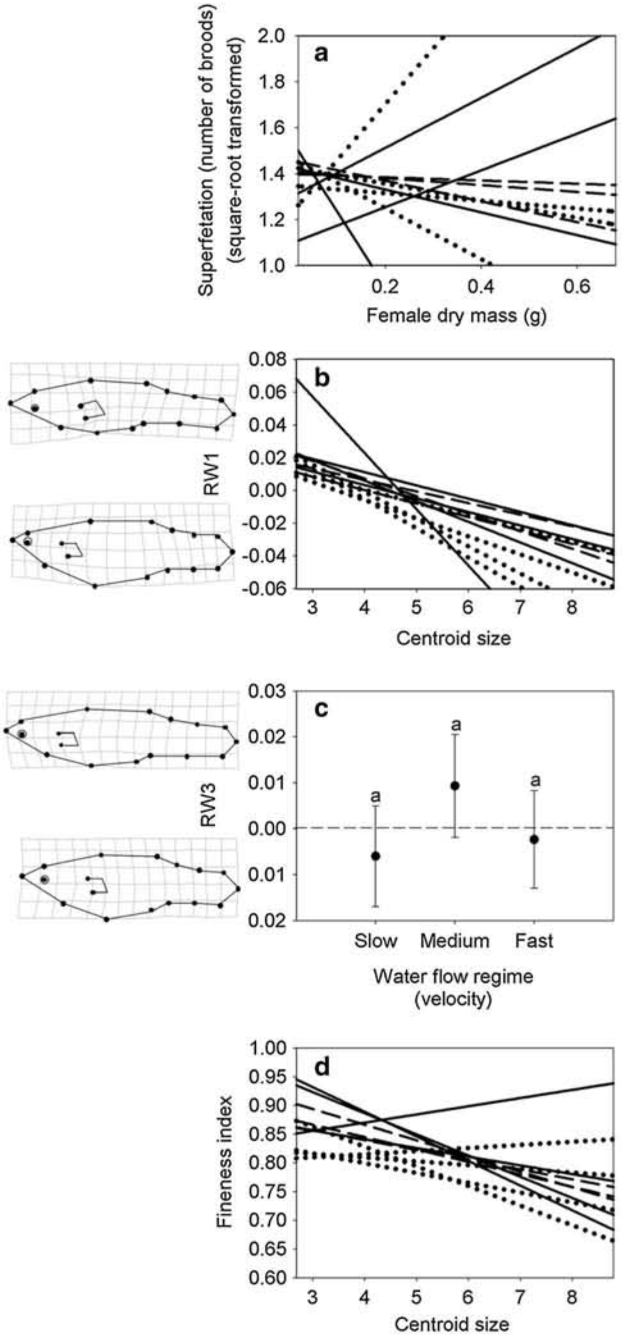
Also in the case of *P. infans*, the second RW (16.1 % of explained variance) depicted an artifact generated by including photographs of bent females. Therefore, we did not analyze this second axis of shape variation. The third RW (11.4 % of explained variance) represented additional variation in body depth and length of the caudal peduncle. The main effect of water flow regime was not significant ($F_{1,398} = 0.48$, $P = 0.49$; Table 3), whereas the interaction between centroid size and source population significantly affected this RW3 ($F_{10,398} = 3.32$, $P = 0.0004$; Table 3). Wide interpopulation variation was observed in the estimated slopes without clear patterns in populations sharing the same category of water velocity (Fig. 2c).

Table 2 Results of the general linear models that examined variation in superfetation, body shape, brood size, individual embryo mass, total number of embryos, and reproductive allotment of *P. gracilis*

Response variable	Effect	*df*	*F*	*P*
Superfetation	Water flow regime	2	1.08	0.34
	Population(water flow regime)	8	3.57	0.0005
	Female dry mass	1	0.3	0.59
	Total number of embryos	1	96.82	<0.0001
	Population(water flow regime) × female dry mass	10	6.68	<0.0001
	Error	401		
First axis of shape variation (RW1)	Water flow regime	2	5.32	0.005
	Population(water flow regime)	8	3.82	0.0002
	Centroid size	1	123.43	<0.0001
	Population(water flow regime) × centroid size	10	2.89	0.002
	Error	402		
Third axis of shape variation (RW3)	Water flow regime	2	1.78	0.17
	Population(water flow regime)	8	2.05	0.04
	Centroid size	1	4.24	0.04
	Population(water flow regime) × centroid size	10	1.56	0.12
	Error	402		
Fineness index (FI)	Water flow regime	2	2.34	0.10
	Population(water flow regime)	8	3.08	0.002
	Centroid size	1	20.66	<0.0001
	Population(water flow regime) × centroid size	10	2.44	0.008
	Error	402		
Brood size	Water flow regime	2	6.92	0.001
	Population(water flow regime)	8	3.07	0.002
	Female dry mass	1	33.74	<0.0001
	Population(water flow regime) × female dry mass	10	11.63	<0.0001
	Error	402		
Individual embryo mass	Water flow regime	2	2.69	0.07
	Population(water flow regime)	8	1.74	0.09
	Developmental stage	1	16.44	<0.0001
	Female dry mass	1	10.70	0.001
	Population(water flow regime) × female dry mass	10	2.02	0.03
	Error	401		
Total number of embryos	Water flow regime	2	11.19	<0.0001
	Population(water flow regime)	8	4.44	<0.0001
	Female dry mass	1	75.53	<0.0001
	Population(water flow regime) × female dry mass	10	13.72	<0.0001
	Error	402		
Reproductive allotment	Water flow regime	2	12.31	<0.0001
	Population(water flow regime)	8	5.03	<0.0001
	Developmental stage	1	14.61	0.0002
	Female dry mass	1	0.05	0.83
	Population(water flow regime) × female dry mass	10	7.02	<0.0001
	Error	401		

The factor population was nested within the factor water flow regime

RW Relative warp



◀**Fig. 1** Variation among populations and water flow regimes [among slow- ($n = 127$), medium- ($n = 135$), and fast-flow habitats ($n = 162$), respectively] in **a** the relationship between female dry mass and degree of superfetation, **b** the relationship between the first axis of shape variation [relative warp axis 1 (*RW1*)] and female size estimated as centroid size, **c** the third axis of shape variation (*RW3*), and **d** the relationship between fineness index (a measure of streamlining) and female centroid size for *Poeciliopsis gracilis*. *Continuous lines* represent slow-flow habitats, *dashed lines* represent medium-flow habitats, and *dotted lines* represent fast-flow habitats. *Deformation grids* depict deviations from the consensus shape for females with positive and negative values in the axes of shape variation (magnified ×2 to improve visualization of the differences). In **c** we only show mean values of RW3 (adjusted for centroid size) for the three different water flow regimes because the interaction between centroid size and source population was not significant. *Error bars* represent 95 % confidence intervals

The main effect of water flow regime on the FI values of *P. infans* was not significant ($F_{1,398} = 0.20$, $P = 0.65$; Table 3). However, the interaction between centroid size and source population significantly affected the FI values ($F_{10,398} = 3.66$, $P = 0.0001$; Table 3). The estimated slopes did not reflect any clear pattern in slow- or medium-flow habitats (Fig. 2d).

Water velocity and life history traits

The interaction between female mass and source population significantly affected brood size, individual embryo mass, total number of embryos, and RA of *P. gracilis* (Table 2). Wide interpopulation variation was observed in the way that all four traits covary with female size (Fig. 3a–d). Populations inhabiting medium-flow habitats exhibited from low to intermediate values of all these life history traits and this pattern was consistent across all female sizes (excepting one medium-flow population where RA decreased markedly as female mass increased; Fig. 3d). In contrast, females from slow- and fast-flow habitats varied widely in the way that these life history traits covaried with size (from negative to highly positive relationships). This wide variation resulted in both large and small values of these four traits occurring in both slow- and fast-flow rivers, with the relative value of the trait (i.e., relatively small or large) being highly dependent on the particular size of the females (Fig. 3a–d).

In the case of *P. infans*, the interaction between female mass and source population significantly affected brood size and total number of embryos (Table 3). In almost all populations larger females produced larger broods and a higher total number of embryos (Fig. 4a, c). No clear difference between slow- and medium-flow habitats was evident in the way that these two traits covaried with female size. The interaction between female mass and source population did not affect individual embryo mass or RA, whereas the main effect of female mass was positive and significant in both cases (Table 3). Water flow regime did not affect either of these two traits (individual embryo mass, $F_{1,397} = 0.23$, $P = 0.63$; RA, $F_{1,397} = 0.22$, $P = 0.64$; Table 3; Fig. 4b, d).

Discussion

Does increased superfetation result from a morphological constraint caused by fast water currents?

In this study we aimed to test the hypothesis that superfetation provides a fitness advantage in environments

Table 3 Results of the general linear models that examined variation in superfetation, body shape, brood size, individual embryo mass, total number of embryos, and reproductive allotment of *P. infans*

Response variable	Effect	*df*	*F*	*P*
Superfetation	Water flow regime	1	7.85	0.005
	Population(water flow regime)	9	2.62	0.006
	Female dry mass	1	0.29	0.59
	Total number of embryos	1	109.12	<0.0001
	Population(water flow regime) × female dry mass	10	2.85	0.002
	Error	397		
RW1	Water flow regime	1	3.55	0.06
	Population(water flow regime)	9	1.73	0.08
	Centroid size	1	334.76	<0.0001
	Population(water flow regime) × centroid size	10	2.98	0.001
	Error	398		
RW3	Water flow regime	1	0.48	0.49
	Population(water flow regime)	9	3.43	0.0004
	Centroid size	1	7.92	0.005
	Population(water flow regime) × centroid size	10	3.32	0.0004
	Error	398		
FI	Water flow regime	1	0.20	0.65
	Population(water flow regime)	9	2.63	0.006
	Centroid size	1	43.03	<0.0001
	Population(water flow regime) × centroid size	10	3.66	0.0001
	Error	398		
Brood size	Water flow regime	1	2.31	0.13
	Population(water flow regime)	9	3.21	0.0009
	Female dry mass	1	76.46	<0.0001
	Population(water flow regime) × female dry mass	10	2.84	0.002
	Error	398		
Individual embryo mass	Water flow regime	1	0.23	0.63
	Population(water flow regime)	9	3.78	0.0001
	Developmental stage	1	26.19	<0.0001
	Female dry mass	1	9.34	0.002
	Population(water flow regime) × female dry mass	10	1.64	0.09
	Error	397		
Total number of embryos	Water flow regime	1	0.1	0.75
	Population(water flow regime)	9	2.05	0.03
	Female dry mass	1	192.33	<0.0001
	Population(water flow regime) × female dry mass	10	5.17	<0.0001
	Error	398		
Reproductive allotment	Water flow regime	1	0.22	0.64
	Population(water flow regime)	9	0.66	0.75
	Female dry mass	1	12.75	0.0004
	Average developmental stage	1	5.85	0.02
	Population(water flow regime) × female dry mass	10	0.9	0.53
	Error	397		

The factor population was nested within the factor water flow regime. For abbreviations, see Table 2

where females should be thin and elongated as a result of fast water currents (Thibault and Schultz 1978; Reznick and Miles 1989). Hence, we expected more simultaneous broods and streamlined phenotypes in fast- and medium-flow habitats compared to slow-flow habitats. However, in both species the relationship between female size and the degree of superfetation was complex and varied widely among populations. Thus, the amount and

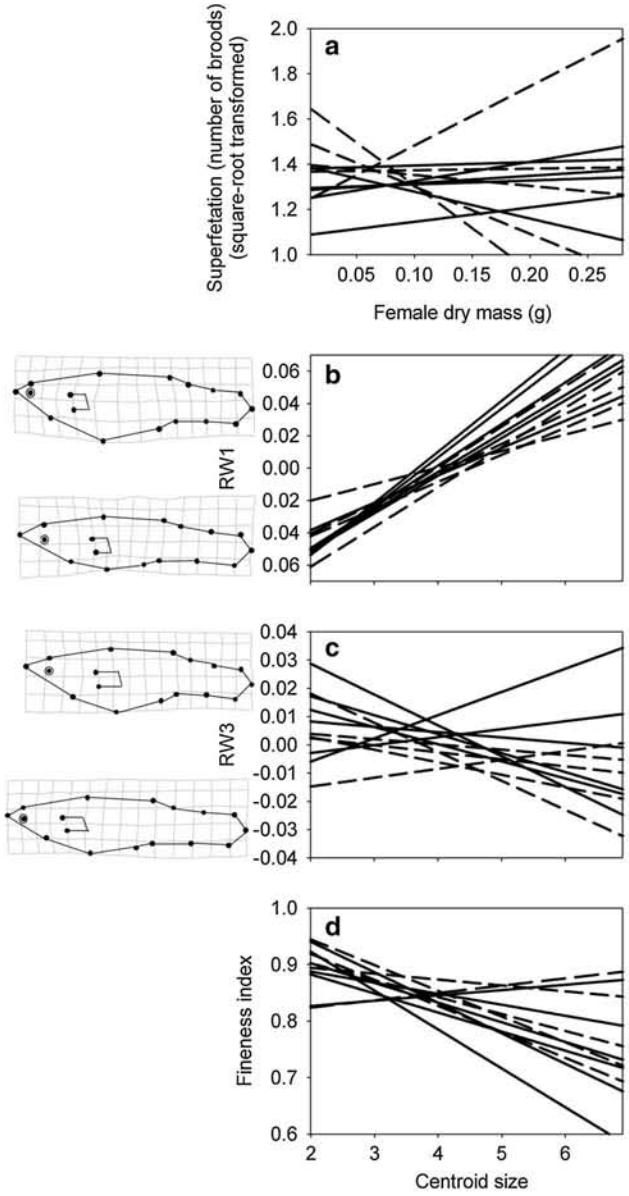


Fig. 2 Variation among populations and between water flow regimes [between slow- ($n = 229$) and medium-flow habitats ($n = 191$)] in **a** the relationship between female dry mass and degree of superfetation, the relationship between female size estimated as centroid size and **b** the first (RW1) and **c** third axes (RW3) of shape variation, and **d** the relationship between fineness index (a measure of streamlining) and female centroid size for *Poeciliopsis infans*. *Continuous lines* represent slow-flow habitats and *dashed lines* represent medium-flow habitats. *Deformation grids* depict deviations from the consensus shape for females with positive and negative values in the axes of shape variation (magnified ×2 to improve visualization of the differences)

direction of the differences in superfetation among populations were highly dependent on the particular size of the females. In general, this wide interpopulation variation did not support the morphological constraint hypothesis in either species. Superfetation was not consistently higher in the fastest water bodies (fast-flow habitats in the case of *P. gracilis* and medium-flow habitats in the case of *P. infans*) across any particular range of female sizes.

In addition, we also found wide variation among populations of both species in the way that female morphology covaries with size, and these patterns of covariation did not support the hypothesis either. The thinnest phenotypes were not consistently observed in the fastest habitats across any particular range of female sizes. Moreover, in *P. gracilis* the FI, which we used as a more accurate estimate of morphological streamlining, was lower in fast-flow habitats for small- and medium-sized females (Fig. 1d). This result is opposite to what we expected because small values of this index indicate a body shape that is less streamlined (Langerhans and Reznick 2010). Thus, females of *P. gracilis* experiencing lower drag (i.e., higher FI values) appear to occur in slow- and medium-flow rivers, rather than in those with the fastest currents (at least for small and intermediate body sizes). In *P. infans*, no clear differences between slow- and medium-flow habitats were observed in the FI for any particular range of female sizes. This result did not support the morphological constraint hypothesis either.

Previously, Zúñiga-Vega et al. (2007) provided strong support for the morphological constraint hypothesis using data on superfetation and body shape of a congeneric species (*Poeciliopsis turrubarensis*). These authors concluded that the velocity of the water current represents a selective factor that shapes intraspecific variation in both morphology and superfetation. Females of *P. turrubarensis* from fast-moving rivers were thinner, more elongated, and produced a higher number of simultaneous broods than their counterparts from slow-moving rivers. In contrast, our evidence from *P. gracilis* and *P. infans* clearly indicates that superfetation is not always the result of a morphological constraint and that the adaptive value of superfetation is not strictly associated with enhanced fitness in fast-flowing rivers.

Neither the thinnest nor the most streamlined phenotypes occurred in the rivers with the fastest water currents. This result observed in both *P. gracilis* and *P. infans* is intriguing because several studies have illustrated a general pattern of increased streamlining in fishes that inhabit fast-flow regimes (Boily and Magnan 2002; Blake 2004; Langerhans 2008; Langerhans and Reznick 2010). However, some studies have reported results that do not match this prediction and some other studies simply point to our lack of a complete understanding in this regard (Langerhans and Reznick 2010). For instance, Neat et al. (2003) studied three populations of *Salaria fluviatilis*, two from lakes and one from a river. They expected morphological similarities between lakes. However, they found the greatest degree of morphological divergence between lake populations, whereas the river population exhibited an intermediate morphology. Haas et al. (2015) found that interpopulation

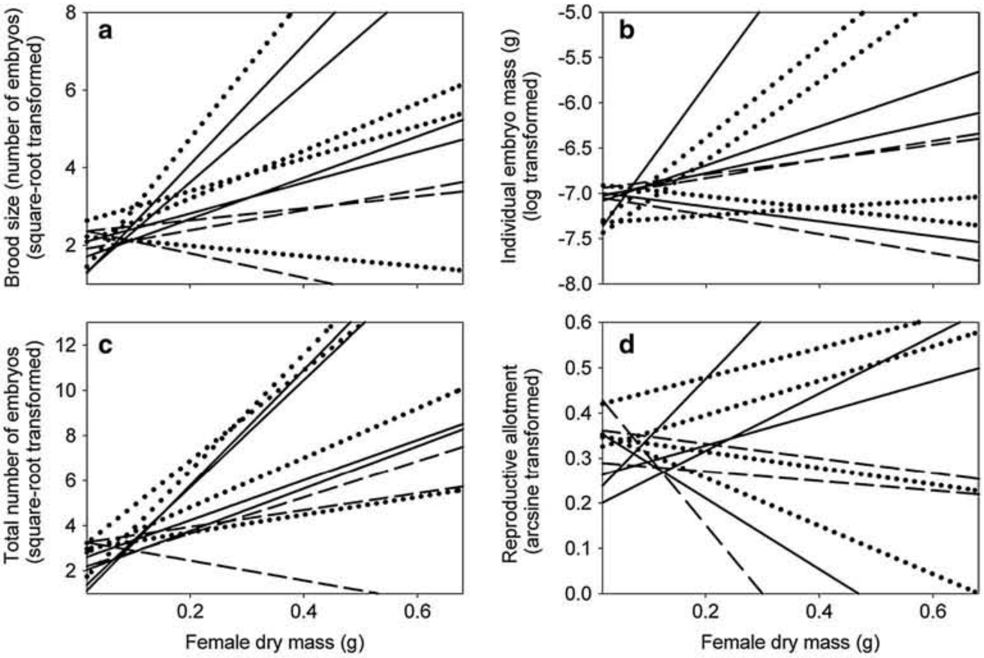


Fig. 3 Variation among populations and water flow regimes [among slow- ($n = 127$), medium- ($n = 135$), and fast-flow habitats ($n = 162$)] in the relationship between female dry mass and **a** brood size, **b** individual embryo mass, **c** total number of embryos, and **d** reproductive allotment for *P. gracilis*. *Continuous lines* represent slow-flow habitats, *dashed lines* represent medium-flow habitats, and *dotted lines* represent fast-flow habitats

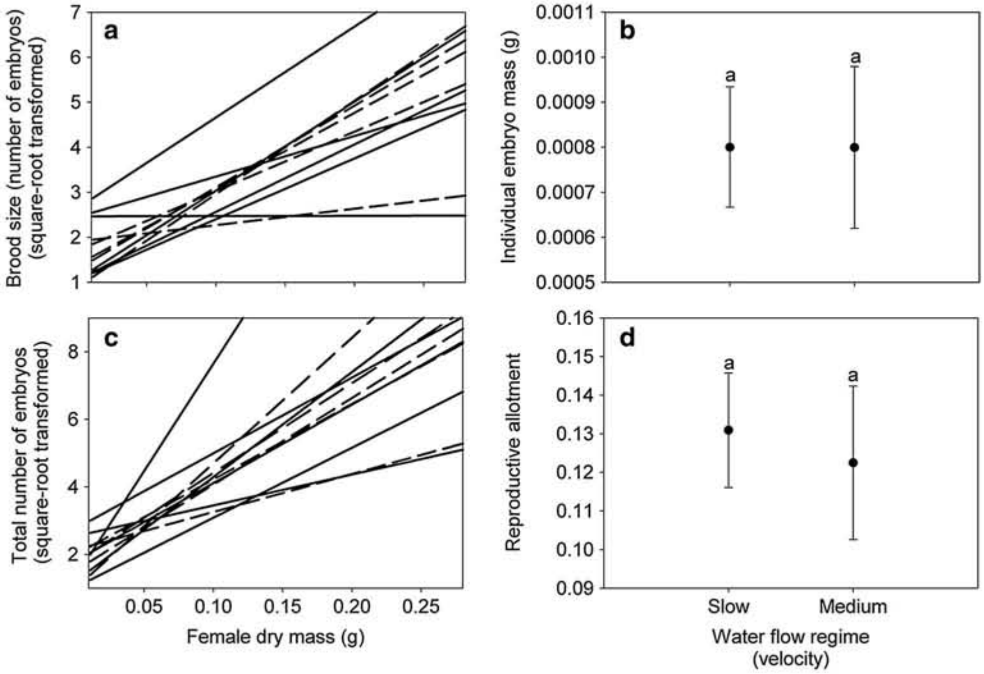


Fig. 4 Variation among populations and between water flow regimes [between slow- ($n = 229$) and medium-flow habitats ($n = 191$)] in **a** the relationship between female dry mass and brood size, **b** individual embryo mass, **c** the relationship between female dry mass and total number of embryos, and **d** reproductive allotment for *P. infans*. *Continuous lines* represent slow-flow habitats and *dashed lines* represent medium-flow habitats. In **b**, **d** we only show mean values of the traits (adjusted for female mass and stage of development) for the two different water flow regimes because in both cases the interaction between female mass and source population was not significant. *Error bars* represent 95 % confidence intervals

variation in body shape of *Cyprinella venusta* was related to the annual amount of water runoff from adjacent lands rather than to average velocity of the water current. Another result that did not support the hypothesis of an association between fast-velocity water and streamlined morphology was observed in rainbow fish (*Melanotaenia eachamensis*), where variation in the morphology of the caudal peduncle was not related to water velocity (McGuigan et al. 2003). Small- and medium-sized females of *P. gracilis* with more streamlined bodies occurred in rivers where the velocity of the water current was slow or intermediate. This result represents additional evidence that rejects this hypothesis.

The potential effect of water velocity on life history traits

In *P. gracilis* we found a complex interaction between female size and source population affecting all the additional life history traits that we studied. No clear differences were observed among water flow regimes for any particular range of female sizes. The only evident pattern consisted of low and intermediate values of all traits (including degree of superfetation) across all female sizes in those populations inhabiting rivers where water velocity is intermediate between slower and faster rivers. However, water velocity is not entirely responsible for this pattern because other sites that were categorized as either slow- or fast-flow habitats resulted in more similar female size-life history relationships than those observed in the medium-flow habitats (Fig. 3). Likely, all three medium-flow sites, as well as those additional sites where the relationship between female size and the particular life history trait was similar, share other ecological factors that promote convergence in female life histories. Several studies have demonstrated that predator-driven mortality, population density, and food availability can drive intraspecific variation in the life histories of poeciliid fishes (Reznick and Endler 1982; Johnson and Belk 2001; Jennions and Telford 2002; Reznick et al. 2002; Johnson and Bagley 2011; Reznick et al. 2012). At least one of these ecological conditions may be similar in those *P. gracilis* sites where the relationship between female size and the particular life history trait was similar. Alternatively, similar relationships between female size and life history traits may be explained by genetic similarities among populations (Hankison and Ptacek 2008; Tobler et al. 2008).

In contrast, in *P. infans* we found less interpopulation variation in the patterns of covariation between female size and life history traits, without any evident effect of water velocity. In most populations, larger females produced larger broods and newborns, a higher total number of embryos, and greater RA compared to smaller females. This effect of size on life history traits has been widely documented in other poeciliid species (e.g., Zúñiga-Vega et al. 2011; Riesch et al. 2012; Weldele et al. 2014). The magnitude of this size effect was fairly consistent across populations and life history traits of *P. infans*, with only a few exceptions (Table 3; Fig. 4). As we have noted before, the rivers where we collected *P. infans* varied less in terms of water velocity compared to those rivers *P. gracilis* inhabits. In turn, the amount of spatial variation in other ecological characteristics may not be large enough to promote strong divergent selection on the life history patterns of *P. infans*. Alternatively, limited genetic variability may explain the moderate life history divergence that we observed among populations of this species.

We observed an ontogenetic change in the body shape of both species that was likely the result of a parallel ontogenetic change in reproductive investment. Despite differences among rivers in water velocity, in all populations of both *P. gracilis* and *P. infans* body depth increased as size increased (Figs. 1b, 2b), whereas in most populations of both species fineness (a measure of streamlining) decreased as individuals grew (Figs. 1d, 2d). Similarly, in most populations of *P. gracilis* and in all populations of *P. infans* the total number of embryos that females produced increased as size increased (Figs. 3c, 4c). Hence, greater body depth and abdominal distension in larger females resulted from higher fecundity, which suggests that positive selection on total fecundity is stronger than those selective pressures that would force females to remain slender and streamlined (e.g., fast water velocity). Some poeciliid fishes disproportionally increase their reproductive effort as they grow, presumably because their opportunities for future reproduction decline as they age (Belk and Tuckfield 2010; Billman et al. 2014). Apparently, superfetation in *P. gracilis* and *P. infans* contributes to a higher reproductive output because the number of simultaneous broods increases as the total number of embryos increases. Females of *Heterandria formosa* also exhibit a positive association between the degree of superfetation and the total number of embryos (Travis et al. 1987).

Alternative hypotheses to explain the adaptive value of superfetation

Based on our evidence from *P. gracilis* and *P. infans*, we conclude that increased superfetation is not strictly linked to the need for elongated and streamlined phenotypes. Therefore, the adaptive value of superfetation may depend on the particular ecological conditions and/or may differ among species. Additional hypotheses have been proposed to explain the potential benefits of superfetation (reviewed in Zúñiga-Vega et al. 2010). One of these suggests that when females cannot choose a mating partner, superfetation might facilitate female control over paternity and increase the genetic variability of the offspring, if every brood is fertilized by a different male (Sandell 1990; Macías-Garcia and González-Zuarth 2005; Pollux et al. 2014). Another hypothesis suggests that superfetation decreases the peak cost of reproduction because superfetating females are able to spread reproduction more evenly over time, instead of carrying many full-term embryos simultaneously. Therefore, superfetation should be favored in environments where reproduction is costly, such as habitats with low food availability (Downhower and Brown 1975). Alternatively, superfetation may be an adaptation to particular demographic pressures. Specifically, this reproductive strategy could be favored by either unpredictable juvenile survival, high life expectancy of adult females, or low and uncertain adult survival probabilities (Thibault 1974; Downhower and Brown 1975; Burley 1980; Travis et al. 1987). The observed variation among populations of *P. gracilis* and *P. infans* in the degree of superfetation and in the relationship between female size and superfetation could be driven by one of these alternative selective factors or, more likely, by their interaction. Future field and experimental studies should test these hypotheses in order to provide additional insight on the adaptive benefits of superfetation.

Acknowledgments This study was supported by the Mexican Research Council (Consejo Nacional de Ciencia y Tecnología) through grant no. 129675 and through a doctorate scholarship awarded to P. F. A. and is a partial fulfillment of the requirements for a doctoral degree (Doctorado en Ciencias Biomédicas, Universidad Nacional Autónoma de México) of P. F. A. under the supervision of J. J. Z. V. This research was conducted with approval from the Mexican Agency of Aquaculture and Fisheries (Comisión Nacional de Acuacultura y Pesca), under permits SDPA/DGVS/03492, DGOPA-07010.210612.1749, and PPF/DGOPA-223/2013. C. Macías Garcia, C. Olivera-Tlahuel, and two anonymous reviewers provided valuable comments and suggestions. K. Villa-Meza helped with photographs. Logistic assistance was provided by E. Ávila-Luna, J. L. Bortolini-Rosales, H. Espinosa-Pérez, M. Hernández-Quiroz, P. Mendoza-Hernández, I. A. Morales-Salas, M. E. Muñiz-Díaz de León, M. E. Pérez-Cruz, G. Ramírez-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, O. Olivares-Loyola, C. Olivera-Tlahuel, H. Pérez-Mendoza, D. Robledo, N. Saleh-Subaie, H. Salinas-Matus, T. Sandoval, I. Solano-Zavaleta, L. Vázquez-Vega, R. Vega-Trejo, K. Villa-Meza, and I. Zapata-Morán.

Author contribution statement J. J. Z. V. formulated the idea for the study; P. F. A. conducted the fieldwork and collected the data; P. F. A. and J. J. Z. V. performed the analyses and wrote the manuscript.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

References

Belk MC, Tuckfield RC (2010) Changing costs of reproduction: age-based differences in reproductive allocation and escape performance in a livebearing fish. Oikos 119:163–169. doi:10.1111/j.1600-0706.2009.17742.x

Billman EJ, Rasmussen JE, Creighton JC, Johnson JB, Belk MC (2014) A multivariate approach to the analysis of within lifetime variation in life history. Methods Ecol Evol 5:797–805. doi:10.1111/2041-210X.12211

Blake RW (2004) Fish functional design and swimming performance. J Fish Biol 65:1193–1222. doi:10.1111/j.0022-1112.2004.00568.x

Boily P, Magnan P (2002) Relationship between individual variation in morphological characters and swimming costs in brook charr (*Salvelinus fontinalis*) and yellow perch (*Perca flavescens*). J Exp Biol 205:1031–1036

Burley N (1980) Clutch overlap and clutch size: alternative and complementary reproductive tactics. Am Nat 115:223–246

Downhower JF, Brown L (1975) Superfoetation in fishes and the cost of reproduction. Nature 256:345. doi:10.1038/256345a0

Frías-Alvarez P, Macias Garcia C, Vázquez-Vega LF, Zúñiga-Vega JJ (2014) Spatial and temporal variation in superfoetation and related life history traits of two viviparous fishes: *Poeciliopsis gracilis* and *P. infans*. Naturwissenschaften 101:1085–1098. doi:10.1007/s00114-014-1247-2

Galindo-Villegas J, Sosa-Lima E (2002) Gonopodial system review and new fish record of *Poeciliopsis infans* (Cyprinodontiformes: Poeciliidae) for lake Patzcuaro, Michoacan, central Mexico. Rev Biol Trop 50:1151–1157

Ghalambor CK, Reznick DN, Walker JA (2004) Constraints of adaptive evolution: the functional trade-off between reproduction and fast-start swimming performance in Trinidad guppy (*Poecilia reticulata*). Am Nat 164:38–50. doi:10.1086/421412

Glantz SA, Slinker BK (2001) Primer of applied regression and analysis of variance, 2nd edn. McGraw-Hill, New York

Gunn JS, Thresher RE (1991) Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Environ Biol Fish 31:323–344. doi:10.1007/BF00002357

Gutiérrez-Cabrera AE, Pulido-Flores G, Monks S, Gaytán-Oyarzún JC (2005) Presencia de *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoidea: Bothriocephalidae) en peces de Metztitlán, Hidalgo, México. Hydrobiologica 15:283–288

Haas TC, Heins DC, Blum MJ (2015) Predictors of body shape among populations of a stream fish (*Cyprinella venusta*, Cypriniformes: Cyprinidae). Biol J Linn Soc 115:842–858. doi:10.1111/bij.12539

Hankison SJ, Ptacek MB (2008) Geographical variation of genetic and phenotypic traits in the Mexican sailfin mollies, *Poecilia velifera* and *P. petenensis*. Mol Ecol 17:2219–2233. doi:10.1111/j.1365-294X.2008.03736.x

Haynes JL (1995) Standardized classification of poeciliid development for life-history studies. Copeia 1995:147–154. doi:10.2307/1446809

Jennions M, Telford S (2002) Life-history phenotypes in populations of *Brachyrhaphis episcopi* (Poeciliidae) with different predator communities. Oecologia 132:44–50. doi:10.1007/s00442-002-0942-4

Johnson JB, Bagley JC (2011) Ecological drivers of life-history divergence. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, pp 38–49

Johnson JB, Belk MC (2001) Predation environment predicts divergent life-history phenotypes among populations of the livebearing fish *Brachyrhaphis rhabdophora*. Oecologia 126:142–149. doi:10.1007/s004420000504

Langerhans RB (2008) Predictability of phenotypic differentiation across flow regimes in fishes. Integr Comp Biol 48:750–768. doi:10.1093/icb/icn092

Langerhans RB, Reznick DN (2010) Ecology and evolution of swimming performance in fishes: predicting evolution with biomechanics. In: Domenici P, Kapoor BG (eds) Fish locomotion: an etho-ecological perspective. Science Publisher, Endfield, NH, pp 200–248

Macías-Garcia C, González-Zuarth CA (2005) Reproductive behavior of viviparous fish and intersexual conflict. In: Uribe MC, Grier HJ (eds) Viviparous fishes. New Life, Homestead, FL, pp 289–302

McGuigan K, Franklin CE, Moritz C, Blows MW (2003) Adaptation of rainbow fish to lake and stream habitats. Evolution 57:104–118. doi:10.1554/0014-3820(2003)057[0104:AORFTL]2.0.CO;2

Miller RR, Mincley WL, Norris SM (2005) Freshwater fishes of Mexico. University of Chicago Press, Chicago

Neat FC, Lengkeek W, Westerbeek EP, Laarhoven B, Videler JJ (2003) Behavioural and morphological differences between lake and river populations of *Salaria fluviatilis*. J Fish Biol 63:374–387. doi:10.1046/j.1095-8649.2003.00159.x

Pires MN, Banet AI, Pollux BJA, Reznick DN (2011) Variation and evolution of reproductive strategies. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, pp 28–37

Plaut I (2002) Does pregnancy affect swimming performance of female mosquitofish, *Gambusia affinis*? Funct Ecol 16:290–295. doi:10.1046/j.1365-2435.2002.00638.x

Pollux BJA, Pires MN, Banet AI, Reznick DN (2009) Evolution of placentas in the family Poeciliidae: an empirical study of macroevolution. Annu Rev Ecol Evol Syst 40:271–289. doi:10.1146/annurev.ecolsys.110308.120209

Pollux BJA, Meredith RW, Springer MS, Reznick DN (2014) The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature 513:233–236. doi:10.1038/nature13451

Reznick DN, Endler JA (1982) The impact of predation on life history evolution in trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177. doi:10.2307/2407978

Reznick DN, Miles DB (1989) Review of life history patterns in Poeciliid fish. In: Meffe GK, Snelson FF Jr (eds) Ecology and evolution of livebearing fishes (Poeciliidae). Prentice Hall, Englewood Cliffs, pp 125–148

Reznick D, Bryant MJ, Bashey F (2002) *r*- and *K*-selection revisited: the role of population regulation in life-history evolution. Ecology 83:1509–1520. doi:10.2307/3071970

Reznick DN, Meredith R, Collette BB (2007) Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beliformes) and Poeciliidae (Cyprinodontiformes). Evolution 61:2570–2583. doi:10.1111/j.1558-5646.2007.00207.x

Reznick DN, Bassar RD, Travis J, Rodd FH (2012) Life-history evolution in guppies. VIII. The demographics of density regulation in guppies (*Poecilia reticulata*). Evolution 66:2903–2915. doi:10.1111/j.1558-5646.2012.01650.x

Riesch R, Martin RA, Bierbach D, Plath M, Langerhans RB, Arias-Rodriguez L (2012) Natural history, life history, and diet of *Priapella chamulae* Schartl, Meyer & Wilde 2006 (Teleostei: Poeciliidae). Aqua Int J Ichthyol 18:95–102

Rohlf FJ (2013a) TpsDig. Department of Ecology and Evolution, State University of New York, Stony Brook

Rohlf FJ (2013b) TpsRelw. Department of Ecology and Evolution, State University of New York, Stony Brook

Sandell M (1990) The evolution of seasonal delayed implantation. Q Rev Biol 65:23–42

Scrimshaw NS (1944) Superfetation in poeciliid fishes. Copeia 1944:180–183. doi:10.2307/1437814

Thibault RE (1974) Genetics of cannibalism in a viviparous fish and its relationship to population density. Nature 251:138–140. doi:10.1038/251138a0

Thibault RE, Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprynodontiformes: Poeciliidae). Evolution 32:320–333. doi:10.2307/2407600

Tobler M, DeWitt TJ, Schlupp I, García de León FJ, Herrmann R, Feulner PG, Tiedemann R, Plath M (2008) Toxic hydrogen sulfide and dark caves: phenotypic and genetic divergence across two abiotic environmental gradients in *Poecilia mexicana*. Evolution 62:2643–2659. doi:10.1111/j.1558-5646.2008.00466.x

Travis J, Farr JA, Henrich S, Cheong RT (1987) Testing theories of clutch overlap with the reproductive ecology of *Heterandria formosa*. Ecology 68:611–623. doi:10.2307/1938466

Turner CL (1937) Reproductive cycles and superfetation in poeciliid fishes. Biol Bull 72:145–164

Weldele ML, Zúñiga-Vega JJ, Johnson JB (2014) Life history of *Gambusia vittata* (Pisces: Poeciliidae). Southwest Nat 59:449–460. doi:10.1894/MP-08.1

Zelditch ML, Swiderski DL, Sheets HD, Fink WL (2004) Geometric morphometrics for biologists: a primer. Elsevier Academic Press, London

Zúñiga-Vega JJ, Reznick D, Johnson JB (2007) Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. Oikos 116:995–1005. doi:10.1111/j.0030-1299.2007.15763.x

Zúñiga-Vega JJ, Macías-Garcia C, Johnson JB (2010) Hypotheses to explain the evolution of superfetation in viviparous fishes. In: Uribe MC, Grier HJ (eds) Viviparous fishes. II. New Life, Homestead, pp 241–253

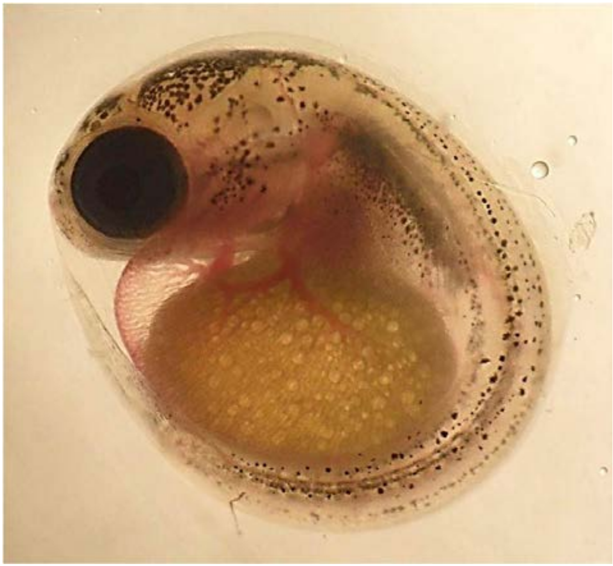
Zúñiga-Vega JJ, Suárez-Rodríguez M, Espinosa-Pérez H, Johnson JB (2011) Morphological and reproductive variation among populations of the Pacific molly *Poecilia butleri*. J Fish Biol 79:1029–1046. doi:10.1111/j.1095-8649.2011.03081.x

CAPÍTULO IV

ARTÍCULO EN PROCESO DE ENVIARSE.

"Exploring the potential relationship between superfoetation and food availability in two poeciliid fishes"

Patricia Frías-Alvarez, Edgar Ávila-Luna, Constantino Macías-Garcia, J. Jaime Zúñiga-Vega



Embrion de *Poeciliopsis gracilis.* Foto: Patricia Frías Álvarez

Submitted to: **Evolutionary Ecology**
Submission date: XX-XX-2016

Exploring the potential relationship between superfeotation and food availability in two poeciliid fishes

Patricia Frías-Alvarez[1,3]

Edgar Ávila-Luna[2]

Constantino Macías-Garcia[2]

J. Jaime Zúñiga-Vega[1]

[1]Departamento de Ecología y Recursos Naturales, Facultad de Ciencias, Universidad Nacional Autónoma de México. Ciudad Universitaria, Distrito Federal 04510, México.

[2]Departamento de Ecología Evolutiva, Instituto de Ecología, Universidad Nacional Autónoma de México. Apartado Postal 70-275, C.P. 04510, Distrito Federal, México.

[3]Corresponding author: pfriasalvarez@ciencias.unam.mx, Phone: (52-55) 5622-4912, Fax: (52-55) 5622-4828.

Running title: Superfoetation, cost of reproduction, and Poeciliid

Keywords Life histories reproductive strategy· *Poeciliopsis gracilis Poeciliopsis infans*· fullness index. food availability

Total word count (excluding references, tables and figures): 3578

Word count by section:

Abstract: 225

Introduction: 524

Materials and methods: 1121

Results: 687

Discussion: 1147

Number of cited references: 38

Number of Figures: 2

Number of Tables: 2

Abstract

Superfoetation is females' ability to simultaneously bear more than one brood at different developmental stages. A reduction of the peak cost of reproduction has been proposed as the adaptive significance of this reproductive strategy. According to this hypothesis, superfoetation should be favoured in environments where reproduction is costly and resources are scarce. We tested this hypothesis using data on reproduction and food availability for several populations of *Poeciliopsis gracilis* and *P. infans,* two livebearing fish species of the family Poeciliidae. We used the fullness index as an indicator of the amount of food available in the environment. We expected to find higher levels of superfoetation in females inhabiting environments with fewer resources (i.e. lower fullness index). Our data partially support the "reduction of the peak cost of reproduction" hypothesis in *P. infans*; in this species some populations had a negative relationship between fullness index (i.e. amount of food ingested) and degree of superfoetation (i.e. the number of simultaneous broods carried by the female). However, in *P. gracilis* the fullness index did not have a significant effect on degree of superfoetation. The results in *P. infans* support the hypothesis that superfoetation is beneficial in environments where reproduction is costly and resources are scarce. Based in our evidence of two species we conclude that superfoetation is not always associated to the amount of resources in the environment.

Introduction

The reproductive mode known as superfoetation is the females' ability to simultaneously bear more than one brood of offspring, each of which is in a different developmental stage (Turner 1937; Scrimshaw 1944; Roellig et al. 2011). Superfoetation is common among livebearing fishes (Turner 1937; Scrimshaw 1944;Thibault and Schultz 1978), and has been reported in three unrelated families: Clinidae (Gunn and Thresher 1991), Zenarchopteridae (Reznick et al. 2007), and Poeciliidae (Pires et al. 2011). The family Poeciliidae includes many genera with superfetating species such as *Poeciliopsis*, *Heterandria,* and *Neoheterandria,* whereas in other genera, such as *Belonesox*, *Brachyrhaphis,* and *Xiphophorus,* superfoetation does not occur (Reznick and Miles 1989; Pollux et al. 2009). This phylogenetic distribution of superfoetation strongly suggests that in the family Poeciliidae superfoetation has evolved independently multiple times (Reznick and Miles 1989; Reznick et al. 2007; Pollux et al. 2009).

The degree of superfoetation (i.e. the number of broods that females can simultaneously bear) can vary both among and within species. For instance, *Heterendria formosa* can bear up to eight broods at different stages of development (Travis et al. 1987), whereas *Poeciliopsis turrubarensis* can bear one to four simultaneous broods (Zúñiga-Vega et al 2007). Frías-Alvarez et al. (2014) reported that *P. infans* can bear one to four simultaneous broods, and the degree of superfoetation in *P. gracilis* varied among several populations, as well as among months within populations. This intraspecific variation as well as multiple independent evolutionary gains of superfoetation suggest that its adaptive value may depend on environmental conditions.

Several hypotheses have been proposed to explain the selective pressures behind the evolution of superfoetation in viviparous fishes (Zúñiga-Vega et al. 2010). One of the most prominent hypotheses is that superfoetation reduces the peak cost of reproduction (Downhower and Brown 1975) by spreading the total costs of reproduction more evenly over time (i.e. a redistribution scheme), allowing females to produce the same number of offspring without affecting the total reproductive output (e.g. females with superfoetation produce one to four small broods spaced in time instead of a single large brood). This would be especially advantageous in environments where reproduction is costly and resources are scarce, a prediction that is supported by theoretical models (Downhower and Brown 1975; Thibault and Schultz 1978). It is therefore expected that as food level decreases and there is less resource availability, reproductive costs increase and there should be a higher degree of superfoetation (Downhower and Brown 1975; Thibault and Schultz 1978; Travis et al. 1987; Zúñiga-Vega et al. 2010). Therefore, superfoetation may allow females to produce the same number of offspring while reducing the peak cost of reproduction.

In this study we used two geographically widespread species of livebearing fish to explore the relationship between food availability in the environment and degree of superfoetation: *Poeciliopsis gracilis* and *P. infans.* We tested the "reduction of the peak cost of reproduction" hypothesis by comparing degree of superfoetation and food availability among populations of each species. We expected to find a higher degree of superfoetation in rivers with lower food availability. In addition, we examined how food availability affects brood size, individual embryo mass, and reproductive allotment in these two poeciliid fishes.

Material and methods

Study species

The species of the family Poeciliidae are endemic to America and the most of its members are widely distributed throughout Central America, Antilles and Mexico (Hrbek et al., 2007). *Poeciliopsis gracilis* is native to México. On the rivers of the Atlantic slope it is found in the Oaxaca and Veracruz states. It has been reported as introduced in some basins of rivers in western and central Mexico (Miller et al. 2005; Gutiérrez-Cabrera et al. 2005; Gómez-Márquez et al. 2008). *P. gracilis* live in a broad array of habitats such as rivers, lagoons, pools, streams, micro reservoirs, lakes, dams, with a wide variety of turbidity and water flow (Miller et al. 2005). Frías-Alvarez et al. (2014) found that the degree of superfoetation in *P. gracilis* varies to 1-4 broods and the mean percentages of pregnant females bearing broods were 68 % in non native populations and 75% in native populations. Gómez-Márquez et al. (2008) found the mean percentages of females bearing brood were only 25% in a non native population. *P. gracilis* is a predominantly lecithotrophic species (i.e. embryos nourished only by the yolk; Reznick et al. 2002).

Poeciliopsis infans is native to Mexico. This specie inhabits the rivers of the Pacific slope and it is found in the basins of the Ameca, Armeria, Coahuayana, and Balsas rivers, as well as the Lerma-Santiago river basin in several states. *P. infans* occurs in several types of water bodies such as springs, lakes, rivers, ponds, canals, ditches of varying turbidity and water flow (Mateos et al. 2002; Galindo-Villegas and Sosa-Lima 2002; Miller et al. 2005). Turner (1937) reported that the degree of superfoetation were two broods, however Frías-Alvarez et al. (2014) found that the degree of superfoetation varies between 1-4 broods and the mean percentages of pregnant females bearing broods were 65 % in native populations. *P. infans* is also a predominantly lecithotrophic species (Reznick et al. 2002).

Study sites and field methods

P. gracilis and *P. infans* are geographically widespread species. We collected females of *P. gracilis* from ten different populations (Table. 1): seven from within *P. gracilis*'s original range (five in the state of Oaxaca state, two in the state of Veracruz) and three from newly colonized sites (in the state of San Luis Potosi; Table 1). Females of *P. infans* were collected from 11 different localities within its original range (nine in the state of Jalisco and two in the state of Zacatecas; Table 1). All females of both species were collected during repeated visits to the study sites, throughout the dry (November to May) and rainy (June to October) seasons, between 2012 and 2013. We collected approximately 20 gravid females per locality on each visit (Table 1) using seine nets (1.3 m depth × 5 m length, 8 mm mesh size). Captured females were anesthetized with 3-amenobenzoic acid ethyl ester (MS-222^{TM}), sacrificed by immersion in 95% ethanol and taken to the laboratory, where they were stored in 70% ethanol. All field and laboratory protocols were carried out with the approval of Mexican fisheries and environmental agencies (Secretaría de Medio Ambiente y Recursos Naturales y Comisión Nacional de Acuacultura).

Quantifying superfoetation and other life history traits

Females were dissected, and if pregnant, we quantified the following life history traits: superfoetation (total number of simultaneous broods in different developmental stages), brood size (number of embryos at each developmental stage), individual embryo mass, and reproductive allotment, following methods described by Reznick and Endler

(1982) and Zúñiga-Vega et al. (2007). All embryos at the same development stage (sensu Haynes 1995) were taken as one brood. We counted the number of embryos per brood to calculate brood size. Females' digestive tracts were removed during dissection to calculate fullness index (methods in following section), and females and their broods were dried in a desiccating oven for 24-48 h at 55°C and weighed on an electronic balance (Sartorius™LA120S, ±0.05 mg). Individual embryo mass was estimated by dividing the brood dry mass by the total number of embryos in that brood. Reproductive allotment (RA) was calculated as the total dry mass (weight) of all broods carried by mother. Female dry mass was used as covariate in the statistical analyses described below.

Quantifying food ingestion

We quantified the amount of food ingested per female using the fullness index (Hyslop 1980; Derweduwen 2012). This index measures the ratio of food mass to body weight and is expressed as: $FI = \left(\frac{SCi}{Pi}\right) 10{,}000$, where SC_i is the dry weight of the stomach contents (mg) and P_i is the dry mass of the female (mg) * (%00 or parts per decimile). We considered high values of the index an indication of high food availability in the environment (i.e. more food ingestion), and low values of the index correspond to low food availability in the localities (i.e. less amount of food ingestion). We measured the mass of the stomach contents by drying each digestive tract (previously removed from females) in a desiccating oven for 24-48 h at 55°C and weighing it on an electronic balance (Sartorius™LA120S, ±0.05 mg).

Statistical analysis

To estimate the relation between superfoetation (number of broods in different development stages), brood size, individual embryo mass, RA and amount of food ingestion by the index of fullness (low values of fullness index suggest that females inhabit environments with scarce food resources, and therefore ingest less food) we used general lineal models. All models also included female dry mass as a covariate, and the models evaluating individual embryo mass had stage of development as an additional covariate. Additionally we included in all models the interaction between female dry mass and population and also the interaction between index of fullness and population to account for spatial differences in the way that life-history traits covary with female size and in the way that superfoetation and the other life-history traits covary with food availability. We generated eight separate models: one for each life history variable for each species.

Analyses were carried out in STATISTICA™ 7.0 (StatSoft Inc.). Since females simultaneously carry more than one brood, brood size and individual embryo mass are not independent. To avoid pseudoreplication due to multiple measures from the same female, we randomly choose one brood from each superfetating female, ensuring that each pregnant female was represented only once in the analyses of brood size and individual embryo mass. Number of simultaneous broods per female (i.e. superfoetation) and brood size were square-root transformed, whereas individual embryo mass, RA and index of fullness were log-transformed to meet assumptions of normality and homogeneity of variances. The graphs that we report per species were obtained from the residuals of linear regressions of superfoetation, brood size, individual embryo mass, and RA on female dry mass in order to correct for differences in female size.

Results

Food ingestion and superfoetation

For *P. gracilis* we did not find a significant effect of the index of fullness (amount of food ingested) on superfoetation ($F_{1,451} = 0.08$, $P = 0.78$; Table 2). In almost all populations superfoetation was independent of food ingestion. In population 1, food ingestion was negatively correlated with degree of superfoetation, however, this result was due to a single data point (Fig. 1). Female dry mass had a significant effect on superfoetation ($F_{1,451} = 17.82$, $P < 0.0001$; Table 2), as did the interaction between index of fullness and population ($F_{9,451} = 2.35$, $P = 0.01$; Table 2). Although significant, the effects of female dry mass (partial $\eta^2=0.04$; Table 2) was weak and the effect of the interaction between index of fullness and superfoetation was also weak (partial $\eta^2=0.04$; Table 2).

In contrast, for *P. infans* we found a significant, although weak, negative effect of the index of fullness (i.e. amount of food ingested) on superfoetation ($F_{1,431} = 14.08$, $P = 0.0002$; partial $\eta^2=0.03$; $\beta=-0.074\pm 0.02$; Table 2). The interaction between index of fullness and population also had a significant effect on superfoetation ($F_{10,432} = 2.90$, $P = 0.002$; Table 2), and again this effect was small ($\eta^2=0.06$; Table 2). In populations three, six, and ten, the lower the quantity of food ingested the higher the degree of superfoetation. Populations four and eleven exhibited a similar pattern (although somewhat weaker), and in population five the relationship was reversed (higher food ingestion, lower degree of superfoetation), although this was due to a single data point. The remaining populations showed no effect of food ingestion on superfoetation (Fig. 1). Female dry mass had a significant effect on superfoetation ($F_{1,432} = 41.7$, $P < 0.0001$; Table 2), however the effect was small (partial $\eta^2=0.09$; Table 2). The interaction between female dry mass and population had a significant effect on superfoetation ($F_{10,432} = 2.53$, $P = 0.006$; Table 2) however the effect of this interaction was small ($\eta^2=0.05$; Table 2).

Food ingestion and other life history traits

For *P. gracilis* the amount of food ingested did not affect brood size or RA, although it did affect individual embryo mass (Table 2). Index of fullness had a weak negative effect on individual embryo mass, and there was a significant effect of the population x fullness index interaction ($\beta=-0.13\pm 0.04$; partial $\eta^2=0.02$; $F_{1,450} = 8.65$, $P = 0.003$; Table 2). In populations two, three, five, six, and seven, there was a weak negative effect of fullness index on individual embryo mass, while in the remaining populations there was no effect (Fig. 1). Brood size and RA were affected by female dry mass, source population and their interaction, and also by the interaction between index of fullness and population (Table 2). Individual embryo mass was significantly affected also by female dry mass and developmental stage (Table 2).

In the case of *P. infans* the amount of food ingested significantly affected brood size ($F_{1,432} = 4.84$, $P = 0.03$) and RA ($F_{1,432} = 22.50$, $P < 0.0001$). In both cases the effect was weak (brood size: $\eta^2=0.01$; RA: $\eta^2=0.05$). Index of fullness covaried negatively with brood size ($\beta=-0.17\pm 0.08$). In populations two, three and six, the lower the amount of food ingested, the higher the number of embryos (Fig. 2). Index of fullness covaried negatively with RA as well ($\beta=-0.28\pm 0.06$). In population six there was a clear negative relationship between food ingested and RA, and in populations two, three and ten there was a similar, although weaker, trend (Fig. 2). The amount of food ingested did not affect individual embryo mass (Table 2). Brood size was affected by female dry mass, source population and their interaction, and also by the interaction between index of fullness and population

(Table 2). Individual embryo mass was affected by female dry mass, development stage, the interaction of female dry mass and source population, and the interaction of index of fullness and population (Table 2). RA was affected by female dry mass, source population and by the interaction between index of fullness and source population (Table 2).

Discussion

Does superfoetation reduce the peak cost of reproduction?

In the present study, we tested the hypothesis that superfoetation provides a fitness advantage in environments where the availability of food is scarce (and, hence, reproduction is costly) by reducing the peak cost of reproduction (Downhower and Brown 1975; Thibault and Schultz 1978). This "reduction of peak cost" hypothesis is one of the most prominent explanations for the adaptive value of superfoetation (Downhower and Brown 1975; Zúñiga-Vega et al. 2010). We expected more simultaneous broods in environments where resources are scarce and females ingest less food. We found partial support for this "reduction of peak cost" hypothesis in *P. infans*. In this species some populations (3, 4, 6, 10, 11; Fig. 1) had a negative relationship between the amount of food ingested (fullness index) and superfoetation, i.e. females in environments with low availability of food resources exhibit higher levels of superfoetation. However in *P. gracilis* we did not found a relationship between the degree of superfoetation and the quantity of food ingestion.

In a previous experimental study with *Heterandria formosa,* Travis et al. (1987) provided evidence that failed to support the "reduction of peak cost" hypothesis. These authors assigned laboratory-reared females to three different food levels: low, medium, and high. Females under medium and high food treatments exhibited higher levels of superfoetation (i.e. females produced more simultaneous broods), than females under low food level treatment. They concluded that superfoetation is a mechanism of temporally overlapping what would have been several reproductive events. According to this mechanism, environments with excess resources allow females to initiate another brood. In a similar food manipulation experiment, however, Pires et al. (2007) found no difference in the degree of superfoetation in *Poeciliopsis prolifica* between high- and low-food treatments. In contrast we found some evidence to support the "reduction of peak cost" hypothesis in some populations of one of the two studied species.

In *P. infans* our evidence indicates that superfoetation may be advantageous in environments with low food resources. This pattern was clearest in populations three, six, and ten (i.e. low food ingested is associated with high levels of superfoetation; Fig. 2). According to this mechanism, superfoetation is a redistribution scheme of a single reproductive event, and therefore reduces the energetic demand made by the female at any particular time, thereby decreasing the peak cost of reproduction (Downhower and Brown 1975). Lowering the cost of reproduction is predicted to increase adult survival as well as the level of iteroparity (Stearns, 1992; Roff 2002).

The effect of food ingestion on other life history traits

In *P. gracilis* we found that the amount of food ingested had a significant effect on individual embryo mass. In this species, females that had ingested less food produced embryos with greater individual embryo mass. In populations two, three, five six and seven this pattern is clear (Fig. 1). Similar results have been found in two other lecithotrophic poeciliids (i.e. embryos nourished only by the yolk): *Poecilia reticulata* and *Priapichthys* *festae.* Females of both species assigned to low food treatments produced larger offspring (Reznick and Yang 1993; Reznick et al. 1996). Since the female's environment may be a good predictor of the environment her offspring will experience, females exposed to resource-poor environments may increase their investment in each offspring to ensure the young have the size and fat stores necessary to survive in poor environments (Reznick and Yang 1993; Reznick et al. 1996).

In the case of *P. infans* we found that the amount of food ingested has a significant effect on brood size and RA. Females that ingested a low quantity of food produced a higher number of embryos (i.e. large brood size), and exhibited a higher RA, as we can observe in population two, three, six, and ten (Fig. 1). Reproductive investment responds to several factors, including food supply (Reznick et al. 1982; Reznick et al. 1992; Zandona et al.2011). A successful strategy of reproductive investment for females inhabiting unpredictable environments is to produce a large number of embryos with a minimal investment (Trexler 1996). Females of *P. infans* inhabiting environments with high, stable food resources should favor survival and future reproductive events over the current event, since increased investment in current reproduction may reduce the probability of survival and subsequent reproductive performance (Stearns 1992; Roff 2002). When female survival is taken into account, the lifetime gain in fitness may be higher by having smaller, rather than larger broods in each reproductive event (Stearns 1992; Roff 2002).

Alternative hypotheses to explain the evolution of superfoetation

In the present study we tested the "reduction of the peak cost of reproduction" hypothesis to explain the adaptive significance of superfoetation. Based on our data from *P. gracilis* and *P. infans,* we can conclude that females inhabiting low resource environments do not always exhibit a higher degree of superfoetation. The results of our study were partially consistent with this hypothesis in *P. infans*, but not in *P. gracilis*, suggesting that the benefits of superfoetation may differ among species or may depend on ecological conditions other than food availability. Other hypotheses have been proposed to explain the adaptive significance of superfoetation (reviewed in Zúñiga-Vega et. al 2010). The first one proposes that superfoetation evolved to facilitate female control over paternity (Sandell 1990; Macías-Garcia and González-Zuarth 2005; Pollux et al. 2014). Due to sexual coercion by males in poeciliids species, females do not exhibit pre-copulatory mate choice, and may be forced to bear offspring of low-quality males (Macías-Garcia and González-Zuarth 2005). Varying the degree of superfoetation might allow females to modulate their investment in offspring of differing quality and/or increase the genetic diversity of offspring (Macías-Garcia and González-Zuarth 2005). The second hypothesis suggests that superfoetation is the result of morphological constraints (Thibault and Schultz 1978, Reznick and Miles 1989). There are environments where it is beneficial to have a streamlined phenotype, such as high-velocity water systems or high-predator habitats. Superfoetation may allow females carry fewer large, full-term embryos at any given time in order to avoid large increases in volume. The third hypothesis suggests that superfoetation increases the rate of offspring production by overlapping different broods (Burley 1980; Travis et al. 1987). According to this hypothesis, abundant resources allow females to overlap more than one reproductive event, leading to superfoetation and, in high-resource environments, higher fecundity. In addition, superfoetation may be favored under certain demographic pressures, such as low and unpredictable juvenile survival, low and uncertain adult survival, and high life expectancy of adult females (Thibault 1974; Downhower and Brown 1975; Burley 1980; Travis et al. 1987).

Our contrasting results in *P. gracilis* and *P. infans* indicate that the adaptive value of superfoetation is not always associated with resource availability. We recommend continuing the exploration of the potential benefits of superfoetation through field studies and controlled experiments. The manipulation of putative selective agents would provide further insight on the adaptive significance of superfoetation.

Acknowledgements

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. The present study is a partial fulfillment of the requirements for the doctoral degree (Doctorado en Ciencias Biomedicas, Universidad Nacional Autonoma de México) of PFA under the supervision of JJZV. D Piñero gave valuable comments and suggestions. Fieldwork was conducted under permits SDPA/DGVS/03492, DGOPA. 07010.210612.1749 and PPF/DGOPA-223/2013. Logistical assistance was provided by E. Ávila-Luna, J.L. Bortolini-Rosales, M. Hernández-Quiroz, P. Mendoza-Hernández, I. A. Morales-Salas, M. E. Muñiz-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-Subaie, H. Salinas-Matus, T. Sandoval, I. Solano-Zavaleta, L.F. Vázquez-Vega, R. Vega-Trejo, K. Villa-Meza, and I. Zapata-Morán.

References

Burley N (1980) Clutch overlap and clutch size: alternative and complementary reproductive tactics. Am Nat 115:223–246

Derweduwen J, Vandendriessche S, Willems T, Hostens K (2012) The diet of demersal and semi-pelagic fish in the Thorntonbank wind farm: tracing changes using stomach analyses data. In: Degraer S, Brabant R, Rumes B (eds) Offshore Wind Farms in the Belgian Part of the North Sea. Heading for an Understanding of Environmental Impacts. Royal Belgian Institute for Natural Sciences, Management Unit of the North Sea Mathematical models, pp 73-84

Downhower JF, Brown L (1975) Superfoetation in fishes and the cost of reproduction. Nature 256:345

Frías-Alvarez P, Macias Garcia C, Vázquez-Vega LF, Zúñiga-Vega JJ (2014) Spatial and temporal variation in superfoetation and related life history traits of two viviparous fishes: *Poeciliopsis gracilis* and *P. infans*. Naturwissenschaften 101:1085-1098

Galindo-Villegas J, Sosa-Lima E (2002) Gonopodial system review and new fish record of *Poeciliopsis infans* (Cyprinodontiformes: Poeciliidae) for lake Patzcuaro, Michoacan, central Mexico. Rev Biol Trop 50:1151-115

Gómez-Márquez JL, Peña-Mendoza B, Salgado-Ugarte IH, Sánchez-Herrera AK, Sastré-Baez L (2008) Reproduction of the fish *Poeciliopsis gracilis* (Cyprinodontiformes: Poeciliidae) in Coatetelco, a tropical shallow lake in Mexico. Rev Biol Trop 56:1801-1812

Gunn JS, Thresher RE (1991) Viviparity and the reproductive ecology of clinid fishes (Clinidae) from temperate Australian waters. Environ Biol Fish 31:323-344

Gutiérrez-Cabrera AE, Pulido-Flores G, Monks S, Gaytán-Oyarzún JC (2005) Presencia de *Bothriocephalus acheilognathi* Yamaguti, 1934 (Cestoidea: Bothriocephalidae en peces de Metztitlán, Hidalgo, México. Hidrobiologica 15: 283-288

Haynes JL (1995) Standardized classification of poeciliid development for life-history studies. Copeia 1995:147-154

Hrbek T, Seckinger J, Meyer A (2007) A phylogenetic and biogeographic perspective on the evolution of poeciliid fishes. Mol Phylogenet Evol 43:986-998

Hyslop EJ (1980). Stomach contents analysis —a review of methods and their application. J Fish Biol 17:411-429

Macías-Garcia C, González-Zuarth CA (2005) Reproductive behavior of viviparous fish and intersexual conflict. In: Uribe MC, Grier HJ (eds) Viviparous Fishes. Homestead: New Life Publications, Florida, pp 289-302

Mateos M, Sanjur OI, Vrijenhoek RC (2002) Historical biogeography of the livebearing fish genus *Poeciliopsis* (Poeciliidae: Cyprinodontiformes). Evolution 56:972-984

Miller RR, Mincley WL, Norris SM (2005) Freshwater fishes of Mexico. University of Chicago Press, Illinois

Pires, MN, McBride KE, Reznick DN (2007) Interpopulation variation in life-history traits of *Poeciliopsis prolifica*: implications for the study of placental evolution. J Exp Zool 307A:113-125

Pires MN, Banet AI, Pollux BJA, Reznick DN (2011) Variation and evolution of reproductive strategies. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago pp 28-37

Pollux BJA, Pires MN, Banet AI, Reznick DN (2009) Evolution of placentas in the family Poeciliidae: an empirical study of macroevolution. Annu Rev Ecol Evol S 40: 271-289.

Pollux BJA, Meredith RW, Springer MS, Reznick DN (2014) The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature 513:233-236

Reznick DN, Endler JA (1982) The impact of predation on life history evolution in trinidadian guppies (*Poecilia reticulata*). Evolution 36:160–177

Reznick DN, Miles DB (1989) Review of life history patterns in Poeciliid fish. In: Meffe GK, Snelson Jr FF (eds) Ecology and Evolution of livebearing fishes (Poeciliidae). Pretice Hall, Englewood Cliffs, New Jersey, pp 125-148

Reznick DN, Miles DB, Winslow S (1992). Life history of *Poecilia picta* (Poeciliidae) from the island of Trinidad. Copeia 1992:782-790.

Reznick D. and Yang, AP (1993). The influence of fluctuating resources on life history: patterns of allocation and plasticity in female guppies. Ecology, 2011-2019

Reznick, D, Callahan H, Llauredo R (1996) Maternal effects on offspring quality in poeciliid fishes. Am Zool 36:147-156

Reznick DN, Mateos M, Springer MS (2002) Independent origins and rapid evolution of the placenta in the fish genus *Poeciliiopsis*. Science 298:1018-1020

Reznick DN, Meredith R, Collette BB (2007) Independent evolution of complex life history adaptations in two families of fishes, live-bearing halfbeaks (Zenarchopteridae, Beliformes) and Pociliidae (Cyprinodontiformes). Evolution 61:2570-2583.

Roellig K, Menzies BR, Hildebrandt TB, Goeritz, F (2011) The concept of superfetation: a critical review on a 'myth'in mammalian reproduction. Biol Rev 86: 77–95

Roff DA (2002) Life history evolution. Sunderland: Sinauer Associates

Sandell M (1990) The evolution of seasonal delayed implantation. Q Rev of Biol 65**:**23-42

Scrimshaw NS (1944) Superfetation in poeciliid fishes. Copeia 1944:180-183

Stearns SC (1992). The evolution of life histories. Oxford: Oxford University Press.

Thibault RE (1974) Genetics of cannibalism in a viviparous fish and its relationship to population density. Nature 251:138-140

Thibault RE, Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprynodontiformes: Poeciliidae). Evolution 32:320-333

Travis J, Farr JA, Henrich S, Cheong RT (1987) Testing theories of clutch overlap with the reproductive ecology of *Heterandria Formosa*. Ecology 68:611-623

Trexler JC (1997) Resource availability and plasticity in offspring provisioning: embryo nourishment in sailfin mollies. Ecology, 78:1370-1381

Turner CL (1937) Reproductive cycles and superfetation in poeciliid fishes. Biol Bull 72:145-164

Zandona E Auer SK, Kilham SS, Howard JL, López-Sepulcre A, O'Connor MP,. Bassar RD, Osorio A, Pringle CM,. Reznick DN. (2011). Diet quality and prey selectivity correlate with life histories and predation regime in Trinidadian guppies. Funct Ecol 25:964-973.

Zúñiga-Vega JJ, Reznick D, Johnson JB (2007) Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. Oikos 116:995-1005

Zúñiga-Vega JJ, Macías-Garcia C, Johnson JB (2010) Hypotheses to explain the evolution of superfetation in viviparous fishes. In: Uribe MC, Grier HJ (eds) Viviparous fishes II. New Life Publications, Homestead, Florida USA, pp 241-253

Table 1 Sampling locations and sample sizes (*N*) of *Poeciliopsis gracilis* and *P. infans*

Population number	Geographic coordinates	Altitude (m)	State	*N*
P. gracilis				
1	21° 58´ N 99° 15´W	295.6	SLP	20
2	21° 59´N 99° 15´W	284.3	SLP	45
3	21° 58´N 98° 57´W	101	SLP	58
4	17° 25´N 95° 01´W	32.1	Veracruz	64
5	17° 12´N 95° 03´W	58.2	Oaxaca	36
6	17° 08´N 95° 07´W	89.7	Oaxaca	8
7	17° 08´N 95° 07´W	97.7	Oaxaca	50
8	17° 26´N 95° 26´W	83.1	Oaxaca	82
9	17° 33´N 95° 32´W	56.4	Oaxaca	72
10	17°50´N 95° 49´W	35.2	Veracruz	46
P. infans				
1	20° 34´N 103° 51´W	1250	Jalisco	43

2	21° 39´N 102° 57´W	1371	Zacatecas	71
3	21° 12´N 103° 22´W	1199	Zacatecas	59
4	21° 14´N 102° 20´W	1709	Jalisco	23
5	21°09´N 102°28´W	1736	Jalisco	45
6	21° 02´N 103° 25´W	1196	Jalisco	50
7	20° 33´N 103° 57´W	1259	Jalisco	40
8	20° 33´N 103° 57´W	1252	Jalisco	21
9	20° 32´N 104° 03´W	1243	Jalisco	44
10	20° 34´N 104° 09´W	1210	Jalisco	12
11	20° 09´N 103° 02´W	1537	Jalisco	57

Table 2 Results of the general linear models that examined variation in superfoetation, brood size, individual embryo mass, and reproductive allotment (RA) of *Poeciliopsis gracilis* and *P. infans*

Effect	SS	*df*	MS	*F*	*P*	*Partial* η^2*
Poeciliopsis gracilis						
Superfoetation						
Female dry mass	0.88	1	0.88	17.82	<0.0001	0.04
Population	0.68	9	0.08	1.53	0.13	0.03
Index of fullness	0.004	1	0.004	0.08	0.78	0.0001
Female dry mass x population	0.83	9	0.09	1.87	0.054	0.04
Index of fullness x population	1.05	9	0.12	2.35	0.01	0.04
Error	22.29	451	0.05			
Brood size						
Female dry mass	43.31	1	43.31	93.53	<0.0001	0.17
Population	22.90	9	2.54	5.49	<0.0001	0.10
Index of fullness	1.15	1	1.15	2.49	0.12	0.005
Female dry mass x population	54.89	9	6.10	13.17	<0.0001	0.21

Index of fullness x population	18.68	9	2.08	4.48	<0.0001	0.08
Error	208.83	451	0.46			
Individual embryo mass						
Female dry mass	3.20	1	3.20	17.37	<0.0001	0.04
Developmental stage	3.19	1	3.19	17.33	<0.0001	0.04
Population	2.37	9	0.26	1.43	0.17	0.03
Index of fullness	1.59	1	1.59	8.65	0.003	0.02
Female dry mass x population	3.01	9	0.33	1.82	0.06	0.04
Index of fullness x population	1.69	9	0.19	1.02	0.42	0.02
Error	82.87	450	0.18			
Reproductive allotment						
Female dry mass	79.06	1	79.06	163.69	<0.0001	0.27
Population	11.96	9	1.33	2.75	0.004	0.05
Index of fullness	1.10	1	1.10	2.27	0.13	0.005
Female dry mass x population	15.95	9	1.77	3.67	0.0002	0.07
Index of fullness x population	13.68	9	1.52	3.15	0.0011	0.06
Error	217.83	451	0.48			
Poeciliopsis infans						

Superfoetation						
Female dry mass	2.09	1	2.09	41.7	<0.0001	0.09
Population	0.76	10	0.08	1.52	0.13	0.03
Index of fullness	0.71	1	0.71	14.08	0.0002	0.03
Female dry mass x population	1.27	10	0.13	2.53	0.006	0.05
Index of fullness x population	1.45	10	0.14	2.90	0.002	0.06
Error	21.65	432	0.05			
Brood size						
Female dry mass	33.06	1	33.06	45.16	<0.0001	0.09
Population	13.67	10	1.37	1.87	0.047	0.04
Index of fullness	3.54	1	3.54	4.84	0.03	0.01
Female dry mass x population	15.28	10	1.53	2.09	0.02	0.05
Index of fullness x population	19.52	10	1.95	2.67	0.004	0.06
Error	316.28	432	0.73			
Individual embryo mass						
Female dry mass	0.72	1	0.72	4.09	0.044	0.01
Developmental stage	3.92	1	3.92	22.42	<0.0001	0.05
Population	2.99	10	0.30	1.71	0.08	0.04
Index of fullness	0.30	1	0.30	1.72	0.19	0.004

Female dry mass x population	3.49	10	0.35	2.00	0.03	0.04
Index of fullness x population	4.31	10	0.43	2.46	0.007	0.05
Error	75.33	431	0.17			
Reproductive allotment						
Female dry mass	64.23	1	64.23	142.66	<0.0001	0.25
Population	13.65	10	1.37	3.03	0.001	0.07
Index of fullness	10.13	1	10.13	22.50	<0.0001	0.05
Female dry mass x population	4.09	10	0.41	0.91	0.53	0.02
Index of fullness x population	19.87	10	1.99	4.41	<0.0001	0.09
Error	194.48	432	0.45			

Figure legends

Fig. 1 Relationships among fullness index (i.e. amount of food ingestion) and the degree of superfoetation, brood size, individual embryo mass, and reproductive allotment for several populations of *Poeciliopsis gracilis*. Life history traits were adjusted for the size of females. Population numbers are shown at the top right corner of each panel

Fig. 2 Relationships among fullness index (i.e. amount of food ingestion) and the degree of superfoetation, brood size, individual embryo mass, and reproductive allotment for several populations of *Poeciliopsis infans*. Life history traits were adjusted for the size of females. Population numbers are shown at the top right corner of each panel

Fig. 1 Frías-Alvarez et al.

Poeciliopsis gracilis

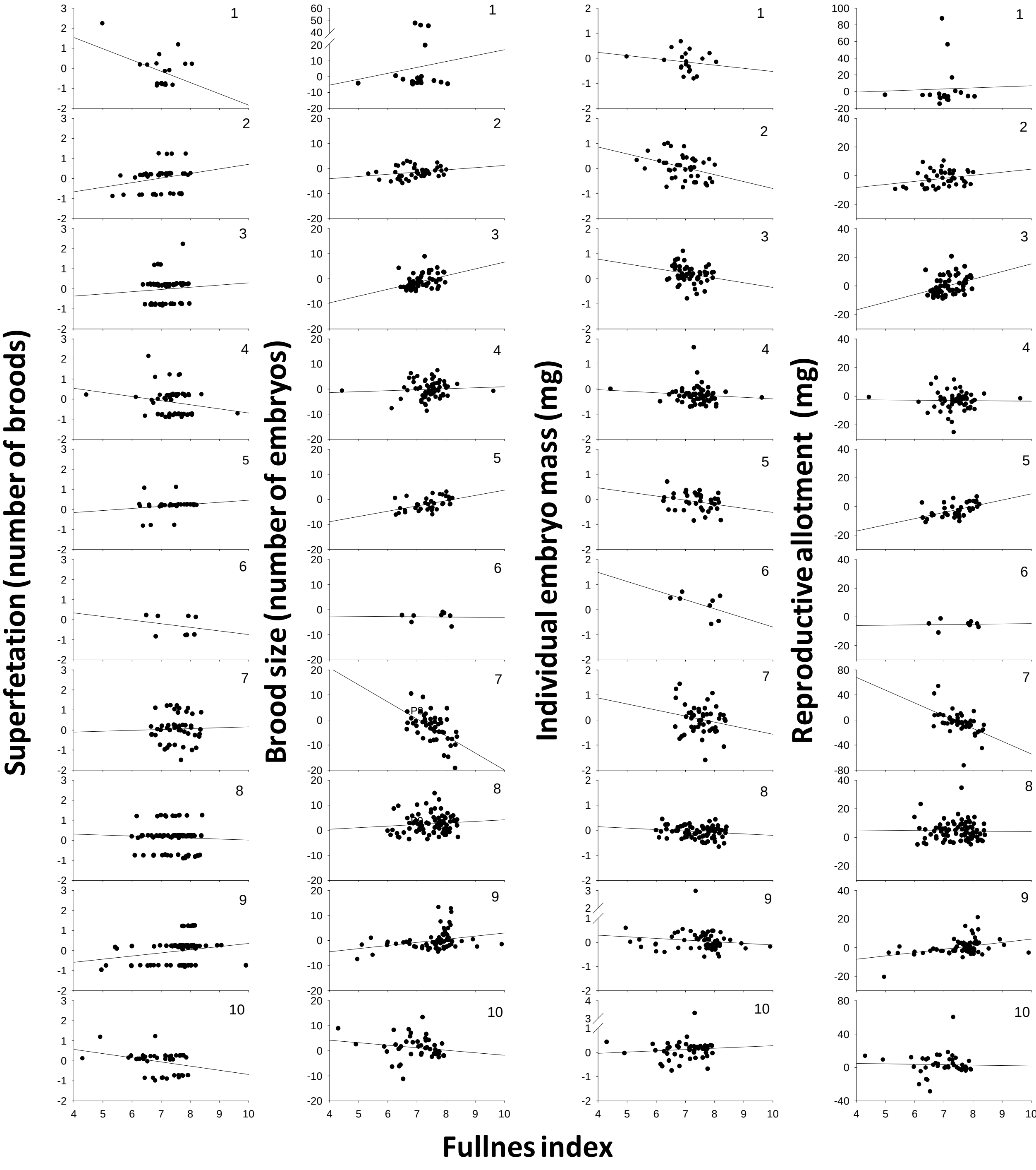
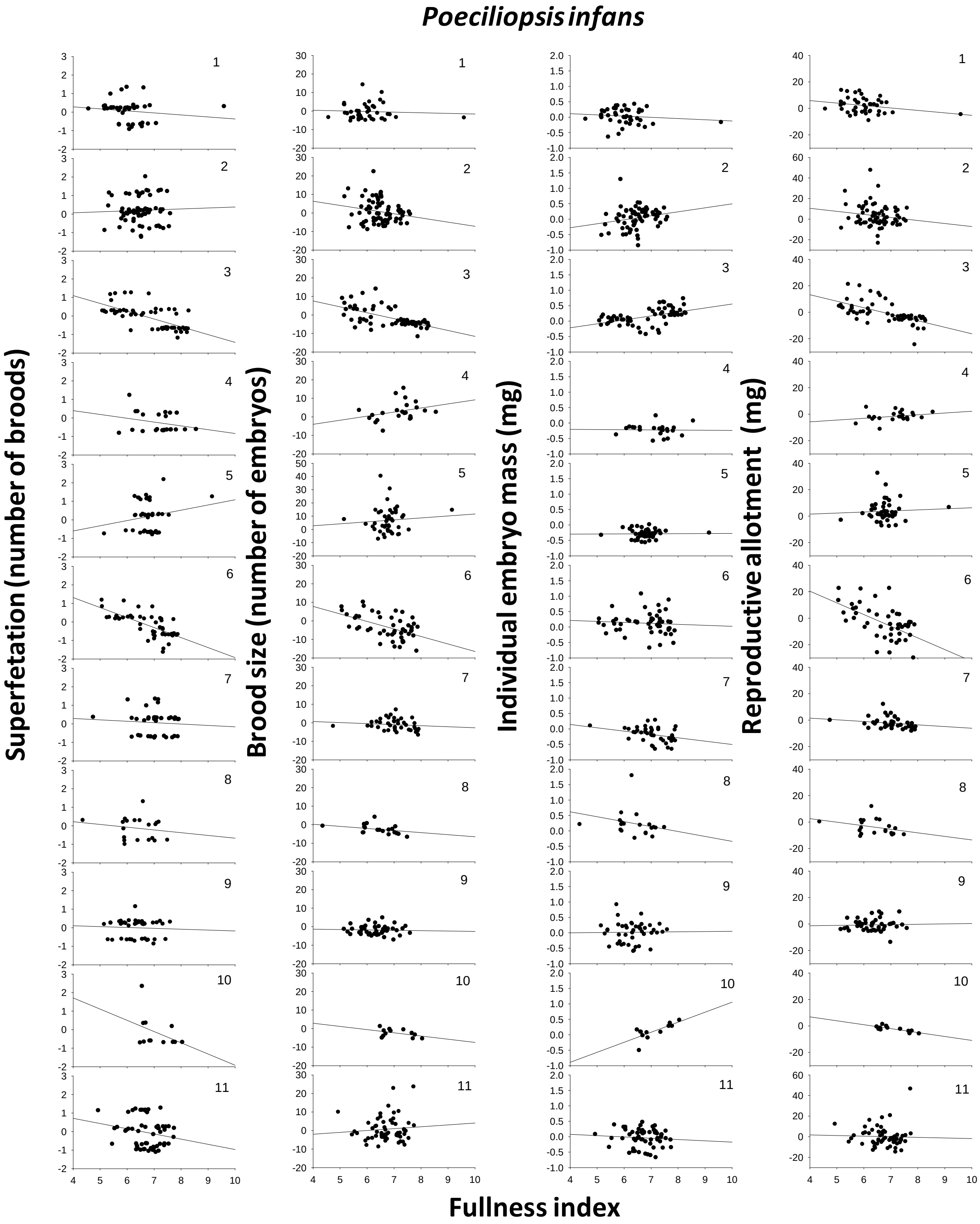


Fig. 2 Frías-Alvarez et al.



CAPÍTULO V

DISCUSIÓN GENERAL

Embriones de *P. gracilis* en diferentes estadios de desarrollo.
Camadas pertenecientes a una hembra.
Foto: Patricia Frías Álvarez

DISCUSIÓN GENERAL

Superfetación: el conocimiento previo e hipótesis desarrolladas acerca de su significado adaptativo

Previo a este trabajo de investigación, únicamente dos estudios mencionan los posibles mecanismos que han impulsado la evolución de la superfetación (Travis et al. 1987; Zúñiga-Vega et al. 2007). Travis et al. (1987) encontraron evidencia en *Heterandria formosa* que sustenta la hipótesis de que la superfetación es una adaptación que incrementa la tasa de producción de crías (i.e. incremento en la frecuencia de producción de crías). La alta mortalidad adulta favorece grandes inversiones reproductivas (para compensar la alta probabilidad de una muerte temprana). Las hembras podrían asignar más de sus recursos disponibles para producir mayor número de crías. Un excedente en los recursos ambientales puede ser utilizado por las hembras para incrementar el número de camadas. Travis et al. (1987) sugieren que un mayor grado de superfetación pude verse favorecida por un aumento en los niveles de comida. Estos autores encontraron evidencia que apoya esta predicción, con el patrón observado en hembras de *H. formosa*. Hembras criadas en laboratorio bajo los tratamientos de nivel de comida alto y medio, produjeron significativamente mayor número de camadas que aquellas hembras criadas bajo un tratamiento con poca alimentación. Estos resultados fueron consistentes con la predicción de que las hembras asignan a la reproducción tantos recursos como les son posibles, debido a que se encuentran en un medio con alta supervivencia. Asimismo Travis et al. (1987) sugieren con base en muestreos realizados en Florida que durante el invierno la mortalidad en adultos de *H. formosa* es alta.

Zúñiga-Vega et al. (2007) encontraron evidencia en *Poeciliopsis turrubarensis* que sustenta la hipótesis de que la superfetación es el resultado de una restricción morfológica. Los autores de este trabajo compararon el grado de superfetación y observaron diferencias en el fenotipo en dos tipos de poblaciones de *P. turrubarensis* en Costa Rica. La primeras poblaciones están presentes en ríos de afluente bajo o lento, los cuales se encuentran cercanos a la costa, mientras que las segundas están presentes en ríos de afluente de alta velocidad que se encuentran alejados de la costa. Los autores encontraron que las hembras lejanas a la costa y que habitan en afluentes de alta velocidad, presentaron un mayor grado de superfetación (i.e. tuvieron mayor número de camadas) y además las hembras presentaban un fenotipos más hidrodinámicos (i.e. hembras más alargadas y delgadas), a comparación de las hembras que habitan en ríos cercanos a la costa con afluentes de baja velocidad, donde las hembras tuvieron un número menor de camadas y su fenotipo fue menos hidrodinámico.

Superfetación: nuevos hallazgos e hipótesis recientemente puestas a prueba acerca de su significado adaptativo

Son escasos los estudios que muestran la variación intraespecífica en el grado de superfetación en peces pecílidos (Johnson y Bagley, 2011). Se ha documentado la variación en el grado de superfetación entre 12 poblaciones de *Poeciliopsis turrubarensis* (Zúñiga-Vega et al. 2007), la variación en la superfetación entre dos poblaciones de *P. prolifica* (Pires et al. 2007) y la variación en la superfetación entre cuatro poblaciones de *Heterandria formosa* (Leips y Travis, 1999). Los resultados obtenidos en este trabajo de investigación con *Poeciliopsis gracilis* y *P. infans* suman, al todavía pequeño número de investigaciones, información relacionada a la variación intraespecífica en el grado de la superfetación. Adicionalmente los resultados del presente trabajo revelan que existe una gran variación en el grado de superfetación (i.e. número total de camadas presentes en una hembra) tanto entre poblaciones de una misma especie como entre especies. La variación observada en el grado de superfetación en las dos especies entre los distintos meses y diferentes estaciones a través de un año indica que la superfetación puede ser el resultado de interacciones complejas entre una respuesta plástica y diferencias genéticas entre los individuos. El estudio de la plasticidad fenotípica es multidisciplinario el cual abarca aspectos ecológicos, evolutivos, conductuales, de desarrollo, y genómicos entre otros (Kelly et al. 2012). Con la habilidad de reconocer regiones genómicas o genes específicos que subyacen a la respuesta plástica se puede examinar las fluctuaciones inter-generacionales en las frecuencias alélicas de los genes que afectan directamente a la plasticidad fenotípica (Kelly et al. 2012). Un acercamiento a estudios en peces y plasticidad fenotípica y genómica se llevo a cabo en *Fundulus heteroclitus* en los cuales los patrones de expresión génica global fueron comparados entre individuos que experimentan diferentes condiciones ambientales o fenotipos (Whitehead et al. 2011).

La falta de información referente a la superfetación ha llevado a que las explicaciones adaptativas sobre este rasgo reproductor ignoren la variación temporal. Lo que podría ocasionar que se subestime el número de especies que presentan este rasgo reproductor o que no se tome en cuenta el verdadero valor del nivel de superfetación por especie. Los estudios de explicaciones adaptativas han tomado el grado la superfetación como un valor fijo, los resultados de los estudios y conclusiones de los estudios podrían cambiar debido a la variación existente en este rasgo entre temporadas, poblaciones y especies. A partir de los resultados obtenidos en el presente trabajo y por lo anteriormente explicado se propone que la investigación referente a la superfetación tanto descriptiva como evolutiva, sea realizada de ser posible en diferentes localidades (variación espacial) y a lo largo de diferentes meses y estaciones del año (variación temporal).

En el presente trabajo pusimos a prueba en varias poblaciones de *P. gracilis* y *P. infans* dos hipótesis que intentan explicar el significado adaptativo de la superfetación. La primera sugiere que la superfetación es el resultado de una restricción morfológica. Esta hipótesis puesta a prueba y corroborada en *Poeciliopsis turrubarensis* (Zúñiga-Vega et al. 2007), nos dice que la superfetación puede verse favorecida en ambientes donde se ejerce una selección de un fenotipo hidrodinámico como son los ambientes con afluentes de velocidad rápida. Bajo esta premisa las hembras que habiten en afluentes de alta velocidad de agua presentarán mayor número de camadas y un fenotipo más hidrodinámico en comparación con aquellas que habitan afluentes de baja velocidad. En el presente trabajo no se encontró evidencia para apoyar la hipótesis. En ambas especies el grado de superfetación fue complejo y varió ampliamente entre las poblaciones. Tanto la cantidad como la dirección de esta variación fue dependiente del tamaño de la hembra y esta variación inter-poblacional no estuvo asociada con la velocidad del agua. Contrario a lo que se esperaba las hembras de *P. gracilis* que presentaron un fenotipo más hidrodinámico fueron aquellas que habitan en lugares con afluentes de baja velocidad. Mientras que para *P. infans* no se encontró una relación entre la velocidad del agua y el fenotipo de las hembras. La superfetación en *P. gracilis* y *P. infans* podría estar contribuyendo a tener una producción reproductiva alta, debido a que el número de camadas simultaneas incrementa tanto como incrementa el número de embriones. Los resultados tan contrastantes encontrados sugieren que está siendo más fuerte una selección positiva en la fecundidad total, que aquellas presiones (i.e. alta velocidad en el flujo de agua) que están forzando a tener un fenotipo más hidrodinámico. Por lo tanto, se concluye que la asociación entre un mayor grado de superfetación y un fenotipo más hidrodinámico en ambientes con afluentes de alta velocidad de agua no debe ser considerada una regla general para los pecílidos

La segunda hipótesis puesta a prueba en este trabajo, menciona que la superfetación evolucionó debido a que disminuye el costo pico de la reproducción (Downhower y Brown, 1975). Esta hipótesis nos dice que la superfetación puede verse favorecida en ambientes donde la reproducción es costosa, como lo son ambientes con baja disponibilidad de recursos (Downhower y Brown, 1975; Thibault y Schultz, 1978). Bajo esta hipótesis las hembras que habiten lugares con escasos recursos presentaran mayor grado de superfetación en comparación con las hembras que habiten en lugares de alta disponibilidad de recursos. Por lo que utilizamos datos de reproducción y disponibilidad de recursos para ambas especies. Los resultados obtenidos en la tesis muestran evidencia que apoya parcialmente la hipótesis para *P. infans*. Sólo algunas poblaciones de esta especie tuvieron una relación negativa entre el grado de superfetación y la disponibilidad de alimento en el ambiente. Mientras que para *P. gracilis* no se encontró evidencia alguna que sustente la hipótesis. Estos resultados contrastan con un estudio previo realizado con *Heterandria formosa* en donde se encontró que hembras sometidas a tratamientos con niveles de comida alto y medio presentaron mayores niveles de superfetación a comparación de las hembras sometidas a tratamientos de comida bajo. Por lo que concluimos que la superfetación no siempre está asociada con la cantidad de recursos disponibles en el ambiente.

Retos futuros y nuevos campos de conocimiento acerca de la superfetación

El creciente interés por indagar el significado adaptativo de la superfetación ha llevado a los investigadores a formular otras hipótesis que tratan de entender el papel que ha jugado la selección natural en la variación intra- e interespecífica en el grado de superfetación. Macías-García y González-Zuarth, (2005) proponen que el conflicto sexual en peces puede ser el causante de la superfetación. Los pecílidos presentan una amplia variación en los rasgos reproductivos asociados con la selección sexual pre-copulatoría (Pollux et al. 2014). En algunas especies de pecílidos los machos poseen ornamentos y realizan despliegues en el cortejo (Luyten y Liley, 1985), estos típicamente tiene un gonopodio pequeño (aleta anal modificada para llevar a cabo la fertilización interna; Macías-García y González-Zuarth, 2005). En otras especies los machos y las hembras son similares y el gonopodio del macho es más largo, los machos no realizan algún tipo de cortejo y la copula se realiza por medio de la coerción (Rosen y Tucker, 1961). Los machos que ejercen coerción con las hembras imposibilitan a las hembras para seleccionar una pareja (machos evitan la selección pre-copulatoria; Bisazza 1993). Bajo este escenario las hembras se ven imposibilitadas de valorar la calidad de los machos que las inseminan, lo que podría ocasionar que las hembras lleven embriones de un macho genéticamente de baja calidad (Zeh y Zeh, 2003; Macías-García y González-Zuarth, 2005). La superfetación puede disminuir la probabilidad de que todas las camadas sean monopolizadas por un solo macho, dividiendo las crías en múltiples y pequeñas camadas separadas temporalmente, donde cada camada es fertilizada por un padre diferente.

Hasta la fecha dos trabajos muestran evidencia que sustenta esta hipótesis. Macias-García y González-Zuarth, (2005) realizaron una búsqueda en la literatura para evidenciar que existe un vínculo entre el potencial de las hembras para elegir pareja (cortejo o coerción sexual) y el modo reproductivo (superfetación o no superfetación). Los autores encontraron que existe una asociación entre el tipo de cortejo y el modo de reproducción. Las especies que presentan coerción sexual (intromisión gonopodial) presentan en su mayoría superfetación, mientras que las especies de pecílidos que presentan cortejo en su mayoría son no-superfetadoras. Pollux et al. (2014) y sus colaboradores por medio de métodos comparativos examinaron el conflicto sexual y su posible relación con la evolución de placentación en la familia Poeciliidae. Los autores encontraron que la superfetación incrementa la probabilidad de cargar camadas de múltiples padres. Esta hipótesis tan interesante puede ser puesta a prueba por medio de experimentos de elección femenina tanto en campo como en laboratorio. Además gracias a las bases teóricas de esta hipótesis se pueden realizar estudios de paternidad múltiple, bajo el supuesto de que las hembras que tengan un mayor grado de superfetación, serán más poliándricas que aquellas hembras con menor grado de superfetación. Estos dos acercamientos pueden ser llevados a cabo tanto inter como intra-poblacionalmente.

Thibault (1974) plantea que la superfetación disminuye la tasa de mortalidad juvenil**.** De acuerdo con la teoría de historias de vida cuando la mortalidad de los jóvenes es alta e impredecible en el tiempo, es selectivamente ventajoso tener una tasa de producción de crías baja y constante a través del tiempo (Hopper, 1999; Menu et al. 2000; Roff, 2002; Einum y Fleming, 2004). Si se reparte una inversión reproductiva total en pequeños eventos reproductivos, podría disminuir el riesgo de perder a toda la descendencia por un evento ambiental fortuito. La superfetación permite la distribución de las crías en pequeñas camadas a lo largo del tiempo y esta estrategia reproductora puede verse como una estrategia que presenta un esfuerzo reproductor bajo y distribuido a través del tiempo. Hasta el momento ningún trabajo empírico ha puesto a prueba esta hipótesis. Para tener una aproximación a esta hipótesis se podría utilizar una investigación demográfica mediante procedimientos de captura y recaptura. La predicción obvia es que en localidades donde exista una mortalidad alta e impredecible de juveniles, las hembras presentarán un mayor grado de superfetación.

Burley (1980) y Travis et al. (1987) plantean que la superfetación se favorece en ambientes con alta mortalidad en los adultos porque podría incrementar la tasa de producción de crías. Burley (1980) sugirió que en aves la superposición de puestas (i.e. superfetación) resulta en mayores tasas reproductivas. En un mismo periodo de tiempo, las aves que superponen huevos en comparación con las que no lo hacen, presentan un aumento en el número de crías, y además la energía total necesaria para mantener a toda la descendencia debe de ser menor. Está hipótesis no considera a la superposición de huevos como un solo evento reproductor separado a través del tiempo. Por el contrario considera a la superposición de huevos como eventos reproductores separados. Incrementar las tasas de producción de crías al costo potencial de perder futuras oportunidades reproductivas podría ser ventajoso si la probabilidades de supervivencia adulta es baja o es incierta (Gadgil y Bossert, 1970; Roff, 1992, 2002; Reznick et al. 1996). Entonces, la superposición de camadas (i.e. superfetación) pudo haber evolucionado como un mecanismo que compensa una alta mortalidad en adultos (Travis et al. 1987).

Hasta el momento no se tiene evidencia empírica que sustente esta hipótesis. Sin embargo Travis et al. (1987) mencionan que existe una alta mortalidad en adultos de

Heterandria formosa durante el invierno, lo cual podría estar asociado con el alto número de camadas que producen las hembras de esta especie (hasta 8 de manera simultánea). Bajo este escenario también una investigación demográfica mediante procedimientos de captura y recaptura, en diferentes estaciones del año, podría ayudar a sustentar la hipótesis.

En conjunto, los resultados obtenidos en el presente trabajo y los datos de investigaciones preliminares nos muestran que la presión selectiva que está actuando sobre la variación en la superfetación es dependiente del hábitat donde se encuentran los organismos y que la superfetación es dependiente de la especie. Por lo tanto, el significado adaptativo de la superfetación puede depender de condiciones ecológicas particulares y/o puede diferir entre especies. La variación tan compleja observada en La velocidad en el flujo de agua parece ser la fuerza selectiva que está actuando en la variación de la superfetación en *P. turrubarensis* (Zúñiga-Vega et al. 2007), mientras que una alta disponibilidad en los recursos está relacionado con la variación en el grado de superfetación en *Heterandría formosa* (Travis et al. 1987). Al contrario ambientes donde puede ser costosa la reproducción como lo son hábitats con recursos escasos están asociados con la variación intraespecifícca en la superfetación en *P. infans*.

La variación en el grado de superfetación tanto intra como interespecifica nos indica que la superfetación podría deberse a factores únicos de cada individuo. Los estudios enfocados en las hormonas nos pueden dar un acercamiento a este enfoque individualizado. Las hormonas en pecílidos influencian su morfo-fisiología y su comportamiento reproductivo, por ejemplo las hormonas pueden regular el cortejo y el comportamiento agresivo en pecílidos (Gabor y Grober, 2010). En hembras de *Poecilia reticulata* los estrógenos promueven la vitalogénesis, el crecimiento de ovocitos y facilitan el parto (Georgescu et al. 2013), mientras que el estradiol mantiene la receptividad en las hembras (Liley, 1972). Hasta el momento no se tiene ningún conocimiento sobre los mecanismos hormonales que promueven la superfetación. Posiblemente cambios en los niveles de ciertas hormonas y/o interacciones entre ellas, pueden estar relacionados con las diferencias en los niveles de superfetación. Otro acercamiento a un enfoque individualizado podría ser el estudio del costo metabólico que conlleva la superfetación a las hembras, tanto los estudios hormonales como de costo metabólico podrían ser llevados a cabo de manera intra como inter-poblacional. Sin duda el estudio de hormonas y el estudio del costo metabólico así como su relación con la superfetación son un nuevo campo de conocimiento por desarrollar.

Hasta el momento no tenemos evidencia suficiente que aclare el origen de la superfetación en pecílidos. Sabemos que existen diferentes fuerzas evolutivas que han mantenido la variación de la superfetación dentro y entre las especies. Estas fuerzas selectivas están actuando de diferentes formas en las especies y posiblemente exista una interacción entre estas fuerzas evolutivas en la variación de la superfetación. Con los conocimientos hasta el momento obtenidos queda un gran camino que recorrer para entender el significado adaptativo de la superfetación. Poner a prueba de nuevo las hipótesis ya investigadas con diferentes especies de pecílidos, así como poner a prueba las hipótesis que aun no han sido exploradas son algunos de los caminos que se pueden realizar para responder esta interesante e intrigante pregunta.

BIBLIOGRAFÍA DEL CAPÍTULO V

Bisazza A (1993) Male competition, female mate choice and sexual dimorphism in Poeciliids fishes. Marine and Freshwater Behaviour and Physiology 23:257-286.

Burley N (1980) Clutch overlap and clutch size: alternative and complementary reproductive tactics. American Naturalist 115:223-246.

Downhower JF, Brown L (1975) Superfoetation in fishes and the cost of reproduction. Nature 256:345.

Hopper HR (1999) Risk-spreading and bet-hedging in insect population biology. Annual Review of Entomoly 44:535-560.

Einum S, Fleming IA (2004) Environmental unpredictability and offspring size: conservative versus diversified bet-hedging. Evolutionary Ecology Research 6:443-455.

Gabor CR, Grober MS (2010) A potential role of male and female androgen in species recognition in a unisexual-bisexual mating complex. Hormones and Behavior 57: 427-433.

Gadgil M, Bossert PW (1970) Life historical consequences of natural selection. American Naturalist 104: 1-24.

Georgescu B, Boaru A, Georgescu CE (2013) Endocrine control of reproduction in the guppy fish (*Poecilia reticulata*). Poecilid Research 3:1-6

Johnson JB, Bagley JC (2011) Ecological drivers of life-history divergence. In: Evans JP, Pilastro A, Schlupp I (eds) Ecology and evolution of poeciliid fishes. University of Chicago Press, Chicago, pp 38-49.

Kelly SA, Panhuis TM, Stoehr AM (2012). Phenotypic plasticity: molecular mechanisms and adaptive significance. Comprehensive Physiology 2:1417-1439.

Leips J, Travis J (1999) The comparative expression of life-history traits and its relationship to the numerical dynamics of four populations of the least killifish. Journal of Animal Ecology 68:595-616.

Liley NR (1972) The effects of estrogens and other steroids on the sexual behavior of the female guppy, *Poecilia reticulata*. General and Comparative Endocrinology. Supplement. 3:542-552.

Luyten PH, Liley NR (1985) Geographic variation in the sexual behaviour of the guppy, *Poecilia reticulata* (Peters). Behaviour 95:164-179.

Macías-Garcia C, González-Zuarth CA (2005) Reproductive behavior of viviparous fish and intersexual conflict. In: Uribe MC, Grier HJ (eds) Viviparous Fishes. Homestead: New Life Publications, Florida, pp 289-302.

Menu F, Roebuck JP, Viala M (2000) Bet-hedging diapause strategies in stochastic environments. American Naturalist 155:724-734.

Reznick DN, Butler MJ IV, Rodd FH, Ross P (1996) Life-history evolution in guppies (*Poecilia reticulate*) 6 Differential mortality as a mechanism for natural selection. Evolution 50:1651-1660.

Roff DA (1992) The evolution of life histories: theory and analysis. New York: Chapman and Hall.

Roff DA (2002) Life history evolution. Sunderland: Sinauer Associates.

Rosen DE, Tucker A (1961) Evolution of secondary sexual characters and sexual behavior patterns in a family of viviparous fishes (Cyprinodontiformes: Poeciliidae). Copeia 1961:201-212.

Pires MN, McBride KE, Reznick DN (2007) Interpopulation variation in life-history traits of *Poeciliopsis prolifica*: implications for the study of placental evolution. Journal of Experimental Zoology 307A:113-125.

Pollux BJA, Meredith RW, Springer MS, Reznick DN (2014) The evolution of the placenta drives a shift in sexual selection in livebearing fish. Nature 513:233-236.

Travis J, Farr JA, Henrich S, Cheong RT (1987) Testing Theories of clutch overlap with the reproductive ecology of *Heterandria Formosa*. Ecology 68:611-623.

Thibault RE, Schultz RJ (1978) Reproductive adaptations among viviparous fishes (Cyprynodontiformes: Poeciliidae). Evolution 32:320-333.

Whitehead A, Galvez F, Zhang S, Williams LM, Oleksiak MF (2011) Functional genomics of physiological plasticity and local adaptation in killifish. Journal of Heredity 102: 499-511.

Zeh JA, Zeh DW (2003) Toward a new sexual selection paradigm: polyandry, conflict and incompatibility. Ethology 109:929-950.

Zúñiga-Vega JJ, Reznick D, Johnson JB (2007) Habitat predicts reproductive superfetation and body shape in the livebearing fish *Poeciliopsis turrubarensis*. Oikos 116:995-1005.

ANEXO I

Se anexan varias preguntas de interés relacionadas con el tema de la superfetación

¿Cuál es el papel de la matrotrofia y la lecitotrofia en la superfetación y cómo se reflejaría en las hipótesis antes propuestas?

Reznick and Miles, (1989) observaron que la mayoría de las especies que superfetan son matrotróficos (la madre provisiona de alimento a los embriones después de la fertilización). Al parecer existe una fuerte asociación entre estos dos rasgos sugiriendo que la evolución de uno de estos dos rasgos facilita la evolución de otro (la matrotrofia facilita la evolución de la superfetación). Sin embargo la excepción rompe la regla y se tiene a *Poeciliopsis monacha* especie superfetadora y además lecitotrófica (la provisión de alimentos ocurre antes de la fertilización) indicando que estos rasgos pueden evolucionar independientemente uno de la otro. Pero también indica que la evolución de un rasgo puede facilitar la subsecuente evolución de otro.

La relación que tiene la madre con los embriones a nivel trófico varía desde especies completamente lecitotróficas (los nutrientes de los embriones son dados exclusivamente por la yema del huevo que fue asignada antes de la fertilización) hasta especies completamente matrotróficas (los nutrientes de los embriones son obtenidos directamente de la madre). En algunas especies la lecitotrofia está presente con la matrotrofia (los embriones obtienen nutrientes tanto de la yema como de la madre).

El índice de matrotrofia IM nos indica que tanto una placenta de pecílido esta transfiriendo sus recursos. En nuestros casos tanto *P. gracilis* (MI =0.69) como *P. infans* (MI=0.86) son especies consideradas como predominantemente lecitrotroficas. El conflicto entre los nutrientes que da la madre a los embriones es nulo debido que la madre asigna los nutrimentos antes de la fertilización.

Hipotesis 1. La superfetación disminuye el costo pico de la reproducción. Bajo esta hipótesis se nos dice que la superfetación debió de haber evolucionado porque reduce la inversión reproductora máxima que deben de hacer las hembras en un determinado momento. Se asume que la superfetación (i.e., superposición de huevos) es un esquema de redistribución de un solo evento reproductor pero en componentes pequeños y dispersos temporalmente. Bajo esta hipótesis se espera que la superfetación se vea favorecida en ambientes donde la reproducción sea costosa, como en ambientes inestables con baja disponibilidad en los recursos alimenticios.

Hipótesis 3. La superfetación disminuye la tasa de mortalidad juvenil.
Bajo esta hipótesis la superfetación debió de haber evolucionado en ambientes donde la probabilidad de sobrevivir de los jóvenes o adultos es muy baja debido a que una tasa baja de producción de crías debe ser ventajosa. Thibault (1974) propone que en peces pecílidos, cuando la supervivencia de las crías es muy variable e impredecible, distribuir la producción de crías a través del tiempo dará como resultado a largo plazo menores tasas de mortalidad juvenil. Las hembras superfetadoras pueden producir el mismo número de crías que una hembra no superfetadora, sin embargo, si la superfetación permite la distribución de las crías en pequeñas camadas a lo largo del tiempo, la superfetación puede verse como una estrategia del tipo “mejor apostador” (i.e., esfuerzo reproductivo bajo y dispersado a través del tiempo; Zúñiga-Vega et al. 2010).

Hipótesis 5. La superfetación incrementa la tasa de producción de crías.

Bajo esta hipótesis ambientes o temporadas con supervivencia adulta baja o estocástica deben de estar asociados a mayores niveles de superfetación en comparación con los ambientes o temporadas con una supervivencia adulta estable. En síntesis, la superfetación, a través de la superposición de diferentes puestas, podría acelerar las tasas de producción de crías, bajo el costo potencial de perder futuras oportunidades reproductoras.

Relación de la lecitotrofia con superfetación

Para un grupo de hembras que tienen la producción de crías lecitotróficas, la superfetación disminuirá la inversión de recursos en cada camada debido a que existe una reducción de tamaño de camada (se relaciona con la hipótesis 1, se asume que la superfetación es un esquema de redistribución de un solo evento reproductor pero en componentes pequeños y dispersos temporalmente y se ve beneficiada en ambientes costosos reduciendo el costo pico de la reproducción) *pero incrementaría la frecuencia a la cual cada camada es iniciada* (se relaciona con la hipótesis 3, se asume que cuando la superfetación permite la distribución de las crías en pequeñas camadas a lo largo del tiempo, esto es que aumenta la frecuencia, la superfetación puede verse como un esfuerzo reproductivo bajo y dispersado a través del tiempo, mientras que se relaciona con la hipótesis 5, la cual nos dice que la superfetación a través de la superposición de diferentes puestas, podría acelerar las tasas de producción de crías, esto es relacionado con el incremento en la frecuencia de cada camada, pero bajo el costo potencial de perder futuras oportunidades reproductoras).

Las dos especies estudiadas en el presente trabajo tiene un IM < 1 por lo cual se relacionan con lo anteriormente descrito. Sin embargo a continuación se describe lo que ocurre para especies matrotroficas. En las especies con un índice de matrotrofia >1 la masa de los embriones aumentaría de una manera no lineal durante el desarrollo de tal manera que durante el desarrollo se observará un incremento de la inversión de la madre, pero se espacian estos periodos de incremento de inversión así menos crías se encuentran en una fase de desarrollo en un determinado en el tiempo.

¿Puede haber competencia de embriones por nutrimentos dentro de la madre?

Si, y esto se ha observado en el pecílido *Heterandria formosa* (Schrader y Travis 2009). Con la evolución de la matrotrofia se provocaron cambios que modifica el momento de la inversión maternal en los embriones, estos cambios fueron desde antes de la fertilización a el periodo entre fertilización y nacimiento e introduce un potencial conflicto genómico entre madre y los embriones. Los embriones matrotróficos pueden influenciar el nivel de inversión que ellos reciben durante el desarrollo y existe un trade-off entre la inversión maternal para cada embrión y entre la supervivencia materna o la fecundidad.

Schrader y Travis (2009) encontraron diferencias en las historias de vida entre dos poblaciones silvestres y cruzas de laboratorio de *H. formosa.* Las hembras silvestres de una población produjeron embriones mucho más grandes que la segunda población y encontraron que la magnitud de este efecto maternal dependía del genotipo de las crías (i.e., del padre). En las cruzas del laboratorio entre individuos de estas dos poblaciones encontraron que el genotipo de las crías (de que padre descendían) ejerce una influencia en el nivel de inversión maternal y afecta la fecundidad a través de tasas altas de abortos entre embriones y un bajo número de embriones que llegan a término. Se refieren a abortos cuando los embriones nacían antes de que la cavidad pericárdica cerrara y por lo general se encontraban muertos.

Ahora bien los efectos observados del padre no ocurren de la misma manera en especies lecitotroficas, donde la influencia del genotipo del padre se observa hasta la siguiente generación, cuando el genotipo de las crías de la hembra ejerce un efecto materno sobre el tamaño de las crías. Podemos decir que en camadas con múltiples padres, la variación observada en el aprovisionamiento de nutrimentos que sucede en intra-camadas podría ser el resultado de selección criptica de la hembra, o aprovisionamiento selectivo de descendencia heterocigota (podría aumentar el crecimiento o supervivencia). Y como se dijo anteriormente también podría ser el resultado de impronta génica.

Schrader y Travis (2012) mencionan que una consecuencia de la superfetación es la competición entre camadas. La combinación de matrotrofia y superfetación crea una situación que es análoga a la eclosión asincrónica en aves (ver hipótesis 5) en este caso la madre simultáneamente provisiona a los jóvenes dependientes, diferentes estados de desarrollo y potencializa diferentes habilidades competitivas. Ellos mencionan que la combinación de matrotrofia con superfetación permite una competición asimétrica entre los embriones de diferentes camadas. Los autores mencionan que en especies matrotróficas con altos niveles de superfetación existe una disminución del tamaño de crías con el orden en que nacen a comparación de aquellas especies con superfetación y lecitrotrofia. Encontraron evidencia a favor con *Heterandria formosa* (la especies con mayor grado de superfetación).

En las especies lecitrotroficas es poco probable que exista competición debido a que no existe aprovisionamiento de alimentos de embriones después de la fertilización.

¿Cómo asegura la hembra el éxito de nacimiento del mayor número de crías, si existe la posibilidad de competencia entre estos? Especialmente entre camadas de diferentes machos.

Estas dos preguntas se encuentran muy de la mano con la primera parte de la respuesta anterior, lo cual está ligado a la paternidad múltiple. La superfetación incrementa el control femenino sobre la paternidad. La superfetación pudo haber evolucionado como un mecanismo para incrementar la variabilidad genética de las crías. Un gran número de especies de pecílidos las hembras se ven imposibilitadas de seleccionar a su pareja, lo cual podría ocasionar que estas terminen cargando una camada grande de una pareja de baja calidad genética. La superfetación podría ser un mecanismo para aumentar la variabilidad genética de las crías por medio de partirlas en pequeños grupos de distintos padres. Cada camada sería de una padre diferente. Estas hembras que no pueden controlar la calidad genética de sus crías, pueden promover la diversidad genética de sus camadas apareándose con diferentes machos y asignando un esfuerzo reproductor bajo a las camadas de cada una de sus parejas reproductoras. Una mayor variabilidad genética podría significar que las crías pueden tener diferentes respuestas al medio ambiente en el que nacen, la hembra no asegura, sin embargo recolecta diferentes genes que aseguran que alguna o varias de sus camadas puedan sobrevivir de un medio determinado.

¿Qué posibilidad hay de “canibalismo”?

En otros peces como es el caso de los tiburones existe la adelfofagía (i.e., el canibalismo intrauterino), y en Godeidos también encontramos el caso de la ovofagia (i.e., la práctica de los embriones de alimentarse de huevos producido por el ovario materno). En el caso de los pecílidos no he se ha tenido algún reporte de canibalismo intrauterino o de ovofagía. Sin embargo se ha reportado para *Heterandria formosa* que las hembras sufren abortos (Schader and Travis, 2009). Bajo mi experiencia de la elaboración de este trabajo tanto en gabinete como en laboratorio, las dos especies observadas no presentaron reabsorción de huevos o de embriones. Varios reportes mencionan la lucha de hermanos por recursos, únicamente en especies matrotróficas. Hoy en día se está haciendo un nuevo modelo que involucre la evolución de historias de vida en las que se tome en cuenta tanto la matrotrofia, la lecitotrofia así como la superfetación.

BIBLIOGRAFÍA DEL ANEXO I

Reznick DN, Miles DB (1989) Review of life history patterns in Poeciliid fish. En: Meffe GK., Snelson Jr. FF. (Editores). Ecology and Evolution of livebearing fishes (Poeciliidae). Pretice Hall, Englewood Cliffs, New Jersey. 125-148 pp.

Schrader M, Travis J (2012) Variation in offspring size with birth order in placental fish: a role for asymmetric sibling competition? Evolution 66:272-279.

Schrader M, Travis J (2009) Do embryos influence maternal investment? Evaluating maternal-fetal coadaptation and the potential for parent-offspring conflict in a placental fish. Evolution, 63: 2805-2815.

Thibault RE (1974) Genetics of cannibalism in a viviparous fish and its relationship to population density. Nature 251:138-140.

Zúñiga-Vega JJ, Macías-García C, Johnson JB (2010) Hypotheses to explain the evolution of superfetation in viviparous fishes. En: Uribe MC., Grier HJ. (Editores). Viviparous Fishes II. New life publications, Homestead, Florida. 13-30 pp.

MI QUERIDO INGENIERO MAICOL S. ATILANO LEY COATI...

... De aquí en adelante solo ... Fiii Fiii Fiii Fiii